Sweet basil (*Ocimum basilicum* L.) improves lipid metabolism in hypercholesterolemic rats

Hicham Harnafi a, Mohammed Aziz b, Souliman Amrani a,∗

a Laboratoire de Biochimie, Faculté des Sciences, Oujda, Morocco
b Laboratoire de Physiologie et d’Ethnopharmacologie, Faculté des Sciences, Oujda, Morocco

1. Introduction

Cardiovascular diseases are the most common cause of death in both western and eastern countries, atherosclerosis and related complications account for the majority of these deaths. Epidemiological and experimental studies have clearly shown the association between high blood LDL-cholesterol concentrations and a high risk of cardiovascular events, the HDL-cholesterol have, in contrast, a protective effect against the risk.1 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.2 Generally, the therapeutic purpose of prevention or treatment of atherosclerosis and related cardiovascular diseases is to reduce elevated levels of plasma lipids, particularly LDL-cholesterol and triglycerides by drug and/or dietary intervention.3 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.4,5 Sweet basil (*Ocimum basilicum*) a member of Labiatae (Ocimoideae) is widely used in cooking for its culinary qualities. This plant is a versatile herb that may be used in an abundant variety of foods. It is excellent in tomato-based dishes, spinach, and large number of squash. It is also widely used in soup and in cream cheese for sandwiches, dips and pasta dishes.5 On the other hand, 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 (Apium graveolens) and green tea. In eastern Morocco, besides its use in cooking, O. basilicum is widely used as a folk medicine to treat hyperlipidemia and prevent atherosclerosis. Its anti-inflammatory and anti-oxidant activities have been demonstrated in many works.

Phytochemical studies have demonstrated that phenolics constitute the major polar compounds of this plant. In our previous study, we demonstrated the anti-oxidant and hypolipidemic effects of this plant in acute hyperlipidemia induced by Triton-X1339 in rats. In this field, the present study was designed to evaluate the polyphenol content and the possible beneficial effect of the aqueous extract from O. basilicum on plasma and liver lipid parameters in cholesterol feeding rats. As far as we know, no such experimental study with the same plant has been conducted.

2. Methods and materials

2.1. Preparation of the aqueous basil extract (ABE)

O. basilicum was purchased from the herbalist in Oujda city (eastern Morocco) and authenticated by a botanist (Pr. A. Khalil, Department of Biology, Faculty of Sciences, Oujda, Morocco). The total aqueous extract from plant aerial parts was prepared as follows: the dried herb was infused 30 min in distilled water (100 °C), filtered and the solution obtained was concentrated in a rotary evaporator under vacuum at 65 °C. The yield of extraction in terms of starting dried plant material was of 20% (w/w). The resulting crude extract was suspended in distilled water and the aliquots were stored at −18 °C before use.

2.2. HPLC analysis

HPLC analysis of the aqueous basil extract was carried out on a Shimadzu LC-10AS apparatus with a Diode Array Detector (SPD10A, Shimadzu) using a Spherisorb ODS II reverse phase (RP18) analytical column (250 × 4.6 mm, particle size 5 μm). Extract (20 μl) was separated at 40 °C at a flow rate of 1 ml/min using the following gradient of aqueous orthophosphoric acid (0.3%) (A) and acetonitrile (B): 0–20 min: 7–17% B, 20–30 min: 17% B, 30–45 min: 17–25% B, 45–60 min: 25–40% B, 60–65 min: 40–10% B, 65–70 min: 10% B.

2.3. Determination of total polyphenol contents

Total polyphenols of O. basilicum extract were determined by Folin–Ciocalteu procedure. 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 absorbance of blue colored mixtures recorded after 40 min at 725 nm against a blank containing 0.5 ml of water, 0.25 ml of Folin–Ciocalteu reagent and 1.25 ml 20% aqueous sodium carbonate solution. The amount of total polyphenols was calculated as a catechin equivalent from the calibration curve of catechin standard solutions and expressed as mg catechin/g dry plant extract. All measurements were done in triplicate.

2.4. Quantification of tannins

Total tannin content was determined by Folin–Ciocalteu procedure as above, after their precipitation by BSA (Bovine Serum Albumin/fraction V, ACROS, New Jersey, USA). 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 2 h at +4 °C. Then the pH was adjusted at 4.6 by HCl 1 N solution. After centrifugation at 4000 rpm/15 min, no adsorbed phenolics in the supernatant were determined by Folin–Ciocalteu procedure as described. 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.

2.5. Dosage of flavonoids

Flavonoid content was determined according to the Jay method. To each 5 ml of analyzed solution, 2.5 ml of AlCl3 reagent were added (133 mg crystalline aluminium chloride and 400 mg crystalline sodium acetate were dissolved in 100 ml distilled water) and absorbances were recorded at 430 nm against a blank (5 ml of analyzed solution plus 2.5 ml of water).

The flavonoid content was determined as rutin equivalent from the calibration curve of rutin standard solutions and expressed as mg rutin/g dry plant extract. All measurements were done in triplicate.

2.6. Preparation of hypercholesterolemic diet (HCD)

HCD consists of standard diet (Société SONABETAIL, Oujda, Morocco) 81.8%, cholesterol 2%, lard 16% and cholic acid 0.2%.

2.7. Animals and treatments

19 Adult female Wistar rats weighing 180–200 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 internationally accepted standard guidelines for use of laboratory animals. At the beginning of the experiment animals were divided into three groups. One of them (n = 7) served as normolipidemic control group (NCG), the second: hyperlipidemic control group (HCG) (n = 6) and the third: basil treated group (BTG) (n = 6). The NCG kept on standard diet and gavaged with distilled water. HCG received hypercholesterolemic diet and gavaged with distilled water. Animals in BTG feeding with HCD were daily gavaged with the ABE at a dose of 0.5 g/kg body weight (BW) for 10 weeks. The body weights of rats from each group were weekly recorded. After 4 weeks treatment, animals were fasted overnight and blood samples were taken from their tail vein using heparinised capillary once 2 weeks. At the end of the experiment (10 weeks), the systolic blood pressure (SBP) of all animals from each group was measured using tail plethysmography. Then, rats were anesthetized with diethyl ether and blood was taken from their abdominal artery after overnight fasting. The blood samples were immediately centrifuged (2500 rpm/10 min) and plasma used for lipid analysis. Liver tissues were quickly dissected, rinsed in ice-chilled normal saline, blotted on filter paper before weighing.

The tissues were cut into small portions and stored at −20 °C until using for measuring cholesterol and triglyceride contents.

2.8. Biochemical assay of plasma total cholesterol (TC) and triglycerides (TGs)

Total cholesterol levels were determined by cholesterol oxidase enzymatic method using Bio Sud 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-aminantipyrine via peroxidase catalysis and...
spectrophotometrically measured at 510 nm. Triglycerides in plasma were quantified by 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-chlorphenol under the catalytic effect of peroxidase was followed spectrophotometrically at 546 nm.

### 2.9. Plasma HDL and LDL-cholesterol

HDL-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 MgCl₂ (Sigma Diagnostic Kit, Inc, USA). The LDL-cholesterol was calculated by the Friedewald formula\(^{14}\): LDL-cholesterol = total cholesterol – [HDL-cholesterol + (triglycerides/5)].

### 2.10. 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-cholesterol to HDL-cholesterol levels.

### 2.11. Extraction and analysis of liver total cholesterol and triglycerides

At the end of the experiment, liver was 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.\(^{15}\) 1 g 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. Total cholesterol and triglycerides were quantified using enzymatic kits as described above.

### 2.12. Statistical analysis

Data obtained were analyzed using Student’s t-test and P values less than 0.05 were considered statistically significant. Our results are expressed as mean ± S.E.M. The statistical analysis was planned from the beginning to the end of the experimentation.

3. Results

#### 3.1. HPLC analysis of the aqueous basil extract

Fig. 1 depicts the 10 principal peaks detected in the extract at 280 nm, their retention times are as follows: peak 1: 12 min, peak 2: 16.2 min, peak 3: 17 min, peak 4: 18.6 min, peak 5: 38.8 min, peak 6: 34 min, peak 7: 41.8 min, peak 8: 42.4 min, peak 9: 44.2 min; peak 10: 50.6 min. These data strongly suggest the predominance of phenolic compounds such as flavonoids and tannins which present characteristic bands at 280 nm.

#### 3.2. Polyphenol content of Ocimum basilicum extract

The determination of phenolic composition of the aqueous \emph{O. basilicum} extract is reported in Table 1. From this result it appears clearly that tannins and flavonoids represent the major polyphenol fractions of the plant. Tannins represent 42% in terms of total phenols. The amount of flavonoids is of 23%.

#### 3.3. Effect of experimental diets and sweet basil consumption on the organ and body weights

During the feeding period, there were no significant differences in relative organ weight between all dietary groups (Table 2). Animals on the experimental diets appeared healthy and any pathological or toxicological sign was noted indicating that the diets given were adequate.

The body weight of all groups increased linearly during the period of experiment (data not shown). After a period of 10 weeks the HCD fed group (HCG) showed a statistically higher body weight than the control group fed with standard diet. The administration of ABE together with the HCD tends to suppress the body weight gain in BTG with respect to the HCG at 10 weeks \((P = 0.073)\) without any reduction in food intake (Table 2).

#### 3.4. Systolic blood pressure (SBP)

The mean SBP of the three groups is shown in Table 2. So, it appears clearly that there were no major differences in this hemodynamic parameter between NCG (120 ± 2.18 mmHg), HCG (118.33 ± 5.88 mmHg) and BTG (120 ± 2.58 mmHg). Data remained similar to that in normotensive rats.

#### 3.5. Induction of hyperlipidemia by the HCD in Wistar rat

Significant increases in the plasma lipid profile in response to the HCD were clearly noticed in HCG with respect to NCG (Tables 3 and 4). Total cholesterol levels in hypercholesterolemic control rats were significantly higher than that in normolipidemic ones after 4, 6, 8 and 10 weeks treatment (+363%, +339%, +539% and +360%; respectively). TG concentration in rat receiving HCD was not significantly different to control group fed rats given standard diet (NCD) after 4, 6 and 8 weeks. However longer treatment of HCD (10 weeks) produces a significant increase in plasma TG levels (+135%) (Tables 3 and 4). It is also clear from our result that after 10 weeks

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Polyphenol content of Ocimum basilicum extract.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extracts</td>
<td>Total phenols(^a)</td>
</tr>
<tr>
<td>Aqueous basil extract</td>
<td>129 ± 6.39</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M. from three assays.
\(^a\) Expressed as mg catechin/g dry extract.
\(^b\) Expressed as mg rutin/g dry extract.
period of treatment, plasma HDL-cholesterol was not statistically changed by the hypercholesterolemic diet (HCD) in hyperlipidemic control group (HCG) when compared with normolipidemic control group (NCG).

At the end of the experiment, we noted also that the change of LDL-cholesterol levels was similar to the changing pattern of total cholesterol and triglycerides, this cholesterol fraction was +1337% higher in rats received HCD than in those kept on standard diet (NCG).

Feeding HCD results in an increase of AI by more than 425% in HCG compared to the NCG (Table 5). Data concerning the LDL-C/HDL-C ratio are summarized in Table 5.

As can be seen, finding showed that the ratio is significantly higher (+815%) in the grouped rats fed with HCD than in those fed with standard diet.

3.6. Effect of ABE on plasma lipid parameters in Wistar rats

Plasma total cholesterol concentrations in BTG were seen to be not significantly decreased after 4 and 6 weeks, however longer treatment by the ABE suppressed the elevated total cholesterol rise produced by HCD at 8 and 10 weeks treatment (37% and 42%, respectively). Our data showed also that the ABE lowered significantly the plasma triglycerides after 10 weeks; the decrease was of 39% with respect to HCG (Tables 3 and 4).

After 10 weeks of chronic treatment we found a significant decrease on plasma LDL-cholesterol (~54%) in basil treated rats when compared to their relative control (HCG). In contrast, sweet basil did not significantly hindered plasma HDL-cholesterol concentrations (Table 4). Concerning the calculated AI and LDL-C/HDL-C ratio, O. basilicum significantly suppressed the elevated values which were nearly returned to basal range. The reductions were of 61% and 59% in AI and LDL-C/HDL-C ratio, respectively (Table 5).

3.7. Effect of O. basilicum administration on liver lipid concentrations in Wistar rat

Table 6 depicts the liver lipid analysis of the three experimental groups. High fat diet resulted in a statistically significant increased concentration of hepatic cholesterol (+509%) and triacylglycerol (+229%) as compared with normolipidemic control rats. The administration of sweet basil together with the fat-enriched diet consistently reduced hepatic total cholesterol and triglycerides by 62% and 57%, respectively.

4. Discussion

It is long known that there is a relationship between the high prevalence of cardiovascular diseases (atherosclerosis, diabetes, hypertension…) and abnormalities in lipid metabolism. In addition, a positive correlation between dietary fat, hyperlipemia and incidence of coronary diseases has been established and documented. Moreover, it is well established that the traditional Mediterranean diets are followed in countries that have a low incidence and prevalence of cardiovascular diseases since the modification of diet composition can prevent cardiovascular events and improve metabolic disorders relative to a western diet. In view of previous evidence that many foods, plant food and spices such as sunflower, virgin-olive and fish oils, celerly (A. graveolens) and garlic (Allium sativum) are successfully prevented hyperlipidemia and atherosclerosis, we assessed the sweet basil (O. basilicum) one of the plant foods for its beneficial effect on plasma and liver lipid profiles after chronic high fat diet treatment regarding to increased triglycerides, total and low-density lipoprotein cholesterol in hypercholesterolemic diet-fed rats’ model. In folk medicine, the herbalist prescribes to patients to infuse 100 g of sweet basil in 100 ml of water and to take about 200 ml of the filtered solution by day. In these conditions, the yield of extraction is about 20% and the quantity of crude extract given is about 4 g/day.
Hyperlipidemic feeding was reported by many workers to induce hyperlipidemic animal models. In our hand, similar results were found; the hypercholesterolemic diet-fed rats have higher levels of TGs, TC and LDL-cholesterol than control group indicating that the hypercholesterolemic model was successfully established. It is evident from our results that the body weights of each dietary group of animals increased linearly from the beginning to the end of the experiment indicating that the diets given were adequate and sweet basil provokes, at the end of the experiment, a significant body weight losing comparatively with animals kept on hypercholesterolemic diet alone (data not shown). This result could be as a result of the plant lipid-lowering activity as demonstrated by Ono et al.,22 but this hypothesis needs to be validated by an experimental study.

Neither hypercholesterolemic diet nor sweet basil modify systolic blood pressure in the experimental groups, values remained similar to that in normotensive animals indicating that 10 weeks treatment was not sufficient to statistically alter this hemodynamic parameter. In the present study, we have demonstrated the hypolipidemic activity of sweet basil. It appears clearly from the results that feeding basil extract along with cholesterol for 10 weeks exhibited a significant hypolipidemic effect decreasing plasma total cholesterol and triacylglycerol levels. This is in agreement with our earlier report demonstrating the hypolipidemic effect of O. basilicum in acute hyperlipidemia induced by Triton WR-1339 in rats. The reduction of plasma total cholesterol by O. basilicum feeding was associated with a decrease of the LDL fraction which represents the first target of a number of hypolipidemic strategies. This finding suggests that the plant extract effect may be due to the improvement of reduced activity of hepatic LDL receptor site in response to the atherogenic diet. 23 The rapid catabolism of LDL-cholesterol through its hepatic receptor (B/E) represents the major pathway for the final elimination of cholesterol in the form of bile acids. This hypothesis was partially validated by the biochemical analysis of liver lipid profile. The plasma cholesterol-suppressive activity of sweet basil was accompanied by a decrease of hepatic cholesterol content which was mainly attributed to its excretion into bile and its conversion into secretory bile acids. It is also recently reported that the triglycerides play a key role in the regulation of lipoprotein interactions to maintain the normal lipid metabolism. Indeed, the elevated plasma TG levels were associated with an increased incidence of coronary artery disease. The consumption of aqueous extract from O. basilicum together with hyperlipidemic diet significantly suppressed the elevated blood and liver concentrations of TGs. This result suggests that the plant is able to restore, at least partially, the catabolic metabolism of this lipid entity.

The underlying mechanisms of the effect found may be due to an increased stimulation of the lipolytic activity of plasma lipoprotein lipase (LPL) and hepatic lipase (HL). On the other hand, administration of sweet basil provides a beneficial action in rat lipid metabolism regarding to the reduction of atherogenic index (AI). In fact, the AI was decreased in basil treated group comparatively to the hyperlipidemic one. Similar results were reported by others when studying the hypolipidemic effect of natural products. This ameliorative action could be due to the plasma lipid-lowering activity of extract. 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 and related cardiovascular events. Again, the administration of aqueous O. basilicum extract significantly suppressed the higher values of LDL-C/HDL-C ratio showing the benefit effect of this herb food on preventing cardiovascular complications incidence. The results found clearly demonstrate that the bioactive substance(s) contained in this plant have a polar character since they are more soluble in water. This result agrees with a previous report showing that plant water soluble extracts possess cholesterol-suppressive capacities and ability to attenuate the accelerated development of atherosclerosis in hypercholesterolemic models. In fact, flavonoids and tannins a heterogeneous group of ubiquitous plant polyphenols, have exhibited a variety of pharmacological activities, including the hypolipidemic effect. The possible mechanism underlying the hypolipidemic of these phytochemicals results generally in three points: firstly these phenolic compounds increase hepatic LDL receptor activity. Secondly, tannins and flavonoids are known to reduce cholesterol synthesis via the suppression of hydroxymethyl glutaryl-CoA (HMG-CoA) reductase. Finally, these molecules inhibit the major enzyme involved in cholesterol metabolism, acyl CoA cholesterol acyl transferase (ACAT).

Besides, HPLC analysis and quantification of total phenolics, tannin and flavonoid contents in the aqueous sweet basil extract confirmed the finding reported by Javanmardi et al. demonstrating that the phenolics are the major polar compounds of sweet basil. In our present study, the presence of this polyphenol fraction in sweet basil may account for the observed lipid-lowering attributes.

In conclusion, these results confirm our previous study demonstrating an acute hypolipidemic effect of the sweet basil and suggest that the plant may be beneficial in preventing hyperlipemia and atherosclerosis confirming the role of the traditional Mediterranean diets regarding to the cardiovascular diseases. However, further phytochemical and pharmacological studies are designed to identify and purify the bioactive compound(s) and to elucidate the possible mechanism for the pharmacological effect observed.

**Conflict of interest**


**Acknowledgment**

This work was supported by “CUD: Commission Universitaire pour le Développement” project between Morocco and Belgium.
(2008–2012). We would especially like to thank Mr. El Mostapha BEDRAOUI and Mr. Karim RAMDAOUI for helping in animal care.

References


2. West KM, Ahuja MMS, Bennet PH. The role of circulating glucose and triglyceride concentration and their interactions with other risk factors as determinants of arterial disease in nine diabetic population samples from the WHO multinational study. Diabetes Care 1983;6:361–9.


10. West KM, Ahuja MMS, Bennet PH. The role of circulating glucose and triglyceride concentration and their interactions with other risk factors as determinants of arterial disease in nine diabetic population samples from the WHO multinational study. Diabetes Care 1983;6:361–9.


