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Effect of polyphenol, flavonoid, and saponin fractions from *Thymus atlanticus* on acute and chronic hyperlipidemia in mice

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Abstract

Background: *Thymus atlanticus* is an endemic plant of the Mediterranean region, which has been used in the Moroccan mountain area to treat several diseases. This study aimed to investigate the effect of polyphenol, flavonoid, and saponin fractions derived from this plant on acute and chronic hyperlipidemia in male albino mice.

Results: The results indicated that the injection of Triton WR-1339 (20 mg/100 g body weight (B.wt.)) and 6-week administration of a high-fat diet (which is an 81.8% standard diet supplemented with 2% cholesterol, 16% lard, and 0.2% cholic acid) significantly increased plasma total cholesterol, triglycerides and low-density lipoprotein cholesterol (LDL-C), but did not affect high-density lipoprotein cholesterol (HDL-C) levels in mice. Administration of a single dose (2 mg/kg B.wt.) of polyphenol, flavonoid, or saponin fractions significantly suppressed the effect of Triton injection on plasma total cholesterol, triglycerides, and LDL-C. In addition, the supplementation of the high-fat diet with polyphenol fraction (2 mg/kg B.wt./day) prevented the increase of total cholesterol, triglycerides, and LDL-C, and effectively increased HDL-C level when compared to mice feeding only the high-fat diet.

Conclusion: In conclusion, phenolic compounds from *Thymus atlanticus* possess a significant hypocholesterolemic and hypotriglyceridemic effects and, therefore, could have an important role in the management of dyslipidemia.

Keywords: Chronic hyperlipidemia, Rosmarinic acid, Saponin, *Thymus atlanticus*, Mice

Background

Cardiovascular diseases are the leading cause of morbidity and mortality worldwide [1]. These diseases are related to several risk factors especially dyslipidemia, which is a metabolic disorder that is manifested by elevated levels of plasma total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C), along with a reduced level of high-density lipoprotein cholesterol (HDL-C) [2]. Dyslipidaemia is closely linked with occurrence and progression of several pathologies including atherosclerosis and its complications such as coronary artery disease [2]. Several clinical trials confirmed that the reduction of plasma levels of TC, TG, and LDL-C induce a significant reduction of the morbidity and mortality rate related to these diseases [3]. Moreover, the main strategy for the treatment of cardiovascular diseases is based on the control and the management of lipid plasma concentrations [4]. Among the current drugs available for treating dyslipidemia, statins, and fibrates constitute the two major classes of hypolipidemic drugs [5, 6]. Although it is effective, the long-term use of statins or fibrates is

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associated with several side effects [7, 8], which can be potentiated when they are co-administered [9].

Taking into account that lipid-lowering drugs are the main strategy in treating atherosclerosis and that their side effects are important [7, 8, 10], the search for new hypolipidemic drugs with fewer side effects has drawn great attention in recent years [11, 12]. In this context, some plants have been studied for their lipid-lowering effect in animal and human models [12, 13]. The principal reason in interest on plants is their effectiveness in traditional medicine as remedy for dyslipidemia and cardiovascular complications [14].

Thymus is an important Mediterranean genus of the Lamiaceae family [15]. This genus comprises more than 300 species [16], which have been widely used in folk medicine to treat several diseases [14]. Among them, *Thymus atlanticus* (*T. atlanticus*) is an endemic herb of Morocco, which has been found to possess potent antioxidant, anti-inflammatory, and anticoagulant proprieties [17, 18]. These activities are related to its high polyphenols content [17, 19]. Moreover, polyphenols are well known to have a wide range of biological functions and can act as inhibitors of pro-inflammatory enzymes and serine proteases of the coagulation [19]. Most studies demonstrated that natural polyphenols exert hypolipidemic effects and can act on lipid metabolism, modulate it and alleviate dyslipidemia [20]. In addition, saponins are phytochemicals that have been found to possess effective hypolipidemic effects [21]. It was reported that the presence of various active saponins in *Thymus* species [22].

Generally, the mouse is the most commonly used model animal in studying human disease [23]. Moreover, the mouse model is widely used to study disorders of lipid metabolism and to investigate the hypolipidemic effect of plant extracts and new drugs since the mouse has many similarities with the human concerning lipid metabolism [24, 25]. In addition, Triton WR-1339 (Triton) is a non-ionic detergent that is capable to develop acute hyperlipidemia by preventing the uptake of lipoproteins from plasma by peripheral tissues resulting in elevated levels of circulating lipid [26]. On the other hand, the high-fat diet-fed mouse is a useful model for impairing lipid levels and chronic hyperlipidemia disease [27].

Therefore, in this study, we used Triton-injected mice and HFD-fed-mice models to evaluate the preventive effectiveness of polyphenol (Pp), flavonoid (Fv), and saponin (Sp) fractions derived from *T. atlanticus* on hyperlipidemia. We also identified the major phenolic compounds present in these fractions.

Methods

Preparation of the plant fractions

Polyphenol (Pp) and saponin (Sp) fractions were extracted from aerial parts of *T. atlanticus* according to the method of Jordán et al. [28] with some modifications. Briefly, the dried powder (50 g) of the aerial parts was defatted with n-hexane (C_6H_{14}) in a Soxhlet system. Afterwards, the residue was air-dried and extracted with methanol 80% (16 h at 45 °C). The methanol extract was re-suspended in distilled water and reextracted with n-butanol (C_4H_9OH). The aqueous fraction (polyphenolic fraction) was concentrated and re-suspended in distilled water. The organic fraction (butanolic fraction) was concentrated for obtained saponin fraction. Flavonoids were extracted according to the method of Lee et al. [29] slightly modified. Briefly, a volume of 40 mL of methanol (70%) was added to 0.5 g of plant powder and then 10 mL HCl (6 M) was added. The solution of extraction consisted of 1.2 M HC1 in 50% aqueous methanol (v/v). After refluxing at 90 °C for 2 h with regular swirling, the extract was allowed to cool and was subsequently made up to 100 mL with methanol and then sonicated for 5 min.

All extraction was repeated three times and the extraction yield was about 8.87 ± 0.61%, 2.83 ± 0.40%, and 7 ± 1.01% (w/w) for Pp, Sp, and Fv fractions, respectively.

Analysis of phenolic profile

Quantitative and qualitative determination of phenolic acids and flavonoids of the three fractions was carried out using the HPLC-DAD method [17]. Gradient HPLC analysis was performed on a Reprosil PUR C18 column equipped with a photodiode array detector. The stationary phase was a C18 analytical column (250 mm × 3 mm) with a particle size of 5 μm thermostated at 28 °C. Fractions (100 μl) were separated at 28 °C. The flow rate was 0.5 mL per min and the absorbance changes were monitored at 215, 250, and 280 nm. The mobile phases were (A) methanol/water (20/80) + 0.2% glacial acetic acid and (B) methanol/water (80/20) + 0.2% glacial acetic acid: 100% (A) and 0% (B) at 0 min, 50% (A) and 50% (B) during 10 min, 17% (A) and 83% (B) during 20 min, which was changed to 100% (A) and 0% (B) in 5 min (35 min, total time). The standards for phenolic identification were rutin, caffeic acid, rosmarinic acid, quercetin, hesperetin, apigenin, daidzein, luteolin-7-glycoside, thymol, and carvacrol.

Acute and chronic hypolipidemic effects

Animals

In vivo experiments were conducted using male albino mice procured from the Animal Experimental Station in Nantes, France. The mice, weighing 25–30 g, were housed in metallic cages (3 mice per cage) under a 12/12-h light/dark cycle and controlled temperature (22-25 °C). The mice were acclimatized for 15 days before experimentation. Food and water were made available ad libitum. At the end of the experimental period, the mice were euthanized with intra-peritoneal injection of

sodium pentobarbital at 60 mg/kg B.wt. All the animal experimental protocols were performed strictly in compliance with the ethical principles of animal experimentation of the Ministry for Food, Agriculture, and Fisheries (France). This study conforms to Directive 2010/63/EU and approved by the local committee (Bretagne-Pays de la Loire committee). The experiments reported in this study adheres to the ARRIVE guidelines for the reporting of animal experiments (http://www.nc3rs.org.uk/page.asp?id=1357).

Preparation of samples

Polyphenol and flavonoid fractions and fenofibrate were prepared and diluted in distillated water, while saponin fraction was prepared in 3% DMSO dissolved in distillated water, before oral administration.

Acute hypolipidemic effect

Forty-two mice were randomly assigned to seven groups (n = 6). Except normal control, all groups were intraperitoneally injected with Triton (20 mg/100 g body weight (B.wt.)) to induce acute hyperlipidemia [30]. One hour after Triton injection, mice have received the appropriate treatment via oral gavage. Normal control and hyperlipidemic control (HC) were received 2 mL of distillated water. DMSO hyperlipidemic control (DMSO-HC), which served as control for Sp-treated group, was received 3% DMSO. Positive control (Fenof) was received fenofibrate at a dose of 65 mg/kg B.wt. The remaining three groups were administered Pp, Fv, or Sp fractions at a dose of 2 g/kg B.wt. Twenty-four hours after fraction administration, blood samples were collected in heparinized tubes for lipid profile analysis.

Chronic hypolipidemic effect

This study was carried out according to the method described by Harnafi et al. [31]. Mice were randomized in four groups with 6 animals each. The control group was fed with standard laboratory diet. Mice fed with HFD for 6 weeks were received daily vehicle (2 mL of distillated water, hyperlipidemic control (HFD)), polyphenol fraction (2 mg/kg B.wt./day, polyphenol group (HFD-Pp)) or fenofibrate (65 mg/kg B.wt./day, positive control (HFD-Fenof)). The HFD is composed of 81.8% standard diet, 2% cholesterol, 16% lard, and 0.2% cholic acid.

The Pp fraction and fenofibrate were administered orally by gavage, once a day, for 6 weeks. After 6 weeks of treatment, animals were slightly anesthetized and blood was collected for analyzing lipid profile.

Biochemical analysis

Blood samples were collected via the orbital sinus of the mice under a slight isoflurane anesthesia after 24 h of the Triton injection in acute hyperlipidemia, or after 6 weeks in a chronic study. Blood was centrifuged and plasma was used for analyzing lipid profile. The levels of TC, TG, LDL-C, and HDL-C in the mice plasma were analyzed using corresponding commercial kits (BiosudSrl, Catania, Italy) according to the previous method of Ramchoun et al. [32].

Statistical analysis

Statistical analysis was made using ANOVA followed by post hoc analysis (Tukey's test). A P value less than 0.05 was considered statistically significant. All results are expressed as mean values ± SD (standard deviation).

Results

Phytochemical analysis

The phytochemical analysis of different fractions was carried out by HPLC-DAD. Figure 1 shows the HPLC chromatograms of three fractions, and identified compounds are given in Table 1.

The results showed that rosmarinic acid was the predominant phenolic acid quantified in all fractions (Table 1). The amount of this acid was about 9.77, 2.74, and 69.38 mg/g of dry fraction in Pp, Fv, and Sp fractions, respectively. Compared to other fractions, saponin fraction was particularly rich in caffeic acid (41.64 mg/g) and quercetin (11.94 mg/g).

Acute hypolipidemic effect

Table 2 illustrates the results concerning plasma lipid concentrations in all groups of mice (six mice in each group). All animals observed in this experiment were in good health. Compared to control, Triton induced a significant increase in the levels of plasma TC (33.35%, $P < 0.05$), TG (80.60%, $P < 0.01$), and LDL-C (54.62%, $P < 0.01$) (Table 2). No significant difference ($P > 0.05$) was observed between control and hyperlipidemic groups (HC and DMSO-HC) concerning HDL-C level. In addition, atherogenic index and LDL/HDL cholesterol ratio were significantly ($P < 0.01$) higher in hyperlipidemic groups than in the control group (Table 2).

Compared to corresponding hyperlipidemic control, the administration of Pp, Fv, and Sp significantly decreased TC by about 52% ($P < 0.01$), 52% ($P < 0.01$), and 66% ($P < 0.001$), and TG by about 71.96% ($P < 0.001$), 78.20% ($P < 0.001$), and 73.40% ($P < 0.001$), and LDL-C by about 69.66% ($P < 0.001$), 76.82% ($P < 0.001$), and 92.56% ($P < 0.001$), respectively (Table 2).

Results are expressed as mean values ± SD (n = 6). AI, atherogenic index; control, not injected with Triton and received distilled water; HC, hyperlipidemic control (Triton + distilled water); DMSO-HC, DMSO hyperlipidemic control (Triton + DMSO 3%); Pp, polyphenol-treated mice; Fv, flavonoid-treated mice; Sp, saponin-treated mice; Fenof, fenofibrate-treated mice. Statistical

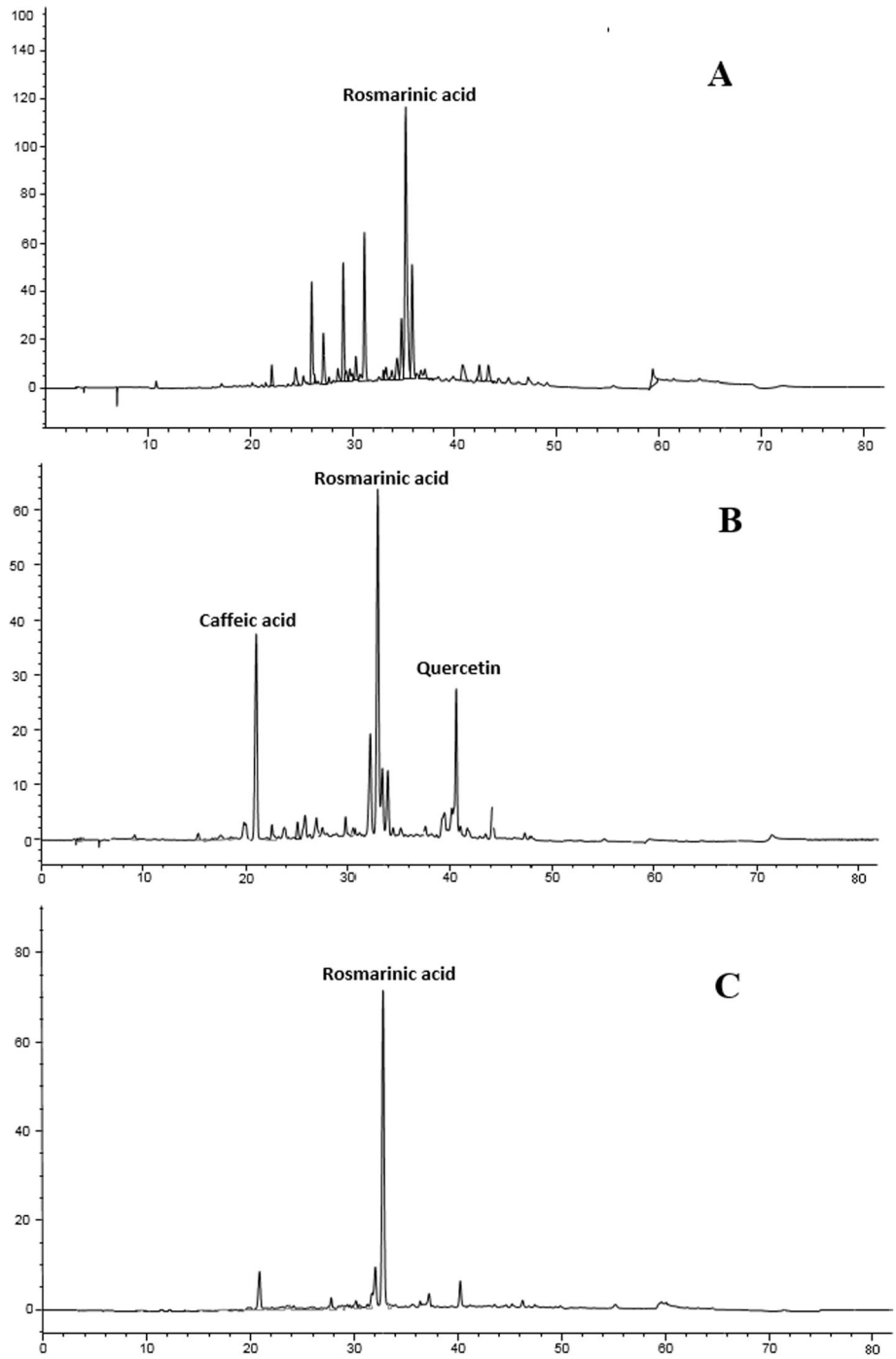

Fig. 1 HPLC chromatogram of *T. atlanticus* fractions. **a** Polyphenol fraction. **b** Saponin fraction. **c** Flavonoid fraction. *x*-axis, retention time (min); *y*-axis, mAU. For peak identification, see Table 1

analysis is made using ANOVA and Tukey's test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; ns, not significant; control, DMSO-HC, Pp, Fv, and Fenof vs. HC; Sp vs. DMSO-HC.

No significant difference in HDL-C level between hyperlipidemic controls and Pp and Sp-treated groups ($P > 0.05$) was noted. However, the administration of Fv significantly increased HDL-C level (50.82%, $P < 0.01$) compared to HC. Atherogenic index and LDL/HDL cholesterol ratio were significantly ($P < 0.01$) lower in Pp, Fv, and Sp treated-groups compared with hyperlipidemic groups (Table 2).

Table 1 Analysis by HPLC-DAD of the three fractions of *T. atlanticus*

Samples	Caffeic acid (mg/g)	Rosmarinic acid (*mg/g*)	Apigenin-7-glycoside (*mg/g*)	Quercetin (*mg/g*)	Content of identified compounds (mg/g)	% of rosmarinic acid in the fraction
Pp		9.77			9.77	100.00
Fv		2.74			2.74	100.00
Sp	41.64	69.38		11.94	122.96	56.42

Pp polyphenol fraction, *Fv* flavonoid fraction, *Sp* saponin fraction, (n = 1)

Compared to HC group, fenofibrate (65 mg/kg B.wt.) prevented the increase of plasma TC, TG, and LDL-C by about 60.37% ($P < 0.001$), 89.02% ($P < 0.001$), and 70.24% ($P < 0.01$), respectively, but did not affect HDL-C level. In addition, fenofibrate induced a marked decrease in the atherogenic index and LDL/HDL cholesterol ratio (Table 2).

Chronic hypolipidemic effect

The results of the levels of plasma lipid and atherogenic indexes in all groups (six animals in each group) are shown in Table 3. All animals were observed and were in good health. The levels of serum TC, TG, and LDL-C were increased by about 19% ($P < 0.05$), 41% ($P < 0.01$), and 44% ($P < 0.01$), respectively, in the group fed with HFD for 6 weeks compared to mice receiving standard diet. In contrast, the HFD did not show any effect on HDL-C level compared to a standard diet (Table 3). In addition, atherogenic index and LDL/HDL cholesterol ratio were increased almost 3.3 times and 1.9 times, respectively, in HFD group compared to control (Table 3).

On the other hand, mice fed with the HFD and daily treated with fenofibrate had their values of TC, TG, and LDL-C significantly lowered by about 24%, 42%, 48%, respectively, when compared to HFD ($P < 0.01$) (Table 3), but fenofibrate treatment did not affect HDL-C level when compared with HFD ($P > 0.05$).

The administration of Pp fraction at the dose of 2 g/kg B.wt./day prevented the increase of TC (28.81%, $P < 0.01$) and TG (44.30%, $P < 0.01$) compared to HFD (Table 3).

Results are expressed as mean values ± SD (n = 6). AI, atherogenic index; control fed a normal diet; HFD group fed a high-fat diet (HFD); HFD-Pp group fed the HFD supplemented with polyphenol fraction (2 g/kg B.wt. per day); HFD-Fenof group fed with HFD supplemented with fenofibrate (65 mg/kg B.wt. per day). Statistical analysis is made using ANOVA and Tukey's test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; ns, not significant vs. HFD.

The LDL-C level was lower in HL-Pp group by about 44% compared to HLC. Interestingly, the Pp fraction significantly increased HDL-C level compared to group feeding HFD (HFD-Pp 89.62 ± 10.04 mg/dL vs. HFD 59.53 ± 6.24 mg/dL, $P < 0.01$) (Table 3).

Discussion

Thymus atlanticus is an endemic herb of Moroccan high Atlas. It has widely been used in folk medicine to treat several diseases including hyperlipidemia and its complications [14]. Moreover, our previous report showed that the aqueous extract derived from this plant alleviated hyperlipidemia induced by Triton in rats, evidenced by a significant decrease in levels of TC, TG, and LDL-C in rats receiving the extract when compared to hyperlipidemic control [32]. The effect of this extract used at a dose of 2 g/kg B.wt. on Triton-induced hyperlipidemia in rats was similar to fenofibrate (65 mg/kg B.wt.). Thus, in the present report, we attempted to evaluate the effect of the same dose of the fractions derived from *T. atlanticus* on acute and chronic hyperlipidemia in mice. Based on previous study on rat, we used here the fractions at a dose of 2 g/kg B.wt. and fenofibrate as positive control.

In the present report, we used the mice as a model to evaluate the effect of *T. atlanticus* fractions on both acute and chronic hyperlipidemia. Acute hyperlipidemic-mice were developed by an intraperitoneally injection of Triton WR 1339, which induced a significant increase in levels of TC, TG, and LDL-C, after 24 h of injection.

Table 2 Effect of *T. atlanticus* fractions in Triton WR-1339-induced hyperlipidemic mice after 24 h

Groups	Total cholesterol	Triglycerides	HDL-C	LDL-C	LDL-C/HDL-C	AI
Control	158.85 ± 6.34*	39.94 ± 3.78***	59.54 ± 3.12^{ns}	72.35 ± 7.63**	1.49 ± 0.01**	1.15 ± 0.12**
HC	238.35 ± 14.45	205.88 ± 16.31	37.74 ± 2.56	159.43 ± 13.64	4.22 ± 0.26	3.94 ± 0.22
DMSO-HC	274.77 ± 9.12^{ns}	302.94 ± 20.67^{ns}	40.39 ± 5.38^{ns}	205.58 ± 13.64^{ns}	5.31 ± 1.24^{ns}	6.70 ± 1.62^{ns}
Pp	114.06 ± 6.12**	57.73 ± 4.23***	54.14 ± 5.97^{ns}	48.38 ± 2.86***	0.98 ± 0.01**	1.21 ± 0.25**
Fv	114.98 ± 12.63**	44.88 ± 3.99***	76.74 ± 8.65**	36.96 ± 3.96***	0.50 ± 0.01***	0.70 ± 0.06**
Sp	93.02 ± 5.03***	80.60 ± 10.34***	57.97 ± 6.14^{ns}	15.30 ± 1.15***	0.28 ± 0.07***	0.46 ± 0.12**
Fenof	94.44 ± 12.02***	22.61 ± 2.24***	42.48 ± 6.63^{ns}	47.44 ± 3.33***	1.18 ± 0.15**	1.29 ± 0.07**

Table 3 Effect of polyphenol fraction supplementation for 6 weeks in high-fat diet-fed mice

Groups	Total cholesterol	Triglycerides	HDL-C	LDL-C	LDL/HDL	AI
Control	158.90 ± 14.73*	39.93 ± 5.33**	59.53 ± 6.24^{ns}	72.35 ± 6.09**	1.49 ± 0.25**	1.14 ± 0.20**
HFD	197.00 ± 19.45	67.52 ± 7.98	50.90 ± 2.96	128.20 ± 16.39	2.81 ± 0.40	3.70 ± 0.62
HFD-Pp	140.25 ± 12.00**	37.60 ± 3.24**	89.62 ± 10.04**	40.62 ± 7.25***	0.45 ± 0.05**	0.57 ± 0.02**
HFD-Fenof	150.62 ± 14.27**	39.21 ± 4.40**	68.06 ± 4.00^{ns}	67.06 ± 10.10**	0.96 ± 0.21**	1.07 ± 0.23**

This evidenced that our model could be effective in developing hyperlipidemia and studying the hypolipidemic effect [26]. It is known that Triton cause hyperlipidemia by blocking the clearance of triglyceride-rich lipoproteins and their uptake from circulation by peripheral cells, which result in hyperlipidemia, after 24 h of Triton injection, characterized by high serum levels of TC, TG, and LDL-C [26]. The oral administration of a single dose of Pp, Fv, or Sp prevented Triton-induced hyperlipidemia, manifested by a significant decrease in TC, TG, and LDL-C levels.

In a chronic study, we observed that the mice fed with the HFD for 6 weeks had high levels of TC, TG, and LDL-C compared with mice receiving the standard diet. The daily administration of Pp fraction (2 g/kg B.wt./day) suppressed the increase of plasma levels of TC, TG, and LDL-C in HFD-induced hyperlipidemic mice.

Both in acute and chronic hyperlipidemia, the reduction of TC, in *T. atlanticus*-treated groups, was accompanied with a significant decline in LDL-C, which are particles that are highly susceptible to acquire atherogenic proprieties [33]. This result suggested that the hypocholesterolemic effect of *T. atlanticus* compounds may be attributed to the enhancement of LDL-C catabolism through hepatic receptors for final elimination of cholesterol in bile acids excretion.

Interestingly, Fv fraction and Pp fraction increased the level of HDL-C fraction in acute and chronic hyperlipidemia, respectively. This indicated a possible atheroprotective effect of *T. atlanticus* compounds since the beneficial role of HDL-C fraction in atherosclerosis is mentioned in many clinical studies [34]. Moreover, it is established an inverse correlation between serum concentration of HDL-C and incidence and progression of atherosclerosis and its complications [35]. However, until now, the role of HDL-C particles in atherosclerosis is still debated [36].

The mechanism of the effect of *T. atlanticus* fractions on increasing HDL-C level may be attributed to the enhancement of lecithin cholesteryl acyl transferase (LCAT), which is a key enzyme in converting free cholesterol into cholesteryl ester and its incorporation into HDL particles [37]. Cholesterol in HDL will transfer back to very low-density lipoproteins (VLDL) or intermediate-density lipoproteins (IDL), which are taken back by the hepatocytes [38].

In addition, all *T. atlanticus* fractions effectively inhibited serum TG elevation after Triton injection in mice. Likewise, the supplementation with Pp fraction decreased TG level in HFD-induced chronic hyperlipidemia in mice. This effect may be due to the enhancement of TG-rich lipoproteins elimination through the improvement of lipoprotein lipase activity, and the enhancement of the uptake of TG carried in VLDL by peripheral organs [39]. Another mechanism may be related to the regulation of intestinal uptake of TG mediated by pancreatic lipase that catalyzes the hydrolysis of TG to mono- and diglycerides, which are uptaken by the intestinal cells [40]. Although the direct implication of TG in atherosclerosis is not confirmed yet, it has been reported that the reduction of serum TG level is effective in preventing hyperlipidemia and treating heart diseases [41].

In our study, the effect of fractions was compared with fenofibrate in two models of hyperlipidemia. Fenofibrate is a medication of the fibrate class mainly used to reduce TG in people with very high TG blood level [5]. Fenofibrate acts through the activation of the nuclear transcription factor peroxisome proliferator-activated receptor-α (PPARα), which is a major regulator of lipid metabolism in the liver and its activation upregulates genes involved in transport and catabolism of lipids [42]. However, the use of fenofibrate has been associated with many side effects, which has drawn attention to search a useful alternative to fenofibrate [43]. In our report, the levels of TC, TG, and LDL-C were decreased upon treatment with fenofibrate in both acute and chronic hyperlipidemia. These findings are in accordance with previous studies reported on fenofibrate effects [8]. Interestingly, in the present study, the administration of fenofibrate at the dose of 65 mg/kg B.wt./day for 6 weeks increased HDL-C level in HFD-induced mice. As a possible mechanism of the action of fenofibrate on HDL-C, it is reported that fenofibrate increases these particles by reducing CETP expression [44]. Comparing the fractions to fenofibrate, we observed that they are similar effects on serum lipid profile in our models of hyperlipidemia, but additional investigations are needed to determine and to compare their precise mechanisms of action. Moreover, histological study of the liver, which is the main organ affected by conventional lipid-lowering drugs, is needed and we would investigate it in future

works. This can permit us to properly compare between *T. atlanticus* extracts and fenofibrate.

Concurrently, in both models, the atherogenic index and LDL/HDL ratio were significantly lower in mice treated with *T. atlanticus* fractions or fenofibrate compared to hyperlipidemic controls. High plasma atherogenic index or LDL/HDL ratio is positively correlated with atherosclerosis and its complications [45]. In fact, these indexes are employed to predict the development of atherosclerosis [46, 47]. This suggests the beneficial effects of *T. atlanticus* in preventing atherosclerosis development.

Moreover, the mechanism of action of the fractions is not investigated at this stage. In addition to the hypotheses mentioned above concerning the possible mechanisms of *T. atlanticus* effects, it is well known that polyphenols inhibit lipid absorption, enhance the fecal lipid excretion, and reduce cholesterol biosynthesis by inhibiting the expression and the activity of 3-hydroxy-3-methylglutaryl-coenzyme A, a critical enzyme in cholesterol synthesis [48]. Additionally, polyphenols inhibit the activity of pancreatic lipase, which mediates the digestion and absorption of TG [49]. HPLC analysis reported here and in previous studies [17, 32] indicated the presence of phenolic acids that have been reported as functional compounds in hyperlipidemia and cardiovascular diseases, mainly through anti-inflammatory, anticoagulant, lipid-lowering, and/or antiatherogenic effects [50, 51], making them the likely effectors of the pharmacological actions of the *T. atlanticus* fractions. The activities of fractions were related to the presence of rosmarinic acid, which was the major compound in our fractions, and is known to have several pharmacological effects including hypolipidemic effect [51]. Rosmarinic acid has been also reported as a potential compound in hyperlipidemia and atherosclerosis, mainly by suppressing lipid accumulation in cells [52] and inhibiting lipid peroxidation [53]. Rosmarinic acid inhibits the formation of membrane cholesterol domains, which lead to the generation of extracellular crystals, a main non-cellular component of the atherosclerosis plaques [54]. In addition, a recent study has reported that Pp fraction and aqueous extract of *T. atlanticus* as well as rosmarinic acid modulated the secretion of monocyte chemoattractant protein-1 (MCP-1) by lipopolysaccharide-stimulated macrophages [18]. MCP-1 is known to play a critical role in the initiation and the development of atherosclerosis. This suggested additional benefits of *T. atlanticus* extracts in atherosclerosis. It has been recently found that Pp fraction of *T. atlanticus* contains, in addition to rosmarinic acid, caffeic acid (1.43 ± 0.06 mg/g dry extract), rutin (0.63 ± 0.01), hyperoside (1.18 ± 0.03), and apigenin-7-*O*-glucoside (2.23 ± 0.03) [18]. These compounds have been reported to have various pharmacological effects including antioxidant, anti-inflammatory, and anticancer effects [15].

A recent study has demonstrated that the aqueous extract of *T. atlanticus* can be classified as a low-toxicity extract according to the organization for economic co-operation and development [18].

The findings found in the present report, together with those observed in a previous study [32], clearly demonstrated that *T. atlanticus* is rich in polyphenols and able to improve acute hyperlipidemia in mice and rats treated with Triton and chronic hyperlipidemia in mice fed with an atherogenic diet. It is likely that the hypolipidemic effects observed in this investigation are related to the presence of high polyphenols content especially rosmarinic acid. According to epidemiologic studies [55], polyphenols intake ameliorates hyperlipidemia and its related diseases by alleviating inflammation and oxidative stress as well as improving cardiovascular risk factors principally lipid profile. However, until now, there are a few clinical implications of plants and polyphenols in hyperlipidemia and its complications.

There are few limitations in this study that could be addressed in future work. Mechanisms of hypolipidemic action of the extracts were not studied since we only focused on biochemical analysis as well as the exact compound responsible for the hypolipidemic effect has not been explored.

Conclusion

In summary, administration of the fractions from *T. atlanticus*, which are rich in phenolic compounds particularly rosmarinic acid, may have a beneficial role in hyperlipidemia through decreasing proatherogenic lipids level and increasing anti-atherogenic HDL-C. Further investigation using in vitro and in vivo models should be conducted to determine possible mechanisms of hypolipidemic action of *T. atlanticus* fractions and confirm their potential as a natural treatment for hyperlipidemia-related diseases.

Abbreviations

Fenof: Fenofibrate; Fv: Flavonoid fraction; HC: Hyperlipidemic control; DMSO-HC: DMSO hyperlipidemic control; HDL-C: High density lipoprotein cholesterol; HFD: High-fat diet; HPLC: High-performance liquid chromatography; IDL: Intermediate density lipoproteins; LDL-C: Low-density lipoprotein cholesterol; Pp: Polyphenol fraction; Sp: Saponin fraction; TC: Total cholesterol; TG: Triglycerides; VLDL: Very-low-density lipoprotein

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Plant identification

T. atlanticus, also known *T. dreatensis*, was collected during March-April 2012, from Grand Atlas Mountains in central Morocco (32° 15′ N, 5° 25′ E, 1995–2012 m). This plant was authenticated by Dr. ibn Tatou. The voucher specimen was deposited at the Herbarium of the Scientific Institute at the University of Mohammed V. Rabat, Morocco (No: RAB 77496).

Authors' contributions
TK, MR, and TS carried out the experimental studies and prepared plant extracts. TK drafted the manuscript. KO and HH helped to design the studies. TK, RM, KO, and SA conceived of the study, CA and MB participated in its design and coordination. All authors have read and approved the final manuscript.

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Availability of data and materials
All data and materials are available upon request.

Ethics approval and consent to participate
Animal experimental protocols conform to Directive 2010/63/EU and have been approved by the local Committee (Bretagne-Pays de la Loire committee).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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