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journal homepage: <http://ees.elsevier.com/apjtm>Original research <http://dx.doi.org/10.1016/j.apjtm.2016.03.038>Evaluation of hypolipidemic *Marrubium vulgare* effect in Triton WR-1339-induced hyperlipidemia in mice

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ABSTRACT

Objective: To evaluate the hypocholesterolemic and hypotriglyceridemic activities of four *Marrubium vulgare* herb extracts using Triton WR-1339-induced hyperlipidemia in mice.

Methods: Hyperlipidemia was developed by intraperitoneal injection of Triton (200 mg/kg body weight). The animals were divided into main four groups of eight mice each: normal control group, hyperlipidemic control group, hyperlipidemic plus tween-40 control and treated group. The fourth one was divided into four subgroups, petroleum ether extract group, chloroform extract group, ethyl acetate extract group and methanol extract treated group each of them contains two sub-sub group for treating animals with two doses at 0.1 and 0.25 LD₅₀.

Results: After 7 h and 24 h of treatment, the intragastric administration of all extracts caused a significant decrease of plasma total cholesterol. Triglyceride levels were also significantly lowered by all extracts while petroleum ether produced the lowest decreasing level. Similar results were observed for LDL-cholesterol concentrations. Furthermore, more polar extracts (methanol and ethyl acetate)-soluble fractions showed a significant ameliorative action on elevated atherogenic index (AI) and LDL/HDL-C ratios, while these atherogenic markers were not statistically suppressed by the chloroform and petroleum ether-soluble extract.

Conclusion: The findings indicated that *Marrubium* may contain polar products able to lower plasma lipid concentrations and might be beneficial in treatment of hyperlipidemia and atherosclerosis.

1. Introduction

Experimental and epidemiological studies have shown that the plasma hypercholesterolemic state could contribute to the development of atherosclerosis and related cardiovascular system diseases (CVD) which are the most common cause of death in both western and eastern societies [1].

Triton WR-1339, a non-ionic detergent (oxyethylated tertiary octyl phenol formaldehyde polymer), has been widely used to produce acute hyperlipidemia in animal models in order to screen natural or chemical drugs [2] and to study cholesterol and triacylglycerol metabolism [3]. The accumulation of plasma

lipids by this detergent appears to be especially due to the inhibition of lipoprotein lipase activity [4].

Hyperlipidemia is a highly predictive risk factor for atherosclerosis, coronary artery diseases and cerebral vascular diseases [5]. Coronary heart disease, stroke, atherosclerosis and hyperlipidemia are the primary cause of death [6]. Hyperlipidemia is characterized by elevated serum total cholesterol. Low density and very low density lipoprotein cholesterol and decreased high density lipoprotein levels. Hyperlipidemia associated lipid disorders are considered to cause atherosclerotic cardiovascular disease [7]. Atherosclerosis (Sclero-hardening) of arteries is a generalized disease of arterial network known as a progressive and silent killer disease characterized by the formation of lesions called atherosclerosis plaques in the walls of large and or medium sized coronary arteries and reduces blood flow to the myocardium called coronary artery diseases (CAD) [8]. Hyperlipidemia is classified into a

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primary and a secondary type, which indicates the complexities associated with disease. The primary disease may be treated using anti-lipidemic drugs but the secondary type originating from diabetes, renal lipid nephrosis or hypothyroidism demands the treatment of the original disease rather than hyperlipidemia [9]. Medicinal plants play a major role in hypolipidemic activity, literature suggests that the lipid lowering action is mediated through, inhibition of hepatic cholesterol biosynthesis and reduction of lipid absorption in the intestine [10].

In many developing countries, most hyperlipidemic individuals use medicinal plants as folk medicine to treat hyperlipidemia and prevent atherosclerosis. Therefore, there is a strong interest, locally, in natural hypolipidemic substances derived from medicinal plants. Vast numbers of plants have received attention in this regard and have been shown to lower plasma lipid levels [11]. *Marrubium vulgare* L. (*M. vulgare*), white horehound, is an aromatic medicinal plant belongs to the family *Lamiaceae* and native to Europe, northern Africa and Asia [12]. Marrubiin is the main active compound isolated from *M. vulgare* L., a medicinal plant used in folk medicine to cure several diseases particularly gastroenteric and respiratory [13]. Aqueous extract of *M. vulgare* L. provides a source of natural antioxidants, which inhibit LDL oxidation and enhance reverse cholesterol transport and thus can prevent cardiovascular diseases development [14]. The goal of this work is evaluation of anti-hyperlipidemic effect of *M. vulgare* successive extracts in Triton WR-1339 model as indicator for their effect on fatty acid biosynthesis and blood lipid removal.

2. Material and methods

2.1. Preparation of *M. vulgare* extracts

The areal parts of the cultivated *M. vulgare* were collected and air dried in the shade, ground and then successively and exhaustively extracted by gradual solvents as follows, petroleum ether (40–60 °C), chloroform, ethyl acetate and methanol. Plant extracts were prepared by continuous extraction using soxhlet apparatus. The four extracts were then concentrated under reduced pressure to remove solvent completely and lyophilized to be free from any solvent and water.

2.2. Antioxidant properties of *Marrubium* extracts

2.2.1. Total polyphenols content

Total polyphenols of *M. vulgare* extracts, petroleum ether, chloroform, ethyl acetate and methanol, were determined by the Folin–Ciocalteu procedure [15] to aliquots of 0.5 mL were added 0.25 mL of Folin–Ciocalteu reagent and 1.25 mL 20% aqueous sodium carbonate solution. Samples were vortexed and absorbance of blue colored mixtures recorded after 40 min at 725 nm against a blank containing 0.5 mL of water or 4% DMSO in water, 0.25 mL of Folin–Ciocalteu reagent and 1.25 mL of 20% aqueous sodium carbonate solution. The amount of total polyphenols was calculated as gallic acid equivalents from the calibration curves of gallic acid standard solutions and expressed as mg gallic/g dry plant extract. All measurements were done in triplicate.

2.2.2. Free radical scavenging activity

The free radical scavenging activity of *M. vulgare* extracts were measured by 1, 1-diphenyl-2-picryl-hydrazil (DPPH•) using the method of Yamaguchi *et al.*, [16]. Briefly, 0.1 mM solution of DPPH• in ethanol was prepared. Then, 1 mL of this solution was added to 3 mL of *M. vulgare* extracts and standards solution at different doses (50, 100, 250, 500 and 1000 µg/mL). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm in a spectrophotometer (Jasco, serial No. C317961148, Japan). Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The reduction in DPPH• radical concentration in the reaction medium was calculated from the following equation:

DPPH• scavenging effect (%) = $100 - [(A_0 - A_1)/A_0] \times 100$, where A_0 was the absorbance of the control reaction and A_1 was the absorbance in the presence of the sample [17].

2.2.3. Reducing power

The reducing power of marrubium extracts was determined according to the method of [18]. *M. vulgare* extracts and standard solution at different concentrations (50, 100, 250, 500 and 1000 µg/mL) in 1 mL of methanol were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 mL, 1%). The mixture was incubated at 50 °C for 20 min. A portion (2.5 mL) of TCA (10%) was added to the mixture, which was then centrifuged for 10 min at $1000 \times g$. The upper layer of solution (2.5 mL) was mixed with methanol (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%), and the absorbance was measured at 700 nm. Vitamin C was used as a control. Higher absorbance of the reaction mixture indicated greater reducing power.

2.2.4. Metal chelating activity

The chelating of ferrous ions by the *M. vulgare* extracts and standards was estimated by the method of [19]. Briefly, *M. vulgare* extracts and standards solution at different concentrations (50, 100, 250, 500 and 1000 µg/mL) were added to a solution of 2 mM $FeCl_2$ (0.05 mL). The reaction was initiated by the addition of 5 mM ferrozine (0.2 mL) and the mixture was shaken vigorously and left standing at room temperature for ten minutes. After the mixture had reached equilibrium, the absorbance of the solution was then measured spectrophotometrically at 562 nm. The percentage of inhibition of ferrozine- Fe^{2+} complex formation was given by the formula:

$$\text{Percent of inhibition} = [(A_0 - A_1)/A_0] \times 100$$

where A_0 was the absorbance of the control, and A_1 was the absorbance in the presence of the sample of *M. vulgare* extracts and standard. The control contains $FeCl_2$ and ferrozine [20].

2.2.5. Lipid peroxidation – ammonium thiocyanate method

The antioxidant activity of *M. vulgare* extracts and standards were determined according to the method of [21] with some modifications. A pre emulsion was prepared by mixing 175 µg Tween 20, 155 µL linoleic acid, and 0.04 M potassium phosphate buffer (pH 7.0). A 1 mL of sample (1 mg/mL) in 99.5% ethanol was mixed with 4.1 mL linoleic emulsion, 0.02 M phosphate buffer (pH 7, 8 mL) and distilled

water (7.9 mL). The mixed solutions of all samples (21 mL) were incubated in screw cap-tubes under dark conditions at 40 °C at certain time intervals. To 0.1 mL of this mixture was pipetted and added with 9.7 mL of 75% and 0.1 mL of 30% ammonium thiocyanate sequentially. After 3 min, 0.1 mL of 0.02 M ferrous chloride in 3.5% hydrochloric acid was added to the reaction mixture. The peroxide level was determined by reading daily of the absorbance at 500 nm. Antioxidant assay of vitamin C was also assessed for comparison. All test data was the average of three replicate analyses. The inhibition of lipid peroxidation in percentage was calculated by the following equation:

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

where A_0 was the absorbance of the control reaction and A_1 was the absorbance in the presence of extracts or standard compounds.

2.3. Acute toxicity study

The acute toxicity test for the *M. vulgare* gradual extracts was carried out to evaluate any possible toxicity. Male albino mice ($n = 8$) of either eight were tested by administering different doses in intraperitoneal route of *Marrubium* extracts by increasing or decreasing the dose, according to the response of animal [22]. The dosing patron was 500, 1000, 1500, 2000, 2500, 3000 and 3500 mg/kg body weight. All groups were observed for any gross effect or mortality during 48 h. Death of half of examined animals was observed at 2, 2.5, 2.5 and 3 g/kg b. wt. for petroleum ether, chloroform, ethyl acetate and methanol extracts, respectively.

2.4. Triton model of hyperlipidemia

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

2.4.1. Animals housing

One hundred and sixty adult male albino mice, weighing 27–30 g, bred in the animal house of National Research Centre, Egypt, were provided ad libitum access only to tap water throughout the experimental duration (24 h). Animals were fed on standard diet; temperature through the housing was controlled at 22–24 °C, relative humidity (60 ± 5)% and light/dark cycles (12/12 hrs). Animals were divided into groups and housed in polypropylene cages; each cage was contained eight mice adapted for one week before starting the experiment.

2.4.2. Experimental design

Overnight fasted mice were randomly divided into twenty groups of eight animals each. The first group, serving as a normal control (NCG), received an intraperitoneal administration of normal saline and water by gavage. The second include two subgroups, gavaged with tween at 0.72% and 25% in saline that used as solvents control –ve (SCG). The third, hyperlipidemic control group (HCG) was treated with Triton and gavaged by distilled water. The fourth include eight treated subgroup, animals administered *Marrubium* extracts at 10% and 25% of LD₅₀ for each extract in intragastric route with

intraperitoneal injection of sterilized saline, served as positive control group. The fifth group contained eight treated subgroups in which animals were administered *Marrubium* extracts at 10% and 25% of LD₅₀ for each extract in intragastric route with intraperitoneal injection of Triton, served as treated group. After treatments (7 h and 24 h), animals were anaesthetized briefly with diethyl ether and blood was taken from their tail vein using a heparinised capillary. The blood samples were immediately centrifuged (2500 rpm/10 min) and the plasma were used for biochemical analysis also sera were collected for determination of liver and kidney function.

2.4.3. Analytical procedures

Triglycerides in plasma were quantified by an enzymatic method using Bio-diagnostic kits (Bio-diagnostic, Egypt). Briefly, after enzymatic hydrolysis with lipases, the formation of quinoneimine from hydrogen peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic effect of peroxidase, was followed spectrophotometrically at 540 nm. Total cholesterol levels were determined by the cholesterol oxidase enzymatic method, using Bio-diagnostic Kits (Bio-diagnostic, Egypt); cholesterol was hydrolyzed and, in the presence of phenol, the quinoneimine as indicator was formed from hydrogen peroxide and 4-aminoantipyrine via peroxidase catalysis and spectrophotometrically measured at 510 nm. HDL-cholesterol concentrations were quantified by the same method as used to determine total cholesterol after removal of other lipoproteins by precipitation with phosphotungstic acid (PTA) and MgCl₂ (Bio-diagnostic kit, Egypt). The total lipid content was determined by the reaction between lipids and sulfuric, phosphoric acid, and vanillin to form pink colored complex quantified spectrophotometrically at 545 nm. The LDL-cholesterol was calculated by the Friedwald formula [23]:

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

The atherogenic index (AI) was calculated by the following formula:

AI = (total cholesterol – HDL-C)/HDL-C and the LDL-C/HDL-C ratio was calculated as the ratio of plasma LDL-C to HDL-C levels.

2.5. Statistical analysis

The results obtained in all analysis were expressed in median \pm SD (standard deviation). The levels of statistical significance ($P < 0.05$) were calculated based one-way ANOVA test for comparisons among means.

3. Results

3.1. Polyphenol content of *M. vulgare* extracts and their antioxidant activity

The polyphenolic compounds content as mg gallic acid/g dry plant extract of *M. vulgare* extracts, radical scavenging activity, reducing power, metal chelation effect as well as lipid peroxidation inhibition activity are shown in Table 1. *M. vulgare* extracts were scavenged DPPH radicals by different effecting level. Methanol and ethyl acetate extracts produced the same

Table 1Polyphenol content and antioxidant activity of *M. vulgare* extracts.

Plant extract	Total polyphenolics content(mg/g)	IC ₅₀ (µg/mL)			
		DPPH scavenging	Reduction capability	Metal ion chelation	Lipid peroxidation
Pet. ether ext.	30.00 ± 2.01	32.32 ± 3.10	26.57 ± 1.06	42.88 ± 2.00	78.15 ± 2.11
Chloroform ext.	140.00 ± 1.08	28.03 ± 2.57	18.41 ± 2.34	39.24 ± 1.46	73.14 ± 1.87
Ethyl acetate ext.	320.00 ± 1.91	23.57 ± 2.64 ^a	15.49 ± 1.85 ^b	25.46 ± 1.34 ^c	67.04 ± 1.65
Methanol ext.	385.00 ± 1.87	22.37 ± 2.91 ^a	13.99 ± 2.46 ^a	24.45 ± 1.76 ^c	50.05 ± 3.11
Vitamin C	–	16.69 ± 3.06	29.00 ± 3.03	67.23 ± 1.28	85.34 ± 1.02

Data are presented IC₅₀ ± Standard deviations, where IC₅₀ is the concentration caused 50% inhibition, chelation or reduction. ANOVA one way was used followed with post Hoc test for multiple comparisons. Vitamin C was used as standards. The same letter was used with insignificant differences, $P < 0.01$.

effect in an insignificant manner (IC₅₀ = 22.37 and 23.57 µg/mL, respectively) also chloroform extract and petroleum ether showed nearly the same effect as radical scavenger (IC₅₀ = 28 and 32 µg/mL, respectively). Methanolic extract was the most powerful extract as reducing agent followed with ethyl acetate extract in an insignificant difference (IC₅₀ = 13.99 and 15.49 µg/mL, respectively) and then chloroform extract (IC₅₀ = 18.41 µg/mL) while petroleum ether extract was the weak one (IC₅₀ = 26.57 µg/mL) but all *Marrubium* extract remained more effective than vitamin C (IC₅₀ = 29 µg/mL). The same trend of radical scavenger results was observed with ferrous ion chelation. Methanolic and ethyl acetate extracts had nearly the same least IC₅₀ (24.45 µg/mL and 25.46 µg/mL, respectively) followed with chloroform extract (IC₅₀ = 39.24 µg/mL) while petroleum ether was the weakest extract in chelating ion metals. These activities were reflected on plant extract effect on lipid peroxidation. The most effective extracts as scavenger, reducing agent and metal chelator were the most inhibitor for lipid peroxidation. Methanol and ethyl acetate extracts inhibited lipid peroxidation with low IC₅₀ (50.05 and 67.04 µg/mL, respectively). However, chloroform produced the middle effect (IC₅₀ = 73.14 µg/mL) followed with petroleum ether (IC₅₀ = 78.15 µg/mL) and all of them were effective than vitamin C (IC₅₀ = 85 µg/mL). Polyphenolic content of different plant fractions were estimated by folin reagent. The methanolic extract contained the maximum amount of polyphenolic compounds (385 mg/g) followed with ethyl acetate (320 mg/g) then

chloroform fraction (140 mg/g) while petroleum ether had the lowest concentration (30 mg/g).

3.2. Effect of *M. vulgare* extracts as hypolipidemic agent in Triton model induced hyperlipidemia in mice

Triton and tween magnify the cholesterol production and LDL concentration in experimental animals after 7 h from administration while *M. vulgare* extracts significantly reduced all determined lipid parameters. Methanol extract at 0.1 and 0.25 LD₅₀ significantly reduced cholesterol production in hyperlipidemic animals (150 and 145 mg/dL) with significant reduction in LDL (30 and 28 mg/dL) and induction of HDL concentration (131 and 122 mg/dL) with reduction in plasma triglyceride concentration (Table 2). The second effective extract in inhibiting cholesterol production is ethyl acetate at the tested two doses (165 and 164 mg/dL for 0.1 and 0.25 LD₅₀, respectively) which showed significant decreasing in LDL level (30 and 22 mg/dL, respectively) and triglycerides level (79 and 67 mg/dL, respectively). Also, chloroform extract at 0.1 and 0.25 LD₅₀ showed favorable effect in reducing the cholesterol level (188 and 153 mg/dL, respectively) with significant reduction in triglyceride (113 and 97 mg/dL, respectively) and decreasing LDL level (28 mg/dL) as well as significant increment in plasma HDL (92 and 87 mg/dL). The lowest mean values in cholesterol level were recorded with petroleum ether extract (220 and 256 mg/dL). It is clearly that increasing pet.

Table 2Effect of *M. vulgare* extracts on lipid parameters in Triton WR-1339-induced hyperlipidemia in mice after 7 h.

Groups	Total cholesterol	Triglyceride	HDL-cholesterol	LDL-cholesterol	
Negative control group	180.00 ± 5.11	60.50 ± 6.23	136.00 ± 5.15	32.50 ± 9.11	
Positive control group	Tween 7%	230.00 ± 6.42	70.00 ± 3.15	124.00 ± 4.25	50.00 ± 8.24
	Triton(200 mg/kg)	452.00 ± 4.68	300.00 ± 5.14	117.00 ± 5.13	280.00 ± 7.34
	Triton and tween 7%	495.00 ± 5.78	306.00 ± 2.45	105.00 ± 4.37	295.00 ± 2.89
Treated groups of different extracts at 0.1 and 0.25 LD ₅₀	Methanol extract	150.00 ± 4.14	71.00 ± 5.24	131.00 ± 3.61	30.00 ± 3.75
	Ethyl acetate extract	145.00 ± 6.17	65.00 ± 5.64	122.00 ± 4.72	28.00 ± 6.10
		165.00 ± 6.82 ^a	79.00 ± 4.71	116.00 ± 4.87	30.00 ± 7.14
		164.00 ± 5.58 ^a	67.00 ± 6.18	107.00 ± 3.25	22.00 ± 9.42
	Chloroform extract	188.00 ± 5.38	113.00 ± 5.01	92.00 ± 5.16	28.00 ± 8.23
		153.00 ± 4.67	97.00 ± 5.32	87.00 ± 6.37	28.00 ± 7.33
	Petroleum ether extract	220.00 ± 4.88	96.00 ± 3.42	74.00 ± 3.47	33.00 ± 1.25
256.00 ± 8.04		100.00 ± 6.19	65.00 ± 5.18	49.00 ± 4.22	

Data are presented mean of eight replicates ± Standard deviations. ANOVA one way was used followed with post Hoc test for multiple comparisons. The same letter was used with insignificant differences, $P < 0.01$. HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol. Lipid parameters determined in mg/dL.

ether extract to 0.25 LD₅₀ produced lower effect than 0.1 LD₅₀, this trend was true with all estimated lipid parameters. It decreased LDL level to reach 33 mg/dl at 0.1 LD₅₀ and reached 49 mg/dL at 0.25 LD₅₀ while increased HDL to be 74 mg/dL at 0.1 LD₅₀ and 65 mg/dL at 0.25 LD₅₀.

Administration of Triton elevated animal cholesterol, triglycerides and low density lipoprotein with a significant reduction in high density lipoprotein. However, treating hyperlipidemic animals with methanol significantly reduced cholesterol after 24 h of administration (160 mg/dL at 0.1 LD₅₀ and 116 mg/dL at 0.25 LD₅₀) and triglycerides (104 and 95 mg/dL for the two doses, respectively) as well as significant reduction in LDL-cholesterol (41.48 and 35.17 mg/dL for the two doses, respectively) accompanied with significant induction in HDL-cholesterol (161 and 169.6 mg/dL for the two doses, respectively) (Table 3). The ethyl acetate extract showed the same effect on all assessed parameters. It increased good lipids, HDL-cholesterol, to be near the -ve control group (146 and 157.8 mg/dL for the two doses, respectively) with significant reduction in cholesterol (175.6 and 168 mg/dL for the two doses, respectively), triglycerides (100 and 90 mg/dL for two used doses, respectively) and LDL-cholesterol (48.6 and 38 mg/dL for two doses, respectively). Cholesterol was also decreased to reach lower level than -ve control when animal treated with chloroform extract at 0.1 and 0.25 LD₅₀ (198.8 and 183 mg/dL for the two doses, respectively). This decreasing was accompanied with significant reduction in triglycerides (130 and 120 mg/dL for the low and high doses, respectively) and LDL-cholesterol (52.49 and 49 mg/dL, respectively) as well as favorable effect on HDL-cholesterol (112 and 117.89 mg/dL). The petroleum ether extract produced the lower effect against hyperlipidemia as compared to the other three extracts with the same trend of data.

It lowered the total cholesterol in hyperlipidemic animals at the 0.1 and 0.25 LD₅₀ (250 and 290 mg/dL, respectively), triglycerides level (200 and 255 mg/dL for the two doses, respectively) and LDL-cholesterol (53 and 79 mg/dL, respectively) with increasing the production of HDL-cholesterol level (104 and 93 mg/dL).

The changes of atherogenic index (AI) and LDL-C/HDL-C ratio in control and treated mice were shown in Table 4. The Triton administration significantly affects the cardiovascular risk markers. Indeed, the AI was statistically increased in both tween

Table 4

Effects of *M. vulgare* on atherogenic index (AI) and LDL/HDL-C ratio in Triton WR-1339-induced hyperlipemia in mice after 7 h and 24 h.

Groups	AI (after 7 h)	AI (after 24 h)	HDL/ LDL (7 h)	HDL/ LDL (24 h)
Negative control group	0.32	0.66	0.24	0.46
Positive control group	0.85	0.72	0.40	0.47
Tween 7%	2.86	3.01	2.39	2.61
Triton	2.67	0.51	2.19	2.59
Treated groups	0.15	0.01	0.23	0.26
Methanol extract	0.19	0.32	0.23	0.21
Ethyl acetate extract	0.42	0.19	0.19	0.33
	0.53	0.07	0.28	0.24
Chloroform ether extract	1.04	0.77	0.30	0.47
	0.76	0.56	0.32	0.42
Petroleum ether extract	1.97	1.39	0.45	0.51
	2.94	2.14	0.75	0.85

Data are presented as mean of eight replicates ± Standard deviations, ANOVA one way was used followed with post Hoc test for multiple comparisons, **P*<0.01. HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol.

control (0.85) and hyperlipidemic with tween group (2.67) when compared with values found in their relative normal lipidemic control (0.32) at 7 h. Similar results were noticed at 24 h.

Besides, there were significant further increases of LDL-C/HDL-C ratios in Triton-injected animals (2.39). In contrast to normal lipidemic mice (0.24), 7 h after Triton treatment produced an elevated ratio either in hyperlipidemic group animals (2.19) by many times. This changing pattern was maintained until 24 h when the ratios were increased in animals compared to normal group. Methanol extract highly reduced AI (0.15 and 0.19 for low and high doses, respectively) lower than normal control group (0.32). The ethyl acetate extract showed the same trend with significant increments than methanol extract (0.42 and 0.53, respectively) with decreasing LDL-C/HDL-C ratios (0.19 and 0.28, respectively).

The atherogenic index was lowered with chloroform administration (1.04 and 0.76 for two doses, respectively) accompanied with reduction in HDL/LDL-cholesterol ratio at 7 h and the same trend of data was recorded at 24 h. The minimum recorded effect was of petroleum ether. It decreased AI at low level of administration (1.97) didn't produce this effect

Table 3

Effect of *M. vulgare* extracts on plasma lipid parameters in Triton WR-1339-induced hyperlipemia in mice after 24 h.

Groups	Total cholesterol	Triglyceride	HDL-cholesterol	LDL-cholesterol
Negative control group	225.00 ± 5.34	139.00 ± 8.31	130.00 ± 3.16	62.20 ± 5.33
Positive control group	223.00 ± 6.42	240.00 ± 8.07	110.00 ± 3.19	65.40 ± 4.12
Tween 7%	538.50 ± 7.10	320.00 ± 7.26	108.00 ± 5.01	350.20 ± 4.08
Triton(200 mg/kg)	580.00 ± 8.12	330.00 ± 6.18	105.00 ± 4.06	360.20 ± 6.22
Triton and tween 7%	160.00 ± 6.05	104.00 ± 6.49	161.00 ± 4.55	41.48 ± 7.16
Treated groups of different extracts at 0.1 and 0.25 LD ₅₀	116.90 ± 6.24	95.00 ± 7.05	169.60 ± 5.28	35.17 ± 4.35
Methanol extract	175.60 ± 5.87	100.00 ± 6.66	146.80 ± 5.94	48.63 ± 3.64
Ethyl acetate extract	168.20 ± 5.68	90.00 ± 5.76	157.80 ± 6.28	38.56 ± 3.87
Chloroform extract	198.80 ± 6.18	130.00 ± 7.017	112.14 ± 7.08	52.79 ± 7.08
	183.90 ± 6.19	120.00 ± 6.38	117.89 ± 6.44	49.20 ± 7.06
Petroleum ether extract	250.10 ± 4.52	200.00 ± 5.49	104.45 ± 4.92	53.40 ± 6.25
	292.20 ± 7.06	255.00 ± 7.08	93.00 ± 5.37	79.40 ± 4.95

Data are presented as mean of eight replicates ± Standard deviations, ANOVA one way was used followed with post Hoc test for multiple comparisons, *P* < 0.01. HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol.

at high dose (2.97) with significant decrease in HDL/LDL-cholesterol ratio (0.45 and 0.75 for two doses, respectively). This trend was also observed at 24 h with minor increments in values.

Data are presented as mean of eight replicates \pm Standard deviations, ANOVA one way was used followed with post Hoc test for multiple comparisons, $*P < 0.01$. HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol.

4. Discussion

Hyperlipidemia is characterized as elevation in triglycerides and cholesterol in the blood therefore the most plausible strategy in prevention or treating hyperlipidemia is using drugs that contains array of compounds which can delay fat digestion and absorption through gastrointestinal tract by inhibiting pancreatic lipase and pancreatic cholesterol esterase activities. The non-ionic detergent, Triton WR-1339, has been widely used to block the uptake of triacyl glycerol-rich lipoproteins from plasma by peripheral tissues in order to produce acute hyperlipidemia in animal models which are often used for a number of objectives, in particular for screening natural or chemical hypolipidemic drugs [2]. With this aim, many medicinal plants have been assessed for their hypolipidemic activity in a Triton WR-1339-induced hyperlipidemic model [11]. This work was aimed to assess the possible hypocholesterolemic and hypotriglyceridemic activities of different *M. vulgare* extracts. It is clear from our results that the methanolic and ethyl acetate fractions from this plant decrease plasma total cholesterol in a marked manner, either 7 h or 24 h after Triton treatment. The reduction of plasma total cholesterol was associated with a decrease of its LDL fraction which is a major, potentially modifiable risk factor of cardiovascular diseases and the target of many hypocholesterolemic therapies. This finding suggests that the cholesterol-lowering activity of these extracts appears to be due to the enhancement of LDL-C catabolism, as demonstrated by [11].

It is evident from recorded results that fractions rich in polyphenolic compounds are those ameliorate the hyperlipidemic effect of Triton, these findings are in accordance of Mäkyinen *et al.* [24]

Recently, a number of clinical studies suggest that the increased risk of coronary heart disease is associated with a high serum concentration of TC, LDL-C and triglyceride. The abnormally high concentration of serum lipids is mainly due to the increase in the mobilization of free fatty acids from the peripheral depots [25]. On the other hand, low serum concentration of HDL-C is also responsible for coronary heart disease [25]. Methanolic and ethyl acetate fractions appears highly ameliorative effect in lowering LDL and increasing HDL-cholesterol followed with chloroform and then the lowest effective one, petroleum ether fraction.

Preclinical observations demonstrate that hyperlipidemia promotes accumulation of oxidatively modified low density lipoproteins in the arterial wall, promoting endothelial dysfunction and development of atherosclerosis and congestive heart diseases [26,27]. Polyphenolic compounds considered as health promoting compounds that included in most functional foods for many diseases as antihyperlipidemic and antihyperglycemic foods [28]. The physiological effect of polyphenolic compounds include possible antioxidant activity,

therefore suggestion their role in prevention of coronary heart disease including atherosclerosis [29]. They may also work by making liver cells more efficient to remove LDL-C from blood by increasing the LDL-C receptor densities in liver and by binding to apolipoprotein B [30].

The methanol and ethyl acetate fractions contained high amount of polyphenol. On the other hand, the *in-vitro* antioxidant estimated properties of four fractions showed the potent effect as radical scavenger hydrogen donor and metal chelator agent that reflected on their effect on inhibiting lipid peroxidation. Plant fractions arranged in their activities as antioxidant had the same pattern of activity in hyperlipidemic animals; methanol, ethylacetate, chloroform and then petroleum ether.

Free radicals and other reactive oxygen species are considered to be important causative factors in the development of diseases of aging such as neurodegenerative diseases, cancer and cardiovascular diseases. Phytochemicals have long been recognized to possess many properties including antioxidant, anti-allergic, anti-inflammatory, antiviral, anti-proliferative and anti-carcinogenic. This relationship has led to considerable interest in assessing the antioxidant capacity of foods, botanicals and other nutritional antioxidant supplements. As plants produce significant amount of antioxidants to prevent the oxidative stress caused by photons and oxygen, they represent a potential source of new compounds with antioxidant activity. Thus, continued research is being undertaken all over the world on different plant species and their therapeutic principles [31]. The four plant fractions showed potent effect as radical scavenger when assessed against DPPH radicals with reduction capability. They also play as excellent metal ion chelator as they tested against ferrous ions. These activities were accompanied with inhibition of lipid peroxidation.

The possible mechanism of this activity may result from the enhancement of lecithin cholesteryl acyl transferase (LCAT) and inhibition of hepatic triglyceride lipase (HTL) on HDL which may lead to a rapid catabolism of blood lipids through extra-hepatic tissues [32]. It is also reported that triglycerides play a key role in the regulation of lipoprotein interactions to maintain normal lipid metabolism [33]. Indeed, the elevated plasma TGs levels were associated with an increased incidence of coronary artery disease. Moreover, these higher plasma TG levels have been attributed mainly to an increased population of small, dense LDL deposits which are very atherogenic and enhanced cholesteryl ester mass transfer from apolipoprotein B-containing lipoproteins (VLDL and LDL). TGs have also been proposed to be a major determinant of cholesterol esterification, its transfer and HDL remodeling in human plasma [34].

Methanol and ethyl acetate fractions from *M. vulgare* significantly suppressed the elevated blood concentrations of TGs. This result suggests that the extracts are able to restore, at least partially, the catabolism of triglycerides. The underlying mechanism of this activity is not elucidated by the present study. However, as hypothesized by many works with other plants [35,36] the restoration of catabolic metabolism of triglycerides could be due to an increased stimulation of the lipolytic activity of plasma lipoprotein lipase (LPL).

Administration of *M. vulgare* provides a beneficial effect on mice lipid metabolism in regard to the reduction of AI. In fact, the AI was decreased in fraction treated groups. This ameliorative action was due to the plasma lipid-lowering activity of different fractions. It is also desirable to have higher plasma HDL and

lower LDL-cholesterol to prevent atherogenesis, since there is a positive correlation between an increased LDL-C/HDL-C ratio and the development of atherosclerosis [37]. The results found clearly demonstrate that the bioactive compound (s) contained in this plant have a polar character since the polar extracts were more active than the other extracts. These results are in accordance with those of [14] who reported that aqueous extract of *M. vulgare* L. inhibit LDL oxidation and enhance reverse cholesterol transport with antioxidant properties which increased the anti-atherogenic potential of HDL. Plant methanol and ethyl acetate extracts possess cholesterol-suppressive capacities and an ability to attenuate the accelerated development of atherosclerosis in hypercholesterolemic models.

Conflict of interest statement

Authors want to declare that they have no conflict of interest.

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