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Original Article

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

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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),

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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).

Experimental design

Following a period of acclimatization (15 days), six animals were randomly designated as the normal group (*N*); these animals received food and water *ad libitum* for the duration of the experiment. Another group of animals ($n = 30$) received a hypercholesterolemic diet (chow pellets for rodents) comprising 1% cholesterol and 0.1% cholic acid (Sigma-Aldrich®, St. Louis, MO, USA). After two weeks under these conditions, the animals were randomly divided into five groups of six animals each ($n = 6$) and then treated orally for 4 weeks as follows: group C (negative control) received 0.5 ml of distilled water; groups CS 150, 300, and 600 received CS at doses of 150, 300, or 600 mg/kg, respectively; group SIMV received 4 mg/kg simvastatin powder (Sigma-Aldrich®, St. Louis, MO, USA) as a positive control. The high cholesterol diet was continued throughout the 30-day treatment period. Doses of CS and simvastatin were based on previous studies (Pankaj et al., 2010). All drugs were given as 0.5 ml/200 g body weight diluted with distilled water (in established doses) and subjected to ultrasonication (20 °C) to facilitate solubility (Vilkhu et al., 2008; Patel et al., 2012). At the end of the experimental period, the animals were fasted for 12 h and anesthetized using a mixture of ketamine (Ketalar; Pfizer®, New York, NY, USA) and xylazine (Bayer® AG, Leverkusen, Germany) (75 mg/kg and 10 mg/kg of body weight, respectively). Blood aliquots were collected for biochemical analyses *via* cardiac puncture and the animals were then euthanized with an overdose of anesthetic.

Biochemical analysis of blood samples for markers of inflammation and atherosclerosis

Upon collection, blood samples were immediately centrifuged at 3000 × *g* for 15 min. Serum TC, HDL-C, IL-1 (sensitivity: 2 pg/ml; range: 1–50 pg/ml), IL-6 (sensitivity: 2 pg/ml; range: 2–200 pg/ml), TNF-α (sensitivity: 4 pg/ml; range: 4–500 pg/ml), IFN-γ (sensitivity: 4 pg/ml; range: 4–200 pg/ml), IL-10 (sensitivity: 2 pg/ml; range: 1–80 pg/ml), CRP, oxLDL, antioxLDL and ALT levels were determined by enzymatic colorimetric and immunological methods (UV/Vis) using commercial Labtest® kits according to the manufacturer's instructions. A semi-automated analyzer (BioSystems®, model BTS 310) was used for all analysis (Li et al., 2012). Serum LDL-C levels were calculated using the Friedewald equation (Friedewald, 1972): LDL-C = TC − [HDL-C − (TG/5)].

Statistical analysis

All results shown are presented as mean values ± standard deviation (SD). The normal distribution of the data was confirmed by Kolmogorov–Smirnov and Levene tests, and results analyzed using one-way ANOVA followed by Tukey's post-test using SPSS 20. A *p*-value of <0.5 was considered statistically significant.

Results

Chemical constituents of aqueous extract of Cynara scolymus

The total phenolic content (expressed as gallic acid equivalents) was found to be approximately 359 ± 2.8 mg (mean ± SD) per gram of CS, while total flavonoid content (recorded in quercetin equivalents) was approximately 48.07 ± 2.8 mg (mean ± SD) per gram of CS.

Determination of DPPH radical scavenging activity

CS showed *in vitro* antioxidant activity by the DPPH method (Fig. 1). The highest scavenging effect was observed for CS with an IC_{50} 57.43 ± 2.05 μg/ml, although it showed lower scavenging abilities than quercetin, ascorbic and gallic acids, which were used as standards (28.04 ± 1.46, 15.02 ± 1.45 and 3.12 ± 1.34 μg/ml, respectively).

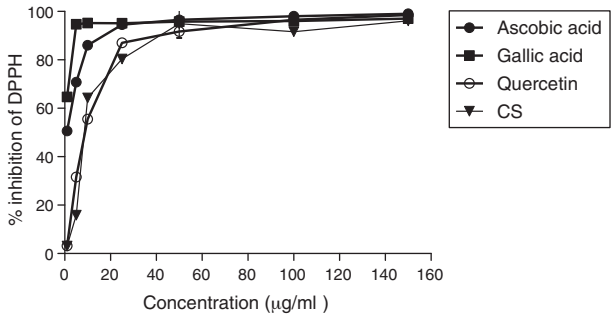

Fig. 1. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenger activity of aqueous extract from *Cynara scolymus* (CS), compared with standards ascorbic and gallic acids and quercetin. Results are expressed as means ± SD ($n = 3$).

Effects of aqueous extracts of Cynara scolymus *on serum lipid profiles*

At the end of the four-week treatment, group C exhibited higher serum levels of TC and LDL-C, compared to group N ($p < 0.0001$). All hyperlipidemic rats treated with CS (150, 300, or 600), or simvastatin (SIMV) exhibited significant and accentuated decreases in TC (−46.9%, −51.9%, −44%, and −41.9%, respectively) (Fig. 2A) and LDL-C (−52.1%, −54.8%, −51.9%, and −46.7%, respectively) levels (Fig. 2B), compared with group C ($p < 0.005$). There were no differences in serum ALT enzyme activity between the groups (data

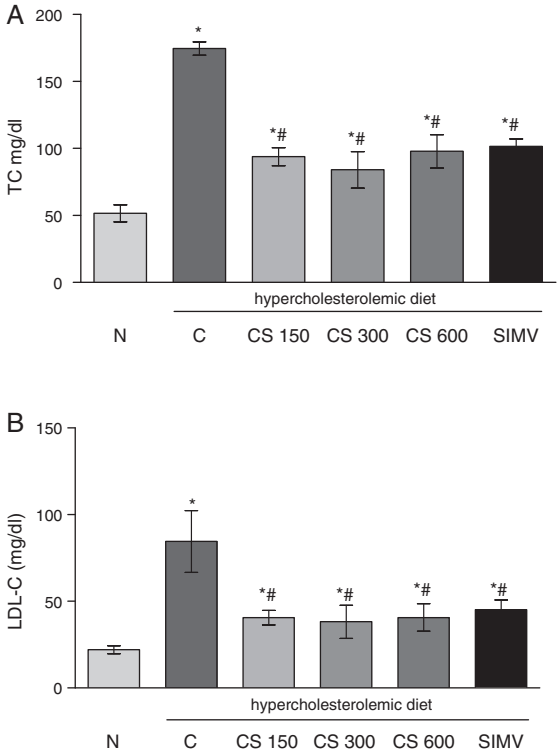

Fig. 2. Effect of aqueous extract from *Cynara scolymus* (CS; 150, 300, and 600 mg/kg), and simvastatin (SIMV; 4 mg/kg) on TC (A) and LDL-C (B), in rats fed a high-fat diet. Rats were either given a normal diet (N) or the following treatment with water (C). Values are expressed as means ± SD ($n = 6$). One way ANOVA $^{*}p < 0.0001$ compared with group N. $^{\#}p < 0.005$ compared with group C.

Table 1
Effect of aqueous extract from *Cynara scolymus* (CS; 150, 300, and 600 mg/kg), and simvastatin (SIMV; 4 mg/kg) on HDL-C, TG and VLDL, in rats fed a high-fat diet. Rats were either given a normal diet (N) or the following treatment with water (C). Values are expressed as means ± SD ($n = 6$).

Groups	HDL-C (mg/dl)	TG (mg/dl)	VLDL (mg/dl)
N	16.14 ± 9.63	65.15 ± 16.92	13.01 ± 7.85
C	42.11 ± 8.61	204.26 ± 38.23	42.85 ± 7.91
CS 150	27.83 ± 6.27	128.35 ± 39.65	25.66 ± 9.72
CS 300	20.48 ± 12.98	127.65 ± 38.85	25.52 ± 9.43
CS 600	27.01 ± 7.13	128.42 ± 40.12	24.95 ± 9.96
SIMV	26.57 ± 8.84	135.23 ± 31.85	26.82 ± 8.11

not show). There were no differences in the serum levels of HDL-C, TG and very low-density lipoprotein cholesterol (VLDL-C) (Table 1) between the groups, as well as, to ALT enzyme activity (data not show).

Markers of atherosclerosis

The analysis of proinflammatory interleukins revealed a significant decrease in IL-1 ($F_{(5,35)} = 44.16$; $p < 0.0001$), IL-6 ($F_{(5,35)} = 13.27$; $p < 0.0001$), TNF-α ($F_{(5,35)} = 8.51$; $p < 0.0001$), and IFN-γ ($F_{(5,35)} = 10.20$; $p < 0.0001$) concentrations following all treatments (CS 150, CS 300, CS 600 and SIMV) compared with group C (Fig. 3A–D, respectively). Conversely, group N and all other treatment groups (CS 150, CS 300, CS 600 and SIMV) exhibited higher levels of anti-inflammatory IL-10 compared with group C ($F_{(5,35)} = 138.20$; $p < 0.0001$) (Fig. 3E). CRP levels were reduced with CS 150, SC 300, SC 600 and SIMV compared with group C ($F_{(5,35)} = 17.18$; $p < 0.0001$) (Fig. 3F).

The concentrations of oxLDL (Fig. 4A) and antioxLDL (Fig. 4B) decreased with all treatments (CS 150, SC 300, SC 600 and SIMV), compared with group C ($F_{(5,35)} = 48.89$ and $F_{(5,35)} = 621.70$; $p < 0.0001$, respectively). The highest reductions were observed

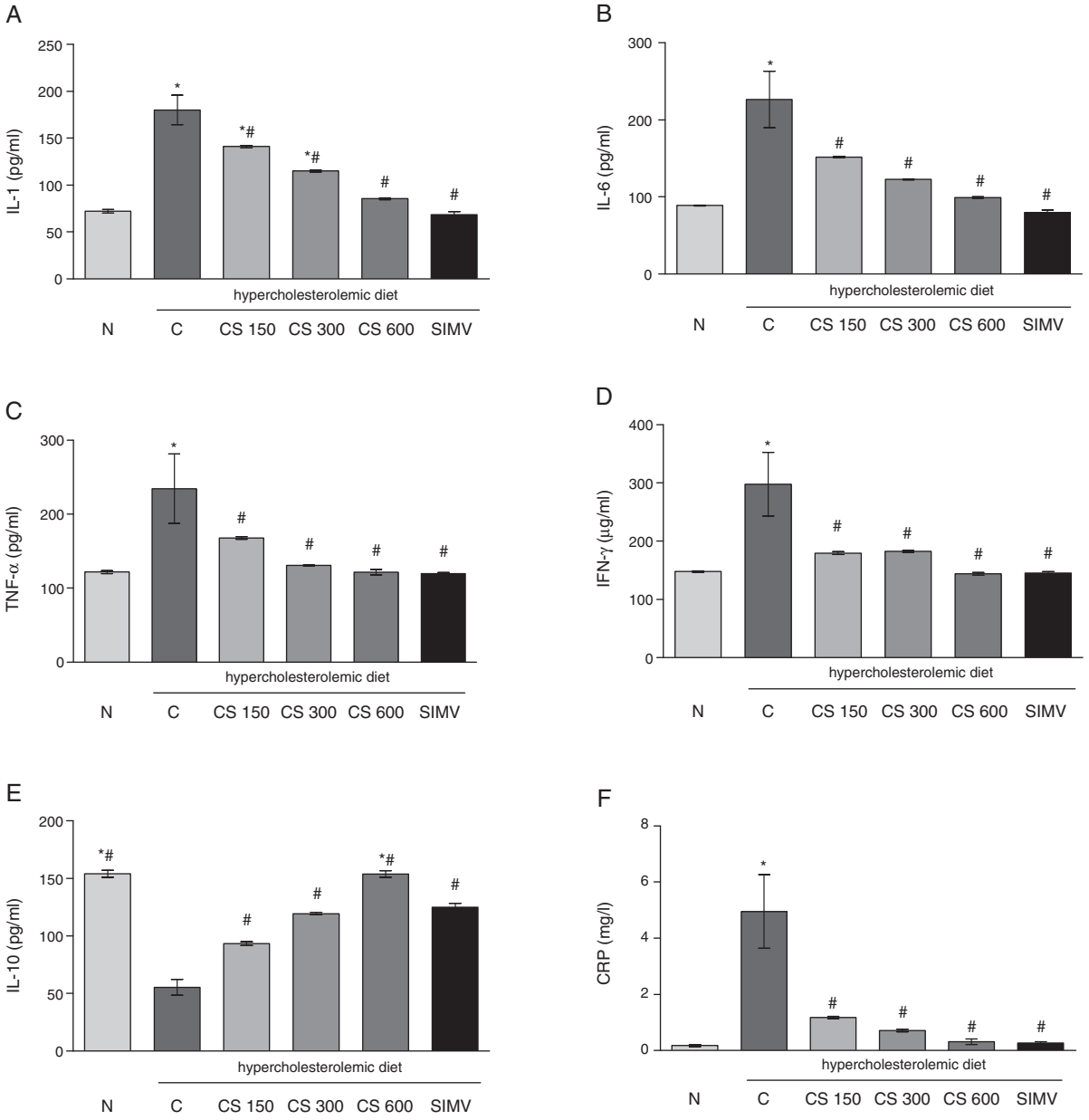

Fig. 3. Effect of aqueous extract from *Cynara scolymus* (CS; 150, 300, and 600 mg/kg), and simvastatin (SIMV; 4 mg/kg) on IL-1, IL-6, TNF-α, IFN-γ, IL-10, and CRP (A, B, C, D, E and F) (mean ± SD; $n = 6$) in rats fed a high-fat diet. Rats were either given a normal diet (N) or the following treatment with water (C). One way ANOVA $^{*}p < 0.0001$ compared with group N. $^{\#}p < 0.005$ compared with the group C.

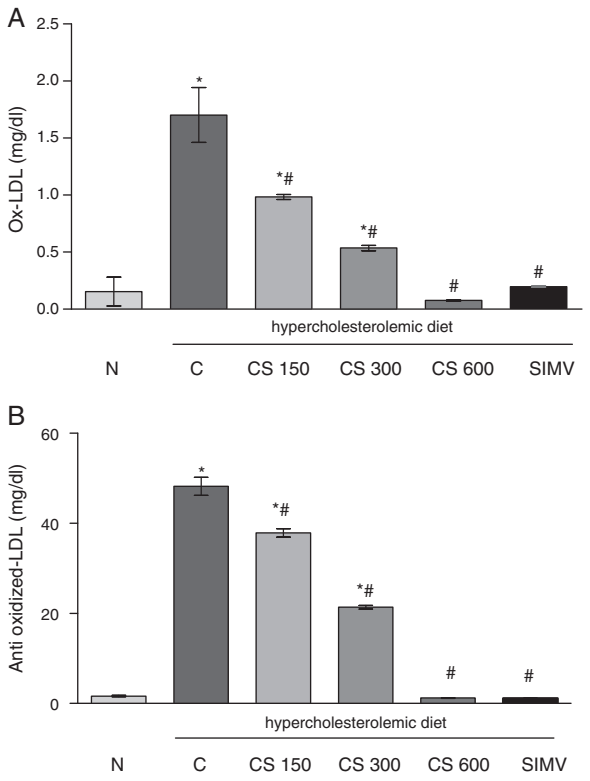

Fig. 4. Effect of aqueous extract from *Cynara scolymus* (CS; 150, 300, and 600 mg/kg), and simvastatin (SIMV; 4 mg/kg), on oxidation (A) and antioxidation (B) of low density lipoprotein (oxLDL and anti oxLDL; mean ± SD; $n = 6$). Rats were either given a normal diet (N) or the following treatment with water (C). One way ANOVA $^{*}p < 0.0001$ compared with group N. $^{\#}p < 0.005$ compared with group C.

in group SC 600 with results very similar to the SIMV and N groups.

Discussion

It is well established that elevated blood lipid levels constitute a major risk factor for atherosclerosis (Keevil et al., 2007; Ascunce et al., 2012). The search for new drugs capable of reducing these molecular species and/or regulating their metabolism has gained momentum over the years, resulting in various reports on significant activities of natural products (Jahromi et al., 1993; Meng et al., 2014). To the best of our knowledge, this is the first report on the hypolipidemic and antiatherogenic effects of an aqueous extract of *C. scolymus* in cholesterol-fed rats. The results of the present study show that a cholesterol-enriched diet causes an increase in serum TC and LDL-C levels, which can be mitigated by CS at dosages ranging from 150 to 600 mg/kg. The elevated values of polyphenols and flavonoids revealed in CS, are possibly related to the hypolipidemic effects that were observed. In a similar study by Bok et al. (1999), these compounds decreased the enzyme activities of 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA reductase) and acyl-CoAacetyltransferase in cholesterol-fed rats, thereby decreasing the levels of cholesterol esters available to form very low density lipoproteins (VLDL), resulting in reduced VLDL secretion by the liver. It is known that quercetin can reduce the activities and mRNA levels of several enzymes involved in the synthesis of fatty acids (Odbayar et al., 2006).

The high antioxidant activity of CS probably contributed to its lipid-lowering effect, as has been reported by Küskü-Kiraz et al. (2010). Studies indicate that *C. scolymus* possesses antioxidant and hypocholesterolemic properties. These properties operate through inhibition of LDL oxidation, increased cholesterol elimination in bile secretions, and reduced cholesterol synthesis *via* inhibition of HMG-CoA reductase (Kraft, 1997). Thus, similar effects may have occurred with CS, contributing to the hypolipidemic activity observed in cholesterol-fed rats.

Preclinical observations have demonstrated that hypercholesterolemia stimulates the accumulation of oxLDL in the arterial wall, promoting endothelial cell dysfunction and the development of atherosclerosis (Aikawa and Libby, 2004). Atherosclerosis is a progressive disease involving both large and medium-sized vessel walls. It is a common factor in cardiac disorders, but can also affect other organs (Auger et al., 2005). The importance of inflammation in all stages of atherosclerosis is well established (Libby, 2002). Various proinflammatory risk factors involving reactive oxygen species can trigger the production of proinflammatory cytokines by NF-κB, which contributes to the development and progression of the disease (Winther et al., 2005; Vallejo et al., 2014). Other markers of atherosclerosis include cytokines that are mainly involved in the early stages of inflammation, such asIL-1, IL-6, and TNF-α, in addition to CRP (Vaddi et al., 1994; Ridker et al., 2002; Tzoulaki et al., 2005; Larsson et al., 2005). Amplification of the inflammatory response *via* activation of macrophages is performed by IFN-γ (Groyer et al., 2006). In this experiment, the group fed a hypercaloric diet and treated with water (C) had higher concentrations of proinflammatory interleukins and treatment with all concentrations of CS (150, 300, and 600 mg/kg) decreased these levels. Similar results were found by Li et al. (2012) whose proposed mechanisms to explain these activities highlighted the inactivation of transcription factors, STAT1 and NF-κB, which are known to precipitate the inflammatory process, by polyphenols (Valerio and Awad, 2011).

Nevertheless, inflammatory processes appear to be regulated by anti-inflammatory cytokines, such as IL-10. The regulatory role of IL-10 was demonstrated by its expression in certain atherosclerotic lesions, as well as the observation that exogenous IL-10 inhibited the release of IL-12-induced LDL. This cytokine can inhibit the action of NF-κB, with consequent decreases in proinflammatory cytokine production, inhibition of macrophage apoptosis, expression of tissue factor and fibrinogen, and proliferation of smooth muscle cells (Heeschen et al., 2003; Zimmerman et al., 2004; Almer et al., 2013). As the control group (C) showed very low levels of this cytokine compared to CS treatments, it can be concluded that the tested substances may have contributed to tissue protection of NF-κB.

Increased LDL-cholesterol levels promote deposition of these particles into arteries with increase in the concentration of reactive species, reduction in the antioxidant defense systems and subsequent oxidation and formation of oxLDL. These substances are toxic to endothelial cells, resulting in lesions that stimulate monocytes and macrophages to become foam cells, eventually leading to atheroma (Groyer et al., 2006; Afonso et al., 2013). In this study, all evaluated substances decreased oxLDL levels compared to group C. One hypothesis for this biological activity is that polyphenolic compounds present in the aqueous extracts of *C. scolymus* revealed antioxidant effects, protective properties and decreased oxidative stress as observed in rat hepatocytes by Gebhardt (1997). These results are in agreement with those obtained by Fuhrman et al. (2000) evaluating the effect of the ethanol extract of *Zingiber officinale* Roscoe. It was also observed that all groups treated with aqueous extract of *C. scolymus* showed reduced levels of autoantibodies against oxLDL compared to group C. According to Young and McEneny (2001), and Dai et al. (2013) high levels of these antibodies are biochemical indicators of the risk of metabolic syndrome and are related to atherosclerosis progression.

It is well-known that polyphenolic compounds exhibit various pharmacological activities, including hypolipidemic and antiatherogenic effects (Harnafi et al., 2008; Schneider et al., 2015).

The results of this study are in agreement with Hyun-Soo et al. (2014), indicating that the function of the aqueous extracts of *C. scolymus* in cholesterol fed-rats is correlated with the decrease of LDL levels, inhibition of a series of proinflammatory cytokines, and likely the antioxidant effects of polyphenols which could contribute in the prevention of atherosclerosis. Levels of ALT did not differ between treatments groups, indicating the absence of CS toxicity at the doses tested. In this context, CS extract could be helpful as dietary supplement to prevent or reduce atherosclerosis which would reduce treatment costs.

Conclusions

Polyphenolic substances present in the aqueous extract of *C. scolymus* L. "artichoke," may be effective in the prevention of hypercholesterolemia, possibly conferring protection against atherosclerosis.

Conflicts of interest

The authors declare no conflicts of interest.

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