Revista Brasileira de Farmacognosia 26 (2016) 233–239

Brazilian Journal of Pharmacognosy
REVISTA BRASILEIRA DE FARMACOGNOSIA
www.elsevier.com/locate/bjp

Original Article

Hypolipidemic and antiatherogenic effects of *Cynara scolymus* in cholesterol-fed rats

Ricieri Mocelin[a], Matheus Marcon[a], Glaucia D. Santo[a], Leila Zanatta[a], Adrieli Sachett[b], Amanda P. Schönell[b], Fernanda Bevilaqua[b], Marta Giachini[b], Rafael Chitolina[b], Silvana M. Wildner[b], Marta M.M.F. Duarte[c], Greicy M.M. Conterato[d], Angelo L. Piato[e], Denise B. Gomes[f], Walter A. Roman Junior[b,f,*]

[a] *Programa de Pós-graduação em Ciências Ambientais, Universidade Comunitária da Região de Chapecó, Chapecó, SC, Brazil*
[b] *Núcleo de Iniciação Científica em Fitoterápicos, Universidade Comunitária da Região de Chapecó, Chapecó, SC, Brazil*
[c] *Departamento de Ciências da Saúde, Universidade Luterana do Brasil, Canoas, RS, Brazil*
[d] *Laboratório de Fisiologia da Reprodução Animal, Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil*
[e] *Programa de Pós-graduação em Farmacologia e Terapêutica, Instituto de Ciências Básicas e da Saúde, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil*
[f] *Programa de Pós-graduação em Ciências da Saúde, Universidade Comunitária da Região de Chapecó, Chapecó, SC, Brazil*

ARTICLE INFO

Article history:
Received 14 July 2015
Accepted 19 November 2015
Available online 31 December 2015

Keywords:
Antihyperlipidemic
Antiatherogenic
Artichoke
Asteraceae
Cholesterol

ABSTRACT

Cynara scolymus L., Asteraceae, are traditionally used to treat dyspepsia. This study evaluated the hypolipidemic and antiatherogenic effects of an aqueous extract prepared from the leaves of *C. scolymus* in rat's model. Hypercholesterolemic rats (1% cholesterol and 0.5% cholic acid for 15 days) were treated (0.5 ml/200 g) with extract of *C. scolymus* (150, 300, or 600 mg/kg *p.o.*; $n = 6$) or simvastatin (4 mg/kg *p.o.*; $n = 6$) once per day for 30 days along with hypercaloric diet. A control group (C) was given water (0.5 ml/200 g; $n = 6$). A high-cholesterol diet was maintained throughout the treatment period. Rats treated with extract of *C. scolymus* (150, 300, or 600 mg/kg) and simvastatin showed significant decreases in serum levels of total cholesterol (−46.9%, −51.9%, −44%, and −41.9%, respectively) and low-density lipoprotein-cholesterol (LDL-C; −52.1%, −54.8%, −51.9%, and −46.7%, respectively), compared with group C ($p < 0.005$). Biochemical analyses revealed significant decrease in the concentration of IL-1, IL-6, TNF-α, IFN-γ, C-reactive protein, oxidized-LDL, and antioxidized-LDL in rats treated with extract of *C. scolymus* (150, 300, or 600 mg/kg). There were no differences in serum ALT enzyme activity between the groups. Our results suggest that hypolipidemic and antiatherogenic effects could be related with the presence of polar substances present in aqueous extract of *C. scolymus*.

Introduction

Cardiovascular disease (CVD) is the main cause of mortality in many countries, accounting for 16.7 million deaths each year (Dahlof, 2010; Lloyd-Jones, 2010). Among the causes of CVD, hyperlipidemia is characterized by increased serum lipids, predominantly low density lipoprotein cholesterol (LDL-C) triacylglycerols, and decreased high density lipoprotein cholesterol (HDL-C) (Raida et al., 2008).

High circulating concentrations of cholesterol, particularly LDL-C, are associated with an increased risk of atherosclerotic CVD (Tabas et al., 2007). Atherosclerosis (AS), hardening (sclerosis) of the arteries (athero), is a slowly progressing, chronic disorder of the large and medium-sized arteries that occurs when fatty substances, cholesterol, cellular waste products, calcium and other substances accumulate as plaques in the walls of the vessels, reducing blood flow (Hansson, 2005; Kakadiya, 2009; Livingston and Lynm, 2012). AS is recognized as a subacute inflammatory condition of the arterial vessel wall, characterized by the infiltration of macrophages and T-cells, which interact with one another and with arterial wall cells (Rocha and Libby, 2009; Wildgruber et al., 2013). Over the course of inflammation, various cytokines have been reported to stimulate the progression of AS, whereas few were found to potentially aid in AS regression (Packard and Libby, 2008). Overproduction of reactive oxygen species has been strongly associated with the development of oxidation-related conditions, such as AS and CVD. AS begins with the transmigration of oxidized low-density lipoproteins (oxLDL) to the intima (subendothelial space),

* Corresponding author.
E-mail: romanwa@unochapeco.edu.br (W.A. Roman Junior).

http://dx.doi.org/10.1016/j.bjp.2015.11.004
0102-695X/

causing injury to endothelial cells (Chen et al., 2010; Melo et al., 2011).

Long-term statin treatment reduces the risk of CVD, decreasing plasma LDL-C and triacylglycerides levels and slowing the progression of AS (Elis et al., 2011). However, in many patients, treatment goals cannot be achieved because of contraindications or poor tolerance, which hinders adherence to treatment (Huijgen et al., 2010; Sjouke et al., 2011). In addition, statins have been associated with an increased risk of developing type 2 diabetes mellitus (Sattar et al., 2010).

The species *Cynara scolymus* L., Asteraceae, popularly known as the globe artichoke, is a perennial plant of Mediterranean origin (Aktay et al., 2000). The leaves of *C. scolymus* are traditionally indicated for the improvement of digestive and urinary tract function (Küskü-Kiraz et al., 2010). Among the main chemical components are the caffeoylquinic acid derivatives (cynarin, chlorogenic and caffeic acids) and flavonoids (luteolin and apigenin) (Llorach et al., 2002; Wang et al., 2003). Recently, it was reported that serum cholesterol and triacylglyceride levels were decreased in hyperlipidemic rats (4% cholesterol and 1% cholic acid supplemented diet for one month) treated with standardized artichoke leaf extract (Küçükgergin et al., 2010) and the aqueous extract of *C. scolymus* presented hypolipidaemic activity in streptozotocin-induced diabetic rats (Heidarian and Soofiniya, 2011). Nevertheless, no studies have assessed the hypolipidemic effects of the aqueous extract of *C. scolymus* in rats by mimicking the popular use of the plant and its relation with the reduction of proinflammatory interleukins. To this end, the objective of this study was to investigate the hypolipidemic and antiatherogenic effects of an aqueous extract of *C. scolymus* in rats.

Material and methods

Standards and chemicals

All chemicals were analytical-reagent grade and the water was distilled. The chemicals included ethanol (Vetec®, Rio de Janeiro, Brazil), 2 N Folin-Ciocalteu reagent, quercetin, aluminum chloride, 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic and ascorbic acids (Sigma-Aldrich®, St. Louis, MO, USA).

Plant material

Leaves of *Cynara scolymus* L., Asteraceae, were collected in Chapecó (SC), Brazil (S 26°50′14″/W 52°59′12″). The plant samples were authenticated by Camila Kissmann, curator of the Herbarium of Chapecó Region Community University, where a voucher specimen (# 3350) was deposited.

Preparation of aqueous extract of Cynara scolymus

A sample of dried leaves of *C. scolymus* (50 g) of the same particle size was collected by passage through a mesh (425 μm; 35 Tyler/Mesch). The sample was extracted by decoction with water (1000 ml) for 15 min (Farmacopeia Brasileira, 2015). The aqueous extract of *C. scolymus* (CS) was concentrated to dryness under reduced pressure at 40 °C, then freeze dried and stored at −20 °C.

Phytochemical analyses

Determination of total phenolic content

Total phenolics were determined using the Folin-Ciocalteu method (Gutfinger, 1981). In brief, the reaction mixture was composed of 0.5 ml of CS (1 mg/ml), 5 ml of distilled water, and 0.5 ml of the Folin-Ciocalteu reagent. After a period of 3 min, 1 ml of saturated sodium carbonate solution (20%) was added. The samples were vortexed and allowed to stand for 1 h. A standard calibration curve of gallic acid was prepared ($0.045x + 0.016$, $r = 0.999$). The absorbance of blue-colored mixtures was measured at 725 nm. Values were reported as the means of triplicate experiments expressed as gallic acid (GA) equivalents (mg gallic acid/g dry extract).

Determination of total flavonoids

Total flavonoid content was determined according to Jay et al. (1975) with some modifications. Briefly, 0.5 ml of 2% aluminum chloride ($AlCl_3$) in ethanol was mixed with an equal volume of CS (1 mg/ml). Absorbance readings were taken at 415 nm after 1 h using ethanol as a blank. Total flavonoid content was determined using a standard quercetin curve ($y = 0.004x + 0.029$, $r = 0.999$) and the results were reported as the means of triplicate analyses expressed as quercetin equivalents (mg quercetin/g of dry extract).

In vitro *2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay*

Free-radical scavenging activity of the vegetal sample was measured using the method described by Brand-Williams et al. (1995) with some modifications. CS (1 ml) (1–150 μg/ml) was added to 2 ml of a solution of DPPH radicals in ethanol (0.004%). The mixture was vigorously shaken and allowed to stand for 30 min at room temperature (RT). The absorbance (A_{sample}) of the resulting solution was measured at 517 nm and the percent antioxidant activity (AA%) was calculated using the following formula: $AA\% = 100 - \{[(A_{sample} - A_{blank}) \times 100]/A_{control}\}$. A solution of ethanol (2 ml) and CS (1 ml) was used as the blank (A_{blank}). A solution of DPPH (2 ml) and ethanol (1 ml) was used as the control ($A_{control}$). Ascorbic and gallic acids and quercetin were used as standards at the same concentrations as CS. Free radical-scavenging activity was expressed as the quantity of antioxidants necessary to decrease the initial DPPH absorbance by 50% (IC_{50}). The IC_{50} value, which is defined as the amount of CS needed to scavenge 50% of DPPH radicals, was determined using non-linear regression of percent inhibition against CS concentration.

Animals

The International Guidelines for Care and Use of Laboratory Animals were followed for all experiments, and the experimental protocol was approved by the Ethics Committee on Animal Use (# 092/PGA/11) of the Integrated Regional University, Brazil. Male Wistar rats ($n = 36$) weighing 250–275 g were used in the study. The animals were housed in wire-bottomed 17 cm × 33.5 cm × 40.5 cm cages in a controlled environment at 22 ± 2 °C with a 12 h light-dark cycle (lights on at 7 am and off at 7 pm) and minimal noise. The rats were given *ad libitum* access to water and commercially prepared chow pellets (Nuvilab® CR-1, Curitiba, Paraná, Brazil) for rodents.

Hyperlipidemic diet

A standard chow diet [composition (w/w): 48.3% carbohydrate, 23.5% crude protein, 5.9% crude fat, 5.9% crude ash, and 3.9% crude fiber Nuvilab® CR-1] was triturated in mill (Tecnal® 650/1) and mixed with cholic acid and cholesterol (989.9:10:0.1). The mixture was moistened, pelleted and dried at greenhouse (Marconi® MAO35/5) (40 °C per 24 h).