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Original Article

Caftaric acid-rich extract from Ocimum basilicum L. modulates lipid profile and prevents lipoprotein oxidation in hyperlipidemic mice

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ABSTRACT

Introduction: The present study aimed to investigate the hypolipidemic and lipoprotein protective effects of a phenolic extract from sweet basil.

Methods: The antihyperlipidemic activity was evaluated using Triton WR-1339 and a high-fat diet (HFD) induced hyperlipidemic mouse models. In the Triton model, plasma lipids were measured after 24h of treatment, whereas in the HFD model, body weight, food intake, plasma and fecal lipids were determined biweekly. After 45 days of treatment, the livers and abdominal adipose tissues were weighed and lipid measurements for each group were performed.

Results: In both models, the phenolic extract at 100 and 200mg/kg significantly reduced plasma total cholesterol (TC), LDL-cholesterol (LDL-C), triglycerides, atherogenic index and LDL-C/HDL-C ratio and increased HDL-C. Besides, the phenolic extract significantly repressed the gain in body, liver and adipose tissue weights while the food intake was not significantly hindered. Moreover, phenolic extract decreases TC and triglycerides in the liver and adipose tissue and increases their fecal excretion. The phenolic extract exhibited a protective effect against plasma lipoprotein oxidation (IC_{50}=4.64±0.42 µg/ml) and neutralized DPPH free radical (IC_{50}=2.83±0.05 µg/ml) in a manner relatively similar to that exerted by butylated hydroxyanisole (synthetic antioxidant). Total phenolics in the extract represent 234.45±0.84 mg/g and HPLC analysis reveals that the extract includes four main phenolics, with caftaric acid being particularly abundant.

Conclusion: This data suggests that sweet basil is an interesting plant food rich in phenolic compounds that might significantly reduce hyperlipidemia and prevent atherosclerosis and related cardiovascular complications.

Keywords:
Ocimum basilicum
Caftaric acid
Hyperlipidemia
Lipoprotein oxidation

Introduction

Hyperlipidemia is a heterogeneous group of metabolic disorders, defined by high plasma levels of total cholesterol (TC), triglycerides (TG) and low-density lipoprotein (LDL) (Xiao, 2016). It is considered to be the principal risk factor for atherosclerosis and cardio-cerebrovascular diseases (Nelson, 2013). Another impact of hyperlipidemia is the high production of reactive oxy-

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gen species as result of lipid peroxidation, which creates an oxidative environment in the body and promotes LDL oxidation, an early event in atherosclerotic process (Amiya, 2016). Various herbs and spices have been intensively studied to treat hyperlipidemia and oxidative stress considering their richness in phenolic compounds, which have shown to be valuable alternatives to prevent and treat cardiovascular diseases (Chang et al., 2013). Indeed, it is demonstrated that the consumption of polyphenol-rich food preparations such as grapes, apples, cacao, olive and tea can help to mitigate the complications of cardiovascular disease such as dyslipidemia, oxidative status, inflammation, endothelial dysfunction and hypertension (Khurana et al., 2013).

Ocimum basilicum L., also known as sweet basil, belonging to the family of Lamiaceae, is an annual culinary herb originated in India, Africa, and southern Asia and is now grown worldwide (Makri and Kintzios, 2008). In many countries, fresh and dried basil leaves are widely used as a flavoring agent in different foods such as salads, pizzas (Kadriye et al., 2019). *O. basilicum* leaves and flowering parts have traditionally been used as a folk remedy to cure various diseases, including poor digestion, nausea and abdominal cramps (Marwat et al., 2011). Furthermore, infusions of *O. basilicum* are used in traditional medicine in some Mediterranean areas, such as Eastern Morocco, to lower plasma lipid content and prevent the risk of atherosclerosis-related diseases (Harnafi et al., 2007). In this context, the present work was designed to demonstrate the possible hypolipidemic and lipoprotein-oxidation preventive activities of sweet basil cultivated in Morocco using high-fat diet (HFD) and Triton-WR-1339-induced hyperlipidemic mice models.

Materials and methods

Preparation of caftaric acid-rich extract

The basil plant was cultivated in the greenhouse of the Faculty of Sciences (University Mohamed I, Oujda). After drying, 30g of aerial parts, finely grounded into a fine powder, were defatted with n-hexane to remove chlorophylls. The resulted marc was air-dried and then extracted with 400ml of boiling distilled water for 30min. After that, the suspension was filtered using Whatman paper no. 2 (pore size 8mm) and the filtrate was washed three times with n-hexane to remove residual non-polar substances. The obtained aqueous fraction was concentrated in the rotatory evaporator at 50°C under reduced pressure and then dried in a ventilated oven at 40°C for 24h to obtain the dried extract. The extraction yielded 15.66%.

Estimation of total phenolic content

The total phenolic content of basil extract was estimated using the method of Folin-Ciocalteu described by Ahmed et al. (2015) with minor modifications. Briefly, 0.25ml of Folin– Ciocalteu reagent and 0.5ml of 20% sodium carbonate were added into 0.5ml of plant extract samples (0.42mg/ml). The mixture was incubated for 30min at room temperature and the absorbance was measured at 725nm. The polyphenol content was calculated from a calibration curve of caftaric acid and expressed in milligrams per gram of extract. All measurements were done in triplicate.

HPLC analysis of phenolic extract from sweet basil

The HPLC analysis of the sweet basil extract was conducted as previously described by Harnafi et al. (2013). Briefly, the separation 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 5m). The extract (10µl) was separated at 20°C at a flow rate of 1ml/min using the following gradient of aqueous trifluoro-acetic acid (pH 2.8) (A) and acetonitrile (B): 0–1 min: 0–3% B, 1–45 min: 3–40% B, 45–55 min: 40% B, 55-56 min: 0% B. The chromatograms were recorded at 340nm. Compounds were identified by their retention times and UV–visible spectra using a database of standard phenolic compounds.

Study of the hypolipidemic effect of basil phenolic extract and Fenofibrate in Triton WR-1339-induced hyperlipidemic mice

Experimental animals

Forty adult male *Albinos* mice weighing 30±2g were bred in the animal house of the University Mohammed I in Oujda, Morocco. The animals were kept at a steady temperature (22±2°C), on a 12h light/dark cycle and they had unlimited access to food and water. The animals were handled and treated according to the internationally accepted standard guidelines for the use of laboratory animals (approved by the local committee of use of laboratory animals, Faculty of Medicine, approv-

al number: 002016).

Experimental schedule

At the beginning of the experiment, animals fasted overnight were divided into five groups of 8 mice each. The first group served as normal control (NCG) received 0.5ml of NaCl 9‰ by intraperitoneal injection and gavaged with distilled water. The second, hyperlipidemic control group (HCG) injected intraperitoneally with 0.5ml of Triton solution (200mg/kg) and gavaged with distilled water. The third and fourth, caftaric acid-rich extract treated groups (CTG), received Triton by intraperitoneal injection and gavaged with the basil extract at a doses of 100 and 200mg/kg, respectively. The fifth, Fenofibrate treated group (FTG), received Triton and gavaged with Fenofibrate drug at a dose of 200mg/kg. The Triton injection and gavage of caftaric acid-rich extract were administered concurrently.

After 24h, experimental animals were slightly anesthetized with diethyl ether and blood samples were withdrawn from the retro-orbital sinus in tubes containing trisodium citrate as an anticoagulant. The plasma was recovered after centrifugation at 2500rpm for 15min in order to determine lipid profile.

Experimental protocol to study the effect of basil phenolic extract and Fenofibrate in high-fat diet-induced hyperlipidemic mice

Preparation of high-fat diet

To investigate the antihyperlipidemic effect of basil phenolic extract, mice were fed a HFD for 45 days. The HFD was prepared as described by Harnafi et al. (2013). It contains the standard mice diet, beef fat, cholesterol, fructose, egg yolk and deoxycholic acid in suitable ratio of 62%, 16%, 1.5%, 10%, 10% and 0.5%, respectively.

Experimental protocol

The mice were divided into groups similar to the Triton-induced hyperlipidemic model, in which all animals except the normal control were fed a high fat diet. The HFD and caftaric acid-rich extract were given simultaneously. Fresh HFD stored at 4°C was provided every day at the same specific time (20g diet/animal). Meanwhile, before adding the daily amount of diet to each animal, the residual diet (if any) from the previous day was weighed for measurement of net food intake. Likewise, every fortnight, body weights of the animals were recorded and blood samples were collected from the retro-orbital sinuses for analysis of plasma lipid parameters. After 45 days treatment, animals were killed by cervical dislocation and then, the liver and abdominal adipose tissues were excised, weighed, and reported as absolute organ weights (g) /100g body weight.

Dosage of total cholesterol

The enzymatic cholesterol assay method is triggered by the hydrolysis of cholesterol ester using a cholesterol ester hydrolase to produce free cholesterol. In the presence of cholesterol oxidase, free cholesterol is oxidised to cholestenone with the release of hydrogen peroxide; the latter reacts with phenol and 4-aminoantipirin to form the red-coloured quinoneimine. One ml of enzymatic reagent (Biosystems Kit, Barcelona, Spain, REF: 12505) was added to 10µl of plasma. After stirring and incubating for 10min at 37°C, the absorbance at 510nm was determined spectrophotometrically. The following formula was used to measure TC concentration.

$$\text{TC (mg/dL)} = \frac{\text{Absorbance sample}}{\text{Absorbance standard}} \times \text{TC concentration of the standard}$$

Dosage of plasma triglycerides

Plasma TG levels were also determined using enzymatic method (Biosystems Kit, Barcelona, Spain, REF: 12528). One mL of assay reagent was applied to 10µl of plasma. The mixture was stirred and incubated for 15min at 37°C, the absorbance was measured at 520nm against a blank containing 10µl of distilled water and 1ml of assay reagent. The following equation was used to determine the TG concentration:

$$\text{TG (mg/dL)} = \frac{\text{Absorbance sample}}{\text{Absorbance standard}} \times \text{TG concentration of the standard}$$

Dosage of high-density lipoprotein- cholesterol (HDL-C)

The HDL-C was measured using the direct method. In the first step, LDL, VLDL particles and chylomicrons release free cholesterol, converted to hydrogen peroxide by an enzymatic reaction. The formed peroxide is consumed by peroxidase reaction yielding a colorless substance. In the second step, a specific detergent solubilizes HDL cholesterol. In conjunction with cholesterol oxidase and cholesterol esterase, peroxidase plus 4-aminoantipyrine develop a colored reaction proportional to HDL-cholesterol concentration. Ten µl of plasma were

reacted with 750µl of reagent A (Biosystems Kit, Barcelona, Spain, REF: 12557) and 250 of reagent B, then allowed to stand for 10min. After that, the absorbance was measured at 520nm against a blank containing 10µl of distilled water, 750µl of reagent A and 250 of reagent B.

The concentration of HDL-C was calculated according to the following formula:

$$\text{HDL} - \text{C (mg/dL)} = \frac{\text{Absorbance sample}}{\text{Absorbance standard}} \times \text{HDL} - \text{C concentration of the standard}$$

Dosage of LDL-C

The LDL-C was also tested in a direct homogeneous system without precipitation using an enzymatic colorimetric method as described in the kit manufacture (Biosystems Kit, Barcelona, Spain, REF: 12585).

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

Lipid indices were calculated by the following formulas:

$$\text{Atherogenic Index} = \frac{(\text{TC} - \text{HDL-C})}{\text{HDL-C}}$$

$$\frac{LDL\text{-}C}{HDL\text{-}C}\ ratio = \frac{concentration\ of\ LDL\text{-}C}{concentration\ of\ HDL\text{-}C}$$

Liver and adipose tissue lipid analysis

Total lipids were extracted from the liver and abdominal adipose tissue according to the method described by Rodríguez and Peinado (2005). The TC and TG contents were assayed by the same commercially available kit used for the plasma TC and TG measurements.

Fecal lipid analysis

Total lipids were extracted from the feces according to the method of (Kraus et al., 2015) with slight modifications. Fecal TC and TG levels were assayed by the same commercially available kit used for the plasma TC and TG measurements.

Determination of lipoprotein-rich plasma oxidation

The plasma lipoprotein oxidation was induced by copper and followed by measuring the amount of thiobarbituric acid reactive substances *(*TBARS) which are the principal secondary products of lipid peroxidation (Barradas et al., 2021). Thus, the lipoprotein-rich plasma ([LDL-cholesterol]= 1250±7.4mg/dl) was taken out from mice treated with Triton WR-1339 (600mg/kg) for 24h and the antioxidant effect of basil extract and butylated hydroxyanisole (BHA) were studied according to the following scheme:

Control: the 50µl of lipoprotein-rich plasma were incubated with 150µl of distilled water; Oxidized lipoproteins: the 50µl of lipoprotein-rich plasma were incubated with 100µl of a solution of copper sulfate $CuSO_4$ (0.33mg/ml) and 50µl of distilled water; CAE-treated lipoproteins: the 50µl of lipoprotein-rich plasma were incubated with 100µl of copper sulfate solution and 50µl of caftaric acid-rich extract (CAE) at different concentrations (0.5, 1, 2.5, 5, 10, 25, 50, 100 and 200µg/ml); BHA-treated lipoproteins: the 50µl of lipoprotein-rich plasma were incubated with 100µl of copper sulfate solution and 50µl of BHA at different concentrations (0.5, 1, 2.5, 5, 10, 25, 50, 100 and 200µg/ml).

The suspensions were vigorously mixed and incubated at 37°C for 24h. Then, 500µl of 20% trichloroacetic acid and 500µl of 0.8% thiobarbituric acid were added. After, the reaction solution was heated in a water bath at 95°C for 30min. After cooling, the solution's absorbance was measured at 532nm. The amounts of TBARS were calculated from the calibration curve of malondialdehyde standard solutions. All measurements were carried out in triplicate.

DPPH free radical scavenging activity

The measurement of 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) scavenging activity was carried out as described by Bouaziz et al. (2010). In succinct terms, 50µl of caftaric acid-rich extract at different concentrations (2.5, 5, 10, 25, 50, 100 and 200µg/ml) were added to 950µl of a methanol solution of DPPH (0.1mM). The samples were incubated for 30min in the dark and then the absorbance was measured at 517nm. Under the same experimental conditions, the synthetic antioxidant BHA was tested as a standard positive control. The radical scavenging effects of caftaric acid-rich extract and BHA were measured as a percentage of DPPH discoloration using the following equation:

$$\%\text{DPPH radical scavenging} = [(A_0 - A_1)/A_0] \times 100$$

Where A_0 is the absorbance of the DPPH solution and A_1 is the absorbance of the sample. The half-maximal inhibitory concentration (IC_{50}) values were determined from the plotted graph representing the antiradical effect against concentrations of the extract or BHA. All experiments were performed in triplicate.

Statistical analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Student's t-test to detect inter-group differences. Statistically significant was considered at $P<0.05$. Data were expressed as mean±SEM.

Results

Total phenol content and HPLC analysis of basil phenol extract

The total phenol content of *O. basilicum* extract was determined to be 234.45±0.84 mg caftaric acid equivalent/g dry extract. The HPLC chromatogram is depicted in Figure 1, as we can see, caftaric acid, caffeic acid, chicoric acid and rosmarinic acid were the principal four phenolic acids contained in the basil extract and the caftaric acid is the major compound.

Effect of caftaric acid-rich extract on lipid profile in Triton WR-1339-induced hyperlipidemic mice

The administration of Triton for 24h led to a significant increase in TC (233.66%, $P=0.00096$), TG (660.5%, $P=0.00081$), and LDL-C (208%, $P=0.00052$) levels, and a marked decrease in HDL-C levels (44.10%, $P=0.00063$) in HCG as compared to NCG. Besides, the atherogenic index (AI) and LDL-C/HDL-C ratio were

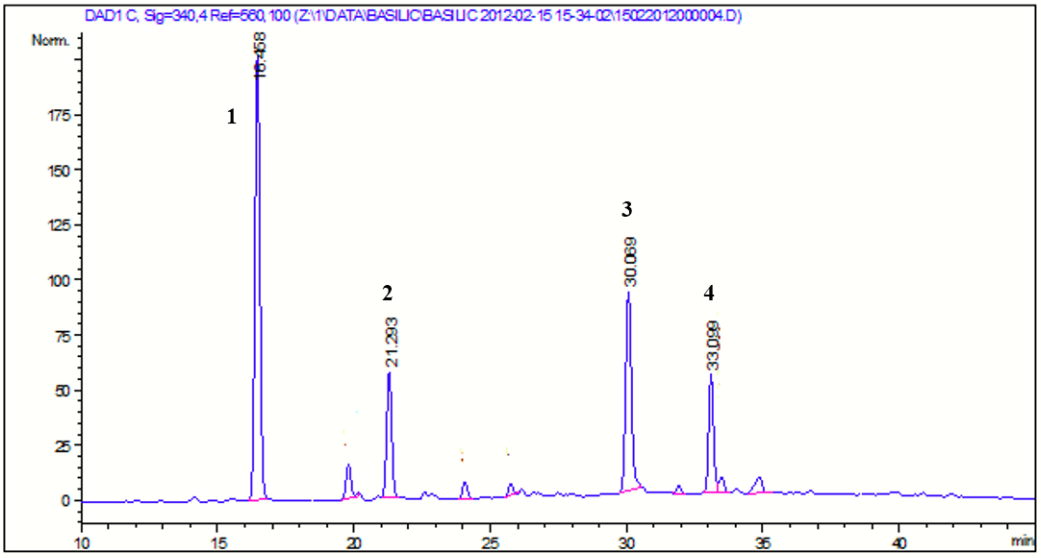

FIGURE 1. HPLC profile of the basil extract. 1: caftaric acid, 2: caffeic acid, 3: chicoric acid, 4: rosmarinic acid.

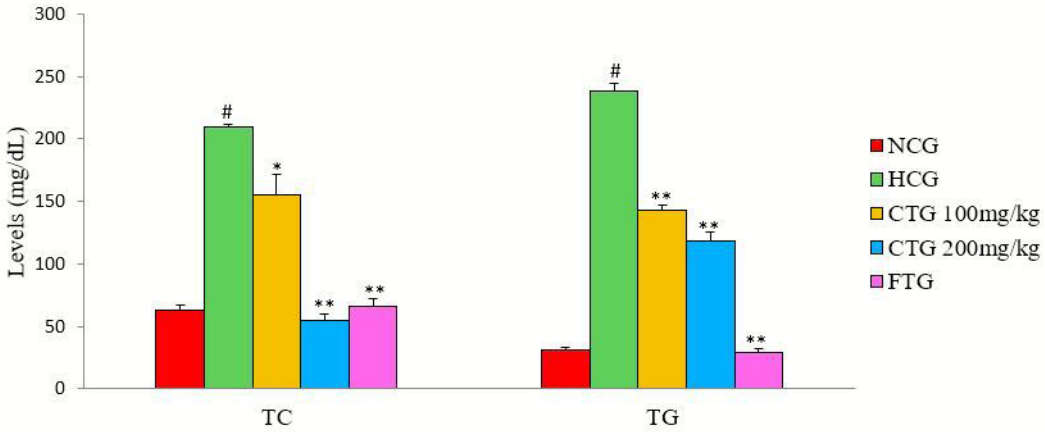

FIGURE 2. Effect of caftaric acid-rich extract and Fenofibrate on total plasma cholesterol and triglycerides content in Triton WR-1339-induced hyperlipidemic mice. Data are mean±SEM (n=8). $^{\#}P<0.001$ (NCG vs. HCG) and $^{*}P<0.05$ and $^{**}P<0.001$ (CTG and HCG vs. FTG). TC: total cholesterol; TG: triglycerides; NCG: normolipidemic control group; HCG: hyperlipidemic control group; CTG: Caftaric acid-rich extract-treated group; FTG: Fenofibrate treated group.

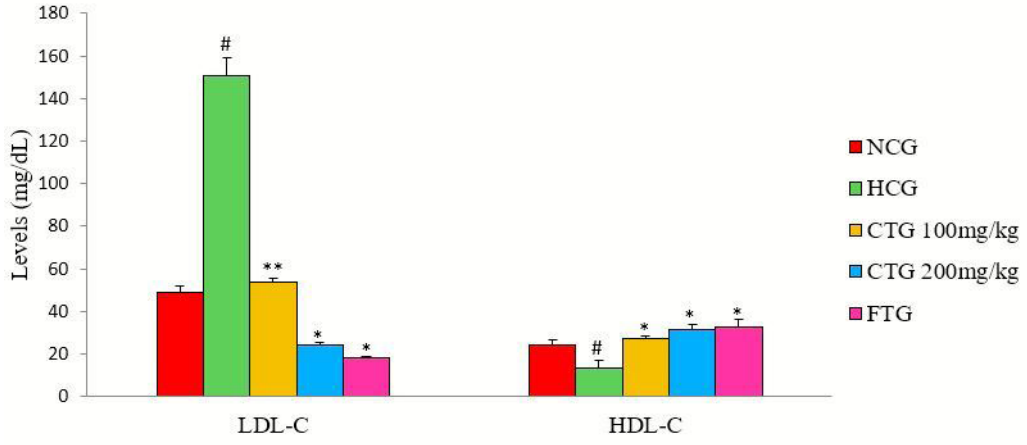

FIGURE 3. Effect of caftaric acid-rich extract and Fenofibrate on plasma LDL-C and HDL-C levels in Triton WR-1339-induced hyperlipidemic mice. Data are mean±SEM (n=8). $^{\#}P<0.001$ (NCG vs. HCG) and $^{*}P<0.001$ and $^{**}P<0.01$ (CTG and FTG vs. HCG). NCG: normolipidemic control group; HCG: hyperlipidemic control group; CTG: caftaric acid-rich extract-treated group; FTG: Fenofibrate treated group.

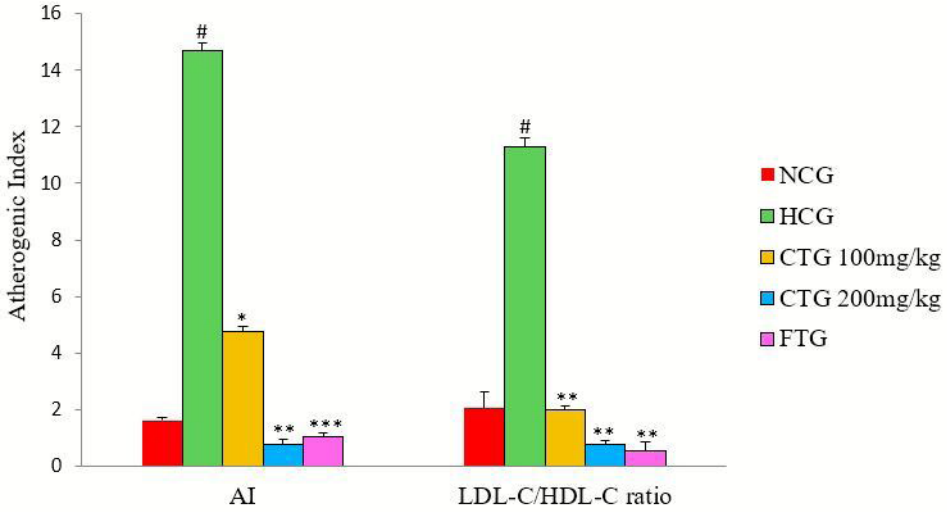

FIGURE 4. Effect of caftaric acid-rich extract and Fenofibrate on atherogenic markers in Triton WR-1339-induced hyperlipidemic mice. Data are mean±SEM (n=8). $^{\#}P<0.001$ (NCG vs. HCG) and $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ (CTG and FTG vs. HCG). AI: atherogenic index; LDL/HDL-C: ratio of low-density lipoprotein-cholesterol to high-density lipoprotein-cholesterol; NCG: normolipidemic control group; HCG: hyperlipidemic control group; CTG: caftaric acid-rich extract-treated group; FTG: Fenofibrate treated group.

significantly affected. Thus, the AI increased by 802% (P=0.00098) and the LDL-C/HDL-C ratio by 450% (P=0.00082) compared to normal mice. The effect of caftaric acid-rich extract at two doses (100 and 200mg/kg) on plasma lipid parameters is dose-dependent. After 24h of triton injection, both doses of caftaric acid-rich extract exerted significant suppression of TC, TG, LDL-C levels and a significant increase in HDL-C level.

In fact, at a dose of 100mg/kg, the caftaric acid-rich extract showed a significant decrease in TC (26%, P=0.039), TG (40%, P=0.00079), LDL-C (64%, P=0.045), and an increase in HDL-C (101%, P=0.00098) compared to HCG. As consequence, the atherogenic index and LDL-C/HDL-C ratio were statistically declined by 67% (P=0.00092) and 82% (P=0.00094), respectively. However, treatment with a higher dose of the extract (200mg/kg) showed a more interesting effect than before. Thus, the TC was reduced by (55% P=0.00082), TG by (50% P=0.00053) and LDL-C by (83% P=0.00086), while the HDL-C was increased by 133%, P=0.00065) when compared to the hyperlipidemic group. This resulted in a more pronounced decrease in atherogenic index (94%, P=0.00077) and LDL-C/HDL-C ratio (93%, P=0.00093).

TABLE 1: Effect of caftaric acid-rich extract and Fenofibrate on plasma total cholesterol and triglyceride levels in HFD-induced hyperlipidemic mice

	TC (mg/dl)				TG (mg/dl)			
	Day 0	**Day 15**	**Day 30**	**Day 45**	**Day 0**	**Day 15**	**Day 30**	**Day 45**
NCG	38,69±2,34	38,54±1,6	38,94±1,3	38,75±3,12	50,21±1,95	50,12±1,6	51,12±1,62	50,82±1,6
HCG	38,45±2,02	49,78±0,8$^{\#\#}$	67,2±1,74$^{\#\#}$	78,81±3,04$^{\#\#}$	50,46±1,51	80,18±3,12$^{\#\#}$	129,2±2,12$^{\#\#}$	138,5±1,2$^{\#\#}$
CTG 100mg/kg	38,65±2,48	40,79±0,15**	53,2±1,75**	52,32±2,19**	51,02±1,38	56,5±8,2*	66,1±1,32**	70,45±1,3**
CTG 200mg/kg	38,63±2,09	50,6±2,5**	50,6±2,5**	41,12±2,32**	50,9±2,6	49,13±12,3*	50,09±1,45**	51,01±1,6**
FTG	38,52±2,16	47,14±1,32**	48,3±1,24**	39,7±2,81**	50.53±2,03	50,63±2,36**	51,83±5,1**	53,53±1,6**

Data are mean±SEM (n=8). $^{\#\#}P$<0.05 (NCG *vs*. HCG) and $^{*}P$<0.05 and $^{**}P$<0.001 (HCG *vs*. CTG and HCG *vs*. FTG). TC: total cholesterol; TG: triglycerides. NCG: normolipidemic control group; HCG: hyperlipidemic control group; CTG: caftaric acid-rich extract-treated group; FTG: Fenofibrate treated group.

TABLE 2: Effect of caftaric acid-rich extract and Fenofibrate on plasma LDL-C and HDL-C levels in HFD-induced hyperlipidemic mice

	LDL-C (mg/dl)				HDL-C (mg/dl)			
	Day 0	**Day 15**	**Day 30**	**Day 45**	**Day 0**	**Day 15**	**Day 30**	**Day 45**
NCG	8,9±2,56	8,2±12,3	8,5±2,65	8,6±1,63	20,12±1,98	20,36±0,65	20,45±0,36	20,1±0,73
HCG	8,5±3,56	35,8±1,1$^{\#}$	48,2±2,86$^{\#\#}$	62,1±1,68$^{\#\#}$	20,1±1,49	12,94±1,4$^{\#\#}$	10,05±0,09$^{\#\#}$	9,02±0,15$^{\#\#}$
CTG 100mg/kg	8,92±3,15	27,5±3,12*	29,21±3,068**	31,29±2,51**	20,13±1,42	19,99±1,32^{ns}	18,02±0,2**	21,94±0,19**
CTG 200mg/kg	8,02±1,54	23,62±2,58**	22,3±1,06**	19,19±1,36**	20,61±1,36	26,364±2,63**	23,2±0,93**	24,32±1,5**
FTG	8,16±2,19	22,16±3,64**	19,23±2,41**	12,02±1,64**	20,69±1,34	27,224±1,52**	23,46±0,6**	27,01±1,64**

Data are mean±SEM (n=8). $^{\#\#}P$<0.05 and $^{\#}P$<0.001 (NCG vs. HCG) and $^{*}P$<0.05 and $^{**}P$<0.001 (HCG vs. CTG and HCG vs. FTG). TC: total cholesterol; TG: triglycerides; HDL-C: high density lipoprotein-cholesterol; LDL-C: low-density lipoprotein-cholesterol; ns: not significant; NCG: normolipidemic control group; HCG: hyperlipidemic control group; CTG: caftaric acid-rich extract-treated group; FTG: Fenofibrate treated group.

On the other hand, this hypolipidemic effect was compared to that of the standard drug Fenofibrate at a dose of 200mg/kg, which showed a remarkable decline in TC, TG and LDL-C by 68% (P=0.00099), 87% (P=0.00076) and 88%(P=0.00093), respectively. Furthermore, a significant rise in HDL-C by 141% (P=0.00089) was also observed. With Fenofibrate, the atherogenic index was reduced by 92% and the LDL-C/HDL-C ratio by 94%. All of the outcomes mentioned above were shown in Figures 2, 3 and 4.

Antihyperlipidemic activity of caftaric acid-rich extract and Fenofibrate in HFD-induced hyperlipidemic mice

In this model, all mice except the normal control group were fed a HFD for 45 days. The plasma lipid parameters measured in all groups of mice on days 0, 15, 30 and 45 were shown in Table 1 and Table 2. The results indicate that on the 15th day, the HFD produced a significant rise in plasma TC (29%, P=0.042), TG (60%, P=0.036), LDL-C (363%, P=0.00065) levels and alleviation in HDL-C levels (36%, P=0.039) in HCG when compared to the NCG. After treating the animals with caftaric acid-rich extract at two different doses (100 and 200mg/kg) a remarkable effect on lipid parameters was observed in a dose- and time-dependent manner.

As we can see, caftaric acid-rich extract at 100mg/kg suppressed the elevated total cholesterol rise produced by HFD on the 30 and 45 days of treatment (20%, P=0.00043 and 33%, P=0.00032, respectively). Furthermore, the same dose of the extract revealed a highly significant lowering effect on TG after 30 and 45 days, the decrease was of 48%, (P=0.00073) and 50% (P=0.00082), respectively with respect to HCG.

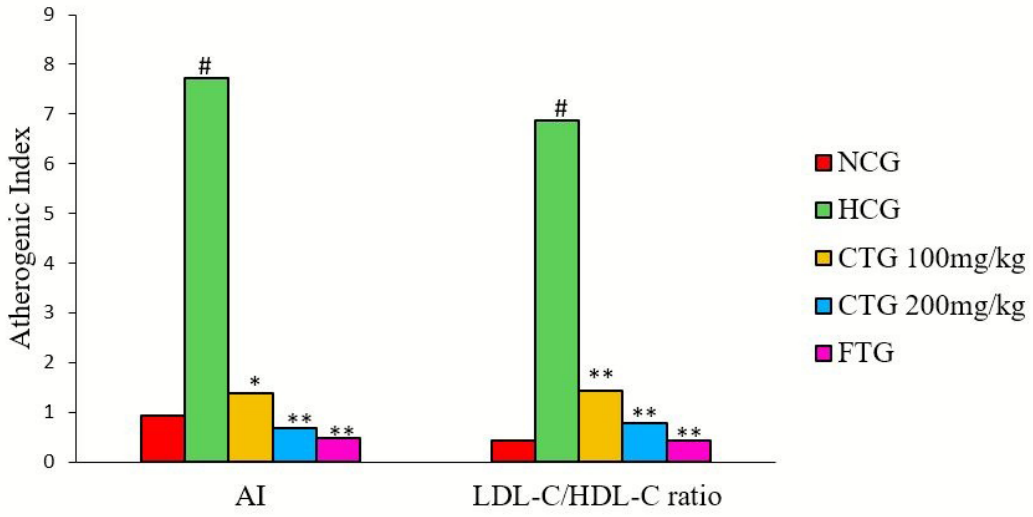

FIGURE 5. Effect of caftaric acid-rich extract and Fenofibrate on atherogenic markers in high fat diet-induced hyperlipidemic mice. Data are mean±SEM (n=8). $^{\#}P<0.001$ (NCG vs. HCG) and $^{**}P<0.001$ (CTG and FTG vs. HCG). AI: atherogenic index; LDL/HDL-C: ratio of low-density lipoprotein-cholesterol to high-density lipoprotein-cholesterol; NCG: normolipidemic control group; HCG: hyperlipidemic control group; CTG: caftaric acid-rich extract-treated group; FTG: Fenofibrate treated group.

After 30 and 45 days of treatment, we found also a significant (*P*=0.00081) decrease in plasma LDL-C (49%, (*P*=0.00081) and 50%, (*P*=0.00089) respectively). The HDL-C was significantly (*P*<0.001) increased after 30 and 45 days treatment by 79% (*P*=0.00091) and 143%, (*P*=0.00082) respectively. However, caftaric acid-rich extract at 200mg/kg promoted a pronounced decrease in the TC after 30 and 45 days (24%, *P*=0.00089) and (47%, *P*=0.00061), respectively). Therefore, the same treatment showed a considerable decrease in TG levels after 30 and 45 days, it was of 61%, *P*=0.00081) and 64%, *P*=0.00096), respectively with respect to HCG. During treatment, we also revealed a significant decrease in plasma LDL-C by 53% (*P*=0.0009) after 30 days and by 70% (*P*=0.00089) after 45 days. However, after the same period treatment, the HDL-C was respectively increased by 131% and 169% (*P*=0.00071).

The results obtained were compared to the Fenofibrate which showed significant modifications in the plasma lipid parameters. As can be seen, finding showed that after 30 days treatment, drug administration resulted in a statistically significant lowering of plasma TC and TG by 30%, (*P*=0.00083) and 53%, (*P*=0.00071), respectively. This drop was followed by a 60%, (*P*=0.00094) decrease in LDL-C and a 134% (*P*=0.00094) rise in HDL-C. Likewise, Fenofibrate suppressed the elevated rise of plasma TC, TG, and LDL-C on the 45 days of treatment by 49%, 69%, and 80% (*P*=0.0005), respectively, and increased HDL-C by 199% (*P*=0.0008). At the end of the experiment, we noted that the feeding HFD results in an increase of AI by more than 8-fold and LDL-C/ HDL-C ratio by more than 16-fold (*P*=0.0082) in HCG compared to the NCG, while this effect was mitigated by treatment with caftaric acid-rich extract in a dose-dependent manner. Thus, at a dose of 100mg/kg, the reductions were 82% (*P*=0.042) and 79% (*P*=0.009) in AI and LDL-C/HDL-C ratio, respectively. While at the same time, 200mg/kg of extract suppressed the elevated values of AI and LDL-C/HDL-C by 92% (*P*=0.0081) and 89% (*P*=0.0083), respectively which were approximately restored to the normal level. Likewise, the Fenofibrate tends to diminish the level of AI by 94% (*P*=0.006) and LDL-C/HDL-C ratio by 95% (*P*=0.0091; Figure 5).

Effect of caftaric acid-rich extract and Fenofibrate on food intake, body weight, liver and adipose tissue weight

During the study, none of the experimental animals died, nor did they exhibit behavioral abnormalities, and no significant difference was observed in the food intake between different treated groups. This indicates that the caftaric acid-rich extract and Fenofibrate have no effect on the appetite of HFD-fed mice (Table 3). As shown in Table 3, compared with the normal group, body weight in hyperlipidemic control group rose significantly from 15 days after HFD supply, and the weight gains after 30

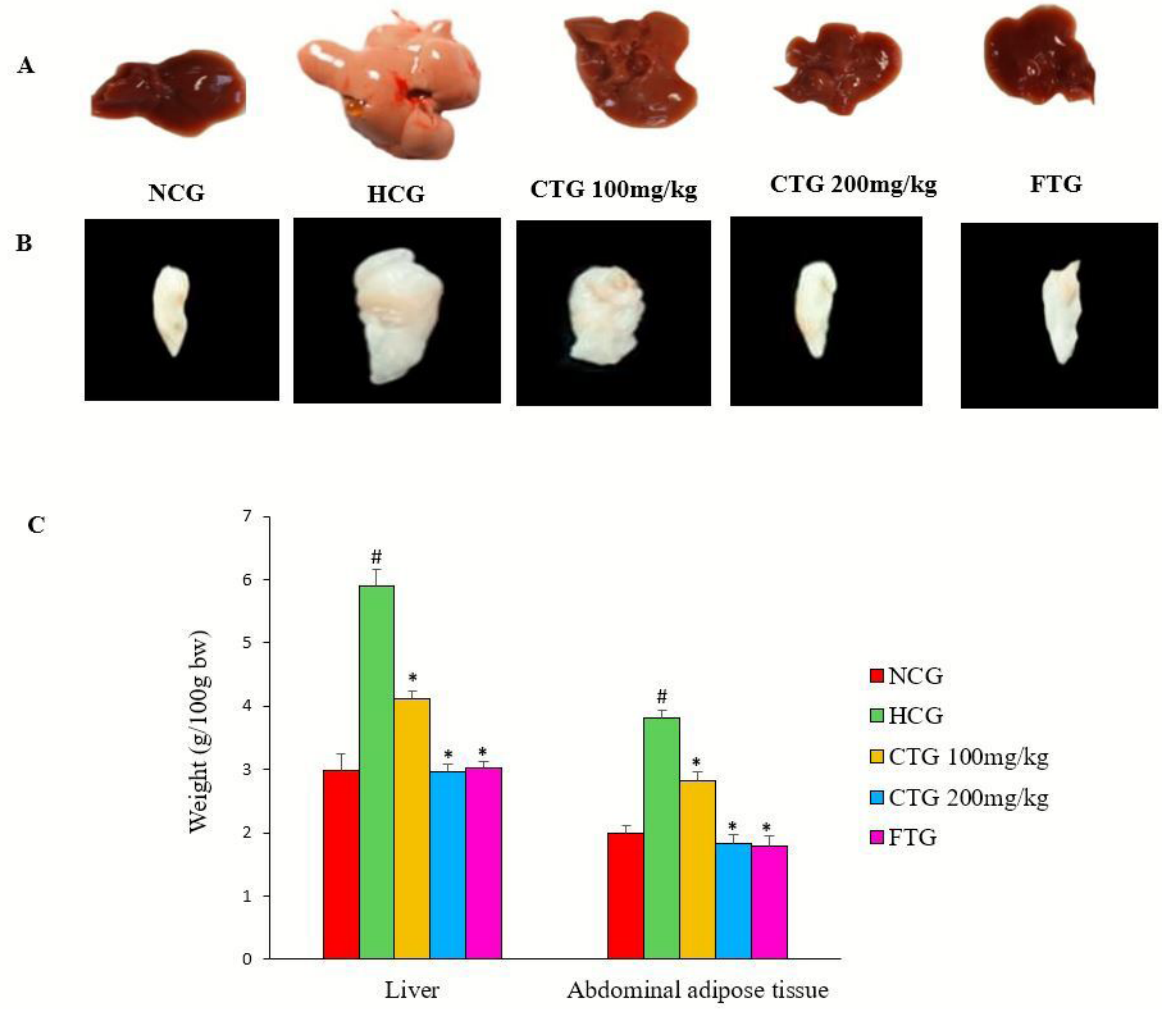

FIGURE 6. Effects of caftaric acid-rich extract and Fenofibrate on the liver and abdominal adipose tissue in hyperlipidemic mice. A: Macroscopic appearance of livers. B: Macroscopic appearance of adipose tissues. C: Liver weight and abdominal adipose tissue weight. The values are mean±SEM (n=8). $^{\#}P<0.001$ (NCG vs. HCG) and $^{*}P<0.001$ (CTG and FTG vs. HCG). NCG: normolipidem-ic control group; HCG: hyperlipidemic control group; CTG: caftatric acid-rich extract treated-group; FTG: Fenofibrate treated group.

and 45 days were more considerable [14%, (P=0.009) and 24%, (P=0.0083) respectively]. However, in mice treated with caftaric acid-rich extract (100 and 200mg/kg) or Fenofibrate, these changes were partially reversed, but not significantly after 15 days of administration. Thus, significant decreases of the body weights were detected in CTG-100 (10%, P=0.031), CTG-200 (12%, P=0.043) and FTG after 30 days treatment as compared with HCG. In addition, we note that the body weight gain was much reduced after 45 days in the CTG-200 mg/kg (17%, P=0.00094) and FTG (19%, P=0.00096).

On the other hand, the livers in normal control group seemed shiny sleek with a dark red color. In contrast, as shown in Figure 6A, the hyperlipidemic mice livers are yellowish enlarged and with irregular surface suggested the fatty liver. After treatment with basil extract and Fenofibrate, the livers were red-brownish with sleek surfaces, similar to the NCG. Furthermore, as displayed in Figure 6B, the relative liver weight of the HFD-fed mice was increased by 31% (P=0.008) as compared to NCG. However, the concomitant administration of caftaric acid-rich extract hindered this increase by 30% (P=0.00098) and 50 % (P=0.00094) at doses of 100 and 200mg/kg, respectively. This is comparable to the effect exerted by Fenofibrate which decreased the relative liver weight by 49% (P=0.00083).

Besides, it is clearly seen in Figure 6C that the abdominal adipose tissue relative weight significantly increased 2- fold (P=0.00073) in the hyperlipidemic mice compared to normolipidemic ones. On the contrary, a significant diminution in the relative mass of adipose tissue was recorded in the group given caftaric acid-rich

TABLE 3: Changes of body weight and food intake in high fat diet fed mice

	Day 15		Day 30		Day 45	
	Body weight (g)	**Food intake**	**Body weight (g)**	**Food intake**	**Body weight (g)**	**Food intake**
NCG	28±0.09	2.63±0.23	28.9±0.022	2.75±0. 14	29.1±0.1	2.69±0.13
HCG	31.2±0.14$^{\#}$	2.87±0.38	33.0±0.09$^{\#}$	2.78±0. 42	36.12±0,5$^{\#}$	2.86±0.21
CTG (100mg/kg)	29.15±1.25^{ns}	2.58±0.11	29.5±0.67**	2.95±0.13	30.82±0.2*	2.71±0.38
CTG (200mg/kg)	29.01±1.11^{ns}	2.62±0.25	29.02±1.2**	2.65±0.19	29.9±0.5*	2.62±0.15
FTG	28.91±1.03^{ns}	2.66±0.12	29.08±0.061*	2.64±0.16	29.18±0.4*	2.67±0.13

Data are mean±SEM (n=8). $^{\#}P$<0.001 (NCG *vs*. HCG) and $^{**}P$<0.05 and $^{*}P$<0.001 (HCG *vs*. CTG and HCG *vs*. FTG). NCG: normolipidemic control group; HCG: hyperlipidemic control group; CTG: caftaric acid-rich extract-treated group; FTG: Fenofibrate treated group; ns: not significant.

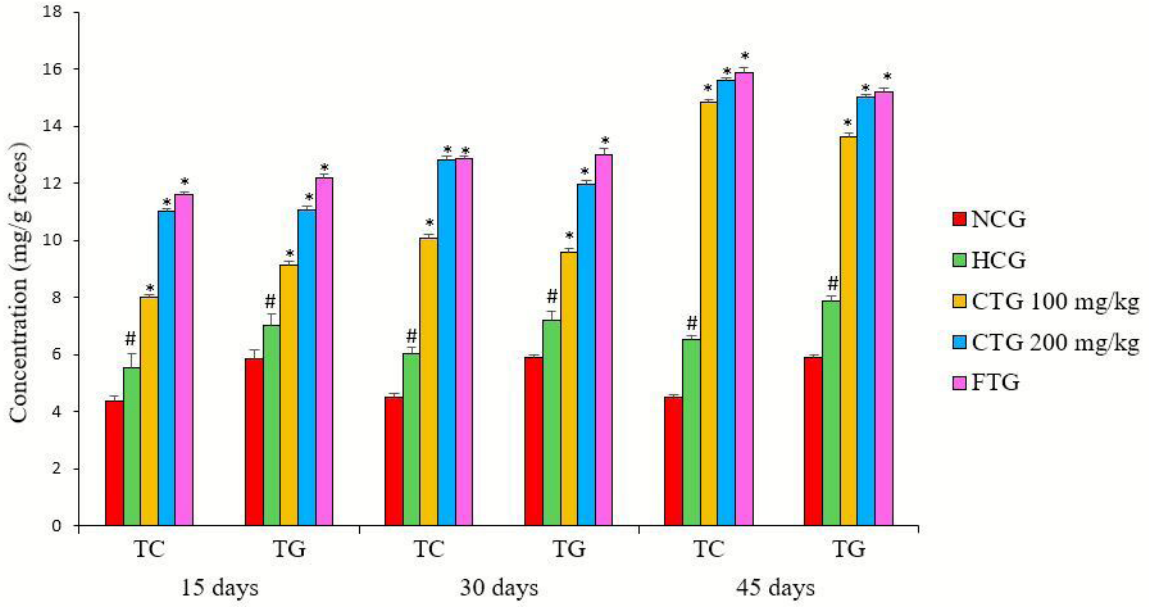

FIGURE 7. Fecal excretion of total cholesterol and triglyceride in hyperlipidemic mice. The values are mean±SEM (n=8). $^{\#}P$<0.001 (NCG vs. HCG) and $^{*}P$<0.001 (CTG and FTG vs. HCG). TC: total cholesterol; TG: triglycerides; NCG: normolipidemic control group; HCG: hyperlipidemic control group; CTG: caftaric acid-rich extract-treated group; FTG: Fenofibrate treated group.

extract at 100mg/kg (26%, *P*=0.00087) and 200mg/kg (52%, *P*=0.00091) as compared with the hyperlipidemic control group. We also note that the Fenofibrate drug acts by 53% (*P*=0.00093) in lowering adipose tissue mass.

Effects of caftaric acid-rich extract and Fenofibrate on mice liver and adipose tissue lipids

The effect of caftaric acid-rich extract on liver lipid concentration in mice fed HFD for 45 days is presented in Table 4. Compared to NCG, the HCG had significantly higher total cholesterol levels in liver tissue homogenate (135%, *P*=0.00091) as well as triglyceride levels (69%, *P*=0.00073). In contrast, the administration of caftaric acid-rich extract at 100mg/kg along with the HFD consistently reduced liver total cholesterol and triglycerides in mice by 22% (*P*=0.009) and 21% (*P*=0.04), respectively. However, at a dose of 200mg/kg, the extract appears to be more efficient and significantly inhibits the increase in hepatic TC (52%, *P*=0.00081) and TG (41%, *P*=0.00096). Fenofibrate showed to be comparable to the extract in reducing liver TC (78%, *P*=0.00093) and relatively more active on triglycerides levels (58%, *P*=0.00089).

Similarly, the consumption of a HFD raised adipose tissue TC by 77% (*P*=0.00021) and TG by 60% (*P*=0.00031) compared to the normal control group. These increased levels promoted by the HFD were significantly lowered by caftaric acid-rich extract treatments in a dose-dependent manner. The levels of TC

TABLE 4: Effect of caftaric acid-rich extract and Fenofibrate on hepatic and adipose tissue lipid profile in high fat diet fed mice

	Parameters	NCG	HCG	CTG 100mg/kg	CTG200mg/kg	FTG
Liver	TC (mg/g)	8,37±0.89	19,72±1,21$^{\#}$	15,34±0.83***	9,43±0,85**	4,16±0,73**
	TG (mg/g)	14,72±0.63	24,84±2.06$^{\#}$	19,54±0.5*	14,52±0.99**	10,96±0.42**
Adipose tissue	TC (mg/g)	1,93±0.05	3,42±0.06$^{\#}$	2,82±0.02**	2,46±0.03**	2,17±0.09**
	TG (mg/g)	4,68±0,73	7,53±0.32$^{\#}$	5.25±0.04**	4,2±0.019**	4,02±0,051**

Data are mean±SEM (n=8). $^{\#}P<0.001$ (NCG *vs*. HCG) and $^{*}P<0.05$, $^{**}P<0.001$, $^{***}P<0.01$ (HCG *vs*. CTG and HCG *vs*. FTG). TC: total cholesterol; TG: triglycerides; NCG: normolipidemic control group; HCG: hyperlipidemic control group; CTG: caftaric acid-rich extract-treated group; FTG: Fenofibrate treated group.

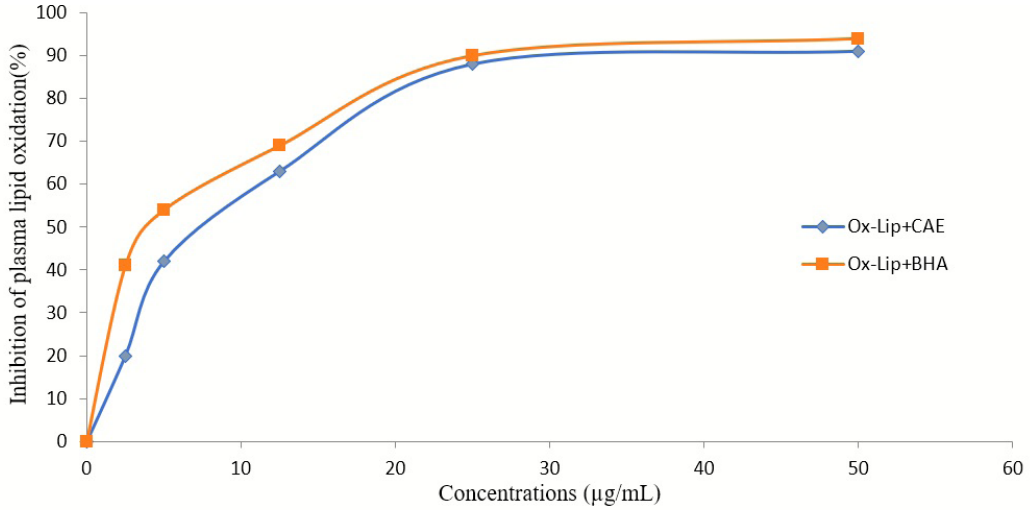

FIGURE 8. Effect of caftaric acid-rich extract and BHA on plasma lipoprotein oxidation. Ox-Lip: oxidized lipoproteins; BHA: butylated hydroxyanisol.

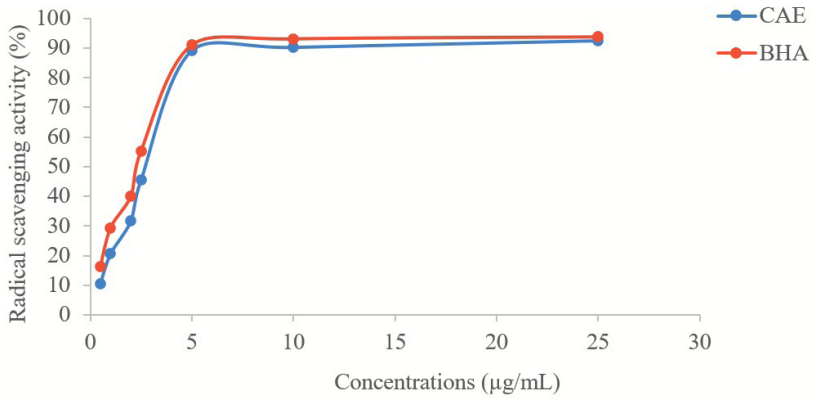

FIGURE 9. Free radical scavenging activity of caftaric acid-rich extract and BHA. CAE: caftaric acid-rich extract; BHA: butylated hydroxyanisol.

and TG were shown to decrease in CTG 100mg/kg by 17% (P=0.00072) and 30% (P=0.0006), respectively. In the CTG 200mg/kg, adipose TC was lowered by 28% (P=0.0009) and TG by 44% (P=0.00083). Fenofibrate also significantly reduced TC and TG levels by 36% (P=0.0005) and 46% (P=0.00076), respectively, in FTG compared to HCG.

Effect of caftaric acid-rich extract and Fenofibrate on fecal cholesterol and triglyceride excretion in mice

The levels of TC and TG excreted into the feces are summarized in Figure 7. The results showed that after

being fed a HFD for 15 days, the TC and TG excretion of mice was significantly increased by 27% (*P*=0.041) and 20% (*P*=0.039) respectively, compared to the NCG. However, treatment with caftaric acid-rich extract at a dose of 100mg/kg significantly increased the total cholesterol content in the feces of hyperlipidemic mice after 30- and 45-days treatment by 67% (*P*=0.0008) and 128% (*P*=0.00092) respectively. Also, the same dose of extract exhibited a significant rise in TG excretion after 30 days by 33% (*P*=0.0009) and 45 days by 73% (*P*=0.00073). At the higher dose of caftaric acid-rich extract (200 mg/kg), the fecal excretion of cholesterol and TG were increased again after 30 days treatment by 113% (*P*=0.00062) and 67% (*P*=0.00081) respectively, and after 45 days by 140% (*P*=0.00073) and 90% (*P*=0.00068), respectively. We can note also that the Fenofibrate drug enhanced fecal excretion of cholesterol and TG with a score relatively comparable to the basil extract. Thus, after 30 days it increases the cholesterol excretion by 114% (*P*=0.00093) and TG by 81% (*P*=0.00062). Likewise, after 45 days treatment, the effect was more marked with 144% raise (*P*=0.00053) in cholesterol and 93% (*P*=0.00061) in TG levels.

Effect of caftaric acid-rich extract and BHA on the oxidation of mice plasma lipoproteins

The effects of copper on plasma lipoprotein oxidation were evaluated under various conditions in the presence or absence of caftaric acid-rich extract and BHA. Based on the findings of the study (as given in Fig. 8), copper produces a significant increase in plasma lipoprotein oxidation at 37°C when compared to control 790% (*P*=0.00091). However, the oxidative process was significantly and dose-dependently reduced when plasma lipoproteins were treated with various concentrates of caftaric acid-rich extract and BHA. Thus, at doses of 1.25, 2.5, 5, 10, 25 and 50µg/ml, caftaric acid-rich extract reduced plasma lipoprotein oxidation by 4% (*P*=0.043), 20% (*P*=0.042), 42% (*P*=0.00094), 63% (*P*=0.0073), 88% (*P*=0.00092) and 91% (*P*=0.00089), respectively. Also, at the doses stated above, BHA suppressed plasma lipoprotein oxidation by 6% (*P*=0.045), 41% (*P*=0.034), 54% (*P*=0.049), 69 % (*P*=0.00063), 90% (*P*=0.0009) and 94% (*P*=0.00081). By comparing the IC50 of the studied compounds, we found that caftaric acid-rich extract (IC50=4.64±0.42 µg/ml, *P*=0.031) was less efficient than BHA (IC50=3.47±0.13µg/ml, *P*=0.036) in preventing plasma lipoprotein oxidation.

Radical scavenging activity of caftaric acid-rich extract and BHA

Figure 9 shows the DPPH radical scavenging rate (%) of caftaric acid-rich extract and BHA at different concentrations. As can be shown, the studied extract is active against free radicals in a concentration-dependent manner. Thus, at 0.5, 1, 2, 2.5, 5, 10 and 25µg/ml, free radical scavenging percentages of the extract were of 10% (*P*=0.0005), 21% (*P*=0.00045), 32% (*P*=0.00065), 46% (*P*=0.00085), 89% (*P*=0.00075), 90% (*P*=0.00063) and 93% (*P*=0.00074), respectively. Alternatively, the BHA scavenges free radicals at the same previous concentrations by 16% (*P*=0.00085), 29% (*P*=0.00087), 40% (*P*=0.00086), 55% (*P*=0.00073), 91% (*P*=0.00081), 93% (*P*=0.00073) and 94% (*P*=0.00082) respectively. Based on a comparison of the IC_{50}, we concluded that there is no significant difference between the antiradical effect of caftaric acid-rich extract (IC_{50}= 2.83±0.05µg/ml) and BHA (IC_{50}= 1.95±0.83µg/ml; *P*=0.072).

Discussion

Cardiovascular diseases (CVD) have received increased attention due to their high prevalence and mortality worldwide (Wang et al., 2015). As previously reported, hyperlipidemia and lipoprotein oxidation are the main contributors to the cascade of events that promote atherosclerotic plaque formation leading to CVD complications (Rafieian et al., 2014). Treatment of CVD was based essentially on synthetic chemical drugs, which are not avoided of harmful side effects. So, herbal medicines and dietary supplements are becoming more and more popular to prevent or cure high cholesterol levels, mainly for person with cholesterol at borderline levels (Villaseñor, 2016). In addition, several epidemiological studies have found that eating a diet rich in natural antioxidants improves the body's antioxidant capacity and lowers the risk of CVDs (Chang et al., 2013). In this context, we aimed at the search of hypolipidemic and anti-lipoprotein oxidation of sweet basil widely used as spice and medicinal plant by Moroccan population. The study was conducted using tyloxapol and HFD induced hyperlipidemic animal models.

The tyloxapol-induced hyperlipidemia model has often been used experimentally to screen natural or chemical hypolipidemic drugs (Harnafi et al., 2007). This

chemical agent (Triton WR-1339) acts as a surfactant that inhibits the lipoprotein lipase activity causing acute hyperlipidemia in several animal species. Furthermore, Triton WR-1339 also stimulates HMG-CoA reductase activity leading to hypercholesterolemia (Huynh et al., 2018). In the current study, Triton WR-1339 given to the mice resulted in a significant rise in plasma TC, TG, LDL-C and AI levels, as well as a decrease in HDL-C. However, the concomitant oral administration of caftaric acid-rich extract significantly hindered lipid levels in experimental hyperlipidemic mice by decreasing all the atherogenic lipid levels (TC, TG and LDL-C) and increasing the anti-atherogenic one (HDL-C). So, this preliminary finding suggested that the basil phenolic extract could be interested in preventing hyperlipidemia and incite us to confirm the results in a model eating HFD.

Thus, it appears clear that feeding the animals with an HFD supplemented with fructose and egg yolk caused a significant increase in plasma level of TC, TG, LDL-C and a decrease in the HDL-C after 45 days, while this effect was reversed by treatment with caftaric acid-rich extract at 100 and 200mg/kg. We also observed that the reduction of plasma cholesterol was associated with a significant decrease in its LDL-C fraction which is the principal atherogenic lipid parameter targeted by hypolipidemic therapies. So, we can suggest that the extract may stimulate the expression and production of hepatic and extra-hepatic LDL- receptors and as a consequence increasing the uptake of plasma LDL-C especially by the liver and peripheral tissues. In the liver, the excess of cholesterol was eliminated freely or as bile salts. This hypothesis was partially validated by the liver, adipose tissue and fecal lipid analysis. Thus, we found that the cholesterol was finally concentrated in treated animal feces. Furthermore, a significant rise in HDL-C levels was noted, which is reported to have an antiatherosclerotic effect (Bandeali and Farmer, 2012). Indeed, a linear inverse association between HDL-C levels and cardiovascular risk has been established and published (Wilkins et al., 2014). So, the increase of HDL-cholesterol by the caftatric acid rich-extract could greatly be important in lipid metabolism regulation. It is possible that the studied extract enhances reverse cholesterol transport by increasing lecithin cholesterol acyl transferase activity and thus ensures enrichment of HDL-2 with cholesterol ester.

Besides, it has been recently discovered that TGs have an essential role in controlling lipoprotein interaction, which is required for lipid metabolism homeostasis (Ackerman et al., 2018). The administration of caftaric acid-rich extract significantly suppressed the elevated plasma, liver and adipocytes concentrations of TG and increased their levels in feces. This hypotriglyceridemic effect might be attributed to an increase in the clearance of TG-rich lipoproteins by peripheral organs via increased lipoprotein lipase activity (Jawed et al., 2019). Another process may be related to the delay of digestion of TG by inhibiting pancreatic lipase that promotes the hydrolysis of TG to mono- and diglycerides, which are then absorbed by enterocytes (Kamoun et al., 2019). On the other hand, it is now established that the AI is a valuable marker of the risk of atherosclerosis development (Uslu et al., 2017). In this study, the administration of caftaric acid-rich extract in both hyperlipidemic mice models has a beneficial effect on lipid metabolism which helps to reduce AI. In addition, it is also preferable to have more significant plasma HDL-C and lower LDL-C to prevent atherogenesis since there is a positive correlation between an increased LDL-C/HDL-C ratio and the development of atherosclerosis (Zhong et al., 2019). In hyperlipidemic mice, treatment with caftaric acid-rich extract significantly reduced the higher values of AI and LDL-C/HDL-C ratio, proving its positive effect on lipid metabolism and atherosclerosis prevention.

Our results are in line with earlier research on the hypolipidemic properties of natural products and extract (Hui et al., 2020; Hashem et al., 2021). On the other hand, an excessive HFD efficiently increases body weight and fat tissue, resulting in an imbalance between energy intake and energy consumption (Hall, 2019). These body alterations may lead to the deactivation of liver and mesenteric genes responsible for beta-oxidation, which increases the hepatic and adipose lipid stocks (Iñiguez et al., 2018). Macroscopically, the HFD enriched with fructose and egg yolk prompts a hypertrophy in liver and adipose tissue. However, significant decreases were observed in body weight gain, hepatic and adipose mass of the treated groups. Despite these changes, daily food intake was similar in all groups, suggesting that the studied extract have no effect on the regulation of appetite. Our findings are in accordance with those reported by Kim et al. (2021), who have found that *Annona muricata* leaf extract is able to reduce body weight, adipocyte

size and hepatocyte ballooning by reducing fatty acid synthesis and uptake in the liver. Besides, the possible mechanism underlying the body weight loss exerted by the basil extract was seemingly attributable to the inhibition of lipogenic enzymes including fatty acid synthase and diacylglycerol acyltransferase 2 as reported by (Bin-Jumah, 2018; Neuschwander-Tetri, 2010). On the other hand, the plant extract could activate fatty acid oxidation enzymes such as carnitine palmitoyltransferase 1 as demonstrated by Tacherfiout et al. (2018). In this study, Fenofibrate was used as known reference hypolipidemic drug. We found that the drug significantly decreased plasma, liver and adipocytes lipid concentrations with a score relatively comparable to that showed by the tested phenolic-rich extract.

As evoked previously, oxidative stress constitutes a pivotal step in the development and progression of atherosclerosis, notably by causing the oxidative change of LDL (Yang et al., 2017). So, the current trends in preventing atherosclerosis aim to regulate blood lipids levels and decrease problems induced by oxidative stress (Wadhera et al., 2016). Thus, in addition to its lipid-lowering effect, the caftaric acid-rich extract showed significant protection of plasma lipoprotein against oxidation induced by copper ions in a dose-dependent manner. The observed effect suggests that the mechanism of action of caftaric acid could be associated with its ability to scavenge free radicals, chelate copper ions or both. In fact, our experimental study revealed that the basil extract significantly neutralizes DPPH radicals, which could be considered as a possible mechanism in the inhibition of lipid oxidation. We also note that the effect is relatively comparable to that exerted by standard antioxidant BHA, which confirms the importance of the antioxidant activity exerted by the basil extract.

Our results agree with several studies demonstrating a significant relationship between antioxidant activity and total phenolic content in many plant species (Yang et al., 2018). Indeed, many investigators have suggested that phenolic compounds act as primary antioxidants or free radical scavengers (Martinez et al., 2020). They even have redox abilities, which allow them to serve as reducing agents, hydrogen donors, singlet oxygen quenchers, and metal chelators (Martinez et al., 2020). According to the HPLC profile, it appears that the caftaric acid (caffeoyltartaric acid) is the most prevalent phenolic component in the studied extract, which is known in the literature by its interesting pharmacological activities (Liu et al., 2017). Therefore, the caftaric acid might be acting alone or in synergy with other phenolic compounds present in the basil extract (rosmarinic, chicoric and caffeic acids) to promote its hypolipidemic and antioxidant effects.

Conclusion

In conclusion, sweet basil provides a rich source of hypolipidemic and antioxidant phenolic acids and could be an alternative nutritional approach for preventing or treating hyperlipidemia, body weight gain, lipid oxidation, and associated cardiovascular events.

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Conflict of interest

The authors declare no conflicts of interest.

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