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Original article

Phenolic acid-rich extract of sweet basil restores cholesterol and triglycerides metabolism in high fat diet-fed mice: A comparison with fenofibrate

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ABSTRACT

Many spices are often added to foods as additives to enhance organoleptic qualities, such as flavor, aroma and color. Sweet basil (*Ocimum basilicum* L.) family of Lamiaceae is widely used in cooking for its culinary attributes. In this study, we aimed at the investigation of the hypocholesterolemic and hypotriglyceridemic activities of the basil phenolic acid-rich extract in high fat diet-induced hyperlipemic mice. Hyperlipemia was developed by a high fat diet containing cholesterol, lard and cholic acid. At the beginning of the experiment, animals were divided into three groups, one of them served as normolipidemic control group (NCG), the second hyperlipidemic control group (HCG) and the third basil-treated group (BTG). After 5 weeks of treatment, basil phenolic acid-rich extract significantly decreased plasma total cholesterol, triglycerides and LDL-cholesterol (−42%,−42% and −86%, respectively, $P < 0.001$). However, HDL-cholesterol was increased (+79%, $P < 0.001$). The extract reduced the atherogenic index and LDL/HDL-C ratio (−88% and −94%, respectively, $P < 0.001$). The reductions of liver total cholesterol and triglycerides were of −50% ($P < 0.01$) and −58% ($P < 0.01$), respectively. The hypolipemic effect of the phenolic acid-rich extract is comparable to that exerted by fenofibrate. This drug significantly reduced plasma total cholesterol, triglycerides and LDL-cholesterol (−25.5%, −51%, and −83.5%, respectively, $P < 0.001$) and increased plasma HDL-cholesterol (+136%, $P < 0.001$). On the other hand, fenofibrate significantly decreased atherogenic index and LDL/HDL-cholesterol ratio (−91% and −93%, respectively, $P < 0.001$). The fenofibrate decreased hepatic total cholesterol by 59.5% and triglycerides by 72%, respectively ($P < 0.01$). HPLC analysis led to identify four major compounds: caftaric acid, cafeic acid, chicoric acid and rosmarinic acid. In conclusion, the Sweet basil contains phenolic products that are able to lower hyperlipidemia and prevent atherosclerosis.

1. Introduction

Cardiovascular diseases are the most common cause of death in both developed and developing countries [1], atherosclerosis and related complications account for the majority of these deaths [2]. Epidemiological and experimental studies have clearly shown the association between high blood LDL-cholesterol (LDL-C) concentrations and a high risk of cardiovascular events, the HDL-cholesterol (HDL-C) have, in contrast, a protective effect against the risk [2]. Furthermore, high serum triglyceride levels are also important as risk factor, especially in diabetic individuals since this lipid fraction influences lipid deposition and clotting mechanisms [3]. Generally, the therapeutic purpose of prevention or treatment of atherosclerosis and related cardiovascular diseases is to reduce elevated levels of plasma lipids, particularly LDL-C and triglycerides by drug and/or dietary intervention [4]. In this field, dietary polyphenols are to receive considerable interest for their presumed role in the prevention of various degenerative diseases, such as cancers and cardiovascular diseases [5,6].

Ocimum basilicum L. commonly known as sweet basil (family of Lamiaceae) is a half-hardy annual or short-lived perennial herb, native to Asia, Africa, South America and the Mediterranean but widely cultivated worldwide [7,8]. This is an important culinary herb and medicinal plant [9]. It is a versatile herb that may be used in an abundant variety of food [6]. Many cultivars and varieties are used and some are cultivated especially for the manufacture of pesto [10].

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In Morocco, as in many developing countries, most hyperlipidemic subjects use traditional medicine as the alternative therapeutic tool to treat hyperlipidemia and prevent cardiovascular disease complications. In this regard, several plants generally used in food or beverage preparation were investigated for their lipid-lowering activities, as examples, we note the celery [11] and green tea [12]. In eastern Morocco, besides its use in cooking, *O. basilicum* is widely used as a folk medicine to treat hyperlipidemia and prevent atherosclerosis.

The plant, with an anti-inflammatory activity, has been noted previously [13]. In our earlier studies, we demonstrated the vasorelaxant and anti-platelet aggregation effects of the aqueous basil extract [14]. We also studied the anti-oxidant and hypolipidemic activities of the crude aqueous extract and polar fractions from Sweet basil in acute hyperlipidemia induced by Triton-1339 and in high fat diet (HFD)-induced hyperlipidemic rats and mice [15–18]. Phytochemical studies showed that phenolics constitute the major polar secondary metabolites of this plant [19,20].

In this regard and in order to continue our previous research, the present study was designed to evaluate the possible beneficial effects of phenolic acid-rich extract from *O. basilicum* on plasma and liver lipid parameters in HFD feeding mice.

2. Material and methods

2.1. Preparation of the phenolic acid-rich extract

O. basilicum was purchased from the herbalist in Oujda city (eastern Morocco) and authenticated by a botanist (Prof 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). The phenolic acid-rich extract from the plant aerial parts was prepared as follows: the dried herb was defatted with *n*-hexane to remove chlorophyll and liposoluble substances. The marc obtained was air dried and infused in distilled water for 30 min. The extract was filtered and the solution obtained was concentrated in a rotator evaporator under vacuum at 50 °C. The yield of extraction, in terms of the starting dried plant material, was of 9% (w/w).

2.2. HPLC analysis of phenolic acid-rich extract

HPLC analysis of the phenolic acid-rich extract was carried out on an Agilent 1100 series chromatograph with a Diode Array Detector, using an hepersil ODS reverse phase (RP18) analytical column (250 × 4 mm, particle size 5 μm). The extract (10 μl) was separated at 20 °C at a flow rate of 1 mL/min using the following gradient of aqueous trifluoro-acetic acid (pH 2.8) (A) and acetonitrile (B): 0–1 min: 0–3% B, 1–45 min: 3–40% B, 45–55 min: 40% B, 55–56 min: 0% B. The chromatograms were recorded at 340 nm. Compounds were identified by their retention times and UV–visible spectra using a database of phenolics. A quantitative analysis was recorded using a calibration curve of cafeic and rosmarinic acids.

2.3. Preparation of high fat diet (HFD)

The HFD was prepared daily. It consists of a standard diet (Société SONABETAIL, Oujda, Morocco) 81.8%, cholesterol 2%, lard 16% and cholic acid 0.2%.

2.4. Animals and treatments

Twenty four adult male albinos mice, weighing 20–30 g, bred in the animal house of the Department of Biology (Faculty of Sciences, Oujda, Morocco) were housed in a controlled room with a 12 h light–dark cycle at room temperature of 22 ± 02 °C and given free access to diet and water ad libitum. Animal maintenance and handling were in accordance to the internationally accepted standard guidelines for use of laboratory animals. At the beginning of the experiment, animals were divided into four groups of six animals each as follows:

- normolipidemic control group (NCG): kept on standard diet and daily gavaged with distilled water;
- hyperlipidemic control group (HCG): received HFD and daily gavaged with distilled water;
- basil phenolic acid-rich extract treated group (BTG): received HFD and daily gavaged with plant extract at a dose of 200 mg/kg body weight for 5 weeks;
- fenofibrate-treated group (FTG): received HFD and daily gavaged with fenofibrate at a dose of 200 mg/kg body weight for 5 weeks.

At the end of the experiment, the animals were anaesthetized with diethyl ether and blood was taken from their retro-orbital sinus in the heparinized tube. The blood samples were immediately centrifuged (2500 rpm/15 min) and the plasma was used for lipid analysis.

2.5. Dosage of plasma total cholesterol and triglycerides

Total cholesterol (TC) levels were determined by cholesterol oxidase enzymatic method using enzymatic Kit (Bio Sud Diagnostici S.r.l Italy); cholesterol was hydrolyzed and in the presence of phenol, the quinoneimine as indicator was formed from hydrogen peroxide and 4-aminantipyrine via peroxidase catalysis and was spectrophotometrically measured at 510 nm.

Triglycerides in plasma were also quantified by enzymatic method using Bio Sud Diagnostici Kits. Briefly, after enzymatic hydrolysis with lipases, the formation of quinoneimine from hydrogen peroxide, 4-aminophenazone, and 4-chlorphenol under the catalytic effect of peroxidase was followed spectrophotometrically at 546 nm.

2.6. Determination of plasma HDL and LDL-cholesterol

HDL-C concentrations were quantified by the same method used to determine total cholesterol after the removal of other lipoproteins by precipitation with phosphotungstic acid (PTA) and $MgCl_2$ (Sigma Diagnostic Kit, Inc, USA). The LDL-C was calculated by the Friedwald formula [21]: LDL-cholesterol = total cholesterol – [HDL – cholesterol + (triglycerides/5)].

2.7. Calculation of atherogenic index (AI) and LDL-C/HDL-C ratio

The AI was calculated by the following formula: AI = (TC – HDL-C)/HDL-C. The LDL-C/HDL-C ratio was calculated as the ratio of plasma LDL-C to HDL-C levels.

2.8. Extraction and analysis of liver total cholesterol and triglycerides

At the end of the experiment, livers were removed, rinsed in ice-chilled normal saline and blotted on filter paper, and then, the tissues were cut into small portions and stored at −20 °C before use. Extraction of liver for analysis of total cholesterol and triglycerides was carried out according to Haug and Hostmark method [22]. One gram of liver portions from each animal was homogenized in 10 mL isopropanol. The homogenate was allowed to stand for 48 h at 4 °C. The mixture was centrifuged 15 min at 2500 rpm and the supernatant was used for lipid analysis. TC and triglycerides were quantified using enzymatic kits as described above.