Phytomedicine 18 (2011) 769–775

Contents lists available at ScienceDirect

Phytomedicine

journal homepage: www.elsevier.de/phymed

Crataegus laevigata decreases neutrophil elastase and has hypolipidemic effect: A randomized, double-blind, placebo-controlled trial

E. Dalli[a,*], E. Colomer[a], M.C. Tormos[b], J. Cosín-Sales[a], J. Milara[c], E. Esteban[a], G. Sáez[b]

[a] *Department of Cardiology, Associated University Hospital Arnau de Vilanova, Valencia, Spain*
[b] *Department of Biochemistry and Molecular Biology, School of Medicine, University of Valencia, Valencia, Spain*
[c] *Department of Pharmacology, School of Medicine, University of Valencia, Valencia, Spain*

ARTICLE INFO

Keywords:
Crataegus laevigata (Rosaceae)
Neutrophil function
Elastase
Coronary heart disease
Randomized, double blind placebo controlled trial

ABSTRACT

Crataegus laevigata is a medicinal plant most commonly used for the treatment of heart failure and psychosomatic disorders. Based on previous experimental findings, this double-blind placebo-controlled study was aimed at finding beneficial effects of *C. laevigata* on biomarkers of coronary heart disease (CHD). The study included 49 diabetic subjects with chronic CHD who were randomly assigned to the treatment for 6 months with either a micronized flower and leaf preparation of *C. laevigata* (400 mg three times a day) or a matching placebo. Blood cell count, lipid profile, C-reactive protein, neutrophil elastase (NE) and malondialdehyde were analyzed in plasma at baseline, at one month and six months. The main results were that NE decreased in the *C. laevigata* group compared to the placebo group. In the *C. laevigata* group, baseline figures (median and interquartile range) were 35.8 (4.5) and in the placebo group 31 (5.9). At the end of the study, values were 33.2 (4.7) ng/ml and 36.7 (2.2) ng/ml, respectively; $p < 0.0001$. *C. laevigata*, added to statins, decreased LDL cholesterol (LDL-C) (mean ± SD) from 105 ± 28.5 mg/dl at baseline to 92.7 ± 25.1 mg/dl at 6 months ($p = 0.03$), and non-HDL cholesterol from 131 ± 37.5 mg/dl to 119.6 ± 33 mg/dl ($p < 0.001$). Differences between groups did not reach statistical significance at 6 months. No significant changes were observed in the rest of parameters. In conclusion, *C. laevigata* decreased NE and showed a trend to lower LDL-C compared to placebo as add-on-treatment for diabetic subjects with chronic CHD.

Introduction

Reviews of herbal remedies recently published, including references to *Crataegus laevigata* (Poir.) DC, have claimed to increase the information about their efficacy, safety and potential interactions with conventional therapies before establishing specific therapeutic recommendations (De Smet 2002; Miller et al. 2004; Expert Consensus Document 2005). The beneficial effects of *C. laevigata* observed in basic and animal research on cardiovascular disorders justifies more human studies.

Flowers, leaves and fruit of *Crataegus* spp. contain polyphenols, mainly procyanidins and flavonoids. Standardizing preparations depending on their procyanidin concentration has been recommended, since these molecules are related to many of its pharmacological effects (Chatterjee et al. 1997). *Crataegus* has a positive inotropic effect and, unlike other synthetic inotropic drugs, prolongs the refractory period (Joseph et al. 1995). Its antiarrhythmic effect has been observed *in vitro* and in ischemia–reperfusion animal models (Pöpping et al. 1995). Myocardial protection has also been demonstrated during ischemic conditions (Al Makdessi et al. 1996; Jayalakshmi et al. 2006; Veveris et al. 2004). One potential therapeutic use could be the atherothrombotic disease. In fact, *in vitro* inhibition of neutrophil elastase (NE) (Chatterjee et al. 1997), and secretory phospholipase A2 (sPLA2) (Ahumada et al. 1997), as well as anti complementary activity (Shahat et al. 1996), have been shown. A tincture of *Crataegus* was shown to have hypocholesterolemic action in atherogenic diet fed rats by increasing bile acid excretion and depressing hepatic cholesterol synthesis (Rajendran et al. 1996). *C. laevigata* also decreased oxidative stress and lipid peroxidation, not only with the entire plant but also with their isolated compounds (Quettier-Deleu Voiselle et al. 2003). We have previously reported that a *C. laevigata* dry extract

Abbreviations: NE, neutrophil elastase; sPLA2, secretory phospholipase A2; Lp-PLA2, lipoprotein-associated phospholipase A2; fMLP, N-formyl-methionyl-leucyl-phenylalanine; NYHA, New York Heart Association; EF, ejection fraction; MI, myocardial infarction; CHD, coronary heart disease; HbA1c, glycosylated haemoglobin A1C; CRP, C-reactive protein; MDA, malondialdehyde; ACEI, angiotensin-converting enzyme inhibitors.

* Corresponding author at: Servicio de Cardiología, Hospital Asociado Universitario Arnau de Vilanova, C/San Clemente 12, 46015 Valencia, Spain.
Tel.: +34 963868466; fax: +34 963868466.
E-mail address: edalli@hotmail.es (E. Dalli).

0944-7113/$ – see front matter © 2010 Elsevier GmbH. All rights reserved.
doi:10.1016/j.phymed.2010.11.011

modified *in vitro* neutrophil functions, with a dose-dependent reduction of fMLP-induced NE release, superoxide anion generation, leukotriene B4 production and lipopolysaccharide-induced generation of tumour necrosis factor-alpha and interleukin-8. *C. laevigata* also produced a dose-dependent inhibition of *in vitro* neutrophil migratory capacity (Dalli et al. 2008). In humans, the *Crataegus* extract WS 1442 improved symptoms and exercise parameters in heart failure (Weikl et al. 1996; Tauchert 2002; Zapfe 2001). A meta-analysis of randomized, placebo-controlled trials suggested a benefit favouring the treatment with *Crataegus* over placebo (Pittler et al. 2003). Recently, the SPICE trial randomized 2681 patients with NYHA class II or III congestive heart failure to treatment with the *Crataegus* extract WS 1442 or placebo, added on top of conventional medication. No differences were observed in the primary end point (a composite of sudden cardiac death, death due to progressive heart failure, fatal myocardial infarct (MI), nonfatal MI, or hospitalization due to heart failure progression), after one year of treatment. Nevertheless, in the subgroup of patients with ejection fraction (EF) between 25 and 35%, a 39.7% reduction of sudden cardiac death was observed (Holubarsch et al. 2008). Neutrophils infiltrate the postischemic myocardium and cause much of the myocardial dysfunction associated with this condition (Kyne et al. 2000). In the coronary arteries, they are involved from the initial steps of fatty streak formation until the rupture of the advanced atherosclerotic plaques. NE has the potential to preferentially disrupt the elastic network but also collagen, fibronectin, proteoglycan and laminin fibres (Owen and Campbell 1999). NE is elevated in patients with coronary heart disease (CHD) and is correlated with the complexity and severity of the coronary stenosis in patients with unstable angina (Amaro et al. 1995). The present study was aimed at finding new therapeutic options for *C. laevigata* in secondary prevention, by measuring *C. laevigata* treatment-induced changes compared to placebo in lipid parameters, oxidative stress, and plasmatic NE levels, in diabetic patients with chronic CHD, in which the atherothrombotic process is more aggressive.

Subjects and methods

Patients

Subjects were eligible for participation if they were between 45 and 75 years old and had been previously diagnosed of type 2 diabetes mellitus and chronic CHD. Patients were recruited from the outpatient clinic of our hospital. Diagnosis of diabetes mellitus was established with a fasting blood sugar test $\geq$126 mg/dl, HbA1c > 6.5% or hypoglycaemic treatment. Chronic CHD was considered with prior diagnosis of unstable angina or myocardial infarction, and at least one coronary lesion $\geq$70%, revascularized or not. Patients had been asymptomatic within the previous 6 months (NYHA class I). Exclusion criteria were: HbA1c > 10%, malignancy, cerebrovascular, kidney, pulmonary or liver disease, treatment with non steroidal anti-inflammatory drugs, and having taken vitamins, dietary or herbal supplements three months before entry. All types of alcohol beverages, fruit juices or tea infusions were prohibited during the study period. The habit of smoking was defined as a consumption of at least one cigarette a day in the last three months, hypertension with blood pressure >140/90 mm Hg or pharmacological treatment, dyslipemia was considered if cholesterol >200 mg/dl, triglycerides >150 mg/dl or pharmacological treatment. The last dosage change of hypolipemic or hypotensive treatment was made at least 3 months before the start of the study. The study was conducted in accord with the Declaration of Helsinki and following guidelines for Good Clinical Practice. All subjects gave written informed consent to participate. The study was approved by the Local Ethics Committee and Sanitary Authorities.

Protocol

Visits and blood tests took place the morning after an overnight fasting at baseline, and one month and six months after treatment. A medical history check and a physical exam were carried out in the first visit. The analytical variables included were blood cell count, and levels of glucose, urea, creatinine, electrolytes, high sensitive C-reactive protein (CRP), lipids, neutrophil elastase (NE) and malondialdehyde (MDA). Eligible patients were assigned to *C. laevigata* or placebo in a double-blind fashion by computer-generated numbers. *C. laevigata* was administered by means of micronized flowers and leaves, standardized for a content of 5% of procyanidins and 2% of flavonoids (Crataesor®, Soria Natural SL, Spain). According to the information provided by the manufacturer, procyanidin quantification was made as follows: 1 g of the *C. laevigata* preparation was extracted four times with 25 ml of acetone/water (70/30) by stirring at room temperature for 15 min. Extraction was followed by centrifugation at 200 g for 5 min and the supernatant was collected. The precipitate was re-extracted. All extracted material was mixed and filtered on paper. 10 ml of extraction was evaporated to dryness, not exceeding 30 °C. Dried residue was dissolved in a mixture of n-BuOH–HCl (95:5). After heating in a water bath under reflux and stirring for 110 min, the solution was cooled to room temperature and made up to 100 ml with n-BuOH–HCl 37% (95:5). The absorbance was measured in the spectrophotometer at λ = 540 nm, considering the n-BuOH–HCl as a white reference. Procyanidin content (expressed as cyanidin chloride) was derived from the formula:

$$\%\,\text{procyanidins} = \frac{E \times 4.115}{b}$$

where E = absorbance and b = weight of dried powdered tissue (Kartnig et al. 1993).

The HPLC analysis of the flavonoids in *C. laevigata* was performed with an apparatus Agilent 1100 with programmable gradient. The column used was an Agilent XOB-C18. The flow rate was 1 ml/min. The concentration of the main flavonoids was: vitexin-2-O-rhamnoside 14.2 mg/g, chlorogenic acid 3.1 mg/g, hyperoside 2 mg/g, hesperidin 1.4 mg/g, quercitrin 1.1 mg/g, caffeic acid 0.7 mg/g, myricitrin 0.2 mg/g, isovitexin 0.1 mg/g. The HPLC profile analysis is shown in Fig. 1. Placebo consisted of microcrystalline cellulose with appropriate masking conditions. Cellulose powder was coloured green, similar to *Crataegus*, and both treatments were identically packaged and supplied to study subjects by the Hospital Pharmacy Department. Each patient took 400 mg of either *C. laevigata* or placebo, three times a day, for 6 months added to their conventional treatment. No modification of concomitant medications was allowed during the study period. All participants underwent counselling to ensure their adherence to the study medication and maintenance of their usual life style (diet and physical activity) throughout the study period. Compliance was carried out by pill counting.

Laboratory data

Blood cell count and biochemical parameters were analyzed in the core laboratory of the hospital. Levels of cholesterol and triglycerides in plasma were measured by automatic procedures using Vitros diagnostic products (Ortho-Clinical Diagnostics, Rochester, NY). The methodologies for triglycerides (Vitros TRIG Slides) and total cholesterol (Vitros CHOL Slides) are based on enzymatic methods as previously described (Spayd et al. 1978; Allain et al. 1974). HDL cholesterol (HDL-C) was measured by means of the VIT-