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Effects of *Campomanesia xanthocarpa* on inflammatory processes, oxidative stress, endothelial dysfunction and lipid biomarkers in hypercholesterolemic individuals

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ABSTRACT

In Brazil, the edible plant *Campomanesia xanthocarpa* Berg. (Myrtaceae), popularly known as "guavirova," has been studied in hypercholesterolemic individuals. The present work investigated the effects of *C. xanthocarpa* on inflammatory processes, oxidative stress, endothelial dysfunction and lipid biomarkers in hypercholesterolemic individuals. A total of 156 individuals were selected in a double-blind fashion and randomly divided into two groups in accordance with the intervals used in the criteria for hypercholesterolemia: individuals with total cholesterol (TC) levels between 200 and 240 mg/dL (undesirable level individuals – UL) and individuals with TC levels >240 mg/dL (hypercholesterolemic individuals – HL). Both groups had a control group (CG), which received placebo treatment, an encapsulated excipient group (lactose) and an experimental group that received 500 mg (EG 500), 750 mg (EG 750) or 1000 mg (EG 1000) of encapsulated *C. xanthocarpa*. The inflammatory processes (high-sensitivity C-reactive protein – hs-CRP), oxidative stress (advanced oxidation protein products–AOPPs; ischemia-modified albumin–IMA), endothelial dysfunction (nitric oxide – NOx) and biochemical (TC, triglycerides, high-density lipoprotein – HDL, low-density lipoproteins – LDL, and very low-density lipoprotein – VLDL) parameters were measured before and 90 days after the initiation of treatment. A significant decrease in TC and LDL levels was observed in HL individuals from the EG 500 group (reduction of 29 ± 3% and 41 ± 5% to levels before treatment) compared to the CG group individuals. A significant reduction in oxidative stress and inflammatory process components (reduction of 52 ± 11% in AOPPs, 32 ± 10% in IMA and 57 ± 7% in hs-CRP) and a significant increase in NOx (increase of 84 ± 27%) was observed in HL individuals in the EG 1000 group when compared to the CG group individuals. Treatment with encapsulated *C. xanthocarpa* reduced blood TC and LDL levels in hypercholesterolemic individuals. In addition to its effect on cholesterol levels, this plant reduced oxidative stress in hypercholesterolemic individuals and improved the levels of NOx.

1. Introduction

Hypercholesterolemia, which manifests as an increased level of low-density lipoproteins (LDL), results in an elevated risk of coronary heart disease (CHD) and leads to a rise in fatal cardiovascular events [9,45]. This condition is recognized as a high serum total cholesterol (TC) concentration above 240 mg/dL [21]

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and is usually accompanied with an increase in LDL levels above 160 mg/dL [41]. Multiple studies have indicated that hypercholesterolemia is a crucial factor in atherosclerosis, and the elevated cholesterol levels are correlated with an increased risk of death from CHD [57].

Atherosclerosis is a chronic inflammatory condition associated with diverse effects on inflammatory processes and acute phase responses, including neutrophil and monocyte activation, secretion of cytokines, such as interleukin-6 (IL-6), and hepatic synthesis of acute phase proteins, such as fibrinogen and C-reactive protein (CRP) [51]. It has been shown that inflammation plays a pivotal role in the development of atherosclerotic changes and that hypercholesterolemia can initiate and enhance the inflammatory response. Moreover, atherosclerosis is associated with endothelial cell activation, oxidative stress, and accumulation of leukocytes in artery walls [32].

Several lines of evidence indicate that oxidative protein modifications and the accumulation of oxidized proteins could be an early indication of oxygen radical-mediated tissue damage and have been found in cells during oxidative stress and atherosclerosis [55,56]. In addition, a novel oxidative stress biomarker, referred to as advanced oxidation protein products (AOPPs), was detected in the plasma of chronic uremic individuals. AOPP levels are a measure of highly oxidized proteins, especially albumin [64]. AOPPs have been highly correlated with carotid intima-media thickness and may be related to atherosclerotic cardiovascular events [11]. There is growing evidence that oxidative stress contributes to mechanisms of vascular dysfunction in the atherogenic process [40].

However, overproduction of free radicals may produce chemical modifications to human serum albumin and result in increased ischemia-modified albumin (IMA). Currently, IMA is regarded as a biomarker of oxidative stress related to ischemia–reperfusion in different clinical conditions associated with oxidative stress, such as myocardial ischemia [5], metabolic syndrome [20] and hypercholesterolemia [15]. A central feature of these events is impaired endothelial function with the presence of cardiac risk factors, and under pathological conditions, leads to the impairment of endothelium-derived nitric oxide (NOx) bioactivity [2]. NOx is produced in endothelial cells from the conversion of L-arginine to L-citrulline through the tightly regulated activity of (endothelial) NOx synthase [40].

Many therapeutic agents are available for the management of hypercholesterolemic individuals and are employed in treatment. A number of studies have demonstrated that the use of lipid-lowering drugs can reduce the number of cardiovascular events and mortality from coronary disease [3]. Moreover, a diet restricted in foods high in cholesterol and regular physical exercise should be proposed in the treatment of these individuals [10]. However, due to resistance to dietary restrictions and financial limitations to use lipid-lowering drugs, many individuals have turned to alternative treatments to control cholesterol levels. Many of these alternative treatments have been used empirically and lack the scientific studies that allow for more reliable conclusions [13].

In Brazil, the edible plant *Campomanesia xanthocarpa* Berg. (Myrtaceae), popularly known as “guavirova,” is found in the southern regions and in Argentina, Paraguay, and Uruguay [26]. In addition, the plant has been studied in hypercholesterolemic individuals [29]. Studies have shown that *C. xanthocarpa* possesses a wide spectrum of physiological effects: the leaves of this plant are used as infusions in folk medicines to treat inflammatory diseases and hypercholesterolemia [1]. Moreover, *C. xanthocarpa* is used for weight loss and control of a number of conditions associated with obesity [13].

One of the most recent studies demonstrated that *C. xanthocarpa* produced an effect that is similar to the mechanism of oral hypolipemiants, and this plant contains a high concentration of saponins [29], which are widely distributed in plants and have many biological activities, including antiplatelet activity [47,54]. Moreover, *C. xanthocarpa* showed antiplatelet, antithrombotic and fibrinolytic activities in mice [30]. However, these studies were either performed with a small number of individuals or in animals. Thus, clinical studies with a larger number of participants are necessary to demonstrate the possible effect of this plant on the atherosclerotic process in hypercholesterolemic individuals. The major aim of this present work was to investigate the effects of *C. xanthocarpa* on inflammatory processes, oxidative stress, endothelial dysfunction and lipid biomarkers of hypercholesterolemic individuals.

2. Materials and methods

2.1. Trial organization

This study included hypercholesterolemic volunteers from the southern Brazil city of Cruz Alta, Rio Grande do Sul state, who were found through announcements in the local media to participate in the study on guavirova as a new possible phytotherapeutic. The inclusion criteria used for selection were: age between 18 and 90 years, hypercholesterolemic, presenting TC levels above 200 mg/dL and LDL levels above 160 mg/dL with or without the presence of other cardiovascular risk factors. Individuals were excluded based on the following criteria: pregnancy, breastfeeding or the potential to become pregnant (not using adequate contraceptive methods); alcohol dependence (2 or more drinks per day); myocardial infarction or vascular cerebral accident occurrence in the last 3 months before the onset of treatment; presence of hepatopathies, nephropathy, chronic pancreatitis, thyroid diseases or other diseases that could put the individual at risk or interfere with the results of the study; and the concomitant use of hormones, bile salts, immunosuppressants or lipid-lowering drugs.

2.2. Participants

Of the 200 individuals who were accepted to participate in the study, 156 were selected in a double-blind fashion and randomly divided into two groups in accordance with the intervals used in the criteria for hypercholesterolemia: individuals with total cholesterol levels between 200 and 240 mg/dL (undesirable level individuals) and individuals with total cholesterol levels >240 mg/dL (hypercholesterolemic individuals). Both groups consisted of a control group, which received placebo treatment, an encapsulated excipient group (lactose) and experimental groups that received 500 mg (EG 500), 750 mg (EG 750) or 1000 mg (EG 1000) of encapsulated *C. xanthocarpa*. Forty-four individuals abandoned the study before starting.

As infusions with quantities of water and leaves of *C. xanthocarpa* are prepared empirically by population and no information about dosages used in traditional medicine was found, we evaluated the ability of our treatment to identify the most successful dose using the dose escalation method [8]. Thus, doses of 500, 750 and 1000 mg per day were selected and used for 3 months. All participants provided informed consent to participate. The study protocol was approved by the Ethics Committee of the Universidade de Cruz Alta (protocol number: 086/08). Our research followed the guidelines of the Declaration of Helsinki and Tokyo for humans and informed consent was obtained for all individuals.