

Original Article

Rosmarinic acid-rich extract from *Ocimum basilicum* L. decreases hyperlipidemia in high fat diet-induced hyperlipidemic mice and prevents plasma lipid oxidation

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Abstract

Introduction: In this study, we investigated the effect of basil rosmarinic acid-rich extract on mice lipid metabolism, low-density lipoprotein oxidation and antiradical property.

Methods: The rosmarinic acid rich-extract was used to treat male mice. Mice were divided into four groups of seven mice and treatment was performed daily and orally for 9 weeks. The antihyperlipidemic effect was evidenced by the measurement of plasma and liver lipid profiles. Thiobarbituric acid reactive substances assay was used to measure the antioxidant capacity of the phenolic extract using mice plasma rich in low density lipoprotein (LDL). The antioxidant activity of the extract was assessed by 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging capacity and the measurement of oxidative β -carotene bleaching.

Results: The extract exerts a significant decrease in plasma total cholesterol, triglycerides and LDL-cholesterol. Similar results were observed in liver total cholesterol and triglycerides. The phenolic extract prevents lipoprotein oxidation by 93% at a dose of 25 μ g/ml. We also note that the extract scavenges DPPH radical in a dose-dependent manner with an IC_{50} = 12.45 \pm 0.18 μ g/ml. Besides, the extract inhibits the oxidation process of β -carotene, the IC_{50} was 12.16 \pm 0.19 μ g/ml. HPLC analysis shows that the extract contains caftaric acid (2.9%), caffeic acid (4.3%), chicoric acid (5.5%) and rosmarinic acid (87.3%) which is the major compound.

Conclusion: The results obtained suggest that the extract may be an important source of natural compounds that can be exploited as a substrate to develop new treatment of hyperlipidemia and atherosclerosis.

Keywords:

Ocimum basilicum L.;
Rosmarinic acid;
Lipid metabolism;
Lipoprotein oxidation;
Free radical scavenging

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Introduction

Hypercholesterolemia and oxidative stress have become a significant health concern in recent years

(Siti et al., 2015; Viktorinova et al., 2016). These parameters are known to be the major risk factors contributing to the development and progression of atherosclerosis and related cardiovascular and cerebrovascular diseases (Krauzová et al., 2016).

Furthermore, the relationship between dietary cholesterol and these diseases has been a topic of intense research. In the fact, the consumption of a high-diet directly affects lipid metabolism and changes serum lipid profile increasing total cholesterol, triglycerides and low density lipoprotein (LDL)-cholesterol and decrease of high density lipoprotein (HDL)-cholesterol (Harnafi et al., 2010). Large clinical and experimental studies have demonstrated that high serum LDL-cholesterol levels are consistently associated with increased risk of cardiovascular disease and are widely used for predicting cardiovascular complications (Deedwania et al., 2016).

Actually, it is well known that dietary habits play a central role in the prevention of some human diseases, including hyperlipidemia and atherosclerosis. Indeed, a positive relationship has been established between certain diets rich in vegetables, fruits, herbs and spices such as the traditional Mediterranean diets and the low risk of cardiovascular diseases (Noites et al., 2015). Because of this, prevention and treatment of hyperlipidemia need, firstly, the diet control regarding lipid quality and quantity, and secondly, the use of lipid-lowering diets and/or drugs (Stone, 1996). Thus, it is very important to pay attention to dietary prevention and control of hyperlipidemia. In this field, dietary compounds are to receiving considerable interest in their presumed role in the prevention of cardiovascular pathologies (Du et al., 2016; Zhang et al., 2016). On the other hand, much attention is being paid to dietary antioxidant substances because many pathological conditions are associated with oxidative stress (Wang et al., 2016). In fact, the oxidation of plasma LDL was reported to be the key process in the premature development of atherosclerosis which can be treated with an association between hypolipidemic and antioxidant therapies (Wadhera et al., 2016).

Sweet basil (*Ocimum basilicum*) a genus from the *Lamiaceae* family is cultivated for the fresh market as a culinary herb, condiment or spice in the dried or frozen leaf form, and as a source of aromatic essential oil for use in foods, flavors and fragrances. Besides its use in cooking, this herb can serve as a component for a wide variety of foods (Miele et al., 2001). In the east of Morocco, sweet basil was considered as an alternative medicine to treat

hyperlipidemia. Recently, several biological activities of the plant were demonstrated, such as anti-inflammatory (Szymanowska et al., 2015), anti-biofilm and antibacterial (Snoussi et al., 2016). Umar et al. (2014) demonstrated that the sweet basil inhibits cyclo-oxygenase isoforms and prostaglandins involved in thrombosis.

Previous studies in our laboratory demonstrated the antihyperlipidemic effect of the crude aqueous extract and polar fractions from this plant in acute hyperlipidemia induced by Triton WR-1339 and by hyperlipidemic diet in rats and mice (Harnafi et al., 2008; Harnafi et al., 2013). In order to determine the possible nature of active compounds of *Ocimum basilicum*, we hypothesized that rosmarinic acid and other phenolic acids may be the major ones. So, the present study was designed; firstly, to identify and quantify the phenolic acids in the n-butanol fraction from *O. basilicum* and to investigate their possible beneficial effect on plasma and liver lipid parameters in high fat diet-induced hyperlipemic mice in comparison with a standard hypolipidemic drug (fenofibrate). Secondly, we assessed the preventive activity against lipoprotein oxidation and the free radical scavenging property.

Materials and methods

Preparation of rosmarinic acid-rich extract

O. basilicum was purchased from an herbalist in Oujda city. The dried powder from aerial parts of the plant was defatted with n-hexane, in a Soxhlet extractor, to remove chlorophyll and liposoluble substances. The marc was air-dried and extracted with methanol (16h). The methanol extract was then filtered and concentrated under reduced pressure until semi-solid substances were obtained. The extract obtained was placed in a drying oven (40°C) to obtain the crude material. The yield of extraction, in terms of the starting dried plant material, was 17.34%. The crude extract was dissolved in distilled water and phenolic acids were extracted by n-butanol using the liquid-liquid partition. The extraction was repeated several times until the solvent becomes transparent. The extract was filtered and n-butanol subsequently evaporated. The extract was desiccated in drying oven (40°C) to obtain a fraction rich in phenolic acids. The extraction yielded 7.93%.

Determination of total phenol content

Total polyphenols of *O. basilicum* extract were determined by the Folin–Ciocalteu (Dewanto et al., 2002) procedure with some modifications. The 0.5ml of each sample was mixed with 0.25ml of Folin–Ciocalteu reagent and 0.5ml of the sodium carbonate solution 20%. After stirring, the preparation was adjusted with distilled water to a final volume of 5ml and mixed thoroughly. The coloring was allowed to grow for 30min in the dark. The blue color was measured spectrophotometrically at 725nm against a blank where the sample was replaced by the same volume of distilled water.

The amount of total polyphenols was calculated from the calibration curve of rosmarinic acid standard solutions and expressed as mg rosmarinic acid/g dry extract. All measurements were done in triplicate.

HPLC analysis of rosmarinic acid-rich extract

HPLC analysis of the rosmarinic 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 (250×4mm, particle size 5µm). The extract (10µl) was separated at 20°C at a flow rate of 1ml/min using the following gradient of aqueous trifluoroacetic 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 340nm. Compounds were identified by their retention times and UV–visible spectra using a database of analytical standards. Individual phenolic acid concentrations were quantified by comparison of integrated peak areas to calibration curves prepared with standards.

Preparation of high-fat diet (HFD) and animal treatment

The antihyperlipidemic effect of rosmarinic acid-rich extract was studied in HFD-induced hyperlipidemic mice. The diet was prepared daily as described by Harnafi et al. (2009). It consists of a standard diet 81.8%, cholesterol 2%, lard 16% and cholic acid 0.2%.

Twenty-eight adult male *albinos* mice weighing 22–30g, were bred in the animal house of the Department of Biology (Faculty of Sciences, Oujda, Morocco) and maintained in a controlled room at a temperature of 22±02°C with a 12h light–dark cycle.

The mice were given free access to diet and water *ad libitum* and experimented in accordance to internationally accepted standard guidelines for use of laboratory animals (approved by the local committee of use of laboratory animals, Faculty of Medicine, approval number: 002016). The mice were randomly divided into four groups as follows: 1- the normolipidemic control group (NCG, n=7): feeding with standard diet and daily gavaged with distilled water; 2- the hyperlipidemic control group (HCG, n=7): feeding with HFD and daily gavaged with distilled water; 3- the rosmarinic acid-rich extract treated group (RTG, n=7): feeding with HFD and daily gavaged with plant extract at a dose of 200mg/kg body weight for 9 weeks and 4- the fenofibrate treated group (FTG, n=7): feeding with HFD and daily gavaged with fenofibrate at a dose of 200mg/kg body weight for 9 weeks.

At the end of the experiment, the animals were fasted overnight and lightly anesthetized with diethyl ether, the blood samples were then taken from their retro-orbital sinus into heparinized tubes. The blood samples were immediately centrifuged (2500 rpm/15min) and plasma was used for lipid analysis.

Plasma lipid analysis

The plasma total cholesterol, triglycerides and HDL-C were quantified by enzymatic methods using commercial kits (Bio Sud Diagnostici S.r.l Italy). The levels were expressed as mg/dl. Total cholesterol was analyzed by cholesterol oxidase, the quinoneimine formed was measured at 510nm. Triglycerides levels were determined after hydrolysis by lipases, the red color of the chromophore was quantified at 520nm. HDL-cholesterol concentrations were determined by the same method used to quantify total cholesterol except that the HDL-C fraction was measured after precipitation of LDL and VLDL by phosphotungstic acid in the presence of magnesium chloride (MgCl₂) using Sigma Diagnostic kit. LDL-cholesterol levels were calculated using the formula of Friedewald et al. (1972) :LDL-C= TC–(HDL-C + TG/5).

Liver lipid analysis

The livers of each animal were taken away, rinsed in ice-cold normal saline and transferred to filter paper. Then the tissues were cut into small pieces and stored at -20°C before use. Liver lipids were

extracted according to the method of Haug and Hostmark (1987) slightly modified. The 1g liver from each animal was homogenized in 10ml of cold isopropanol in an ice bath and the homogenate was allowed to macerate for 48h at 4°C. The mixture was centrifuged at 2500rpm for 15min and the supernatant was used for lipid quantification. Total cholesterol and triglycerides were measured by the enzymatic method as described above.

Determination of plasma lipid oxidation *in vitro*

Thiobarbituric acid reactive substances (TBARS) assay was used to measure the antioxidant capacity of rosmarinic acid-rich extract using mice plasma rich in lipoproteins ([LDL-cholesterol]= 97±8.49mg/dl) as a substrate of the oxidative process. The TBARS were quantified using the method described by Ramchoun et al. (2015) slightly modified. The lipoprotein-rich plasma was removed in mice treated with Triton WR-1339 (600mg/kg) for 24h. The study was performed according to the following schedule: 1- control: 40µl of lipoprotein-rich plasma were incubated with 2460µl of distilled water; 2- oxidized lipoprotein-rich plasma: 40µl of plasma rich in lipoproteins were incubated with 10µl of CuSO₄ solution (0.33mg/ml) and 2450µl of distilled water; 3- RAE: 40µl of lipoprotein-rich plasma were incubated with 10µl of CuSO₄ solution (0.33mg/ml) plus 5µl of rosmarinic acid-rich extract (RAE) at various concentrations (0.05, 0.5, 5, 10 and 25µg/ml) and 2445µl of distilled water and 4- BHT: 40µl of lipoprotein-rich plasma were incubated with 10µl of CuSO₄ solution (0.33mg/ml) plus 5µl of butylated hydroxytoluene (BHT) solution at various concentrations (0.05, 0.5, 5, 10 and 25µg/ml) and 2445µl of distilled water.

The tubes were shaken well and then incubated at 30°C for 16h. After, 500µl of trichloroacetic acid 20% and 500µl of thiobarbituric acid 0.8% were added to the reaction mixture. After stirring, the mixture was heated in a water bath at 95°C for 30min. The tubes were then cooled and added with 2ml of n-butanol, after vortexing, the tubes were centrifuged at 4500rpm for 15min. The absorbance of the colored red phase was measured at 532nm. The amounts of TBARS were calculated from the calibration curve of malondialdehyde (MDA) standard solutions. All measurements were done in triplicate.

DPPH free radical scavenging activity of

rosmarinic acid-rich extract

As a radical form, 2,2-diphenyl-1-picrylhydrazyl (DPPH) absorbs visible light at 517nm and after a reduction by antioxidants species, its absorbance decreases. The anti-radical effect was determined by the DPPH test according to the method described by Blois (1958) with slight modifications. The 5µl of rosmarinic acid-rich extract at different concentrations (0.05, 0.5, 5, 10 and 25µg/ml) were mixed with 2495µl of methanolic solution containing DPPH radical (0.06mg/ml). The mixture was shaken vigorously and left to stand for 30min at room temperature. The reduction of the DPPH-radical was measured by monitoring the decrease of absorption at 517nm. Positive control was carried out using BHT as a standard antioxidant substance in the same experimental conditions. The radical scavenging activity (RSA) was calculated according to the following formula: $RSA (\%) = (A_{blank} - A_{sample}) / A_{blank} * 100$. The IC₅₀ (concentration providing 50% inhibition) values were calculated from the plotted graph of scavenging activity against concentrations of the samples. All tests were done in triplicate.

Determination of β-carotene oxidative bleaching

The determination of the effect of rosmarinic acid-rich extract on β-carotene oxidative bleaching was carried out according to the method described by Leouifoudi et al. (2015) slightly modified. A stock solution of β-carotene–linoleic acid mixture was prepared as following: 2mg of β-carotene dissolved in 1ml of chloroform were mixed with 20mg of linoleic acid and 200mg of Tween 80; after evaporation of chloroform, 100ml of distilled water were added with vigorous stirring to form the emulsion linoleate-β-carotene. The 2495µl of this reaction mixture were mixed with 5µl of rosmarinic acid-rich extract at different concentrations (0.05, 0.5, 5, 10 and 25µg/ml). The emulsion system was incubated up to 24h and absorbance of the mixtures was measured at 490nm. The BHT was used as a positive control in the same experimental conditions. The inhibition of β-carotene oxidative bleaching was calculated according to the following formula: $\% \text{ inhibition} = 100 - [(A_{blank} - A_{sample}) / A_{blank}] * 100$. The IC₅₀ values were calculated from the plotted graph of antioxidant activity against concentrations of the samples.

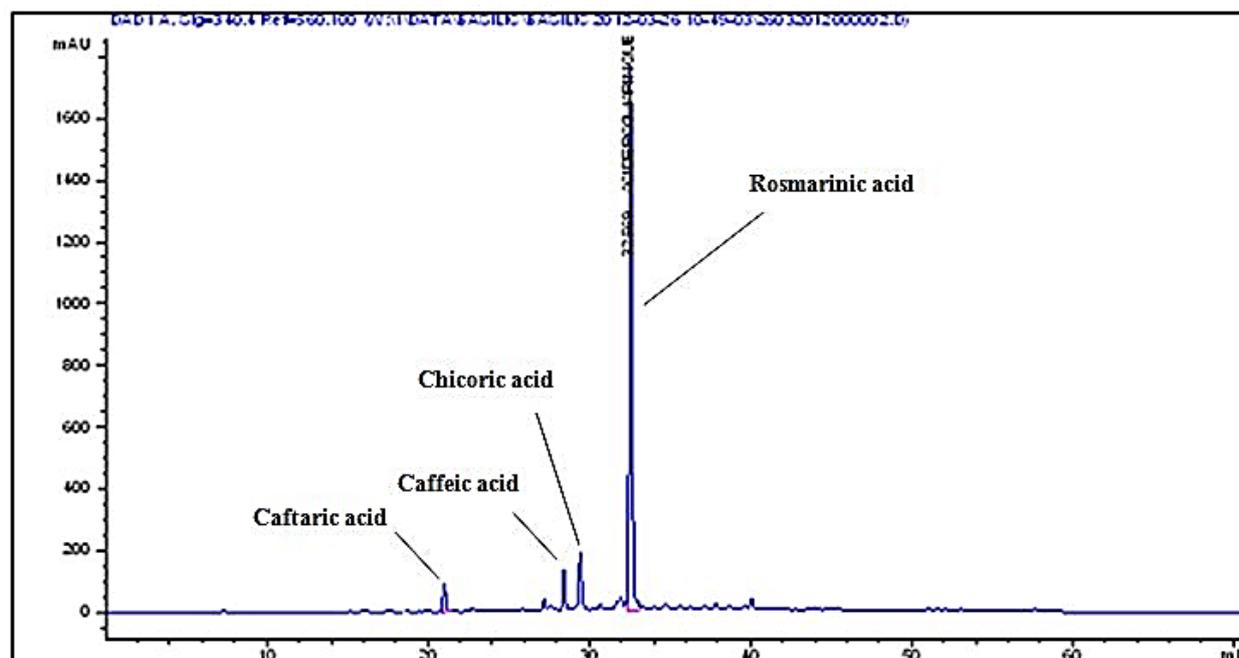


Fig.1. HPLC analysis of rosmarinic acid rich-extract from *Ocimum basilicum*.

Statistical analysis

Data obtained were analyzed using student t-test and one way ANOVA. The P values less than 0.05 were considered as statistically significant. Our results are expressed as mean \pm SEM.

Results

Total phenol content and HPLC analysis of basil phenol extract

The determination of total phenol content shown that *O. basilicum* extract is rich in phenolics, the amount was 174.39 ± 0.92 mg rosmarinic acid equivalent/g dry extract. The Figure 1 depicts the HPLC chromatogram of the basil extract. We identified four phenolic acids: peak 1, caftaric acid: 3.6 ± 0.001 mg/g dry extract (2.9%); peak 2, caffeic acid: 5.31 ± 0.12 mg/g dry extract (4.3%); peak 3; chicoric acid: 6.84 ± 0.36 mg/g dry extract (5.5%) and peak 4, rosmarinic acid: 108.02 ± 0.019 mg/g dry extract (87.3%).

Induction of hyperlipidemia by the high-fat diet in mice

The high-fat diet significantly changed plasma lipid parameters in mice after 9 weeks (Fig.2). In fact, total cholesterol levels were significantly increased in mice receiving the high-fat diet comparatively to control (+188%, $P<0.001$). Triglycerides were also

statistically modified (+215%, $P<0.05$). In addition, the LDL-cholesterol was 30 times ($P<0.001$) higher in mice feeding the high-fat diet compared to control. However, plasma HDL-cholesterol was not statistically changed ($P=0.09$).

Effect of rosmarinic acid-rich extract and fenofibrate on mice plasma lipid profile

Plasma total cholesterol concentration in rosmarinic acid-rich extract treated mice was significantly decreased after 9 weeks of treatment (-42%, $P<0.01$). The extract also lowered triglycerides by 70% ($P<0.05$, Fig. 2). Furthermore, we found a significant reduction in plasma LDL-C (-75%, $P<0.001$) in the extract-treated mice when compared to their relative hyperlipidemic control. The HDL-C was also relatively decreased ($P=0.022$).

As can be seen, fenofibrate administration resulted in a statistically significant diminution in plasma total cholesterol (-43%, $P<0.01$). Triglyceride concentrations were reduced by 64%, but the change was not statistically significant ($P=0.08$, Fig. 2). The drug also suppressed the elevated rise of LDL-C (-79%, $P<0.001$) but the change in HDL-C was not statistically significant ($P=0.68$).

Effect of rosmarinic acid-rich extract and fenofibrate on mice liver lipids

The high-fat diet resulted in a significantly augmented

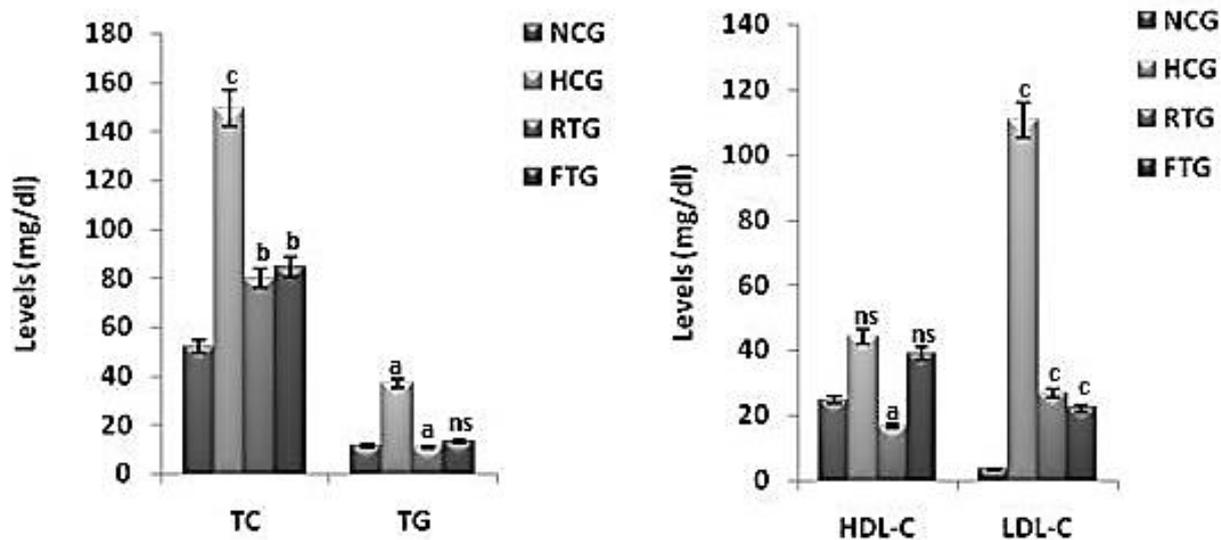


Fig.2. Effect of rosmarinic acid-rich extract and fenofibrate on mice plasma lipid and lipoprotein parameters. Values are expressed as mean±SEM (n=7). ^a*P*<0.05, ^b*P*<0.01, ^c*P*<0.001, ns: not significant (HCG versus NCG; RTG and FTG versus HCG). TC: total cholesterol; TG: triglycerides; HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol; NCG: normolipidemic control group; HCG: hyperlipidemic control group; RTG: rosmarinic acid-rich extract treated group, FTG: Fenofibrate treated group.

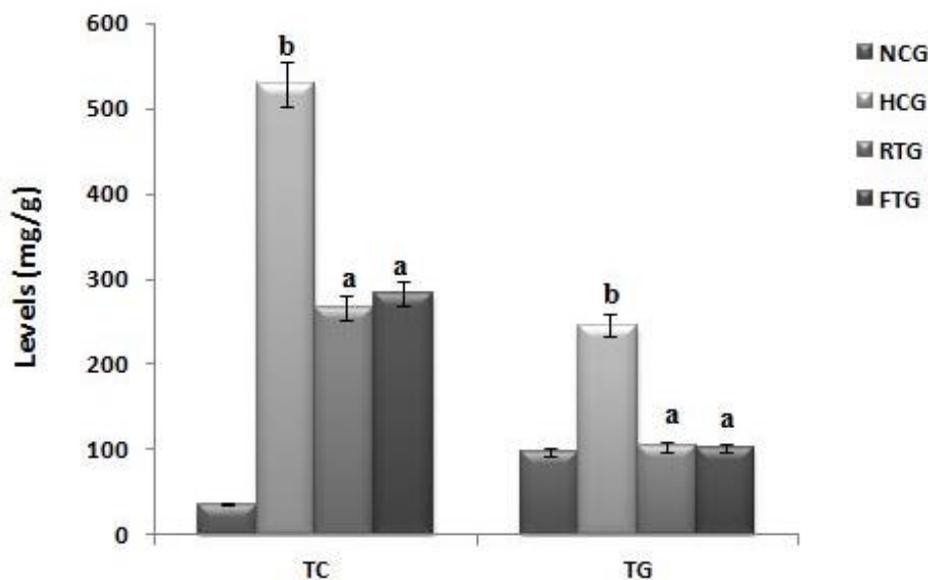


Fig.3. Effect of rosmarinic acid-rich extract and fenofibrate on mice liver lipid profile. Values are expressed as mean±SEM (n=7). ^a*P*<0.01, ^b*P*<0.001, (HCG versus NCG; RTG and FTG versus HCG). TC: total cholesterol; TG: triglycerides; NCG: normolipidemic control group; HCG: hyperlipidemic control group; RTG: rosmarinic acid-rich extract treated group, FTG: Fenofibrate treated group.

levels of hepatic cholesterol (+1300%, *P*<0.001) and triglycerides (+150%, *P*<0.001) in hyperlipidemic mice when compared to control. However, oral administration of rosmarinic acid-rich extract for 9 weeks consistently decreased the mice liver total cholesterol (-49%, *P*<0.01) and triglycerides (-57%,

P<0.01). Furthermore, fenofibrate significantly reduced hepatic total cholesterol by 46% and triglycerides by 58% (*P*<0.01, Fig. 3).

Effect of rosmarinic acid-rich extract on mice plasma lipoprotein oxidation

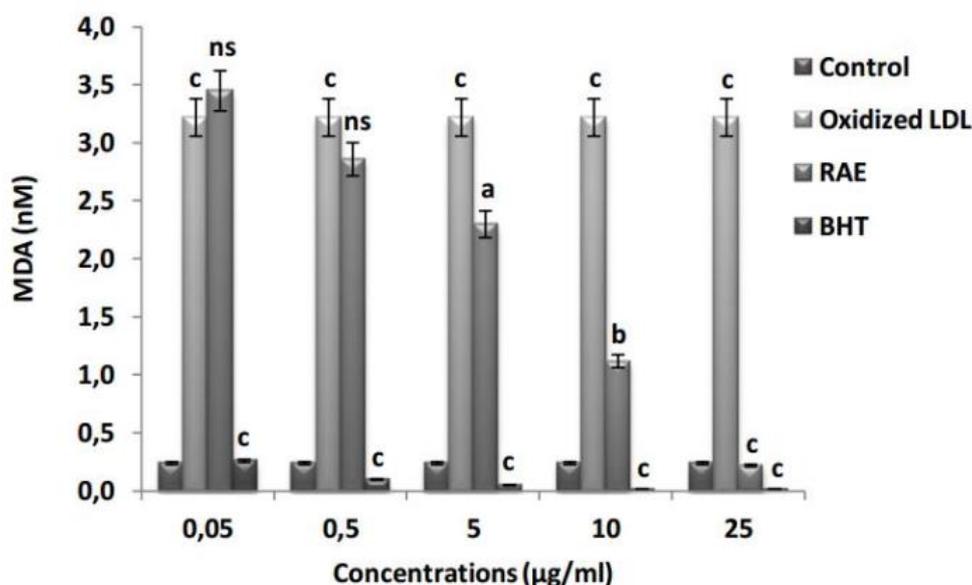


Fig.4. Effect of rosmarinic acid-rich extract and BHT on mice plasma LDL oxidation *in vitro*. Data are mean±SEM (n=3). ^a $P<0.05$, ^b $P<0.01$; ^c $P<0.001$ (oxidized plasma *versus* control; RAE and BHT *versus* oxidized plasma). RAE: rosmarinic acid-rich extract; BHT: butylated hydroxytoluene.

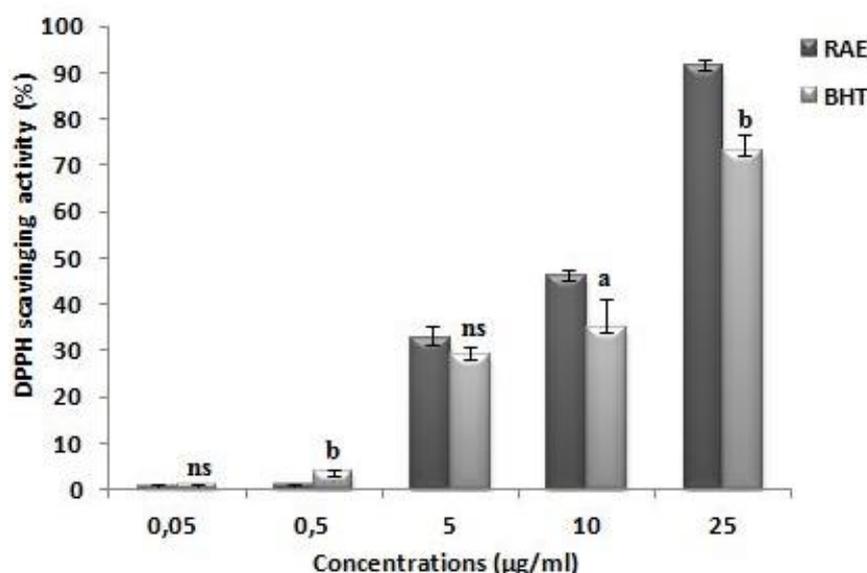


Fig.5. DPPH scavenging activity of rosmarinic acid-rich extract and BHT. Values are expressed as mean±SEM (n=3). ^a $P<0.05$, ^b $P<0.001$, ns: not significant (RAE *versus* BHT). RAE: rosmarinic acid-rich extract; BHT: butylated hydroxytoluene.

The quantification of TBARS is a well-established assay for screening and monitoring lipid peroxidation. The results obtained are given in Figure 4. The oxidation of plasma lipoproteins was measured as the amount of MDA. We observed that the CuSO_4 solution statistically increased the oxidation of lipoprotein-rich plasma by more than 13 times ($P<0.001$). However, the treatment with rosmarinic acid-rich extract inhibits the oxidation process in a dose-dependent manner. The scores are 11%

($P>0.05$), 22% ($P<0.05$), 65% ($P<0.01$) and 93% ($P<0.001$) at doses of 0.5, 5, 10 and 25 µg/ml, respectively, with an IC_{50} of 10.26 ± 0.87 µg/ml. The BHT exerted a strong antioxidant activity at the same concentrations with percentages of 91% ($P<0.001$), 96% ($P<0.001$) and 98% ($P<0.001$).

DPPH scavenging activity of rosmarinic acid-rich extract from *O. basilicum*

The radical scavenging activity of rosmarinic acid-rich

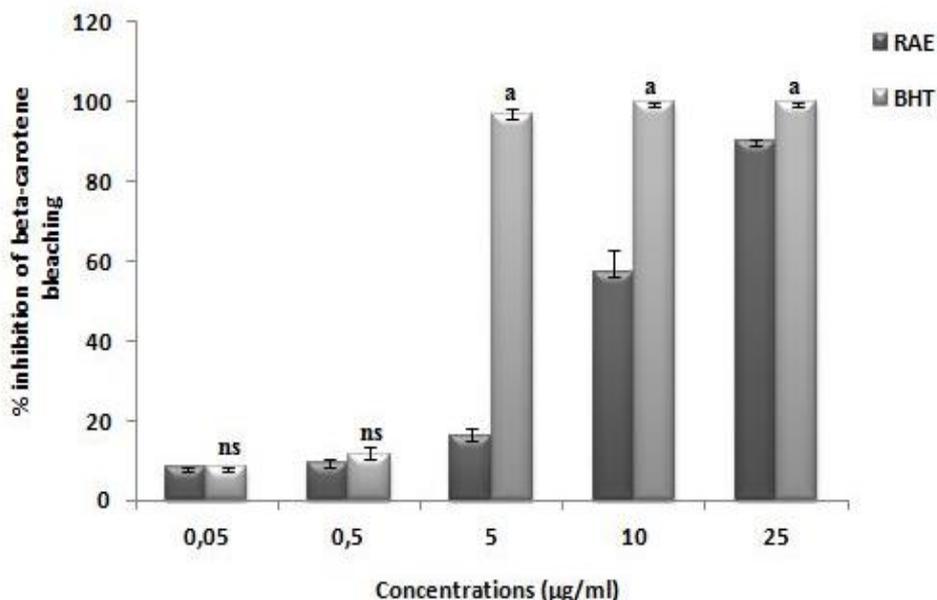


Fig.6. Effect of rosmarinic acid-rich extract on β -carotene oxidative bleaching. Values are expressed as mean \pm SEM (n=3). ^a P <0.001, ns: not significant (RAE *versus* BHT). RAE: rosmarinic acid-rich extract; BHT: butylated hydroxytoluene.

extract was summarized in Figure 5. We note that the extract scavenges DPPH radical in a dose-dependent manner. The percentages of inhibition were 0.6%, 1%, 32%, 46% and 91.5% at doses of 0.05, 0.5, 5, 10 and 25 μ g/ml, respectively. The BHT exerted an effect relatively comparable to that shown by the extract. The percentages of inhibition were 1%, 4%, 29%, 35% and 73%, at the same concentrations, respectively. When comparing IC_{50} of the tested compounds, we concluded that the phenolic extract has good anti-radical scavenging activity against the DPPH radical with an IC_{50} = 12.45 \pm 0.18 μ g/ml compared to that recorded by BHT (IC_{50} = 15.91 \pm 0.71 μ g/ml, P <0.01).

Effect of rosmarinic acid-rich extract on β -carotene oxidative bleaching

The β -carotene bleaching method measured the ability of a substance to inhibit lipid peroxidation. Figure 6 shows the antioxidant activity of rosmarinic acid-rich extract in comparison with BHT. At lower concentrations, the antioxidant effects of such extract and BHT were comparable but at doses higher than 5 μ g/ml, BHT was very effective. In fact, the extract inhibits oxidation process by 8%, 9%, 16%, 57% and 90% at doses of 0.05, 0.5, 5, 10 and 25 μ g/ml, respectively. However, the BHT inhibits oxidation by 8%, 11% and 97% at concentrations of 0.05, 0.5 and 5 μ g/ml, respectively. At 10 and 25 μ g/ml, the inhibition

reached a maximum of 100%. The inhibition of linoleic acid β -carotene-system oxidation has shown considerable activity for the basil phenolic extract with an IC_{50} = 12.16 \pm 0.19 μ g/ml which is statistically lower than that of BHT having an IC_{50} = 4.19 \pm 0.12 μ g/ml (P <0.001).

Discussion

Atherosclerosis and related cardiovascular diseases are a growing health concern both in developed and developing countries resulting from known risk factors such as hyperlipidemia and oxidative stress (Wu et al., 2016). In fact, it is well established that elevated blood lipid levels and LDL oxidation constitute the major risk factor for atherosclerosis (Keevil et al., 2007). Sellam and Bour (2016) showed that hypercholesterolemia represents 22.9% and hypertriglyceridemia 18.6% in Oujda city (eastern Morocco). The search for new treatments capable of reducing blood lipid concentrations or regulating their metabolism has gained momentum over the years resulting in various reports on significant activities of functional foods and natural products (Sanz-Buenhombre et al., 2016). Therefore, the development of lipid-lowering strategies from natural sources is the best option and is in great demand. Many species, drinks, and functional foods, medicinal and aromatic plants have been widely used

traditionally for the treatment of various cardiovascular diseases (Irudayaraj et al., 2013). In this work, we assessed the rosmarinic acid-rich extract of sweet basil (*Ocimum basilicum*), cultivated in Oujda city, for its antioxidant, antiradical and beneficial effects on plasma and liver lipid profiles after chronic high-fat diet treatment in mice model.

Several studies in the past have shown that a diet with high-fat content caused a considerable increase in the plasma cholesterol and triglycerides concentrations in rats and mice (Ramchoun et al., 2012). Using the same experimental model, we demonstrated that treatment for 9 weeks with the rosmarinic acid-rich extract reduced significantly total cholesterol, triglycerides and LDL-cholesterol levels. So, the phenolic extract exerts a protective action on lipid metabolism in hyperlipidemic mice. This is in agreement with previous reports showing that the methanol and aqueous extracts from basil have an antihyperlipidemic effect in high-fat diet induced hyperlipidemic mice (Harnafi et al., 2013).

It appears clearly from the results that the reduction of plasma total cholesterol by rosmarinic acid-rich extract was associated with a decrease of the LDL fraction which carries cholesterol from the liver to the peripheral cells and smooth muscle cells of the arteries. A rise in LDL may cause deposition of cholesterol in the arteries and aorta and hence is bad for health and a direct risk factor for coronary heart disease (Deedwania et al., 2016). The possible mechanism of the plasma LDL-C lowering effect may be due to the enhancement of the activity of lecithin-cholesterol acyl-transferase. Besides, the extract may stimulate the rapid catabolism of LDL-C through its hepatic receptor (B/E) as a major pathway for the final elimination of cholesterol in bile (Mbikay et al., 2014). This hypothesis was partially validated by the liver lipid analysis. The plasma cholesterol-suppressive activity exerted by the rosmarinic acid-rich extract was accompanied by a decrease of hepatic cholesterol content, which was mainly attributed to its conversion into bile acids.

Besides, it has been reported in recent studies that triglycerides are independently related to coronary heart disease and most of the antihypercholesterolemic drugs do not decrease triglycerides levels (Bleda et al., 2016). However, the rosmarinic acid-rich extract significantly lowered

plasma and liver triglycerides and this effect might be related to the increase in lipoprotein lipase activity involved in the catabolism of triglycerides as suggested by Demonty et al. (2002).

In this study, fenofibrate was used as a known reference hypolipemic treatment. Our results showed that the drug significantly decreased plasma and liver lipid concentrations and we suggested that its lipid-lowering property was relatively comparable to the tested rosmarinic acid-rich extract from basil. In fact, fenofibrate suppressed the elevated rise of plasma cholesterol and liver triglycerides in a marked manner. This is in agreement with the mechanism by which fibrates act on lipid metabolism (Rizvi et al., 2003; Ou et al., 2005). On the other hand, oxidation of LDL-cholesterol is a risk factor and plays a key role in several steps of atherosclerosis. A decrease in oxidative stress and protection of LDL from oxidation might, therefore, be a strategy with great promise for the prevention of atherosclerosis associated with cardiovascular disease (Wadhwa et al., 2016). Our experimental study demonstrated that rosmarinic acid-rich extract from sweet basil protects significantly the lipoprotein-rich plasma oxidation in a dose-dependent manner. The extract has, also, a powerful anti-radical effect against DPPH and radicals produced by oxidative degradation of linoleic acid. Previous works reported that there is a highly positive relationship between total phenolics and antioxidant activity of many plant species, because of the radical scavenging ability of their hydroxyl groups (Shen et al., 2016). In the HPLC profile of sweet basil extract, we clearly identified four major phenolic compounds that are caftaric acid, caffeic acid, chicoric acid and rosmarinic acid. The quantitative analysis shows that rosmarinic acid is the most abundant phenolic compound in the extract representing 87.3%. The finding agrees with the previous study reporting the same phenolic acids profile in *O. basilicum* (Kwee and Niemeyer, 2011). This result led to suggest that rosmarinic acid, acting alone or in synergy with other phenolic acids, might be the principal compound responsible for the antihyperlipidemic and antioxidant activities demonstrated above.

Conclusion

In conclusion, we suggest that the phenolic extract of

Ocimum basilicum rich in rosmarinic acid can be beneficial as folk medicine on human health regarding the prevention and treatment of hyperlipidemia, lipid oxidation and related atherosclerotic process. The extract might be also exploited as an additive to prepare functional foods or a substrate to develop natural and safety antiatherogenic preparations.

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Conflict of interest

The authors declare no conflicts of interest.

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