Author's response to reviews

Title: Oral treatment with Euterpe oleracea Mart. (acai) extract improves cardiac dysfunction and exercise intolerance in rats subjected to myocardial infarction

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Author's response to reviews: see over
Answers to reviewers

Reviewer 1 (PL):

1. The reviewer commented: Clarify: "acai" OR "açaí" (text vs reference list);

The word “açaí” has been altered in the manuscript.

2. The reviewer commented: Explain the choice of the dose and treatment period;

The dose selected (100 mg/kg) was based on the fact that 50 mg/kg did not alter the parameters observed after MI. The duration of treatment was chosen based on previous work in which the reduction of diastolic dysfunction induced by MI was reduced after 4 weeks of treatment with an inotropic agent (Gabriel et al., 2010).

3. The reviewer commented: Concerning the "oral treatment": in drinking water or by gavage?

Oral administration was done by gavage. That information has been included in Methods.

4. The reviewer questioned: Can this oral treatment change water or food intake?

Unfortunately, those parameters were not evaluated.

5. The reviewer questioned: Did açaí treatment alter adiposity?

Unfortunately, adiposity was not evaluated.

Reviewer 2: (EB)

1. The reviewer commented: in the Abstract, it should be stated which coronary artery was occluded;

The authors agreed with the reviewer and the phrase “anterior descendent coronary artery” has been included in the abstract.

2. The reviewer commented: if the number of rats with MI is 6 and this group was subdivided into 2 subgroups, one has only 3 rats per group;

Twelve rats were submitted to MI and after surgery were randomly subdivided into 2 groups (6 for each). The reviewer well noted the error in the abstract and the number 6 has been altered to 12.
3. The reviewer questioned: is there an increase in mean arterial pressure in MI rats with the treatment with açai: 115.3 +/- 7.24 mmHg (placebo) versus 130.0 +/- 8.16 mmHg (açai)?

There was not significant increase of systolic pressure in the MI-treated group when compared to sham group.

4. The reviewer questioned: why sham rats were not treated?

Previously, Costa et al described that systolic blood pressure, which was measured by using the tail-cuff plethysmography, was not altered in normotensive rats by treatment of extract (200 mg/kg) during 6 weeks (Naunyn-Schmiedeberg's Arch Pharmacol (2012) 385:1199). Thus, the authors had previous demonstration that açai extract did not affect blood pressure, which was the reason for not testing in sham group.

5. The reviewer commented: one table should be added with weight, mean blood pressure, heart frequency, etc for each subgroup;

All parameters evaluated in the experimental groups were shown as graphics. The inclusion of a table with those data should be repetitive.

6. The reviewer stated: the discussion should be shortened. As it stands it is confuse and somewhat repetitive.

As suggested by the reviewer, the discussion has been altered and shortened.

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“In the present study, we demonstrated that the development of deleterious effects in MI rats was prevented by oral treatment with açai extract for 4 weeks. Treatment with açai prevented the reduction of exercise resistance during the exercise test procedure, in addition to preventing cardiac hypertrophy, LV fibrosis, and hemodynamic changes, such as reductions of systolic arterial pressure, LV systolic pressure, and relaxation rate (-dp/dt), and enhancement of the LV end-diastolic pressure.

In the exercise test protocol, MI rats ran for shorter distances compared to sham rats. Intolerance to physical exercise has been characterized in different animal models of heart failure [15], as well as in humans [16-18]. In this study, açai extract treatment of the MI group for 4 weeks prevented a reduction in exercise tolerance, with rats performing at a similar level to sham rats. This beneficial effect might be partly related to the vasodilator action of açai through the NO/sGC/cGMP pathway [12]. This action might improve blood flow in the skeletal muscle and, hence, exercise capacity. In addition, diastolic dysfunction is the primary mechanism responsible for dyspnea and muscle fatigue in heart failure subjects [19]. The role of açai in preventing exercise intolerance might also be related to the delayed development of cardiac hypertrophy, reduction in the LV end-diastolic pressure, and an increase in the rate of relaxation (-dp/dt) of cardiac muscle from MI rats.
Plasma levels of proinflammatory cytokines seem to increase with heart failure, and have a predictive prognostic value [20]. MI induces leukocyte recruitment to the injured myocardium, which contributes to myocardial damage [5] which could be reduced by açai extract. Several studies have demonstrated the anti-inflammatory action of *E. oleracea*. More recently, Moura et al. [14] demonstrated that *E. oleracea* extract reduces acute lung inflammation in mice, by decreasing the numbers of alveolar macrophages and neutrophils in lung sections and decreasing TNF-α expression in lung homogenates. Another important action of *E. oleracea* in the controlling inflammatory process is the inhibition of NO production by reducing the expression of iNOS [13].

ROS and oxidative stress might also exacerbate myocardial damage after MI. Increasing numbers of hydroxyl radicals and superoxide anions during heart ischemia lead to destruction of the cell membrane, lipid peroxidation, and damage to the antioxidative defense system [2,3]. Experimental and clinical studies have shown that the infarct size of myocardial necrosis may be limited by antioxidant agents [4]. Several researchers have demonstrated the beneficial effects of açai as an antioxidant agent [11,21-23]. Açai juice increases the expression of antioxidant enzymes, such as glutathione reductase and glutathione peroxidase 3, in the aorta of apolipoprotein E-deficient mice.

Myofibroblast persistence after MI promotes fibrosis and myocardial remodeling, leading to increased myocardial stiffness, systolic and diastolic dysfunction, LV hypertrophy, arrhythmia, neurohormonal activation, and, ultimately, heart failure [6]. In the current study, the development of cardiac fibrosis was prevented in MI rats that were treated orally with açai extract, based on the observed reduction of collagen deposition in the LV. Thus, treatment with açai extract has beneficial effects in delaying cardiac remodeling, and represents a novel therapeutic agent to prevent heart failure resulting from MI.”