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Original Article

AJUGA IVA TREATMENT INCREASES POST-HEPARIN LIPASE ACTIVITY AND DECREASES SERUM AND VLDL-TRIACYLGLYCEROLS IN RATS FED A CHOLESTEROL-RICH DIET

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ABSTRACT

Objective: Hypercholesterolemia is among the most common health problems treated with traditional remedies. The role of lipoprotein lipase in hypercholesterolemia has been the subject of many reviews. We hypothesized that administration of *Ajuga iva* (*Ai*) aqueous extract to rats fed a cholesterol-rich diet would induce hypotriglyceridaemia by decreasing lipolytic activity.

Methods: Male wistar rats (n=12) were fed on 1% cholesterol-enriched diet for 15d. After this phase, hypercholesterolemic rats (HC) were divided into two groups fed the same diet supplemented or not with *Ai* for 15d.

Results: compared with the HC group, serum triacylglycerols (TG) and unesterified cholesterol (UC) values were respectively 1.4-fold lower and 1.8-fold higher in *Ai*-HC. VLDL amount (which represented the sum of apolipoproteins (apos)+TG+cholesteryl esters (CE)+UC+phospholipids (PL)), apos, TG and CE contents were, respectively, 2.2-, 2.6-, 4.4-and 1.9-fold lower, whereas that of UC were 1.9-fold higher in *Ai*-HC. LDL-HDL_1-TG value was 1.5-fold lower, and that of PL was 2-fold higher in *Ai*-HC. The HDL_2, amount, TG and UC values were respectively, 2.2-, 8-and 1.2-fold higher and the PL contents were 1.2-fold lower in the *Ai*-HC. The HDL_3, TG, UC and CE values were 3-, 1.6-and 2-fold higher in the *Ai*-HC group, whereas, PL contents were 1.4-fold lower. Hepatic lipase activity was similar and that of post-heparin lipases was increased by+15% in *Ai*-HC.

Conclusion: cholesterol-enriched diet supplemented with a lyophilised aqueous extract from *Ajuga iva* induces hypotriglyceridemia concomitantly with decreased VLDL-TG, by stimulating post-heparin lipoprotein lipase activity.

Keywords: Hypercholesterolemic rat, *Ajuga iva*–lipoproteins, Hepatic lipase, Post heparin lipase.

INTRODUCTION

The role of lipoprotein lipase in atherosclerosis has been the subject of recent reviews. Lipases are water-soluble enzymes that hydrolyze the ester bonds of water-insoluble substrates such as triacylglycerol (TG), phospholipids (PL) and cholesteryl esters (CE). The lipase gene family originally induced lipoprotein lipase (LPL), hepatic lipase and pancreatic lipase [1]. LPL is a rate-limiting enzyme that hydrolyzes circulating TG-rich lipoprotein such as very low density lipoprotein (VLDL) and chylomicrons [2, 3].

Hepatic lipase is a glycoprotein that is synthesized and secreted by the liver, and which binds to heparan sulphate proteoglycans on the surface of sinusoidal endothelial cells and on the external surface of parenchymal cells in the space of Disse. Hepatic lipase catalyses the hydrolysis of TG and PL in different lipoproteins, contributing to the remodelling of VLDL remnants, as well as intermediate density lipoprotein (IDL), low density lipoprotein (LDL) and high density lipoprotein (HDL) [1]. Hepatic lipase plays a major role in promoting the scavenger receptor B1-mediated uptake of HDL-CE. Thus, Hepatic lipase may contribute to the process of reverse cholesterol transport. These effects possibly influence the process of atherosclerosis [4].

Medicinal plants are relied upon by 80% of the world population for their basic health care needs [5]. Various medicinal properties have been ascribed to natural herbs and constitute the main source of new pharmaceuticals and health care products [6].

Ajuga iva (L.) Schreiber (Lamiaceae), locally known as ''Chendgoura, '' in Algeria is used in phytomedicine around the world for several diseases. *Ajuga iva* possesses hypoglycaemic [7], vasorelaxant [8] and hypolipidemic [9] effects, which have been experimentally demonstrated.

In our ongoing research project on the medicinal plants used in Algeria traditional medicine for the treatment of hypercholesterolaemia, we undertook the present study in order to reveal and elucidate the traditional use of these plants from the view of scientific point. The effect of *Ajuga iva* on post-heparin lipase, hepatic lipase activities and serum lipoproteins amounts and composition in rats fed a cholesterol-rich diet has never been examined.

Therefore, the hypothesis that administration of *Ai* aqueous extract from Algeria to rats fed a cholesterol-rich diet would induce hypotriglyceridaemia by decreasing lipolytic activity was tested following 2 objectives. The first was to study the influence of *Ai* on serum lipoproteins amount and composition. The second was to study the changes in hepatic and post-heparin lipases activities in rats fed a cholesterol-rich diet.

MATERIALS AND METHODS

Plant material

Mature whole *Ajuga iva (Ai)* (L) Schreber plants were collected in November 2004 from Béchar, southwest of Algeria, and identified by A. Marouf, in Botanical Laboratory (Faculty of Nature and Life Sciences, University of Oran). A voucher specimen has been deposited in the herbarium of the Laboratory of Clinical and Metabolic Nutrition, Faculty of Nature and Life Sciences, University of Oran, under the number 2345.

Preparation of *Ajuga iva* aqueous extract

The aerial parts of *Ajuga iva* were dried at ambient temperature. Then, 500 ml of distilled water was added to 50 g of finely powered leaves and the mixture heated under reflux for 60 min, and the decoction was filtered. The filtrate was frozen at-20ºC and then lyophilized (*Ai*). The crude yield of the lyophilized material was approximately 18% (wt/wt).

Ai phytochemical analysis

The aqueous extract (*Ai*) was analyzed by thin layer chromatography (TLC, Silicycle) and high performance thin layer

chromatography (HPTLC Merck) on a precoated silica gel plates 60 F 254, [solvent system: $CHCl_3$/MeOH/H_2O 60:32:7, detection with vanillin reagent (1% vanillin in EtOH/H_2SO_4)]. This extract was screened for the presence of phenolic compounds such as flavonoids, tannins, other phenols, and for the presence of terpenoids, alkaloids, and carbohydrates using standard procedures [10]. The major class of chemical groups was carried out by TLC on silica gel as follows: for flavonoids (AcOEt-formic acid-acetic acid, water, 100:11:11:26, detection under UV before and after spraying with 1% $AlCl_3$), for tannins (test with $FeCl_3$). The phytochemical screening of the aqueous extract revealed the presence of some compounds including, flavonoids, tannins and sugar. Furthermore a tentative of isolation of some pure secondary metabolites was achieved by using several chromatography techniques. The aqueous extract (20 g) was submitted to vacuum liquid chromatography (VLC) on a normal silica gel eluted by $CHCl_3$/MeOH/H_2O 60:32:7 yielded a soluble material which was concentrated (fraction *AiS*, 4.05 g) and an important red brown colored precipitate (14.61 g). This precipitate gives a positive reaction with 0.1 % $FeCl_3$ (brownish green coloration) characteristic of the presence of tannins. A part of this precipitate (500 mg) was submitted to acidic hydrolysis (2h in 2N trifluoroacetic acid at 120 °C) yielding sugars components. The fraction *AiS* containing an important number of secondary metabolites was fractionated by flash chromatography yielding 6 subfractions as *AiS* 1-*AiS* 6. The 6th fraction *AiS* 6 (700 mg) was submitted to successive medium pressure liquid chromatography (MPLC) on silica gel ($CHCl_3$/MeOH/H_2O 60:32:7) and on reverse phase RP-18 silica gel (MeOH/H_2O gradient) yielding three compounds 1 (49 mg), 2 (187 mg) and 3 (19 mg) purified at 90, 95 and 100%, respectively. Theirs characterisation was carried by spectroscopic methods. They were identified as iridoids and especially 8-O-acetylharpagide by comparison with literature values [11]. The antioxidant (AOX) activity of the *Ai* extract and its iridoids were conducted by using KRL test [12]. This test evaluates the resistance to free radical aggression by measuring the capacity of total blood to withstand free radical-induced hemolysis. Trolox is a water-soluble synthetic analogue of vitamin E. Trolox was used as standard and the AOX activity of the extract was compared with those of trolox and given as mmol/l trolox.

Animals and diets

The General Guidelines on the Use of Living Animals in Scientific Investigation [13] were followed and, the protocol and use of rats were approved by our institutional committee on animal care and use. Male Wistar rats (n= 24) (Iffa Credo, l'Arbresle, Lyon, France) weighing 120±5 g were used in this study. Experimental hypercholesterolemia was induced by feeding normocholesterolemic rats (with total cholesterol (TC) value of 2.40±0.62 mmol/l) a 1% cholesterol-enriched diet (casein, 200 (95% purity) (Prolabo, Paris, France) combined with Isio 4 oil, 50; sucrose, 40; cornstarch, 585; cellulose, 50; vitamins, 20; minerals 40; cholesterol, 10; cholic acid 5 (Merck, Darmstadt, Germany) (to facilitate cholesterol absorption) for 15 days [14]. After this phase, serum TC concentration was measured and the value was 2.7-fold higher than the beginning of the study (6.5±0.6 mmol/l). Hypercholesterolemic (HC) rats were divided into two groups fed for 15 days (d15) the same diet with or without Ajuga iva (Ai) lyophilised aqueous extract (0.5%). Diets and tap water were freely available. Animals were kept in wire bottom cages at temperature of 24 °C, relative humidity of 60% and light were automatically turned on from 07:00 to 19:00.

Blood samples

At d15, rats were food deprived for 12h and anaesthetized with sodium pentobarbital (60 mg/kg BW) and 6 rats of each group were injected with heparin (200UI/kg BW). Blood was collected from abdominal aorta into EDTA tubes and centrifuged at 4 °C, 6000 x g for 15 minutes. Liver was quickly excised in ice-cold saline, blotted on filter paper and weighed.

Isolation and characterisation of plasma VLDL, LDL-HDL_1, HDL_2 and HDL_3

Serum VLDL and LDL-HDL_1 were isolated by precipitation using $MgCl_2$ and phosphotungstate (Sigma Chemical Company, France) by the method of Burstein *et al.*, (1970) [15]. HDL_2 and HDL_3 were performed by differential dextran sulphate magnesium chloride precipitation, according to Burstein *et al.*, (1989) [16]. To estimate the validity of this method, ultracentrifugation was performed according to Havel *et al.* (1955) [17].

Lipid parameters

Total cholesterol (TC) and triacyglycerols (TG) concentrations were determined by enzymatic colorimetric methods (kits Human, GmbH, Wiesbaden, Germany) and unesterified cholesterol (UC) was estimated by enzymatic method (kits Boehringer, Meylan, France). Cholesteryl esters (CE) were calculated by the difference between total and unesterified cholesterol. CE amounts were estimated as 1.67 times the esterified cholesterol amount. Analysis of phospholipids (PL) was assessed by enzymatic determination of phospholipids (BioMerieux, Lyon, France).

Substrate preparation for lipoprotein lipase activities

The substrate emulsion was prepared according to Nelsson-Ehle & Eckman (1977) method [18]. A total of 7 mg unlabeled triolein (Sigma Chemical, St Louis, MO) was mixed with 5.4 µCi glycerol tri [9–10(n)-3H] oleate (NEN, Boston, MA) and 0.3 mg lysophosphatidylcholine (Sigma Chemical, St Louis, MO). After solvent removal under N_2 stream, the mixture was sonicated with 2.4 ml of 0.2 M-Tris buffer pH 8.2. After sonication, 0.3 ml of 4% bovine serum albumin fraction V and 0.3 ml of heat inactivated serum (providing apo C-II, an activator of LPL) were added to the emulsion.

Lipolytic activity determination

Hepatic lipase activity was measured according to the method of Bengtsson-Olivecrona & Olivecrona, (1992) [19]: one hundred mg of liver was homogenised in ultraturax (Basic T25 Ika Werke) with 0.9 ml ice-cold buffer containing 0.025 M-NH_3, antiproteasics (10 µg/ml leupeptin, 1µg/ml pepstatin, 25 IU/ml aprotinin), 5 mM-EDTA, 5 IU/ml heparin, 100 mg/ml CHAPS and 0.08% (w/v) sodium dodecyl sulphate substituted by 0.4% (w/v) triton X100) and adjusted at pH 8.2. The homogenate was centrifuged at 1200 x g, at 4 °C for 20 minute. One hundred µl of the fraction between the upper fat layer and the bottom sediment was removed for lipoprotein lipase assay and incubated in duplicate for 1h at 28 °C with 100 µl of substrate prepared according to the method of Nelsson-Ehle & Eckman, (1977) [18]. The supernatant was adjusted to 1 M-NaCl to inhibit the LPL activity and heat inactivated serum was omitted in the substrate preparation. At the end of the incubation period, liberated fatty acids were extracted according to the Belfrage & Vaughan, (1969) [20] method with a 2-phase solvent system: 3.5 ml CH_3OH: CH_3Cl: heptane (1.41:1.25:1, v/v/v) and 1.05 ml 0.1 M-tetraborate-carbonate, pH 10.5.

[^{3}H] radioactivity in 1.5 ml aliquots of the methanol/water upper phase was measured in 10 ml of scintillant (Ultima gold XR; Perkin Elmer, Boston, MA) in a 7500 LS scintillation counter (Beckman, Palo Alto, CA). Enzyme expressed as mU/mg proteins (1 mU of enzyme activity corresponded to 1 nmole of fatty acid released per minute at 28 °C). Proteins were estimated according to the method of Lowry *et al.*, (1951) [21] using bovine serum albumin as standard.

Post-heparin lipase activity

Serum lipoprotein lipase activity was measured according to Nilsson-Ehle & Eckman, (1977) [18] method. Radioactivity was determined in 1.5 ml aliquots of upper solvent phase. Enzyme activity was expressed as mmol/ml/h.

Statistical analysis

Results were expressed as means±SEM. Statistical evaluation of the data was carried out by the parametric Student '*t*' test. The limit of statistical significance was set at $P<0.05$ between the both groups treated and untreated with *Ajuga iva* extract.

RESULTS

Body weight and food intake

At d15, a similar body weight was noted in the both hypercholesterolemic (HC) rats treated or not with *Ai*. Food intake

was increased by 11% in *Ai* treated compared with the untreated group (table 1).

Serum and liver lipid parameters

At d15, serum TG values were 1.4-fold lower in *Ai*, whereas UC contents were 1.8-fold higher in treated than untreated hypercholesterolemic groups. Serum PL and CE contents and liver TG, UC, PL and CE values were not sensitive to *Ai* administration (table 1).

Table 1: Body weight, food intake and serum and liver lipid contents in untreated and *Ai*-treated hypercholesterolemic rats

	HC	*Ai*-HC
Body weight (g)	196.00±22.00	204.00±24.00
Food intake (g/d/rat)	16.00±0.20	18.00±0.50*
Serum (mmol/l)		
UC	0.64±0.14	1.16±0.17*
CE	4.70±1.50	6.20±1.50
PL	4.05±0.61	4.13±0.61
TG	2.00±0.11	1.40±0.20*
Liver (µmol/g)		
UC	21.10±2.10	21.33±1.60
CE	42.60±1.30	43.60±12.00
PL	32.70±3.29	33.40±4.50
TG	46.58±2.88	47.46±8.84

Rats were fed a diet containing 1% cholesterol for 15 days. After this phase, rats were fed the cholesterol-enriched diet and treated with 0.5% *Ai* (*Ai*-HC) or not (HC). Statistical evaluation of the data was carried out by the parametric Student t test. Values are means±SEM of 6 rats per group. * P<0.05, *Ai*-HC treated *vs* untreated HC group, CE, cholesteryl esters; PL, phospholipids; TG, triacylglycerols; UC, unesterified cholesterol.

Cholesterol and triacylglycerols distribution between lipoproteins

The distribution of serum CT and TG among the different fractions of lipoproteins showed that the greater part was carried by LDL-HDL_1 and represented, respectively, 63% and 44% in the HC *Ai* treated group and 68% and 50% in the HC untreated group. The CT and TG carried by VLDL represented 7% and 14% in *Ai*-HC group *vs* 19% and 44% in HC group. The part of TG carried by HDL (the sum of HDL_2 and HDL_3) represented 30% and 42% in *Ai*-HC group and 13% and 6% in HC group, respectively (table 2).

Table 2: Cholesterol and triacylglycerols distribution between different lipoproteins in untreated and *Ai*-treated hypercholesterolemic rats

	HC (%)	*Ai*-HC (%)
C-VLDL	19	7
C-LDL-HDL_1	68	63
C-HDL_2	8	12
C-HDL_3	5	18
TG-VLDL	44	14
TG-LDL-HDL_1	50	44
TG-HDL_2	3	27
TG-HDL_3	3	15

Rats were fed a diet containing 1% cholesterol for 15 days. After this phase, rats were fed the cholesterol-enriched diet and treated with 0.5% *Ai* (*Ai*-HC) or not (HC). Statistical evaluation of the data was carried out by the parametric Student t test. Values are means±SEM of 6 rats per group. * P<0.05, *Ai*-HC treated *vs* untreated HC group.

Serum VLDL and LDL-HDL_1 composition

VLDL amount (which represented the sum of apolipoproteins (apos)+TG+CE+UC+PL), apos, TG, and CE contents were, respectively, 2.2-, 2.6-, 4.4-and 1.9-fold lower in *Ai*-HC treated group than untreated HC group. VLDL-UC values were 2.9-fold higher in *Ai* treated group compared to the untreated hypercholesterolemic group (table 3).

LDL-HDL_1-TG value was 1.5-fold lower, whereas, that of PL was twofold higher in *Ai* treated group compared to untreated group (table 3).

Table 3: Serum VLDL and LDL+HDL_1 amounts and composition in untreated and *Ai*-treated hypercholesterolemic rats

	HC	*Ai*-HC
VLDL		
Amount (g/l)	1.88±0.58	0.85±0.17*
Apolipoproteins (g/l)	0.62±0.27	0.24±0.07*
TG (mmol/l)	0.88±0.17	0.20±0.03*
PL (mmol/l)	0.71±0.06	0.74±0.006
UC (mmol/l)	0.09±0.016	0.26±0.12*
CE (mmol/l)	1.70±0.60	0.9±0.10*
LDL-HDL_1		
Amount (g/l)	2.85±0.83	3.08±0.65
Apolipoproteins (g/l)	1.12±0.41	1.45±0.29
TG (mmol/l)	1.00±0.16	0.66±0.06*
PL (mmol/l)	0.50±0.04	1.00±0.27*
UC (mmol/l)	0.15±0.04	0.20±0.05
CE (mmol/l)	3.10±1.10	4.00±1.10

Rats were fed a diet containing 1% cholesterol for 15 days. After this phase, rats were fed the cholesterol-enriched diet and treated with 0.5% *Ai* (*Ai*-HC) or not (HC). Statistical evaluation of the data was carried out by the parametric Student t test. Values are means±SEM of 6 rats per group. * P<0.05, *Ai*-HC treated *vs* untreated HC group. Amount = apolipoproteins + triacylglycerols (TG)+phospholipids (PL)+unesterified cholesterol (UC)+cholesteryl esters (CE).

Serum HDL_2 and HDL_3 amounts and composition

In the HDL_2, amount, TG and UC values were, respectively, 2.2-, 8- and 1.2-fold higher and the PL contents were 1.2-fold lower in the *Ai*-HC group compared to the untreated HC group.

In the HDL_3, TG, UC and CE values were 3-, 1.6-and 2-fold higher in the *Ai*-HC group, whereas, PL contents were 1.4-fold lower in the *Ai*-HC group compared to the untreated HC group (table 4).

Table 4: Serum HDL_2 and HDL_3 amounts and composition in untreated and *Ai*-treated hypercholesterolemic rats

	HC	*Ai*-HC
HDL_2		
Amount (g/l)	0.33±0.06	0.74±0.01*
Apolipoproteins (g/l)	0.11±0.03	0.08±0.01
TG (mmol/l)	0.05±0.01	0.40±0.09*
PL (mmol/l)	0.67±0.01	0.56±0.06*
UC (mmol/l)	0.10±0.02	0.25±0.01*
CE (mmol/l)	0.33±0.08	0.42±0.02
HDL_3		
Amount (g/l)	4.46±0.35	4.25±0.35
Apolipoproteins (g/l)	4.20±0.30	3.60±0.50
TG (mmol/l)	0.07±0.01	0.22±0.05*
PL (mmol/l)	1.50±0.20	1.09±0.16*
UC (mmol/l)	0.18±0.02	0.30±0.03*
CE (mmol/l)	0.40±0.01	0.80±0.02*

Rats were fed a diet containing 1% cholesterol for 15 days. After this phase, rats were fed the cholesterol-enriched diet and treated with 0.5% *Ai* (*Ai*-HC) or not (HC). Statistical evaluation of the data was carried out by the parametric Student t test. Values are means±SEM of 6 rats per group. * P<0.05, *Ai*-HC treated *vs* untreated HC group.

Amount = apolipoproteins+triacylglycerols (TG)+phospholipids (PL)+unesterified cholesterol (UC)+cholesteryl esters (CE).

Hepatic and post-heparin lipase activity

Hepatic lipase activity was similar, and that of post-heparin lipases was increased by+15% in *Ai* treated group than the untreated group (table 5).

Table 5: Hepatic and post-heparin lipase activity in untreated and *Ai*-treated hypercholesterolemic rats

	HC	*Ai*-HC
Hepatic lipase activity **(**mU/mg protein**)**	8.28±1.55	7.32±0.56
Post-heparin lipase activity (mmol/ml/h)	13.00±0.16	15.28±1.29*

Rats were fed a diet containing 1% cholesterol for 15 days. After this phase, rats were fed the cholesterol-enriched diet and treated with 0.5% *Ai* (*Ai*-HC) or not (HC). Statistical evaluation of the data was carried out by the parametric Student t test. Values are means±SEM of 6 rats per group. *P<0.05, *Ai*-HC treated *vs* untreated HC group.

DISCUSSION

The purpose of this study was to investigate the influence of aqueous extract of *Ajuga iva* on serum lipoprotein composition and changes in hepatic and post-heparin lipases activities in rats fed a cholesterol-rich diet rats.

In this study, food intake was higher in hypercholesterolemic rats treated with aqueous extract of *Ajuga iva* (*Ai*-HC), but their body weight was similar to that of the untreated HC group. These results are consistent with those of [22] who's showed no significant difference, in rats submitted to a diet supplemented with cholesterol (2%) and treated orally with 500 mg of *Morus alba* L. extract for 36 days.

Lipid metabolism is a complex process involving lipoproteins, lipoprotein receptors and enzymes that play an important role in lipid regulation. The liver regulates its intra-hepatic cholesterol homeostasis, by maintaining an appropriate balance between the regulatory free cholesterol and the more inert cholesterol ester pool [23].

In the liver, the values of TG and UC+CE showed no significant difference in both groups treated or not with the *Ajuga iva*, probably due to a similar synthesis and/or catabolism TG and cholesterol in the liver. In addition, the unchanged content of TG in this tissue in the both groups could be explained by the similar value in hepatic lipase activity. On the other hand, chemical studies on *Ajuga iva* aqueous extract have revealed the presence of several flavonoids, tannins, terpenes and steroids [24]. Since tannins administered orally (100 mg/rat) during 10 weeks have been reported to present a reduced serum TG and fat deposition and lowered hepatic lipase activity in high fat diet-fed rats [25], our study indicated that other active principle(s) of *Ai* could be responsible for the observed effects of aqueous *Ai* extract on liver TG values and hepatic lipase activity in hypercholesterolemic rats.

On the other hand, LDL takes the cholesterol from the liver to tissues, whereas high-density HDL facilitates the translocation of cholesterol from the peripheral tissues to liver for catabolism [26]. Therefore, HDL has a useful effect in reducing tissue cholesterol, and increasing the ratio in serum is suggested, while decreasing level that for LDL-cholesterol to reduce the risk of cardiovascular diseases [27].

Our result showed that *Ajuga iva* lowered VLDL-C. A diet enriched in cholesterol (2%) and cholic acid (0.5%) increased the serum value of VLDL cholesterol [23]. Hypotriglyceridemic effect noted in the *Ai*-HC group might be due to increased catabolism of VLDL, lipoprotein which is responsible for hepatic TG export. This hypotriglyceridemia was decreased with concomitantly VLDL-TG. [28] noted that in hypercholesterolemic rats, treatment with 500 and 1000 mg of aqueous extract of *Hibiscus sabdariffa L*. (roselle) for 6 weeks resulted in a decrease in serum TG value. Indeed, in *Ai* treated group, the VLDL amount was decreased, reflecting the reduction of their surface (apo and UC) and central (TG and CE) components. Moreover, the decrease of serum TG might be the result of the increase post-heparin lipoprotein lipase activity in *Ai*-HC group. The high in post-heparin lipolytic activity seemed to be main preventive of hypertriglyceridemia development induced by dietary cholesterol in *Ai*-HC group. The effect of feeding cholesterol on serum TG level is well established. Osada *et al.*, (1994) [29] showed that dietary cholesterol induces hypertriglyceridaemia in rat. Previously, Othani *et al.*, (1990) [30] observed the same result without any change in the synthesis of apo B. The increase in serum TG could be due to the decreased activity of LDL receptors activity, when the diet is supplemented with cholesterol [31]. Our results showed that the amount of cholesterol added to the diet of rats treated with aqueous extract of *Ajuga iva* at the dose 0.5% not mask the effect of this extract on triacylglycerols levels.

Tsutsumi, (2003) [3] showed that the decrease in serum lipoprotein lipase activity was associated with the increased in serum TG levels and the reduced HDL-C, both risk factor of cardiovascular disease. It is well clear that the risk of atherosclerosis is inversely associated with plasma levels of HDL-C [32]. In our previous study we showed that HDL_2-C and HDL_3-C values were higher, in *Ai*-HC than the HC group [14].

After intravascular hydrolysis of TG rich lipoprotein by lipoprotein lipase, surface remnant components such as UC, PL and apos may provide substrates for a generation or modification of plasma HDL. The contribution of the lipolysed lipoprotein components to HDL formation has been reinforced by several studies where the activity of the enzyme lipoprotein lipase LPL was shown to correlate with HDL cholesterol levels in human plasma [33]. In addition, HDL acts much like heparin to liberate hepatic lipase from cell surface proteoglycans and stimulate TG clearance [34].

PL enriched HDL would be more efficient in promoting free cholesterol efflux from cell membranes, hence accelerating the reverse cholesterol transport. These may provide the basis for the mechanism that accounts for the inverse correlation between HDL and TG plasma levels found in epidemiological studies in human populations as well as in several circumstances, where plasma lipid levels are modified by pharmacological and dietary means [35]. Our result showed that PL-HDL were lower in *Ai* treated group than the untreated group. These finding might be explained: firstly by the presence of all compounds at the same time, because the active principle (iridoids) extract from *Ajuga iva* increased PL-HDL contents in rats receiving intraperitonealy different doses (5, 10 or 15 mg/kg BW) of iridoids compared to hypercholesterlemic untreated rats [36], secondly by the unchanged hepatic lipase activity which was sufficient to hydrolyze PL-HDL in this group. Hepatic lipase is recognised to hydrolyze PL and TG in HDL [27].

The effect of antioxidant capacity on blood lipid metabolism and LPL activity of rats fed a high-fat diet (HFD) treated with lipoic acid or N-acetylcysteine (0.1%) showed that HFD induced abnormal increases in lipid peroxidation, serum concentrations of TC, C-LDL and a decrease in C-HDL concentration [37]. Decreased LPL activity, accompanied by a depressed antioxidant defense system, was observed in HFD-fed rats. In our previous study [14] *Ai* treatment decreased lipid peroxydation and increased C-HDL and it was able to reduce the oxidative stress, in hypercholesterolemic rats by increasing the antioxidant enzyme activity. It may be suggested that *Ai* can improve the antioxidant capacity and activity of LPL.

In conclusion, in spite of the similar value in lipid composition of the liver between *Ajuga iva* treated group and untreated group, serum lipids as well as lipoprotein amounts and composition were significantly different. In addition, the aqueous extract of *Ai* leads to hypotriglyceridaemia with a decreased lipolytic activity, reflecting the lower levels of VLDL-TG in the HC group.

ABBREVIATION

Ai, Ajuga iva; Apos, apolipoproteins; CE, cholesteryl esters; HC, hypercholesterolemic; HDL, high density lipoproteins; LDL, low density lipoproteins; PL, phospholipids; TC, total cholesterol; TG, triacylglycerols; UC, unesterified cholesterol; VLDL; very low density lipoproteins.

CONFLICT OF INTERESTS

The authors declared no conflict of interest. All authors critically reviewed the manuscript and approved the final version submitted for publication.

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