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Study of oxidative and inflammatory parameters in LDLr-KO mice treated with a hypercholesterolemic diet: Comparison between the use of *Campomanesia xanthocarpa* and acetylsalicylic acid

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ABSTRACT

Background: : Atherosclerosis is an inflammatory disease that affects the arterial wall leading to myocardial, cerebral, and peripheral ischemic syndromes. The use of low doses of aspirin inhibits platelet aggregation and inflammation and prevents cardiovascular mortality. However, ASA may produce hemorrhagic events. Thus, several studies have sought new natural compounds to suppress platelet aggregation without causing serious adverse effects.
Purpose: : In this sense, this study aims to compare the effects of *Campomanesia xanthocarpa* plant extract with those of acetylsalicylic acid (ASA) on inflammatory parameters observed in homozygous mice knockout for the low-density lipoprotein receptor (LDLr-KO) treated with a hypercholesterolemic diet.
Material and Methods: : In this study, 28 male LDLr-KO mice were divided into three groups and fed a hypercholesterolemic diet for 4 weeks. Thereafter, the animals that received the hypercholesterolemic diet were treated for 5 days with (1) distilled water, (2) *C. xanthocarpa* extract, or (3) acetylsalicylic acid. The levels of inflammatory markers were assessed in the blood samples. The gastric tolerability of the animals after oral administration of the treatments was assessed through quantification of the lesions in the gastric mucosa.
Results: : The levels of proinflammatory cytokines IL-1, IL-6, TNF-α, and INF-γ were reduced to 19.2 ± 3%, 20.4 + 1.3%, 24.7 ± 1.2%, and 20.8 ± 1.7%, respectively, in the group treated with *C. xanthocarpa*, when compared to control group. Furthermore, treatment with plant extract significantly increased the levels of the anti-inflammatory cytokine IL-10 by 27.3 ± 5.9%, but ASA showed no significant effect on the same cytokines when compared to the control group, with the exception of IL-10, which presented an increase of 8.6 ± 3.5%. Treatments with *C. xanthocarpa* and ASA also caused significant reductions of 26.4 ± 3% and 38.4± 6% in the serum levels of oxLDL, respectively. However, only treatment with *C. xanthocarpa* reduced the levels of

Abbreviations: LDL, low-density lipoprotein; oxLDL, oxidized low-density lipoprotein; ASA, acetylsalicylic acid; COX, cyclooxygenase; PGI, prostaglandin; LX, lipoxin; TNFα, tumor necrosis factor-alpha; IL, interleukin; NFκB, nuclear factor kappa B; ROS, reactive oxygen species; HMGr, 3-hydroxy-3-methyl-glutaryl-CoA reductase; LDLr-KO, knockout for the low-density lipoprotein receptor; IFN-γ, interferon gamma; 15R-HETH, 15R-hydroxyeicosatetraenoic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; HPLC, high performance liquid chromatography.

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anti-oxLDL antibodies when compared with the control (25.8 ± 6%). In addition, the analyzed extract did not induce ulcerogenic activity, while ASA induced the formation of lesions.
Conclusion: : In conclusion, treatment with *C. xanthocarpa* causes anti-inflammatory activity in hypercholesterolemic animals, with results superior to those obtained with the use of ASA.

Introduction

Cardiovascular diseases are the main cause of death and morbidity throughout the world, representing 40% of the mortality rate in western societies (Soehnlein, 2015). According to the World Health Organization (WHO), in 2015, approximately 30% of the world mortality was attributed to cardiovascular diseases, with atherosclerosis being the main pathological cause (Countries et al., 2010). Atherosclerosis is an inflammatory disease that affects the arterial wall leading to myocardial, cerebral, and peripheral ischemic syndromes (Maranhao and Leite, 2015). One of the main risk factors associated with atherosclerosis is the accumulation of low-density lipoprotein cholesterol (LDL) in the intima-media layer of the artery (Maranhao and Leite, 2015).

The endothelial dysfunction caused by cardiovascular risk factors causes structural changes that allow the accumulation and retention of LDL in the arterial intima, thereby leading to the development of atheromatous plaques (Nouri et al., 2015). In this context, the LDL was oxidized, leading to the formation of oxidized LDL (oxLDL), which triggers the expression of adhesion molecules and secretion of proinflammatory cytokines by endothelial cells, along with the deposition of cytokines derived from platelets. These effects lead to infiltration of immune cells in the intima, such as monocytes and macrophages. These release more cytokines, chemokines, and adhesion molecules that contribute to atherogenesis, in addition to the rupture or erosion of advanced atherosclerotic lesions, thus exacerbating the activation and aggregation of platelets on the ruptured plaque surface (Ridker and Lüscher, 2014).

In addition to playing a key role in hemostasis, platelets aid in inflammatory and immune processes, as they release proinflammatory cytokines after activation (Rondina et al., 2013). Thus, effective treatments that seek a secondary prevention of atherosclerotic disease have been proposed (Ridker and Lüscher, 2014), such as the use of the nonselective cyclooxygenase (COX) inhibitor acetylsalicylic acid (ASA) (Cyrus et al., 2002). Studies have revealed that the use of low doses of aspirin (81 mg, recommended by the American Heart Association) inhibits platelet aggregation and inflammation and prevents cardiovascular mortality (Clarke et al., 1991; Cyrus et al., 2002).

However, despite the consensus that ASA can prevent cardiovascular ischemic disorders, this drug may produce hemorrhagic events (Johnson, 2008). Thus, several studies have sought new natural compounds to suppress platelet aggregation without causing serious adverse effects (Lau et al., 2009; Ryu et al., 2009). Therefore, different studies with plants have demonstrated efficacy against atherosclerosis, with emphasis on *in vivo* and *in vitro* experimental studies. (Viecili et al., 2014; Papoutsi et al., 2008). *Campomanesia xanthocarpa* Berg. (Myrtaceae), popularly known as “guavirova,” which is one of the plants used in studies, exhibits a broad spectrum of physiological effects (Lorenzi, 2008), including antiplatelet, antithrombotic, and fibrinolytic activity in mice when administered orally, compared with ASA (Klafke et al., 2012).

It has also been demonstrated that *C. xanthocarpa* exerts a hypolipidemic effect, by inhibiting hydroxy-3-methylglutaryl-coenzyme A reductase (HMGr), besides decreasing oxidative stress and improving the levels of nitric oxide (Klafke et al., 2010, Viecili et al., 2014). However, although this plant has demonstrated anti-atherosclerotic activity, its anti-inflammatory activity, with a focus on atherosclerosis, has not yet been studied, since inflammation is one of the main factors involved in the initiation, progression, and complications of the disease (Hansson and Hermansson, 2011; Weber and Noels, 2011).

Therefore, the objective of this study was to evaluate the anti-inflammatory and antioxidant effects of *C. xanthocarpa* and ASA in knockout mice for the low-density lipoprotein receptor (LDLr-KO) (Ishibashi et al., 1994, Zadelaar et al., 2007).

Materials and methods

Preparation of the plant and extract of C. xanthocarpa

The leaves of the *C. xanthocarpa* plant were collected from a tree in the city at Cruz Alta, RS -Brazil (RS; 28º38’19”S, 53º36’23”W, 452 m) and were identified by the herbarium of the University of Cruz Alta (number 1088). This material was sanitized in a process that involved a 0.4% sodium hypochlorite solution bath during 1 h, followed by washing in running potable water for 15 minutes, according method previously described by Klafke et al. (2012). Then, the material was dried at 40–45 °C in oven and triturated to a fine powder. In order to carry out the tests, a *C. xanthocarpa* extract was prepared using 500 mg of dry leaves that was added to 30 ml of water and kept under 37 °C and constant agitation for 30 minutes. After, this solution was filtered and evaporated to determine total dry content. The final powder was diluted in water and then adjusted to the desired concentration to perform the tests. Drug: extract ratio of 500:12 mg/ml.

High performance liquid chromatography (HPLC)

Chromatographic analysis was performed using an HPLC system (Shimadzu, Kyoto, Japan) (SIL-20A) equipped with a C18 reverse phase column (4.6 mm × 250 mm, particle size 5 μm), Shimadzu LC-20AT alternative pumps linked to a DGU 20A5 degasser, a CBM 20A integrator, a SPD-M20A DAD (diode array) detector, and LC Software 1.22 SP1. Chromatographic analyzes were performed under the following conditions: gradient flow rate, 0.6 ml/min; injection volume, 40 μl, and mobile phase consisting solvent A, acetic acid 2% and solvent B, methanol 100 %. The elution profile was: 0–2 min 5% solvent B, followed by a gradual increase in solvent B to 25%, 40%, 50%, 60%, 70%, and 100% at 10, 20, 30, 40, 50, and 80 min, respectively, according to the method described by Laghari et al. (2011), with minor modifications. The three wavelengths used were 270, 320, and 360 nm.

The compounds were identified with a chromatographic profile, co-chromatography with authentic standards, and DAD spectra with authentic standards (gallic acid, quercetin, rutin, chlorogenic acid, and kaempferol). All chromatographic analyzes were carried out at room temperature and in triplicate.

Animals and treatment

To perform the experiments, 28 male homozygous mice knockout for the LDL receptor (LDLr-/-, C57BL 6 mice), weighing 25–29 g (age, 4.0–4.5 months), were purchased from the Faculty of Pharmaceutical Sciences of the University of Sao Paulo (USP - Sao Paulo, Brazil). The animals knockout for the LDLr presented signs of intense vascular inflammation. The choice of the model was based on studies showing