Phenolic extract from *Ocimum basilicum* restores lipid metabolism in Triton WR-1339-induced hyperlipidemic mice and prevents lipoprotein-rich plasma oxidation

Ilham Touiss, Saloua Khatib, Oussama Bekkouch, Souliman Amrani, Hicham Harnafi *

Laboratory of Biochemistry and Biotechnologies, Department of Biology, Faculty of Sciences, 60000 Oujda, Morocco

Received 8 September 2016; received in revised form 15 January 2017; accepted 17 February 2017

Available online 4 March 2017

Abstract

In this study we investigated the hypolipidemic and anti-lipoprotein-oxidation activities of phenolic extract from sweet basil a popular culinary herb. The hypolipidemic activity was studied in mice model injected intraperitoneally with Triton WR-1339 at a dose of 200 mg/kg body weight. The animals were grouped as follows: normolipidemic control group (n = 8), hyperlipidemic control group (n = 8) and phenolic extract-treated group (n = 8) at a dose of 200 mg/kg body weight. After 7 h and 24 h treatment, the oral administration of the phenolic extract exerts a significant reduction of plasma total cholesterol, triglycerides and LDL-cholesterol concentrations (P < 0.001). On the other hand, we demonstrated that the phenolic extract prevents plasma lipid oxidation by 16% (P < 0.001), 20% (P < 0.001), 32% (P < 0.001) and 44% (P < 0.001) at a doses of 10, 25, 50 and 100 μg/mL, respectively. The results were compared with ascorbic acid used as standard synthetic antioxidant. HPLC analysis shows that the extract contains 4 major phenolics and is especially rich in rosmarinic acid. This finding indicates that the phenolic extract might be beneficial in lowering hyperlipidemia and preventing atherosclerosis.

© 2017 Beijing Academy of Food Sciences. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Keywords: Sweet basil; Phenolic acids; Hypocholesterolaemia; Hypotriglyceridaemia; Lipoprotein-rich plasma oxidation

1. Introduction

Hypercholesterolemia and oxidative stress represent the major risk factors for the development and progression of atherosclerosis and related cardiovascular diseases [1,2]. A number of experimental studies have clearly demonstrated that high plasma LDL-cholesterol levels are positively correlated with atherosclerosis process [3]. However, HDL-cholesterol is an antiatherogenic fraction [4]. In addition, a long-standing relationship exists between high plasma triglyceride levels and cardiovascular disease [5].

Lifestyle and dietary habits are crucial in the prevention of some human diseases such as hyperlipidemia and atherosclerosis. Indeed, the Mediterranean diet rich in fruits and vegetables is negatively correlated with cardiovascular risk [6]. Thus, the management of hyperlipidemia needs a strategy based on diet control and hypolipidemic treatment [7]. In this context, it is very important to search functional foods and dietary phytochemicals with hypolipidemic and antioxidant virtues, because hyperlipidemia and low density lipoprotein oxidation are known to be the key process in the physiopathology of atherosclerosis which can be treated by hypolipidemic and antioxidant therapies [3].

Sweet basil (*Ocimum basilicum*) from Lamiaceae family is an aromatic plant cultivated as a culinary herb, condiment or spice and as food aromatizing [11]. In the east of Morocco, sweet basil is not only used for cooking but also considered as medicinal plant to treat hyperlipidaemia, atherosclerosis and related
pathologies. Recently, sweet basil has been proved to demonstrate a number of biological activities, such as antidiabetic [12] and anti thrombotic effects [13].

In the present study we demonstrated that the phenolic acid-rich extract from O. basilicum exerted a significant hypolipidemic activity in Triton WR-1339-induced hyperlipemic mice and prevents efficiently against lipoprotein-rich plasma oxidation. This results could be used to develop natural antiatherogenic treatments from sweet basil.

2. Materials and methods

2.1. Preparation of basil phenolic extract

O. basilicum was purchased from an herbalist in Oujda city (Morocco) and authenticated by a botanist, a voucher specimens has been deposited at the department of Biology (Collection n° LO15). The dried leaves of the plant were ground into a fine powder and treated with LO15). The dried leaves of the plant were ground into a fine powder and treated with

2.2. Determination of total phenol content

Total polyphenols of O. basilicum extract were determined by the Folin–Ciochateau procedure [14] with some modifications. 0.5 mL of samples was mixed with 0.25 mL of Folin–Ciocalteau reagent and 0.5 mL of the sodium carbonate solution 20%. The mixture was stirred and adjusted to 5 mL with distilled water. The blue coloration was developed for 30 min in the dark. Then, it was measured spectrophotometrically at 725 nm against a blank.

2.2. Determination of total phenol content

Total polyphenols of O. basilicum extract were determined by the Folin–Ciochateau procedure [14] with some modifications. 0.5 mL of samples was mixed with 0.25 mL of Folin–Ciocalteau reagent and 0.5 mL of the sodium carbonate solution 20%. The mixture was stirred and adjusted to 5 mL with distilled water. The blue coloration was developed for 30 min in the dark. Then, it was measured spectrophotometrically at 725 nm against a blank.

2.2. Determination of total phenol content

Total polyphenols of O. basilicum extract were determined by the Folin–Ciochateau procedure [14] with some modifications. 0.5 mL of samples was mixed with 0.25 mL of Folin–Ciocalteau reagent and 0.5 mL of the sodium carbonate solution 20%. The mixture was stirred and adjusted to 5 mL with distilled water. The blue coloration was developed for 30 min in the dark. Then, it was measured spectrophotometrically at 725 nm against a blank.

2.2. Determination of total phenol content

Total polyphenols of O. basilicum extract were determined by the Folin–Ciochateau procedure [14] with some modifications. 0.5 mL of samples was mixed with 0.25 mL of Folin–Ciocalteau reagent and 0.5 mL of the sodium carbonate solution 20%. The mixture was stirred and adjusted to 5 mL with distilled water. The blue coloration was developed for 30 min in the dark. Then, it was measured spectrophotometrically at 725 nm against a blank.

2.2. Determination of total phenol content

Total polyphenols of O. basilicum extract were determined by the Folin–Ciochateau procedure [14] with some modifications. 0.5 mL of samples was mixed with 0.25 mL of Folin–Ciocalteau reagent and 0.5 mL of the sodium carbonate solution 20%. The mixture was stirred and adjusted to 5 mL with distilled water. The blue coloration was developed for 30 min in the dark. Then, it was measured spectrophotometrically at 725 nm against a blank.

2.3. HPLC analysis of phenolic extract from sweet basil

HPLC analysis of the basil phenolic extract was carried out on an Agilent 1100 series chromatograph (Agilent, Palo Alto, CA) with a Diode Array Detector, using an Inerbil ODS II reverse phase (RP18) analytical column (250 x 4.6 mm, particle size 5 µm).

The extract (20 µL, 1 mg/mL in methanol) was separated at 30 °C at a flow rate of 1 mL/min using the following gradient of aqueous orthophosphoric acid (pH3) (A) and acetonitrile (B): 0–5 min: 0% B, 5–7 min: 0%–5% B, 7–10 min: 5%–7% B, 10–15 min: 7%–10% B, 15–20 min: 10%–15% B, 20–25 min: 15%–18% B, 25–30 min: 18%–20% B, 30–33 min: 20%–25% B, 33–38 min: 25%–28% B, 38–42 min: 28%–34% B, 42–45 min: 34%–38% B, 45–65 min: 38%–45% B, 65–75 min: 45%–55% B, 75–85 min: 55%–0% B. The chromatogram was recorded at 280 nm. Compounds were identified by their retention times and UV–vis spectra using a database of standard phenolics.

2.4. Animals and treatments

2.4.1. Experimental animals

Twenty four adult male albinos mice, aged 5 weeks (25–30 g), bred in the animal house of the Department of Biology (Faculty of Sciences, Oujda) were provided access only to water during the experimental duration. Their housing was maintained at a temperature of 22 ± 2 °C with a 12 h light–dark cycle. Animal maintenance and handling were in accordance to the accepted standard guidelines for use of laboratory animals.

2.4.2. Experimental schedule

Triton WR-1339 (Tyloxsophol, Sigma–Aldrich, USA) was dissolved in normal saline (pH 7.4). The experimental mice were fasted overnight and divided into three groups of eight animals each:

- Normal lipemic control group (NCG): injected with normal saline (pH 7.4) and gavaged with distilled water;
- Hyperlipemic control group (HCG): injected with Triton WR-1339 (200 mg/kg BW) and gavaged with 4% aqueous DMSO solution;
- Phenolic extract treated group (PTG): injected with Triton and gavaged with the extract at a dose of 200 mg/kg BW dissolved in 4% aqueous DMSO.

After 7 h and 24 h of treatments, blood was taken from the retro-orbital sinus of animals after brief anaesthesia with diethyl ether. The blood samples were immediately centrifuged at 2500 r per min for/10 min and the was used for lipid analysis.

2.5. Dosage of plasma total cholesterol and triglycerides

Total cholesterol was analyzed enzymatically using Bio sud Diagnostici Kits (Italy); 10 µL of plasma were mixed with 1 mL of buffered enzymatic solution. The coloured quinoneimine produced was measured at 510 nm. Triglycerides in plasma were also quantified by an enzymatic method using Bio Sud Diagnostici kits (Italy); 10 µL of plasma were added to 1 mL of enzymatic solution to develop a chromophore which is spectrophotometrically measured at 540 nm.

2.6. Determination of plasma HDL and LDL-cholesterol

Plasma HDL-cholesterol was assessed after precipitation of other lipoprotein (LDL and VLDL) using a mixture of phosphotungstic acid and MgCl2 (Sigma Diagnostic kit, Inc., USA). Briefly, 10 µL of plasma were added to 10 µL of the mixture. 10 µL of supernatant were used to analyse cholesterol as described above by the same method used to determine total cholesterol.
The plasma LDL-cholesterol was calculated using the Fried- 
wald formula [15]:

$$LDL-\text{Cholesterol} = \text{Total cholesterol} - \left[ \text{HDL-} \text{Cholesterol} + \frac{\text{triglycerides}}{5} \right].$$

2.7. Determination of lipoprotein-rich plasma oxidation

The thiobarbituric acid reactive substances (TBARS) levels were analysed as marker of plasma lipoprotein oxidation according to the method described by Park et al. [16]. Lipoprotein-rich plasma used as substrate for oxidative process was obtained from mice injected with Triton WR-1339 at a dose of 600 mg/kg body weight for 24 h, the plasma contains 100 ± 8.5 mg/dL of LDL-cholesterol (analysed by enzymatic kit as described above). In the control tube, lipoprotein-rich plasma (40 µL) was incubated with distilled water only. In a second control tube, lipoprotein-rich plasma (40 µL) was incubated with 10 µL of copper sulphate (CuSO$_4$·5H$_2$O) solution (0.33 mg/mL). In the third assay, lipoprotein-rich plasma (40 µL) was incubated with 10 µL of copper sulphate and the phenolic extract was added at 10, 25, 50 and 100 µg/mL. The preparations were mixed vigorously and incubated 24 h at 30°C. Then, 100 µL of 8.1% (w/v) sulphate dodecyl sodium (SDS) was added to each assay. The mixture was stirred and incubated for 60 min at room temperature. Each tube was added of 250 µL of 20% trichloroacetic acid (pH 3.5) and 250 µL of 0.8% (w/v) thiobarbituric acid (TBA), the reaction mixture was heated at 95°C for 60 min. After cooling, 1 mL of n-butanol was added and the tubes were vortexed. The solution was centrifuged at 4500 r per min for 15 min. The absorbance of the coloured layer was recorded at 532 nm. The amounts of TBARS (thiobarbituric acid reactive substances) were calculated and expressed as 1,1,3,3-tetramethoxypropane equivalent from the calibration curve of standard solutions. The ascorbic acid was used as standard known antioxidant. All measurements were done in triplicate.

2.8. Statistical analysis

Data were analysed using Student’s t-test and one-way ANOVA and differences with P values less than 0.05 were considered statistically significant. The results are expressed as mean ± SEM.

3. Results

3.1. HPLC analysis of basil phenolic extract

The determination of total phenol content showed that O. basilicum extract is rich in phenolics, the amount was 174.39 ± 0.92 mg rosmarinic acid equivalent/g dry extract. The Fig. 1 depicts the HPLC chromatogram of the basil extract. We identified four phenolic acids: caftaric acid, chicoric acid, rosmarinic acid and cafeic acid.

3.2. Induction of hyperlipemia by Triton WR-1339

The intraperitoneal injection of Triton resulted in a marked increase in plasma lipid concentrations. Total cholesterol levels in Triton-injected mice were statistically higher than those in control after 7 h (+175%; P < 0.001) and 24 h (+133%; P < 0.001). Triglyceride concentrations were also significantly elevated in Triton-injected mice comparatively to the control. The levels were increased by 387% (P < 0.001) after 7 h treatment and 107% (P < 0.001) after 24 h (Figs. 2 and 4).

On the other hand, the Triton produced no statistically significant changes in plasma HDL-cholesterol after 7 h and 24 h treatment. However, LDL-cholesterol was increased by +830%
Fig. 2. Effect of basil phenolic extract on mice total cholesterol and triglycerides after 7 h treatment.
TC: total cholesterol, TG: triglycerides, NCG: normolipidemic control group, HCG: hyperlipidemic control group, PTG: phenolic extract treated group.
*aP < 0.001 (HCG versus NCG and PTG versus HCG).

Fig. 3. Effect of basil phenolic extract on mice plasma HDL and LDL-cholesterol after 7 h treatment.
HDL-C: high density lipoprotein-cholesterol, LDL-C: low density lipoprotein-cholesterol, NCG: normolipidemic control group, HCG: hyperlipidemic control group, PTG: phenolic extract treated group.
*aP < 0.001; ns: not significant (HCG versus NCG and PTG versus HCG).

Fig. 4. Effect of basil phenolic extract on mice total cholesterol and triglycerides after 24 h treatment.
TC: total cholesterol, TG: triglycerides, NCG: normolipidemic control group, HCG: hyperlipidemic control group, PTG: phenolic extract treated group.
*aP < 0.001 (HCG versus NCG and PTG versus HCG).

(P < 0.001) and +495% (P < 0.001) after 7 h and 24 h, respectively (Figs. 3 and 5).

3.3 Effect of basil phenolic extract on mice plasma lipid parameters

Plasma total cholesterol concentrations in phenolic extract-treated group appear to be statistically decreased both after 7 h and 24 h treatment (−81%; P < 0.001). Furthermore, the extract also suppressed the high plasma triglycerides rise after 7 h and 24 h by 65% (P < 0.001) and 83% (P < 0.001), respectively (Figs. 2 and 4). LDL-cholesterol was seen to be significantly decreased in treated group in comparison to hyperlipidemic control after 7 h (−86%; P < 0.001) and 24 h (−81%; P < 0.001). In addition, the phenolic extract relatively hindered plasma HDL-cholesterol in treated mice both at 7 h and at 24 h (−69%; P < 0.001 and −80%; P < 0.001, respectively) (Figs. 3 and 5).

3.4 Effect of basil phenolic extract on the oxidation of lipoprotein-rich plasma

The evaluation of the oxidation products clearly showed that the levels of TBARS were significantly increased in lipoprotein-rich plasma submitted to copper sulphate induction when compared with control (+41%, P < 0.001). In contrast, the phenolic extract from O. basilicum at a doses of 10, 25, 50 and 100 μg/mL led to suppress the TBARS production by 16% (P < 0.001), 20% (P < 0.001), 32% (P < 0.001) and 44% (P < 0.001), respectively (Fig. 6). We note also that the ascorbic acid, used as standard known antioxidant, exerted a marked inhibition of lipoprotein oxidation. In fact, it decreases the production of TBARS by 15.5% (P < 0.001), 48% (P < 0.001), 59%
demonstrated that phenolic extract from sweet basil has exerted free radical-related pathologies [29]. Our experimental study due to their free hydroxyl groups and protect efficiently against medicinal and aromatic plants possess high antioxidant potential gations have demonstrated that phenolic compounds from diet, systemic of the human body. Besides, many experimental investi-

gations have shown that the phenomenon of modification and very atherogenic LDL deposits [25,26]. Our experimental study shows that the phenolic extract from O. basilicum significantly reduced triglycerides levels. This lowering activity can be explained by the enhancement of the plasma lipoprotein lipase and hepatic lipase activities as shown with other plant extracts [27].

On the other hand, free radicals were shown to cause oxidative modification of biomolecules contributing to oxidative stress which is believed to be the origin of a many diseases including atherosclerosis risk [24]. The elevated plasma triglyceride concentrations have been also associated with cholesterol esterification and very atherogenic LDL deposits [25,26]. Our experimental study shows that the phenolic extract from O. basilicum significantly reduced triglycerides levels. This lowering activity can be explained by the enhancement of the plasma lipoprotein lipase and hepatic lipase activities as shown with other plant extracts [27].

We observed that the extract significantly decreased the mice plasma total cholesterol. This activity was associated with a diminution in LDL-cholesterol representing the atherogenic fraction [19]. As demonstrated by Mbikay et al., [20], this finding led us to suggest that the cholesterol-lowering activity of the phenolic extract might be due to the activation of LDL hepatic receptors (B/E) for the final clearance as bile acids. We suggest also, on the basis of other studies, that the hypolipidemic effect can be explained by other mechanisms involving modulation of enzymes implicated in cholesterol metabolism: HMG-CoA reductase [21], acyl CoA cholesterol acyl transferase (ACAT) [22] and lecithin-cholesterol acyl transferase (LCAT) [23].

Triglycerides play a central role in the regulation of lipoprotein interactions to maintain lipid metabolism. These lipids are not directly atherogenic but represent an important marker of atherosclerosis risk [24]. The elevated plasma triglyceride concentrations have been also associated with cholesterol esterification and very atherogenic LDL deposits [25,26]. Our experimental study shows that the phenolic extract from O. basilicum significantly reduced triglycerides levels. This lowering activity can be explained by the enhancement of the plasma lipoprotein lipase and hepatic lipase activities as shown with other plant extracts [27].

We conclude that this work provides an important biological activities of the culinary herb O. basilicum. The plant is rich in rosmarinic acid which can be the major compound, acting alone or in synergy with other phenolic acids, responsible for the hypolipidemic and antioxidant activities. These results could be exploited to develop a natural preparation able to prevent hyperlipidemia and atherosclerosis.

**Conflicts of interest**

The authors declare no conflicts of interest.

**Acknowledgment**

The authors would like to thank El mostapha BEDRAOUI for helping in animal care.

**References**


[8] M. Miele, R. Dondero, G. Ciarallo, M. Mazzei, Methyleugenol in Oci-


