Original Article

***Euterpe Oleracea* (Açaí) Modifies Sterol Metabolism and Attenuates Experimentally-Induced Atherosclerosis**

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Aim: *Euterpe oleracea* (açaí) is a fruit from the Amazon region whose chemical composition may be beneficial for individuals with atherosclerosis. We hypothesized that consumption of *Euterpe oleracea* would reduce atherosclerosis development by decreasing cholesterol absorption and synthesis.

Methods: Male New Zealand rabbits were fed a cholesterol-enriched diet (0.5%) for 12 weeks, when they were randomized to receive *Euterpe oleracea* extract ($n=15$) or water ($n=12$) plus a 0.05% cholesterol-enriched diet for an additional 12 weeks. Plasma phytosterols and desmosterol were determined by ultra-performance liquid chromatography and mass spectrometry. Atherosclerotic lesions were estimated by computerized planimetry and histomorphometry.

Results: At sacrifice, animals treated with *Euterpe oleracea* had lower levels of total cholesterol ($p=0.03$), non-HDL-cholesterol ($p=0.03$) and triglycerides ($p=0.02$) than controls. These animals had smaller atherosclerotic plaque area in their aortas ($p=0.001$) and a smaller intima/media ratio ($p=0.002$) than controls, without differences in plaque composition. At the end of the study, campesterol, β -sitosterol, and desmosterol plasma levels did not differ between groups; however, animals treated with *Euterpe oleracea* showed lower desmosterol/campesterol ($p=0.026$) and desmosterol/ β -sitosterol ($p=0.006$) ratios than controls.

Conclusions: Consumption of *Euterpe oleracea* extract markedly improved the lipid profile and attenuated atherosclerosis. These effects were related in part to a better balance in the synthesis and absorption of sterols.

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Key words; *Euterpe oleracea* (açaí), Atherosclerosis, Lipids, Desmosterol, Phytosterols

Introduction

Euterpe oleracea (açaí) is a typical fruit from the Amazon region, largely consumed in Brazil and exported to many countries in Europe, Asia, and the Americas.

Chemical studies revealed that *Euterpe oleracea* is rich in anthocyanic compounds and several polyphenols¹⁻³.

Beyond the presence of compounds with antioxidant properties, *Euterpe oleracea* improves endothelial function through the action of nitric oxide and release of endothelium-derived hyperpolarizing factor⁴.

The oil from *Euterpe oleracea* is composed of fatty acids of nutritional value (60% monounsaturated and 13% polyunsaturated) in addition to fiber and vitamin E⁵.

Recently, some beneficial effects of *Euterpe oleracea* on the lipid profile were reported in a study of hypercholesterolemic rats⁶; however, these properties for lipid metabolism and atherosclerosis are poorly reported and understood.

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Thus, we decided to investigate the potential benefits of *Euterpe oleracea* in rabbits with diet-induced atherosclerosis. Beyond the effects of *Euterpe oleracea* on the lipid profile and atherosclerosis, its effects on markers of cholesterol absorption and synthesis were also examined. These markers and their ratios have been studied in prospective studies and are considered reliable surrogates for measures of cholesterol absorption and synthesis, and predictors of cardiovascular events⁷⁻¹⁰.

Methods

Extract of *Euterpe Oleracea*

Fruits from *Euterpe oleracea* were obtained in the Amazon region (Para State, Brazil) at the same food store. Briefly, the extract was prepared daily by combining water and fruit in a blender. The extract was filtered, separated from the seeds, packed in plastic bags, which were sealed immediately to avoid contamination, and sent daily to the animal facility. Experimental and control animals were immediately offered bowls containing water with and without fresh extract (80 mL) from *Euterpe oleracea*, respectively.

The chemical composition of *Euterpe oleracea* was provided by EMBRAPA⁵ and the plant sterol content was determined in samples of the *Euterpe oleracea* extract by gas chromatography with flame ionization detection (GC FID)¹¹ (presented in **Table 1**). *Euterpe oleracea* has 15.4 mg plant sterols in 100 g extract, of which 85.0% is β -sitosterol (13.1 mg/100 g), 8.2% stigmasterol (1.3 mg/100 g), and 2.0% campestanol (0.3 mg/100 g), with traces of other plant sterols.

Animals and Diet

Adult male New Zealand white rabbits ($n=27$, age=3 months, weight=2,600-3,000 g) were studied. The study protocol was approved by the local ethics committee and all animals received proper care, in compliance with guidelines of the Brazilian College of Experimental Animals. The animals were individually housed and provided with a light/dark cycle. Every day, the cages were cleaned and chow and water (with or without the *Euterpe oleracea* extract) were changed.

The rabbits were weighed at the beginning of the study and thereafter at 12 and 24 weeks. Before sacrifice, the animals were anesthetized with xylazine (5 mg/kg, Rompun; BayerAG, SP, Brazil) and ketamine (35 mg/kg, Ketalar; Parke-Davis, USA). The animals initiated the study protocol after an adaptation period of 15 days. During the first 12 weeks, the animals were fed a regular diet (Nuvilab; Nuvital, Brazil) plus 0.5% cholesterol (C8503; Sigma-Aldrich, San Diego,

Table 1. Chemical composition of *Euterpe oleracea* [5]

Content	Amount*
Dried matter (%)	15.0
Proteins (g/100 g)	13.0
Lipids (g/100 g)	48.0
Monounsaturated fat (g/100 g)	28.8
Polyunsaturated fat (g/100 g)	6.24
Carbohydrates (g/100 g)	1.50
Fructose (g/100 g)	0.00
Glucose (g/100 g)	1.50
Sucrose (g/100 g)	0.00
Fibers (g/100 g)	34.0
Energy (kcal/100 g)	66.3
Ash (g/100 g)	3.50
Sodium (mg/100 g)	56.4
Potassium (mg/100 g)	932
Calcium (g/100 g)	286
Magnesium (g/100 g)	174
Iron (g/100 g)	1.50
Copper (g/100 g)	1.70
Zinc (g/100 g)	7.00
Phosphorus (g/100 g)	124
Vitamin B ₁ (g/100 g)	0.25
α -tocopherol (g/100 g)	45.0

*in dried form

CA, USA). After this period, the cholesterol content of the diet was reduced to 0.05% and the animals were randomized to receive the *Euterpe oleracea* extract ($n=15$) or water ($n=12$) for an additional 12-week period.

Biochemical Analyses

After a 12-h fasting period, blood samples were collected from the central ear artery of all animals and assayed. Samples were stored at -80°C and defrosted at room temperature for 2 h prior analysis. Measurements were assayed by automated techniques in an Olympus AU-640 system (Olympus, Nagano, Japan). Serum lipids were determined by enzymatic colorimetric methods using appropriate reagents (Olympus) for total cholesterol (TC), HDL-cholesterol (HDL-C) and triglycerides. Non-HDL-cholesterol was calculated by differences between TC and HDL-C. Blood glucose levels were determined by an automated enzymatic method (Advia 1650; Bayer, Germany).

β -sitosterol and campesterol (markers of sterol absorption) as well as desmosterol (precursor of endogenous cholesterol synthesis) were measured at week 24 by ultra-performance liquid chromatography (UPLC) and mass spectrometry (MS). Plasma samples

were evaluated for these sterols by a method developed at Synchrophar (Campinas, SP, Brazil)¹². This method consisted of liquid-liquid extraction, followed by separation in a UPLC system and detection in an Atmospheric Pressure Chemical Ionization (APCI) ion-source MS operating on "single-ion monitoring" for each sterol (β -sitosterol, campesterol, and desmosterol). Extraction was carried out with diethyl ether and n-hexane (80/20; v/v) by vortex mixing followed by evaporation of the organic phase at 50°C under a gentle N₂ stream. After evaporation, the residue was reconstituted with isopropanol and injected onto the UPLC column (Acquity Waters Co., Milford). The MS system (Quattro Premier-XE; Waters Co., Manchester, UK) was adjusted to monitor single ions formed by an APCI ion source. Sterols were detected in their free (non-esterified) forms, monitoring the ions for desmosterol ($m/z=367.30$), β -sitosterol ($m/z=397.25$), and campesterol ($m/z=383.60$). Compound concentrations were determined by comparing the peak response with the linear portion of the calibration curve (0.5–10.0 $\mu\text{g/mL}$). Samples with concentrations higher than 10.0 $\mu\text{g/mL}$ were properly diluted for comparison with the calibration curve. Results are presented in mg/dL. To evaluate the relation between cholesterol absorption and synthesis, the ratios between desmosterol/campesterol and desmosterol/ β -sitosterol were calculated⁷.

Histopathology and Immunohistochemistry

Specimens of the aortas were excised from the ascending arch to the iliac bifurcation and examined as previously reported¹³. The aortas were cut longitudinally, fixed in 10% buffered (pH 7.4) formalin (Labsynth, SP, Brazil), and the lipid-enriched areas were identified with 1-[4-(Phenylazo) Phenylazo]-2-Naphthol (Sudan red III) dye (Science Lab, TX, USA). Plaque-containing areas in the aortas were estimated by computerized planimetry using Image Tool software version 3.0 (University of Texas Health Science Center at San Antonio (UTHSCSA), TX, USA). The percentage of the lesion area was calculated as the ratio between the lesion and the total areas of the aorta. Fragments from the arch, thoracic and abdominal aortas were processed for histology with hematoxylin and eosin (Nuclear, Alkimia, PR, Brazil) staining. Sections (4- μm thick) of these specimens were stained with Verhoeff's elastic fiber (Nuclear, Alkimia), evaluated for intima (I) and media (M) areas using Image Tool software version 3.0 and the I/M ratio was then calculated. Monoclonal antibodies were used to determine macrophages (RAM-11; DAKO Corp., CA, USA) and smooth muscle actin (HHF-35; DAKO Corp.).

The streptavidin-biotin-peroxidase complex (C-004; DAKO Corp.) method was used; the sections were stained by adding diaminobenzidine solution and counterstained with Harris hematoxylin (Nuclear, Alkimia). For quantification of macrophages and smooth muscle cells in the intima layer, the greatest lesion observed in the histological section was analyzed. Digitalized images of two atherosclerotic plaque sections (magnification: 400) including the most stained areas were recorded using an imaging system QColor3 (Olympus America Inc.), and positively stained areas were determined by morphometric Image Tool software version 3.0. The histological structure of the atherosclerotic lesions was analyzed in HE-stained sections, the collagen content was observed under polarized light in sections stained by Sirius red, and collagen was quantified by a computer-assisted image system. All histological analyses were performed in a blinded fashion.

Statistical Analysis

Numerical data were expressed as the mean (SD). Continuous variables were examined for normal distribution by the Kolmogorov-Smirnov test. Variables with normal distribution were compared between groups using Student's unpaired *t* test; in the case of non-Gaussian distribution, the Mann-Whitney *U*-test was used. The Friedman test was used to compare repeated measures of weight throughout the study. All tests were two-tailed and statistical significance was set at $p < 0.05$. Analyses were performed using SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Food Intake and Weight Gain

The animals consumed an average of 100 g/day of the diet and 80 mL water with/without *Euterpe oleracea* extract. The diet and the extract of *Euterpe oleracea* were well tolerated and the animals gained weight throughout the study ($p < 0.0005$, Friedman test) in both groups. No difference in animal weight was observed between groups at randomization in week 12 and week 24 (Table 2).

Biochemistry

The biochemical analyses performed at week 12 (before randomization) showed that the cholesterol-enriched diet (0.5%) promoted marked hypercholesterolemia. All lipid values were comparable between groups at this time point. Conversely, at the end of the study, animals treated with *Euterpe oleracea* extract showed lower serum levels of total cholesterol, non-

Table 2. Body weight, lipids and glucose serum levels at 12 and 24 weeks, by group

	<i>Euterpe oleracea</i> (n = 15)	Control (n = 12)	p values
12 weeks			
Body weight, mean (SD)	2,893 (137)	2,867 (171)	0.44
Total cholesterol, mean (SD)	1,124 (96)	1,087 (84)	0.31
HDL-C, mean (SD)	36 (11)	37 (12)	0.85
Non HDL-C, mean (SD)	1,088 (91)	1,050 (86)	0.28
Triglycerides, mean (SD)	305 (234)	424 (282)	0.25
Glucose, mean (SD)	124 (13)	139 (36)	0.68
24 weeks			
Body weight, mean (SD)	3,108 (197)	2,930 (267)	0.07
Total cholesterol, mean (SD)	167 (113)	341 (224)	0.03
HDL-C, mean (SD)	28 (9)	30 (10)	0.56
Non HDL-C, mean (SD)	139 (109)	310 (220)	0.03
Triglycerides, mean (SD)	83 (91)	164 (112)	0.02
Glucose, mean (SD)	118 (19)	112 (17)	0.86

Abbreviations: HDL-C: high-density lipoprotein-cholesterol; SD: standard deviation; TC: total cholesterol. Mean values and SD are expressed in mg/dL; comparisons between groups were made using Student's *t* test, except for body weight, glucose levels and triglycerides, compared using the Mann-Whitney *U*-test.

Table 3. Plasma markers of cholesterol absorption and synthesis at week 24, by group

	<i>Euterpe oleracea</i> (n = 15)	Control (n = 12)	p value
Campesterol, mean (SD)	10.5 (11.0)	16.7 (15.6)	0.260
Sitosterol, mean (SD)	3.0 (2.0)	3.9 (3.2)	0.370
Desmosterol, mean (SD)	0.5 (0.6)	1.2 (1.3)	0.140
Desmosterol/campesterol, mean (SD)	0.04 (0.04)	0.08 (0.04)	0.026
Desmosterol/ β -sitosterol, mean (SD)	0.12 (0.12)	0.25 (0.11)	0.006

SD: standard deviation. Mean values and SD are expressed in mg/dL; comparisons between groups were made using the Mann-Whitney *U*-test.

HDL-C and triglycerides than control animals. Serum glucose levels did not differ between groups at weeks 12 and 24 (**Table 2**).

At the end of the study, plasma levels of campesterol, β -sitosterol, and desmosterol did not differ between groups; however, lower desmosterol/campesterol and desmosterol/ β -sitosterol ratios were observed in animals treated with *Euterpe oleracea* extract (**Table 3**).

Histopathology and Immunohistochemistry

In our model, classic atherosclerosis was observed in the aortas of control rabbits challenged by the cholesterol-enriched diet; however, marked attenuation in the degree of atherosclerosis was observed among animals treated with *Euterpe oleracea* extract. In these animals, a smaller plaque area was observed, especially in the aortic arch and thoracic aorta, as well as smaller

intima/media ratios in the same regions than in controls (**Fig. 1** and **2**). No differences were observed between groups regarding the media layer in the areas of the arch, thoracic or abdominal aortas and the percent areas occupied by macrophages and smooth muscle cells in their aortas (**Table 4**). No difference was observed either in the histological aspect of the plaques or in plaque composition regarding elastic fibers, collagen, smooth muscle cells, and macrophage content when analyzed by histomorphometry (**Table 4**). **Fig. 3** shows pictures of representative specimens of the aortas from animals in both groups and photomicrographs of the aorta sections.

Discussion

The present study confirmed our initial hypothe-

Fig. 1. (A) Box plots of aortic arch plaque area. A marked reduction in this parameter was observed in animals treated with *Euterpe oleracea* extract ($p=0.001$ vs control group, Mann-Whitney *U*-test). (B) Box plots of the aortic arch intima/media ratio. Animals treated with *Euterpe oleracea* extract had lower intima/media ratios ($p=0.002$ vs control group, Mann-Whitney *U*-test).

Fig. 2. Box plots of plaque area as measured by planimetry. (A) Box plots of plaque area. Smaller plaque areas were observed in the aortas of animals treated with *Euterpe oleracea* extract ($p=0.005$ vs control group, Mann-Whitney *U*-test). (B) Box plots of the aortic plaque percent area. Reduced plaque areas were observed in the aortas of animals treated with *Euterpe oleracea* extract ($p=0.028$ vs control group, Mann-Whitney test).

sis that consumption of *Euterpe oleracea* can attenuate atherosclerosis. In addition to the previously reported possible antiatherosclerotic properties of *Euterpe oleracea*, mainly based on the high content of antioxidant compounds in their fruits¹⁴⁻¹⁶, our study has shown that consumption of *Euterpe oleracea* extract improved

the balance between cholesterol absorption and synthesis, decreased serum cholesterol and triglycerides, and attenuated atherosclerosis. It has been reported by Matthan *et al.* that impaired cholesterol homeostasis, reflected by increased cholesterol absorption and low synthesis markers, is the profile associated with preva-

Fig. 3. Representative images of the aorta stained by Sudan III of rabbits fed a high-cholesterol diet-*Euterpe oleracea*-treated animal (A) and Control (B). The photomicrographs show histological aorta fragments stained by HE (C and H), Verhoeff for elastic fibers (D and I); immunohistochemical reaction for macrophages (E and J) and SMA (F and K) and picrosirius polarized stain for collagen (G and L) (magnification 100x). Photomicrographs C to G are from *Euterpe oleracea* group and H to L are from Control group.

HE=hematoxylin & eosin; SMA=smooth muscle actin

Table 4. Plaque composition of aortas at week 24, by group

Plaque component	<i>Euterpe oleracea</i> (n = 15)	Control (n = 12)	p value
Macrophages, mean (SD)	20.4 (15.5)	22.7 (18.3)	0.56
Smooth muscle actin, mean (SD)	13.3 (4.8)	12.7 (7.0)	0.66
Elastic tissue, mean (SD)	0.5 (0.6)	1.2 (1.3)	0.26

Macrophages, smooth muscle actin and elastic tissue are expressed as the percentages of aortic areas. Comparisons between groups were made using the Mann-Whitney *U*-test.

lent cardiovascular disease in the Framingham Offspring population⁸⁾. In addition, in the Prospective Cardiovascular Munster Study high β -sitosterol or campesterol levels were markers of cardiovascular events among middle-aged males⁹⁾; however, recent data from the Spanish EPIC cohort have shown that phytosterols levels were inversely associated with incident cardiovascular disease¹⁷⁾. Furthermore, Gylling *et al.* have demonstrated that cholesterol absorption and synthesis are inherited traits¹⁸⁾.

The classic rabbit model of diet-induced atherosclerosis is highly dependent on the rates of cholesterol absorption and the levels of serum cholesterol achieved. Thus, attenuation of atherosclerosis by the *Euterpe oleracea* extract could be a consequence of decreased absorption of dietary cholesterol; however, this hypothesis appears insufficient to explain our findings, since there were no significant differences between the two groups of animals for markers of cholesterol absorption (β -sitosterol and campesterol).

Inhibition of cholesterol synthesis could be the second mechanism involved in the reduced development of atherosclerosis among animals receiving the *Euterpe oleracea* extract. Again, attenuation of atherosclerosis in animals treated by the *Euterpe oleracea* extract may not be explained by reduced synthesis of cholesterol, since these animals did not differ in the levels of desmosterol; however, differences in the desmosterol/campesterol and desmosterol/ β -sitosterol ratios were observed between groups. Thus, consumption of *Euterpe oleracea* seems to contribute to a better balance between cholesterol synthesis and absorption.

We investigated the content of phytosterols in *Euterpe oleracea* and found that 15 mg in 100 g extract were plant sterols, of which β -sitosterol was the most abundant. This amount of plant sterols may have contributed, at least in part, to the lower absorption of cholesterol added to the diet, explaining the reduction in total cholesterol observed in our study¹⁹⁾. In fact, previous studies have shown that a high content of fiber and phytosterols can decrease the rate of cholesterol absorption^{20, 21)}. The composition of the extract

of *Euterpe oleracea*, which has these two constituents, may be associated with a lower rate of cholesterol absorption by the small intestine.

In addition, part of the benefits in the lipid profile could also be attributed to the fatty acids present in the *Euterpe oleracea* extract⁵⁾. One or more compounds in the chemical composition of *Euterpe oleracea*, with a high content of monounsaturated and polyunsaturated fatty acids, can reduce cholesterol levels due to increased expression of LDL receptors in the liver^{22, 23)} followed by cholesterol excretion via the biliary tract.

It is also possible that other cholesterol-independent mechanisms of absorption and synthesis are involved in decreased atherosclerosis development, as observed among animals treated with *Euterpe oleracea* extract. In this context, the high content of antioxidants, mainly polyphenolic compounds, lignans, and other constituents, may have contributed to the decrease in plaque formation²⁴⁾.

Improved endothelial function is another mechanism that might be involved in atherosclerosis attenuation. Rocha *et al.*⁴⁾ showed that *Euterpe oleracea* extract increases endothelium-dependent vasodilation, an effect that is attenuated by the addition of N(G)-nitro-L-arginine methyl ester (L-NAME) and abolished by the addition of KCl + L-NAME.

We previously demonstrated improved endothelial function by statins²⁵⁾ and by angiotensin converting-enzyme inhibitor²⁶⁾, accompanied by attenuation of atherosclerosis development in rabbits, independent of changes in serum cholesterol levels.

Recently, favorable effects on glucose levels and insulin resistance were reported following the use of *Euterpe oleracea* extract in an experimental model of metabolic syndrome²⁷⁾.

Based on the findings of our study and the mechanisms discussed above, the reduction of atherosclerosis observed in these animals probably results from the sum of multiple beneficial actions of *Euterpe oleracea*, including decreased ratios of markers of cholesterol synthesis and absorption, reduced oxidative

modification of lipoproteins, increased expression of LDL receptors, and improved endothelial function. In addition, preliminary data involving the use of *Euterpe oleracea* extract in humans have shown favorable findings among subjects with metabolic syndrome, which includes improvement in the lipid profile and decreased post-prandial glucose levels²⁸⁾. Furthermore, a novel anti-inflammatory property of *Euterpe oleracea* was recently reported, involving inhibition of the gene expression of adhesion molecules and nuclear factor kappa B activation induced by glucose and lipopolysaccharide²⁹⁾.

Study Limitations

Our study examined the effects of *Euterpe oleracea* in animals challenged by an atherogenic diet. It is possible that the beneficial effects observed on atherosclerosis in this model could be of a higher magnitude than on atherosclerosis related to other classic risk factors observed in humans.

Our study did not evaluate endothelial function or the antioxidant actions of *Euterpe oleracea*. Regarding these parameters, the known beneficial effects of antioxidant compounds present in the *Euterpe oleracea* extract may have contributed to our histological findings.

We did not assess intestinal expression of NPC1L1 due to the unavailability of commercial antibodies for rabbits.

Conclusion

Consumption of *Euterpe oleracea* extract by rabbits with diet-induced hypercholesterolemia markedly improved the lipid profile and attenuated aortic atherosclerosis. These effects were related, at least in part, to a better balance between synthesis and absorption of sterols, surrogate markers of cholesterol homeostasis.

Conflicts of Interests

None.

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