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

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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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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

as mg catechin/g dry plant extract. All measurements were done in triplicate.

2.6. Dosage of flavonoids

Flavonoids content was determined by the method of Jay, Gonnet, Wollenweber, and Voirin (1975). To each 5 ml of analyzed solution, 2.5 ml of $AlCl_3$ reagent were added (133 mg crystalline aluminium chloride and 400 mg crystalline sodium acetate were dissolved in 100 ml of extracting solvent) and absorbances were recorded at 430 nm against a blank (5 ml of analyzed solution plus 2.5 ml of water). The flavonoids content was determined as rutin equivalents from the calibration curve of rutin standard solutions and expressed as mg rutin/g of dry plant extract. All measurements were done in triplicate.

2.7. Animals and treatments

Sixty four adult male albino mice, weighing 27–30 g, bred in the animal house of the department of biology (Faculty of Sciences, Oujda, Morocco), were provided *ad libitum* access only to tap water throughout the experimental duration (24 h). Their housing was maintained at a temperature of 22 ± 02 °C with a 12 h light-dark cycle.

2.8. Triton model of hyperlipideamia

Triton WR-1339 (Tyloxapol, Sigma–Aldrich, USA) was dissolved in normal saline (pH 7.4) and administered intraperitoneally to the mice (200 mg/kg B.W) in order to develop an acute hyperlipidemia in them.

2.9. Experimental design

Overnight fasted mice were randomly divided into eight groups of eight animals each. The first group, serving as a normal control (NCG), received an intraperitoneal administration of normal saline and water by gavage; the second, hyperlipidemic control group (HCG) was treated with Triton and gavaged by distilled water; the third, hyperlipidemic plus DMSO control group (HDCG) received an intraperitoneal injection of Triton and was gavaged with DMSO 4% (in distilled water). In the fourth treated group (CETG), animals were intraperitoneally injected with Triton, followed by an intragastric administration of crude aqueous basil extract (0.5 g/100 g BW); the fifth grouped mice (DMETG) were also treated by Triton and gavaged by the dichloromethane-soluble extract dissolved in DMSO-distilled water mixture (4% v/v) at a dose of 0.2 g/100 g BW; in the sixth, ethyl acetate extract-treated group (EETG), administration of Triton was followed by an intragastric treatment with ethyl acetate-soluble extract (0.2 g/100 g BW) suspended in distilled water; the mice of the seventh treated group (METG) were gavaged by the methanol-soluble extract (0.2 g/100 g BW) dissolved in distilled water after Triton injection; the last group (AFTG) was also intraperitoneally injected with Triton and intragastrically treated with the aqueous basil fraction (0.2 g/100 g BW).

After treatments (7 h and 24 h), animals were anaesthetised briefly with diethyl ether and blood was taken from their tail vein using a heparinised capillary. The blood samples were immediately centrifuged (2500 rpm/10 min) and the plasma was used for lipid analysis.

2.10. Analytical procedures

2.10.1. Plasma triglycerides, total cholesterol, HDL and LDL-cholesterol

Triglycerides in plasma were quantified by an enzymatic method using Bio Sud Diagnostici kits (Bio sud Diagnostici S.r.l. Italy). Briefly, after enzymatic hydrolysis with lipases, the formation of quinoneimine from hydrogen peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic effect of peroxidise, was followed spectrophotometrically at 540 nm.

Total cholesterol levels were determined by the cholesterol oxidase enzymatic method, using Biosud Diagnostici Kits (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-aminoantipyrine via peroxidase catalysis and spectrophotometrically measured at 510 nm.

HDL-cholesterol concentrations were quantified by the same method as used to determine total cholesterol after removal of other lipoproteins by precipitation with phosphotungstic acid (PTA) and $MgCl_2$ (Sigma Diagnostic kit, Inc, USA).

The LDL-cholesterol was calculated by the Friedwald formula (Friedwald, Levy, & Fredrickson, 1972):

$$\text{LDL-Cholesterol} = \text{totalcholesterol} - [\text{HDL-Cholesterol} + (\text{triglycerides}/5)].$$

2.10.2. Atherogenic index (AI) and LDL-C/HDL-C ratio

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

2.11. Statistical analysis

Data obtained were analyzed using the Student's *t*-test and a *P* values less than 0.05 was considered statistically significant. Our results were expressed as means ± SEM.

3. Results

3.1. Polyphenol content of O. basilicum extracts

The determination of polyphenol composition of aqueous and organic *O. basilicum* extracts is shown in

Table 1
Polyphenol content of *Ocimum basilicum* extracts

Extracts	Total phenols[a]	Tannins[a]	Flavonoids[b]
Crude aqueous extract	129 ± 6.39	54.2 ± 4.01	30 ± 1.00
Dichloromethane extract	24 ± 0.57	4.49 ± 0.49	14.9 ± 3.92
Ethyl acetate extract	83 ± 1.52	56 ± 3.21	12.8 ± 3.04
Methanol extract	110 ± 0.57	46.5 ± 0.29	21.8 ± 1.51
Aqueous fraction	36.3 ± 0.88	4.66 ± 0.25	5.7 ± 0.43

Values are expressed as means ± SEM from three assays.
[a] Expressed as mg catechin/g dry extract.
[b] Expressed as mg rutin/g dry extract.

Table 1; it appears clear that the crude and methanol extracts contain more phenolics than do other extracts. Besides, the result shows that the tannins and flavonoids represent the major polyphenol fractions of this plant. Tannins represent, in terms of total phenols, 42%, 18%, 67%, 42% and 13%, in the crude, dichloromethane, ethyl acetate, methanol and aqueous extracts, respectively. The amounts of flavonoids in the same extracts were 23%, 62%, 15%, 20% and 16%, respectively.

3.2. Induction of hyperlipidemia by Triton WR-1339

The plasma total cholesterol and triglyceride levels of all groups 7 h and 24 h after treatments are shown in Tables 2 and 3. In comparison with the normal control group (NCG), Triton WR-1339 caused a marked increase of plasma total cholesterol and triglyceride levels of the hyperlipidemic control group (HCG) and HCG+DMSO 4% (HDCG), at both 7 h and 24 h after injection. In fact, 7 h after Triton administration the increases of plasma total cholesterol concentration were 149% in HCG and 176% in HDCG with respect to the NCG. Triglycerides levels were also elevated by 380% and 387% in HCG and HDCG, respectively. Again, 24 h after treatment, the elevated plasma lipid profile was maintained, either in the hyperlipidemic group mice (HCG) or in those receiving Triton, followed by 4% DMSO (HDCG). In the HCG, the increase of total cholesterol was 140% and that of triglycerides was 112%. In the HDCG, a similar significant pattern of change was observed for blood total cholesterol (+134%) and triacylglycerol levels (+107%) when compared to the NCG.

HDL and LDL-cholesterol concentrations are shown in Tables 2 and 3. Neither at 7 h nor at 24 h was the HDL-cholesterol significantly changed in both HCG and HDCG with respect to their relative control group (NCG), while a significant increase on LDL-cholesterol levels occurred at 7 h and was maintained until 24 h from Triton injection. LDL-cholesterol concentrations in HCG and HDCG were respectively, 784% and 830% higher than those in normal control grouped animals after 7 h. Also, the increase of this parameter was partially reduced to +474% and +496% in HCG and HDCG, respectively, 24 h after the beginning of the experiment.

Table 4 shows the changes of atherogenic index (AI) and LDL-C/HDL-C ratio in control and treated mice. It appears clear from these results that the Triton administration significantly affects the cardiovascular risk markers.

Indeed, the AI was statistically increased in both HCG (+840%) and HDCG (+637%) when compared with values found in their relative normolipidemic control at 7 h. Similar results were noticed at 24 h. The AI of HCG was 389% higher than that of NCG and this risk marker was more than seven times increased in HDCG with respect to NCG.

Besides, there were significant further increases of LDL-C/HDL-C ratios in Triton-injected animals (HCG and HDCG). In contrast to normolipidemic mice, 7 h after Triton treatment produced an elevated ratio either in hyperlipidemic group animals (817%) or in HDCG (more than 13 times). This changing pattern was maintained until 24 h when the ratios were increased by 509% and 850% in HCG and HDCG, respectively, compared to NCG.

Table 2
Effect of *Ocimum basilicum* on plasma lipid levels in Triton WR-1339-induced hyperlipemic mice after 7 h

Lipid parameters	TC (mg/dl plasma)	TG (mg/dl plasma)	HDL-C (mg/dl plasma)	LDL-C (mg/dl plasma)
NCG	181 ± 5.17	61.5 ± 3.56	137 ± 5.70	31.2 ± 4.50
HCG	450 ± 22.3[b]	295 ± 3.83[b]	115 ± 9.61[NS]	276 ± 27.2[b]
HDCG	498 ± 32.4[b]	300 ± 5.43[b]	132 ± 14.3[NS]	290 ± 22.3[b]
CETG	190 ± 14.5[b]	71.6 ± 4.36[b]	106 ± 5.17[NS]	70.3 ± 16.9[b]
DETG	414 ± 9.86[a]	284 ± 5.92[NS]	99.8 ± 10.6[NS]	257 ± 10.4[NS]
EETG	192 ± 9.18[b]	156 ± 14.4[b]	90.7 ± 11.0[NS]	60.3 ± 7.31[b]
METG	211 ± 6.99[b]	87.8 ± 7.26[b]	117 ± 6.98[NS]	76.0 ± 10.5[b]
AFTG	196 ± 9.97[b]	80.2 ± 8.55[b]	134 ± 6.45[NS]	45.8 ± 13.0[b]

Values are means ± SEM from eight animals in each group. NCG: normal control group; HCG: hyperlipidemic control group; HDCG: hyperlipidemic+4% DMSO control group; CETG: total aqueous basil extract-treated group; DETG: dichloromethane extract-treated group; EETG: ethyl acetate extract-treated group; METG: methanol extract-treated group; AFTG: aqueous fraction-treated group; TC: total cholesterol; TG: triglycerides; HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol.
HCG and HDCG are compared with NCG. DETG is compared with HDCG. TETG, EETG, METG and AFTG are compared with HCG.
NS: not significant.
[a] $P < 0.05$.
[b] $P < 0.001$.

Table 3
Effect of *Ocimum basilicum* on plasma lipid levels in Triton WR-1339-induced hyperlipemic mice after 24 h

Lipid parameters	TC (mg/dl plasma)	TG (mg/dl plasma)	HDL-C (mg/dl plasma)	LDL-C (mg/dl plasma)
NCG	222 ± 7.86	137 ± 6.16	136 ± 3.54	60.1 ± 7.72
HCG	533 ± 12.7[b]	289 ± 7.62[b]	130 ± 4.95[NS]	345 ± 14.7[b]
HDCG	518 ± 10.2[b]	283 ± 9.05[b]	104 ± 13.3[NS]	358 ± 16.4[b]
CETG	227 ± 7.92[b]	95.6 ± 6.33[b]	142 ± 6.39[NS]	59.5 ± 13.0[b]
DETG	465 ± 20.3[a]	282 ± 11.0[NS]	98.6 ± 21.9[NS]	311 ± 30.0[NS]
EETG	254 ± 4.89[b]	129 ± 12.4[b]	186 ± 5.34[NS]	48.8 ± 11.8[b]
METG	240 ± 9.39[b]	106 ± 12.7[b]	169 ± 11.6[NS]	49.6 ± 7.94[b]
AFTG	226 ± 10.4[b]	61.2 ± 2.69[b]	175 ± 10.3[NS]	41.2 ± 11.6[b]

Values are means ± SEM from eight animals in each group. NCG: normal control group; HCG: hyperlipidemic control group; HDCG: hyperlipidemic+4% DMSO control group; CETG: total aqueous basil extract-treated group; DETG: dichloromethane extract-treated group; EETG: ethyl acetate extract-treated group; METG: methanol extract-treated group; AFTG: aqueous fraction-treated group; TC: total cholesterol; TG: triglycerides; HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol.
HCG and HDCG are compared with NCG. DETG is compared with HDCG. TETG, EETG, METG and AFTG are compared with HCG.
NS: not significant.
[a] $P < 0.05$.
[b] $P < 0.001$.

Table 4
Effects of *Ocimum basilicum* on atherogenic index (AI) and LDL/HDL-C ratio in Triton WR-1339-induced hyperlipemic mice after 7 h and 24 h

Groups	AI (7 h)	AI (24 h)	LDL/HDL (7 h)	LDL/HDL (24 h)
NCG	0.32 ± 0.04	0.64 ± 0.07	0.23 ± 0.08	0.44 ± 0.06
HCG	3.01 ± 0.70[c]	3.13 ± 0.19[d]	2.11 ± 0.17[c]	2.68 ± 0.18[c]
HDCG	2.36 ± 0.26[d]	4.83 ± 1.00[d]	3.13 ± 1.03[a]	4.18 ± 0.87[c]
CETG	0.84 ± 0.15[a]	0.63 ± 0.11[d]	0.70 ± 0.20[c]	0.42 ± 0.10[c]
DETG	3.45 ± 0.33[NS]	6.57 ± 2.32[NS]	2.83 ± 0.37[NS]	5.61 ± 1.64[NS]
EETG	1.39 ± 0.26[NS]	0.37 ± 0.13[d]	0.86 ± 0.26[b]	0.26 ± 0.06[c]
METG	0.85 ± 0.11[b]	0.45 ± 0.08[d]	0.69 ± 0.14[c]	0.31 ± 0.06[c]
AFTG	0.48 ± 0.11[c]	0.30 ± 0.06[d]	0.36 ± 0.11[c]	0.24 ± 0.06[c]

Values are means ± SEM from eight animals in each group. NCG: normal control group; HCG: hyperlipidemic control group; HDCG: hyperlipidemic+4% DMSO control group; CETG: total aqueous basil extract-treated group; DETG: dichloromethane extract-treated group; EETG: ethyl acetate extract-treated group; METG: methanol extract-treated group; AFTG: aqueous fraction-treated group; TC: total cholesterol; TG: triglycerides; HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol.
HCG and HDCG are compared with NCG. DETG is compared with HDCG. TETG, EETG, METG and AFTG are compared with HCG.
NS: not significant.
[a] $P < 0.05$.
[b] $P < 0.02$.
[c] $P < 0.01$.
[d] $P < 0.001$.

3.3. Effect of aqueous and organic Basil extracts on mice plasma lipid profile

3.3.1. Total cholesterol and triglycerides

The plasma total cholesterol and triglyceride levels of aqueous-organic basil extract-treated mice are shown in Tables 2 and 3. Importantly, the elevated total cholesterol concentrations produced by Triton administration after 7 h were significantly (P values <0.001) suppressed by more than 50% in animals gavaged with crude aqueous (CETG), ethyl acetate (EETG), methanolic (METG) and aqueous extracts. However, the reduction of total cholesterol by dichloromethane-soluble extract was not very marked (−17%, $P < 0.05$) in DMETG compared to its relative hyperlipidemic control group (HDCG). Although the plasma TG levels of the mice treated with dichloromethane-soluble fraction were not significantly deceased with respect to the levels found in animals of HDCG, polar solvent-soluble fractions produced a significant reduction ($P < 0.001$) of TG concentrations in CETG (−76%), EETG (−47%) and METG (−70%), as well as in AFTG (−73%). Again, from 7 h, the similar lipid lowering effect of various extracts was maintained until 24 h (Table 3).

3.3.2. HDL and LDL-cholesterol

7 h after treatment, no significant differences in blood HDL-C between any treated groups were observed (Table 2). In contrast, 24 h after treatment, both ethyl acetate-and aqueous-soluble fractions significantly increase of HDL-C levels, by 42% and 34%, respectively. The levels of this cholesterol fraction also tended to increase by the methanol-soluble extract but the changing pattern remained statistically insignificant (Table 3).

Neither after 7 h or at 24 h did the dichloromethane soluble extract statistically decrease LDL-cholesterol, while all other plant extracts showed a significant ameliorative action on plasma elevated LDL-cholesterol caused by Triton WR-1339 (Tables 2 and 3). Indeed, 7 h after the beginning of the

experiment, the significant ($P < 0.001$) decreases of plasma LDL-C were 74%, 78%, 72% and 83% in CETG, EETG, METG and AFTG, respectively. Polar extractant-soluble agents from *O. basilicum* significantly prevented the increases of LDL-C during the period of study. At the end of the experiment (24 h), the elevated values of LDL-C were suppressed in CETG, EETG, METG and AFTG at significance levels similar to that obtained at 7 h with some improvements compared to the HCG (Table 3).

3.3.3. Atherogenic index (AI) and LDL-C/HDL-C ratio

Promising results in lowering of the AI by the polar products extracted from *O. basilicum* in Triton-induced hyperlipidemic mice were found (Table 4). This cardiovascular predictive marker in dichloromethane-soluble agent-treated mice (DETG) was not significantly different from the hyperlipidemic control group mice treated with 4% DMSO (HDCG). However, all polar solvent-soluble extracts showed an improvement of the cardiovascular risk level by the decrease of AI in the treated groups (CETG, EETG, METG and AFTG) by more than 40% at 7 h and 79% at 24 h (P values are less than 0.001) when compared to their corresponding hyperlipemic control (HCG).

The ratio of LDL-C to HDL-C is also a predictive indicator of cardiovascular disease incidence. The Triton injection produced a significant increase of this marker and the dichloromethane extract did not statistically change it, either at 7 h or at 24 h after treatment. In contrast, elevated ratios in CETG, EETG, METG and AFTG nearly returned to basal values after 7 h when the data were compared, in the same period, to data found for hyperlipidemic mice (Table 4).

4. Discussion and conclusions

The non-ionic detergent, Triton WR-1339, has been widely used to block the uptake of triacyl glycerol-rich lipoproteins from plasma by peripheral tissues in order to produce acute hyperlipidemia in animal models which are often used for a number of objectives, in particular for screening natural or chemical hypolipidemic drugs (Schurr et al., 1972). With this aim, many medicinal plants, such as *Phylanthus nuriri,* have been assessed for their hypolipidemic activity in a Triton WR-1339-induced hyperlipidemic model (Khanna et al., 2002). In fact, Schurr et al. (1972) demonstrated that parenteral administration of Triton induced hyperlipidemia in adult rats; maximum blood cholesterol and triglyceride levels were reached at 20 h, followed by a decline to normal values. Similar results were reported by Lauk, Galati, Forestieri, Kirjavainen, and Trovato (1989) when investigating (with the same model), the hypolipidemic activity of *Muccuna pruriens*. In our hands, this model gave similar plasma lipid profile changes, both at 7 h and at 24 h after Triton WR-1339 injection in mice. This result demonstrates the feasibility of using Triton-induced hyperlipidemic mice as an experimental model to investigate the hypolipidemic effect of *O. basilicum* extracts.

This work was aimed at the assessment of the possible hypocholesterolemic and hypotriglyceridemic activities of aqueous and organic *O. basilicum* extracts. So, it is clear from our results that the aqueous and organic fractions from this plant decrease plasma total cholesterol in a marked manner, either 7 h or 24 h after Triton treatment.

The reduction of plasma total cholesterol was associated with a decrease of its LDL fraction which is a major, potentially modifiable risk factor of cardiovascular diseases and the target of many hypocholesterolemic therapies. This finding suggests that the cholesterol-lowering activity of these extracts appears to be due to the enhancement of LDL-C catabolism through hepatic receptors, as demonstrated by Khanna et al. (2002). In addition, the ethyl acetate and aqueous fractions showed protective action by the increase of HDL-cholesterol levels, which is reported to have a preventive function against atherogenesis since an independent inverse relationship between blood HDL-C levels and cardiovascular risk incidence has been documented and reported beyond any doubt (Malloy & Kan, 1994). This lipoprotein called "good cholesterol" facilitates the mobilisation of triglycerides and cholesterol from plasma to liver where it is catabolised and eliminated in the form of bile acids. The possible mechanism of this activity may result from the enhancement of lecithin cholesteryl acyl transferase (LCAT) and inhibition of hepatic triglyceride lipase (HTL) on HDL which may lead to a rapid catabolism of blood lipids through extrahepatic tissues (Anila & Vijayalakshmi, 2002; Sudheesh, Presannakumar, Vijayakumar, & Vijayalakshmi, 1997).

It is also recently reported that triglycerides play a key role in the regulation of lipoprotein interactions to maintain normal lipid metabolism. Indeed, the elevated plasma TGs levels were associated with an increased incidence of coronary artery disease (Hokanson & Austin, 1996). Moreover, these higher plasma TG levels have been attributed mainly to an increased population of small, dense LDL deposits which are very atherogenic (Austin, Hokanson, & Brunzell, 1994) and enhanced cholesteryl ester mass transfer from apolipoprotein B-containing lipoproteins (VLDL and LDL) (Guérin et al., 2001). TGs have also been proposed to be a major determinant of cholesterol esterification, its transfer and HDL remodelling in human plasma (Murakami et al., 1995).

Polar extractant-soluble fractions from *O. basilicum* significantly suppressed the elevated blood concentrations of TGs. This result suggests that the extracts are able to restore, at least partially, the catabolism of triglycerides. The underlying mechanism of this activity is not elucidated by the present study. However, as hypothesised by many works with other plants (Pérez, Canal, Campello, Adelaida, & Torres, 1999; Sudheesh et al., 1997; Xie et al., 2007), the restoration of catabolic metabolism of triglycerides could be due to an increased stimulation of the lipolytic activity of plasma lipoprotein lipase (LPL).

Administration of *O. basilicum* provides a beneficial action on mice lipid metabolism in regard to the reduction

of AI. In fact, the AI was deceased in all polar basil fraction treated groups. Similar results were reported by others when studying the hypolipidemic effect of natural products (Cherng & Shih, 2005). This ameliorative action was due to the plasma lipid-lowering activity of different fractions.

It is also desirable to have higher plasma HDL and lower LDL-cholesterol to prevent atherogenesis, since there is a positive correlation between an increased LDL-C/HDL-C ratio and the development of atherosclerosis. Again, the administration of total aqueous *O. basilicum* extract and its polar fractions (ethyl acetate, methanol and aqueous extracts) significantly suppressed the higher values of LDL-C/HDL-C ratio showing the beneficial effect of this plant in preventing atherosclerosis incidence.

The results found clearly demonstrate that the bioactive compound(s) contained in this plant have a polar character since they are more soluble in water and polar organic solvents. This finding is in agreement with previous reports showing that plant methanol-, ethyl acetate- and water-soluble extracts possess cholesterol-suppressive capacities and an ability to attenuate the accelerated development of atherosclerosis in hypercholesterolemic models.

In fact, flavonoids and tannins, a heterogeneous group of ubiquitous plant polyphenols, exhibit different pharmacological activities, including hypolipidemic and anti-atherogenic effects (Del Bas et al., 2005; Koshy, Anila, & Vijayalakshmi, 2001; Tebib, Besançon, & Rouanet, 1994; Lee et al., 2006). Thus, there is ample evidence to suggest that flavonoids and tannins are the major compounds responsible for the hypolipidemic activity of sweet basil, since polar extracts, including water, methanol and ethyl acetate (containing tannins and flavonoid glycosides), are more effective than are non-polar extracts, e.g. dichloromethane (rich in genins "free flavonoids" and liposoluble compounds).

Furthermore, quantification of total phenol, tannin, and flavonoid contents in plant samples confirmed the results reported by Javanmardia, Stushnoffb, Lockeb, and Vivancob (2003) demonstrating that these phenolic fractions represent major polar compounds of *O. basilicum*. Again, the finding strongly suggests that the hypolipidaemic activity of this plant could be attributed to the presence of valuable polyphenolic compounds.

This result is considered important for the treatment of hyperlipidemia-induced atherosclerosis and apparently validates the folk medicinal use of sweet basil by hyperlipidemic patients in east of Morocco. Therefore, further studies are necessary to elucidate the exact mechanisms of the *O. basilicum* effect on plasma lipid parameters, including the activity of purified compounds and dose-response effects.

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