Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 108 (2008) 205–212
Food Chemistry
www.elsevier.com/locate/foodchem

Hypolipemic activity of polyphenol-rich extracts from *Ocimum basilicum* in Triton WR-1339-induced hyperlipidemic mice

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Received 12 April 2007; received in revised form 30 September 2007; accepted 23 October 2007

Abstract

The hypocholesterolemic and hypotriglyceridemic activities of the aqueous and organic extracts of *Ocimum basilicum* were studied using Triton WR-1339-induced hyperlipemic mice as an experimental model. Hyperlipidemia was developed by intraperitoneal injection of Triton (200 mg/kg body weight "BW"). The animals were divided into eight groups of eight mice each: normolipidemic control group (NCG), hyperlipidemic control group (HCG), hyperlipidemic plus DMSO control (HDCG), crude aqueous basil extract-treated group (CETG), dichloromethane extract-treated group (DETG), ethyl acetate extract-treated group (EETG), methanol extract-treated group (METG), and aqueous fraction-treated group (AFTG). After 7 h and 24 h of treatment, the intragastric administration of all extracts caused a significant decrease of plasma total cholesterol. Triglyceride levels were also significantly lowered but not in DETG. Similar results were observed for LDL-cholesterol concentrations. Although no significant change of HDL-cholesterol was noticed after 7 h of treatments, a significant increase of this cholesterol fraction was observed in EETG and AFTG after 24 h. Furthermore, crude aqueous basil extract and all polar solvent (methanol, ethyl acetate, water)-soluble fractions showed a significant ameliorative action on elevated atherogenic index (AI) and LDL/HDL-C ratios, while these atherogenic markers were not statistically suppressed by the dichloromethane-soluble extract. This finding indicates that *O. basilicum* may contain polar products able to lower plasma lipid concentrations and might be beneficial in treatment of hyperlipidemia and atherosclerosis.

Keywords: Hypocholesterolaemia; Hypotriglyceridaemia; Atherogenic index; *Ocimum basilicum*; Triton WR-1339; Mice

1. Introduction

Experimental and epidemiological studies have shown that the plasma hypercholesterolemic state could contribute to the development of atherosclerosis and related cardiovascular system diseases (CVD) which are the most common cause of death in both western and eastern societies (Epstein,1992). Indeed, clinical trials have demonstrated that the increase in plasma low density lipoprotein cholesterol (LDL-C) levels is implicated in the early development and progression of atherosclerosis. However, high density lipoprotein cholesterol (HDL-C) is an anti-atherogenic fraction (Martin, Hulley, Browner, Kuller, & Wentworth, 1986). Triglycerides (TGs) may also be a risk factor, especially in individuals with diabetes (West, Ahuja, & Bennet, 1983).

A logical strategy, to prevent or to treat atherosclerosis and reduce the incidence of cardiovascular disease events, is to target hyperlipidemia by drugs and/or dietary intervention (La Rosa, Hunninghake, & Bush, 1990). With this aim, efforts to develop effective and better hypolipidaemic

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doi:10.1016/j.foodchem.2007.10.062

drugs have led to discovery of natural products and have stimulated the search for new lipid-lowering agents from this source.

Triton WR-1339, a non-ionic detergent (oxyethylated tertiary octyl phenol formaldehyde polymer), has been widely used to produce acute hyperlipidaemia in animal models in order to screen natural or chemical drugs (Schurr, Schultz, & Parkinson, 1972) and to study cholesterol and triacylglycerol metabolism (Zeniya & Reuben, 1988). The accumulation of plasma lipids by this detergent appears to be especially due to the inhibition of lipoprotein lipase activity (Hayashi, Niinobe, Matsumoto, & Suga, 1981).

In Morocco, as in many developing countries, most hyperlipidemic individuals use medicinal plants as folk medicine to treat hyperlipidaemia and prevent atherosclerosis. Therefore, there is a strong interest, locally, in natural hypolipidemic substances derived from medicinal plants.

Vast numbers of plants have received attention in this regard and have been shown to lower plasma lipid levels (Khanna, Rizvi, & Chander, 2002). Sweet basil, *Ocimum basilicum* (Family of Labiatae), often used in the east of Morocco by hyperlipidemic subjects as an alternative therapeutical tool to treat hyperlipidaemia, is a medicinal plant originating from Asia (Paton, 1992). Among pharmacological activities, only its anti-inflammatory effect has been ascertained (Singh, 1999). However, an effect on blood lipid profile has not yet been shown by this plant. The present study is designed to evaluate the possible beneficial effect of Sweet Basil on plasma lipid parameters in Triton WR-1339-induced hyperlipidaemic mice and to identify the active fraction (s) using different polar and non-polar organic solvents as extractants.

2. Material and methods

2.1. Plant material

O. basilicum was purchased from a herbalist in Oujda city and authenticated by a botanist (Pr. A. Khalil, Department of Biology, Faculty of Sciences, Oujda, Morocco). A voucher specimen has been deposited at the department of biology (collection no LO 15).

2.2. Preparation of crude aqueous basil extract

The crude aqueous extract from *O. basilicum* aerial parts was prepared by the same method as used in folk medicine with some improvements. The dried herb was infused for 30 min in distilled water (100 °C), filtered, and the solution obtained concentrated in a rotatory evaporator under vacuum at 65 °C. The yield of extract in terms of starting dried plant material was of 27% (w/w). The resulting crude extract was suspended in distilled water and the aliquots were stored at −18 °C before use.

2.3. Preparation of organic solvent extracts

The dried powder from aerial parts of the plant was defatted with *n*-hexane (C_6H_{14}) in a Soxhlet extractor. Afterwards, the marc was air-dried and extracted with dichloromethane (CH_2Cl_2; polarity index $P' = 3.1$) until completely exhausted (16 h) to afford the lipophilic extract (yield was of 2% w/w). The resulting marc was again air-dried and exhausted (16 h) with ethyl acetate ($C_4H_8O_2$; $P' = 4.4$) to obtain a relatively soluble extract; the yield of extraction in this case was 5% w/w. The same operation was done to gain the methanol (CH_3OH; $P' = 5.1$) extract from the marc; after the ethyl acetate extraction, this yielded 10% w/w. At the end of the Soxhlet extraction, the residual plant powder was again air-dried and infused in distilled water ($P' = 10.2$) to afford the hydrophilic extract (aqueous fraction) using the same experimental method as designed to obtain the crude aqueous extract. This yielded approximately 9% (w/w) of dried extract. Each solvent extract was filtered and the filtrate was placed in the rotatory evaporator under a reduced pressure to remove the extractant until semi-solid substances were obtained. Then, the extracts were placed in the drying oven (40 °C) to obtain the dried material.

2.4. Determination of total polyphenol contents

Total polyphenols of *O. basilicum* extracts were determined by the Folin–Ciocalteu procedure (Hagerman, Harvey-Mueller, & Makkar, 2000) To aliquots of 0.5 ml were added 0.25 ml of Folin–Ciocalteu reagent and 1.25 ml 20% aqueous sodium carbonate solution. Samples were vortexed and absorbances of blue coloured mixtures recorded after 40 min at 725 nm against a blank containing 0.5 ml of water or 4% DMSO in water, 0.25 ml of Folin–Ciocalteu reagent and 1.25 ml of 20% aqueous sodium carbonate solution. The amount of total polyphenols was calculated as catechin equivalents from the calibration curves of catechin standard solutions and expressed as mg catechin/g dry plant extract. All measurements were done in triplicate.

2.5. Quantification of tannins

Total tannins content was determined by the Folin–Ciocalteu procedure, as described above, after their adsorption onto BSA (bovine serum albumin/ fraction V, ACROS, New Jersey, USA) (Hagerman & Butler, 1978).

In brief, 20 ml of each sample (20 mg/ml) were homogenized with 250 mg of BSA and the mixture was stirred for 30 min; the preparation obtained was stored for 2 h at +4 °C. Then the pH was adjusted to 4.6 (pHi of BSA) by 1 N HCl solution. After centrifugation at 4000 rpm/15 min, no adsorbed phenolics in the supernatant were determined by the Folin–Ciocalteu procedure, as described above. Calculated values were subtracted from total polyphenol contents and the amount of total tannins expressed