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Original article

# Hypolipemiant and antioxidant effects of *Eugenia brasiliensis* in an animal model of coconut oil-induced hypertriglyceridemia

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ABSTRACT

We investigated the effects of chronic administration of crude hydroalcoholic extract (CHE) and crude acetone extract (CAE) obtained from leaves of *Eugenia brasiliensis* species on hypertriglyceridemia and oxidative stress caused by the chronic administration of coconut oil. Rats received CHE or CAE (50, 100 or 150 mg/kg, orally) for 30 days, plus coconut oil (2 mL, orally) or saline for 15th. Triglyceride levels, liver cell lipid accumulation, thiobarbituric acid reactive substances (TBA-RS), total sulfhydryl content and the activities of antioxidant enzymes catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) were evaluated in the blood and liver of rats. Results showed that chronic administration of CHE or CAE was able to prevent hypertriglyceridemia and decrease the lipid droplets in liver cells, as well as the increase in TBA-RS, the reduction in total sulfhydryl content and CAT activity in the blood and prevent total or partial the increase in CAT and reduction in SOD and GSH-Px activities in the liver. These findings indicate that both extracts may have hypolipidemic and antioxidant effects.

## 1. Introduction

Dyslipidemia is a risk factor for cardiovascular diseases, causing lipid peroxidation and contributing to reactive oxygen species (ROS) production, resulting in the deterioration of tissues [1,2]. Epidemiological data show that about 25% of adults in the United States present hypertriglyceridemia (HTG), a lipid disorder usually characterized by elevated plasma triglyceride levels (> 150 mg/dL) and severe HTG (> 500 mg/dL) [3,4]. HTG may be associated with genetic and environmental factors, including lifestyle (alcohol, tobacco, high-carbohydrate diet and obesity) [5,6] and it is recommended that those demonstrating increased plasma triglyceride levels to measures to reduce these levels.

Free radicals are continuously produced and have important biological functions; however, they may also play a role in pathological conditions. These molecules can be degraded by several mechanisms, and a fine balance exists between the generation and degradation of these species for the maintenance of cellular homeostasis. Under some circumstances, however, an imbalance between the formation of reactive species and antioxidant defenses occurs, where the failure to degrade or neutralize these ROS can cause the oxidative stress that is associated with a number of pathological conditions, including dyslipidemia [2,1].

The use of medicinal plants dates from the beginning of humanity, and constitutes an area of research that aims to identify bioactive chemicals with potential for the development of new drugs with therapeutic benefit [7]. The Myrtaceae family comprises about 100 genera, including *Eugenia*, *Plinia* and *Psidium*, and about 3.000 species. The family has two major dispersion centers, in the Americas and Australia, although these plants can be found all over the world [8]. The species,

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*Eugenia brasiliensis (E. brasiliensis)* Lam is also known by the names "grumixama", "grumixameira" and "cereja-brasileira". This species can be found between the Northeast region of Brazil and the pluvial Atlantic forest of south Brazil, as well as in Santa Catarina state, in the region of Florianópolis, Brusque, Ibirama, Itajaí and Joinville [9].

The *E. Brasiliensis* species presents several bioactive compounds and, in addition to its fruit being used as food, it is also consumed for therapeutic purposes, with reports that it displays antidiarrheal and diuretic [10]; antimicrobial [11,12]; antiinflammatory [13], and antioxidant activities [14,15]. Flavonoids such as quercetin, rutin, myricetin and galangin can be extracted from the leaves of this plant, in addition to other phenolic compounds such as catechin, gallocatechin, gallic acid [13], triterpenebetulin, α-amyrin, β-amyrin acid, 29-hydroxy-oleanolic [12] and ursolic acid [16].

Given the high prevalence of HTG [3], and the vast number of diseases, medicines and environmental factors that can cause to HTG in the population, as well as the side effects caused by currently available drugs employed for HTG, the search continues to identify further substances to limit triglyceride levels. Studies regarding the therapeutic potential of the family Myrtaceae are scarce [12], as such the present study aimed to determine the *in vivo* effects of the chronic administration of crude hydroalcoholic and acetonic extracts of *E. brasiliensis* on HTG and oxidative stress in a rat model of HTG induced by the administration of coconut oil.

## 2. Materials and methods

### 2.1. Plant material

Leaves from *E. brasiliensis* were collected in Florianópolis, Santa Catarina state, Brazil (27°36′13.65″S, 48°31′14.75″W), in March 2012. Plant material was identified by Dr. Daniel de Barcellos Falkenberg from the Botany Department of the Universidade Federal de Santa Catarina (UFSC), and a voucher specimen was deposited in the herbarium FLOR of the same institution under registry number 34675.

#### 2.1.1. Preparation of the extracts of E. brasiliensis

Fresh, whole, young and healthy leaves of the species *E. brasiliensis* were used to obtain the extracts. To obtain the crude hydroalcoholic extract (CHE), the plant material was dried and milled, totaling 1813 g of material. This material was macerated in hydroalcoholic solution (92.8%, $w.w^{-1}$) for seven days. The extract was filtered and solvent evaporated in a rotary evaporator (below 60 °C) coupled with a vacuum condenser, and concentrated to a reduced volume. After total evaporation of the solvent, 192.5 g of crude extract was obtained, which represented a yield of 10.62% of plant material.

To obtain the crude acetonic extract (CAE), leaves were subjected to a process of extraction with acetone. Whole leaves, young and healthy (1320 g) were immersed in acetone for 5 min [17]. After this time, the extract was filtered and the solvent evaporated using a rota-evaporator (at a temperature below 60 °C) with a condenser under vacuum attached, until its complete evaporation. After total evaporation of the solvent, 87.0 g of CAE was obtained, which represent a yield of 6.59% of plant material.

### 2.2. Animals and experimental protocols

Male Wistar rats (230–280 g), obtained from the Univali University, Itajaí, Santa Catarina, Brazil, were housed and acclimated for 7 days to allow adaptation to the new environment, and were maintained on a 12 h light/12 h dark cycle at a constant temperature (22 ± 1 °C), with free access to water and food. The animals were housed 6 per cage and the "Principles of Laboratory Animal Care" (NIH publication 85–23, revised 1985) were followed in all experiments. The experimental protocol was approved by the Ethics Committee for Animal Research of the Universidade da Região de Joinville, Joinville, Brazil, under the protocol number 002/2015-PRPPG/CEP. All chemicals were purchased from Sigma Chemical Co., St Louis, MO, USA.

### 2.3. Experimental protocols

The rats were divided into 14 groups (n = 6–7). The CHE, CAE and saline (control group) were administered once time a day, by gavage, totaling 30 days, and in the middle of the treatment (15th), the animals also started to receive coconut oil (2 mL per day, by gavage) or saline divided in 2 administrations per day, for 15 days, as follow (Fig. 1):

The animals were sacrificed by decapitation 24hs after the last administration, in the absence of anesthesia. The blood was collected and the liver removed for the evaluation of oxidative stress parameters, triglycerides and histopathological analysis.

The amount of coconut oil administered followed the protocol established by Jun et al. [18], and the CHE and CAE doses (50, 100 and 150 mg/kg) were chosen based on the studies of Kar et al. [19] and Ravi et al. [20,21].

### 2.4. Erythrocyte and plasma preparation

Erythrocytes and plasma were prepared from total blood samples obtained from rats. For erythrocyte separation, peripheral blood was collected and transferred to heparinized tubes, which were centrifuged

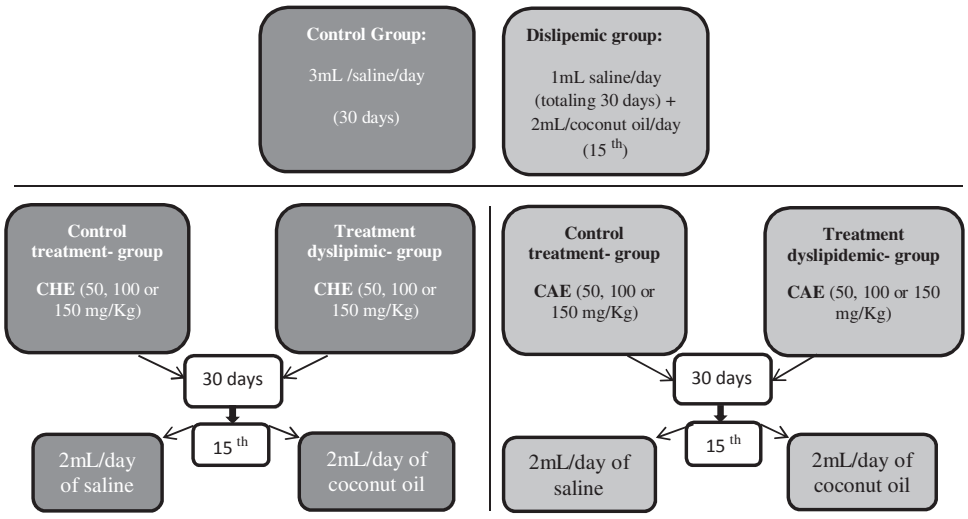


**Fig. 1.** All administrations were orally (by gavage). The rats were divided into Control group that received 1 mL of saline once a day for 15 days and afterwards received 1 mL of saline three times a day (totaling 30 days); Dyslipidemic group that received 1 mL of saline once a day for 15 days and afterwards received (for 15 days) 1 mL of saline once a day and 1 mL of coconut oil twice a day (totaling 30 days); Control treatment group + CHE or CAE that received CHE or CAE of *E. brasiliensis* (50, 100 or 150 mg/kg) once a day for 30 days and from the 15th day received 1 mL of saline twice a day for 15 days; Treatment dyslipidemic-group + CHE or CAE that received CHE or CAE of *E. brasiliensis* (50, 100 or 150 mg/kg) once a day for 30 days andfrom the 15th day received 1 mL of coconut oil twice a day for 15 days. CHE: crude hydroalcoholic extract; CAE: crude acetonic extract.