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Original article

Synthesis of new andrographolide derivatives and evaluation of their antidyslipidemic, LDL-oxidation and antioxidant activity

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ABSTRACT

Andrographis paniculata, native to Taiwan, Mainland China and India, is a medicinal herb, which possesses various biological activities including anti-atherosclerosis. Andrographolide (**1**) has been identified as one of the active constituents against atherosclerosis. In continuation of our drug discovery program we synthesized few novel derivatives of **1** to improve their antidyslipidemic, LDL-oxidation and antioxidant activity. The tosylated derivative **7** has been turned out to be more potent than the parent compound and comparable activity with marketed antidyslipidemic drugs.

1. Introduction

Hypercholesterolemia and hypertriglyceridemia are either, alone or together major risk factors contributing to prevalence and severity of coronary artery disease and the progression of stroke, atherosclerosis, hyperlipidemia [1–3], which are leading cause of death in the developed and developing countries. High levels of low-density lipoprotein (LDL) accumulate in the extracellular sub endothelial space of arteries and are highly atherogenic and toxic to vascular cells thereby leading to atherosclerosis, hypertension, obesity, diabetes, functional depression in some organs, etc [4,5]. Oxidative stress is also a major contributing factor for peroxidative damage to lipoproteins, which is dependable for initiation and progression of atherosclerosis in hyperlipidemic subjects [6]. Hyperlipidemia may also provoke abnormalities with oxidation of fatty acids leading to the development of ketone bodies as well as making liver and muscle resistance to insulin which start and progress diabetes in patients [7]. To rise above these ailments, a drug having multifold properties such as lipid lowering and antioxidant activities mutually is in great demand.

The discovery of new drugs from traditional medicine is not a new phenomenon. It has been reported that traditional systems have immense potential to cure various diseases [8,9]. Plants have always been an ideal source of drugs and many of the currently available drugs have been derived directly or developed from plants (Fig. 1). For example, metformin is currently used as antidiabetic agent in the treatment of type-2 diabetes. Metformin and its analogs [10] were synthesized on the basis of a natural product lead viz, galegine [11], which was isolated from the seeds of *Galega officinali* (Fig. 1). The synthetic cholesterol lowering statins such as fluvastatin [12], cerivastatin [13] were developed on the basis of natural product leads, that is, mevastatin [14]. The fish oils, which contain fatty acids such as eicosapentaenoic acid and docosahexenoic acids (Fig. 1), have been reported for their lowering effect on triglycerides and cholesterol [15].

As a part of our drug discovery program we identified few antidyslipidemic agents such as aegeline from *Aegle marmelos* [16] and 4-hydroxyisoluecine from *Trigonella foenum graecum* seeds (Fig. 1) [17]. In continuation of this program we explored *Andrographis paniculata*. *A. paniculata* (Burm. f.) Nees (Acanthaceae) (*A. paniculata*, Chuanxinlian), native to Taiwan, Mainland China and India, is a medicinal herb with an extremely bitter in taste [18],

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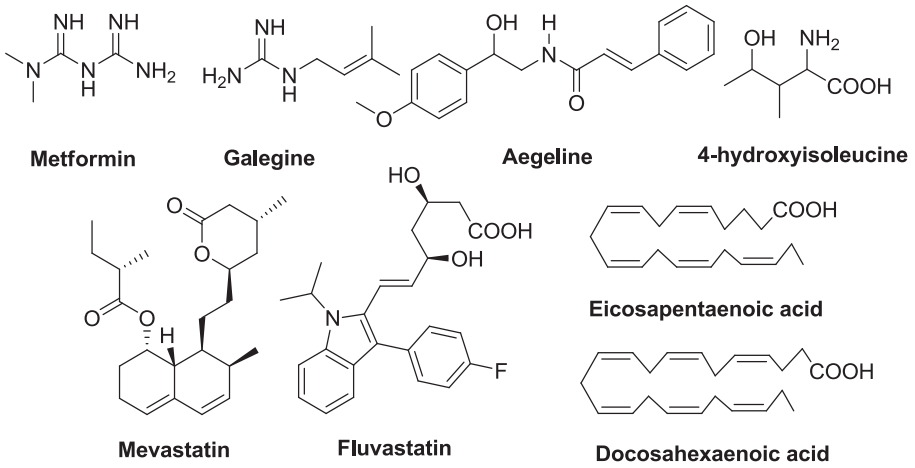


Fig. 1. Naturally occurring and synthetic antidiabetic and antidyslipidemic agents.

which possesses anti inflammation, anti cancer, immunomodulation, anti infection, anti hepatotoxicity, anti atherosclerosis, anti oxidation and anti hyperglycemic effect [19–29]. According to Chinese medicinal system, *A. paniculata* 'cools' and relieves internal heat, inflammation and pain and is used for detoxification [30–32]. Antihyperlipidemic and antioxidant activities of the extracts and the constituents of *A. paniculata* have been explored by several researchers in recent years. Furthermore, an alcoholic extract of the plant and its major constituent **1** have been shown to antihyperlipidemic agent through bioactivity-guided chromatographic fractionation. However, no systematic attempt has been made to improve the antihyperlipidemic and antioxidant activity of **1** and to synthesize novel and potent agents. Towards this goal, we have synthesized a series of new derivatives from **1** and evaluated lipid lowering activity in triton induced hyperlipidemic rat model. Further we have studied the dose dependent lipid lowering activity, lipoprotein cholesterol level, LPL activity, antioxidant and LDL oxidation.

2. Results and discussion

2.1. Chemistry

The structure of andrographolide contains a α-alkylidene γ-butyrolactone moiety, two olefin bonds $\Delta^{8(17)}$ and $\Delta^{12(13)}$, and three hydroxyls at C-3, C-19, and C-14. Of the three hydroxyl groups, the one at C- 14 is allylic in nature, and the others at C-3 and C-19 are secondary and primary, respectively. The derivatives of andrographolide were synthesized by modifying the above structural features.

In order to determine the importance of hydroxyl groups of andrographolide 1 for antihyperlipidemic and antioxidant activity. Initially, the hydroxyl groups at C-3 and C-19 of 1 were protected as an isopropylidene to give 2 and then allylic hydroxyl at C-14 was acetylated with acetic anhydride to provide 3. The compound 3's isopropylidine ring was hydrolyzed to get 14-Acetyl andrographolide (4) with 1N HCl. Sulfonylation reaction was carried out on 14-acetyl andrographolie (4) to generate derivatives 5 and 6 and subsequently hydrolyzed with NaOMe to give deacetylated compound 7 and 8 respectively (Scheme 1).

To understand the role of the exocyclic double bond $\Delta^{12(13)}$ in 1, 9 was prepared by selectively reducing the conjugated double bond with $NaBH_4$ and $NiCl_2$ $6H_2O$. To further study, the role of allylic hydroxyl at C-14 and the conjugated double bond, 10 was prepared, in which the exocyclic double bond $\Delta^{12(13)}$ isomerized to the endocyclic double bond $\Delta^{13(14)}$, with the simultaneous removal of C-14 hydroxyl and compound 11 was synthesized from 1 with $NaBH_4$ and Amberlyst-15 (Scheme 2).

Selective epoxidation of the exocyclic double bond $\Delta^{8(17)}$ of 1 with m-CPBA led to 12. Our efforts to oxidize the C-3, 19 hydroxyl groups of 1 with DMP yielded 13. To understand the role of the two olefin bonds $\Delta^{8(17)}$ and $\Delta^{12(13)}$ in 1, 14 and 15 were prepared by selectively reducing the double bond (Scheme 3).

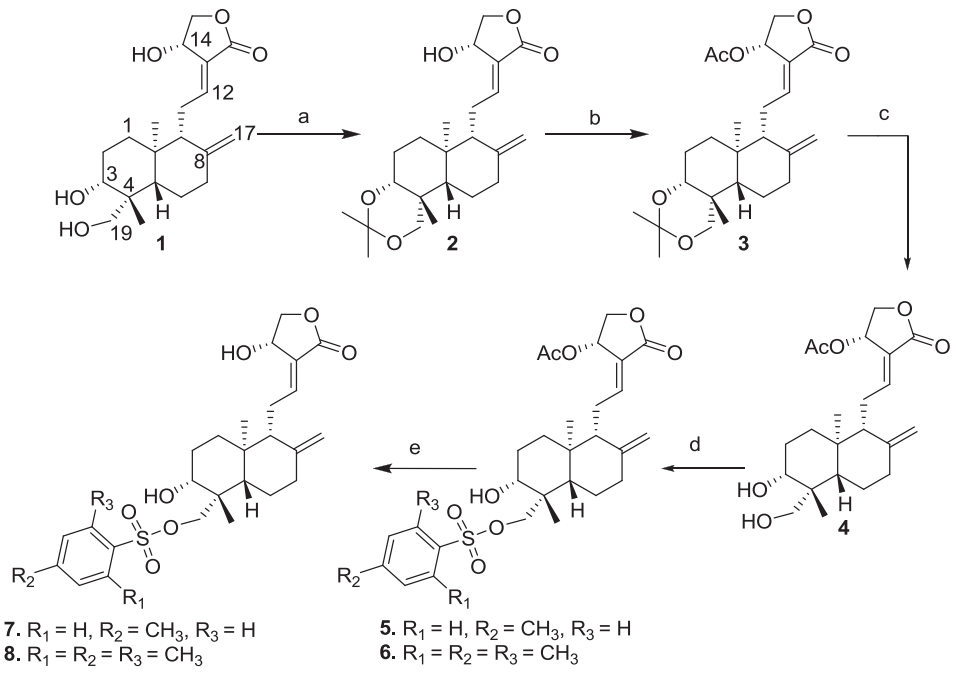
2.2. Biological evaluation

2.2.1. Lipid lowering activity of triton induced hyperlipidemic rat

Administration of triton WR-1339 in rats induced marked hyperlipidemia as evidenced by increase in the plasma levels of TC (+2.11 folds), PL (+2.37 folds), TG (+2.44 folds) followed by decrease in PHLA level (−24%) as compared to control rats. Treatment of hyperlipidemic rats with all the andographolide derivatives (**2**–**16**) at the dose of 100 mg/kg p.o. reversed the plasma levels of lipid but with varying extents. The parent compound, andrographolide (**1**) is causing a decrease in plasma levels of TC, PL and TG by 27%, 26% and 28% respectively followed 16% increase in PHLA level as compared to triton induced rats. The synthesized derivatives inhibited cholesterol biosynthesis and potentiated the activity of lipolytic enzymes to early clearance of lipids from circulation in triton induced hyperlipidemia. Among these derivatives (**2**–**16**), the sulfonyl derivative (**7**) turned out to be most potent lipid lowering agent, causing a decrease in plasma levels of TC, PL and TG by 33%, 37% and 35% respectively followed 18% increase in PHLA level as compared to triton induced rats at the dose of 100 mg/kg, where as the marketed lipid lowering drugs gemfibrozil and fenofibrate decreased the levels of TC, PL and TG in plasma by 30%, 30%, 28% and 32%, 33%, 33% respectively followed 16% and 17% increase in PHLA level as compared to triton induced rats at the same dose. Derivatives **5** and **8** showed moderate activity, caused a decrease in plasma levels of TC, PL and TG levels by 20%, 22%, 22% and 22%, 20%, 20% respectively followed 13% and 12% increase in PHLA level as compared to triton induced rats (Table 1). All other modifications such as hydrogenation of double bonds (**9**, **10**, **14**, **15**), epoxidation (**12**), oxidation (**13**), acetylation (**16**) lead to decreased activity to its parent compound (**1**).

2.2.2. Effect of compounds *1* and *7* on lipid lowering at different doses in triton induced hyperlipidemic rats

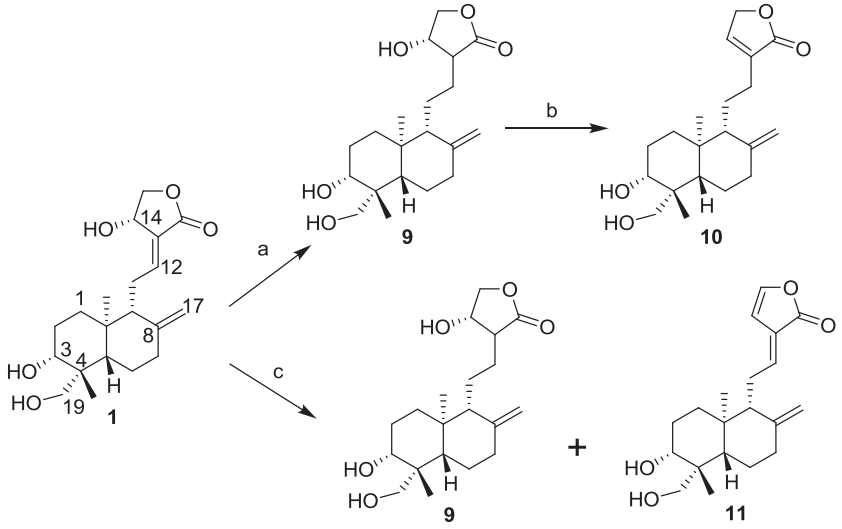
Since the tosylated derivative **7** has shown better activity profile than its parent compound **1** and comparable profile to marketed



Reagents and conditions: a) I_2, Acetone, rt, 2h, 90% b) Ac_2O, Pyridine, rt, 1h, 98% c) 1N HCl, THF, T= 20^0C, 2h, 95% d) Tosyl chloride/ 2- Mesitylene sulfonyl chloride, Pyridine, 60-70^0C, 4h, 70% e) NaOMe, MeOH, rt, 1h, 85%.

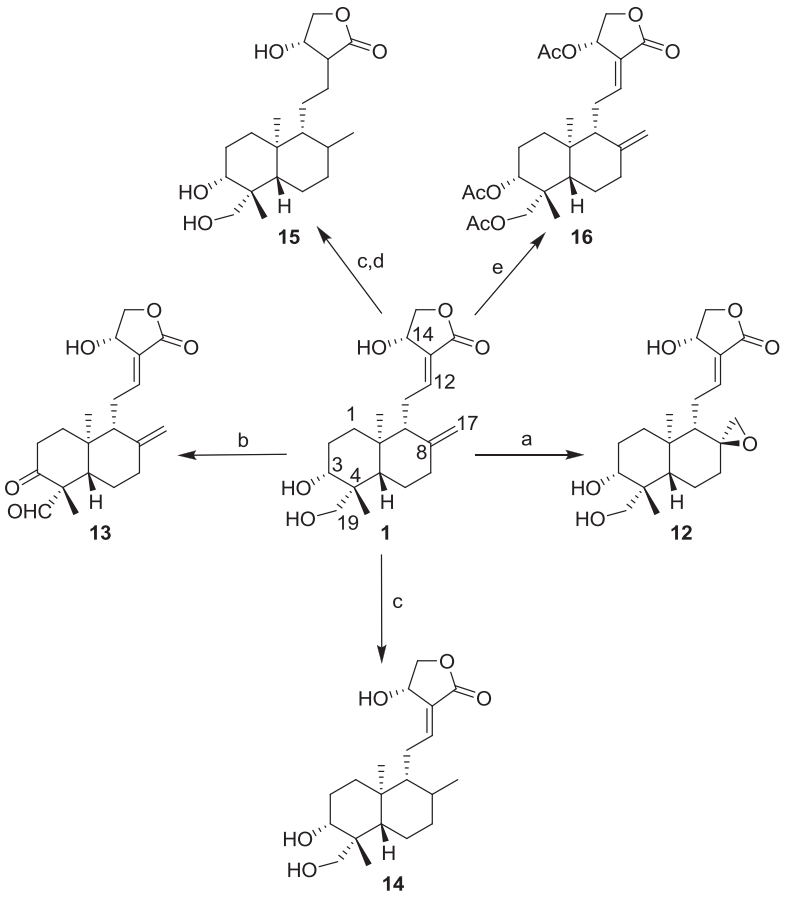
Scheme 1. Synthesis of sulfonyl derivatives **5**–**8** of andrographolide (**1**).

drugs (gemfibrozil and fenofibrate) a dose dependent activity study was carried out. As shown in Table 2, the administration of triton to rats increased their plasma levels of TC (+3.45 folds), PL (+2.86 folds), TG (+3.15 folds) followed by decrease in PHLA level (−41%) as compared to control rats. The efficacy of compounds **1** and **7** were studied at different doses in 25–200 mg/kg body weight. Compound **7** lowered the TC by 16%, PL by 15% and TG by 14% followed by increase in PHLA level by 10% (Table 2) at the dose of 25 mg/kg and at a dose of 50 mg/kg it lowered the TC by 22%, PL by 22%, TG by 20% and increased the PHLA level by 13%. At a higher dose of 100 mg/kg it lowered the TC by 33%, PL by 37%, TG by 35% and increased the PHLA level by 18%. A marginal improvement in the activity profile has been observed at further higher doses such as 150 and 200 mg/kg b.w. (Table 2).



Reagents and conditions: a) $NaBH_4$, $NiCl_2.6H_2O$, MeOH, rt, 30 min, 90% b) Na_2CO_3, MeOH, 50^0C, 2h, 80% c) $NaBH_4$, Amberlyst-15, THF, rt, 2h, Yield: 75: 25.

Scheme 2. Hydrogenation of andrographolide (**1**) by using $NaBH_4/NiCl_2 \cdot 6H_2O$ and $NaBH_4$, amberlyst-15.



Reagents and conditions: a) m-CPBA, MeoH, rt, 12h, 80% b) DMP, DCM, 0^0C, 30 min, 70% c) 10% Pd/C, Dry MeOH, rt, 5h, 90% d) $NaBH_4$, $NiCl_2.6H_2O$, MeOH, rt, 30 min, 90% e) Ac_2O, Pyridine, 60-70^0C, 4h, 98%.

Scheme 3. Acetylation, hydrogenation, oxidation and epoxidation of andrographolide (**1**).

Table 1
Percentage (%) change of plasma lipids with the treatment of andrographolide (**1**) and its derivatives (**2-16**) in Triton-induced hyperlipidemic rats at dose of 100 mg/kg body weight.

Animal groups	TC (mg/dl)	PL (mg/dl)	TG (mg/dl)	PHLA (nmol of free fatty acids formed/h/ml of plasma)
Control	86.49 ± 6.34	85.70 ± 6.41	88.42 ± 7.63	16.02 ± 1.72
Triton	182.89 ± 17.34^{c} (+2.11 Fold)	203.72 ± 17.11^{c} (+2.37 Fold)	215.78 ± 15.12^{c} (+2.44 Fold)	12.04 ± 0.59^{c} (−24)
Triton + Compound **1**	133.50 ± 10.06*** (−27)	150.75 ± 11.08*** (−26)	155.36 ± 11.05*** (−28)	13.96 ± 1.22* (+16)
Triton + Compound **2**	153.62 ± 10.63* (−16)	179.27 ± 12.22* (−12)	183.41 ± 11.73* (−15)	12.76 ± 0.93 ^{NS} (+6)
Triton + Compound **3**	159.50 ± 11.63* (−16)	175.19 ± 13.82* (−14)	187.72 ± 15.81* (−13)	12.64 ± 0.83 ^{NS} (+5)
Triton + Compound **4**	165.20 ± 10.88* (−13)	177.23 ± 12.32* (−13)	183.41 ± 13.79* (−15)	12.88 ± 1.03 ^{NS} (+7)
Triton + Compound **5**	151.91 ± 12.39** (−20)	158.90 ± 9.73** (−22)	168.30 ± 10.46** (−22)	13.60 ± 1.16* (+13)
Triton + Compound **6**	167.10 ± 14.73 (−12)	175.19 ± 14.86 (−14)	192.04 ± 14.68 (−11)	12.76 ± 0.92 (+6)
Triton + Compound **7**	122.53 ± 6.81*** (−33)	128.34 ± 7.53*** (−37)	140.25 ± 9.53*** (−35)	14.20 ± 1.18* (+18)
Triton + Compound **8**	142.65 ± 9.56** (−22)	162.97 ± 9.38** (−20)	172.62 ± 13.48** (−20)	13.48 ± 1.10* (+12)
Triton + Compound **9**	160.94 ± 10.26* (−12)	183.34 ± 11.37* (−10)	192.04 ± 13.72* (−11)	12.64 ± 0.96 ^{NS} (+5)
Triton + Compound **10**	151.79 ± 11.38* (−17)	175.19 ± 12.89* (−14)	189.88 ± 9.81* (−12)	12.76 ± 0.81 ^{NS} (+6)
Triton + Compound **11**	148.14 ± 9.88* (−19)	169.08 ± 14.84* (−17)	176.93 ± 13.09* (−18)	13.24 ± 0.86* (+10)
Triton + Compound **12**	155.45 ± 11.33* (−15)	169.08 ± 9.86* (−17)	185.57 ± 12.89* (−14)	12.76 ± 1.16 ^{NS} (+6)
Triton + Compound **13**	159.11 ± 9.93* (−13)	183.34 ± 13.19* (−10)	194.20 ± 10.13* (−10)	12.52 ± 0.63 ^{NS} (+4)
Triton + Compound **14**	157.28 ± 8.73* (−14)	169.08 ± 9.83* (−17)	183.41 ± 11.46* (−15)	12.64 ± 1.03 ^{NS} (+5)
Triton + Compound **15**	162.72 ± 9.61* (−11)	177.23 ± 11.82* (−13)	183.41 ± 13.32* (−15)	12.52 ± 0.81 ^{NS} (+4)
Triton + Compound **16**	148.14 ± 9.29* (−19)	171.12 ± 10.84* (−16)	176.93 ± 11.18* (−18)	13.36 ± 0.88* (+11)
Triton + Gemfibrozil	125.86 ± 11.72*** (−30)	138.00 ± 12.34*** (−30)	150.22 ± 13.08*** (−28)	14.89 ± 1.41* (+16)
Triton + Fenofibrate	129.12 ± 10.36*** (−32)	136.49 ± 9.59*** (−33)	144.57 ± 10.28*** (−33)	14.08 ± 1.26* (+17)

Each parameter represents pooled data from 6 rats/group and values are expressed as mean ± s.d. $^{c}p < 0.001$, triton treated group compared with control group and $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ triton plus compounds groups compared with triton treated group only. Note: ^{ns} (non significant) and f (fold change over control group). Units are ^{a}mg/dl; nmol of free fatty acids formed/h/ml of plasma.

Table 2
Percentage (%) change of plasma lipids with the treatment of andrographolide (**1**) and **7** in triton-induced hyperlipidemic rats at different doses.

Animal groups	TC (mg/dl)	PL (mg/dl)	TG (mg/dl)	PHLA (nmol of free fatty acids formed/h/ml of plasma)
Control	81.32 ± 6.92	83.42 ± 6.12	85.26 ± 8.00	18.62 ± 1.66
Triton	280.70 ± 25.16^{c} (+3.45 Fold)	238.60 ± 18.63^{c} (+2.86 Fold)	269.15 ± 15.36^{c} (+3.15 Fold)	10.84 ± 0.74^{c} (−41)
Triton + Compound **1**				
25 mg/kg b.w	241.40 ± 19.88* (−14)	209.96 ± 18.82* (−12)	228.77 ± 20.00* (−15)	11.92 ± 0.86* (+10)
50 mg/kg b.w	221.75 ± 20.06** (−21)	190.88 ± 14.06** (−20)	212.62 ± 18.56** (−21)	12.24 ± 1.02* (+13)
100 mg/kg b.w	204.91 ± 18.22*** (−27)	176.56 ± 16.74*** (−26)	193.78 ± 12.02*** (−28)	12.57 ± 0.76* (+16)
150 mg/kg b.w	199.29 ± 16.24*** (−29)	169.40 ± 17.00*** (−29)	188.40 ± 16.21*** (−30)	12.79 ± 1.24* (+18)
200 mg/kg b.w	199.25 ± 15.48*** (−29)	167.02 ± 13.39*** (−30)	188.38 ± 17.72*** (−30)	12.75 ± 0.86* (+18)
Triton + Compound **7**				
25 mg/kg b.w	235.78 ± 12.63* (−16)	202.81 ± 10.42* (−15)	231.46 ± 17.36* (−14)	11.92 ± 0.73* (+10)
50 mg/kg b.w	218.94 ± 15.81** (−22)	186.10 ± 12.26** (−22)	215.32 ± 13.81** (−20)	12.24 ± 0.091* (+13)
100 mg/kg b.w	188.06 ± 13.33*** (−33)	150.31 ± 8.43*** (−37)	174.94 ± 12.53*** (−35)	12.79 ± 1.13* (+18)
150 mg/kg b.w	185.26 ± 12.89*** (−34)	145.54 ± 9.28*** (−39)	169.56 ± 8.82*** (−37)	12.79 ± 0.89* (+18)
200 mg/kg b.w	185.26 ± 11.06*** (−34)	143.16 ± 7.93*** (−40)	169.56 ± 11.32*** (−37)	12.79 ± 0.77* (+18)
Triton + Gemfibrozil (100 mg/kg b.w)	188.06 ± 16.28*** (−33)	155.09 ± 12.86*** (−35)	180.33 ± 16.00*** (−33)	13.00 ± 1.22** (+20)
Triton + Fenofibrate (100 mg/kg b.w)	182.45 ± 13.36*** (−35)	157.47 ± 11.48*** (−34)	177.63 ± 17.05*** (−34)	13.00 ± 1.28*** (+20)

Each parameter represents pooled data from 6 rats/group and values are expressed as mean ± s.d. $^{c}p < 0.001$, triton treated group compared with control group and $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ triton plus compounds groups compared with triton treated group only. Note: ^{ns} (non significant) and f (fold change over control group). Units are ^{a}mg/dl; ^{b}mol of free fatty acids formed/h/ml of plasma.

2.2.3. *Effect of compounds **1** and **7** on plasma lipoprotein lipids in triton induced hyperlipidemic rats*

Compounds **1** and **7** (100 mg/kg b.w) improve the serum lipoproteins cholesterol level in triton induced hyperlipidemic rats. The analysis of hyperlipidemic serum of triton administered rats showed significantly increase in the level of VLDL-C and LDL-C followed by decrease in HDL-C as compared to control rats. Treatment with compounds **1** and **7** significantly reversed the increased levels of VLDL-TC and LDL-TC and decreased HDL-TC in triton induced hyperlipidemic rats (Fig. 2). Here also the tosylated derivative **7** showed better activity profile than its parent compound **1** and the activity is comparable to marketed drugs.

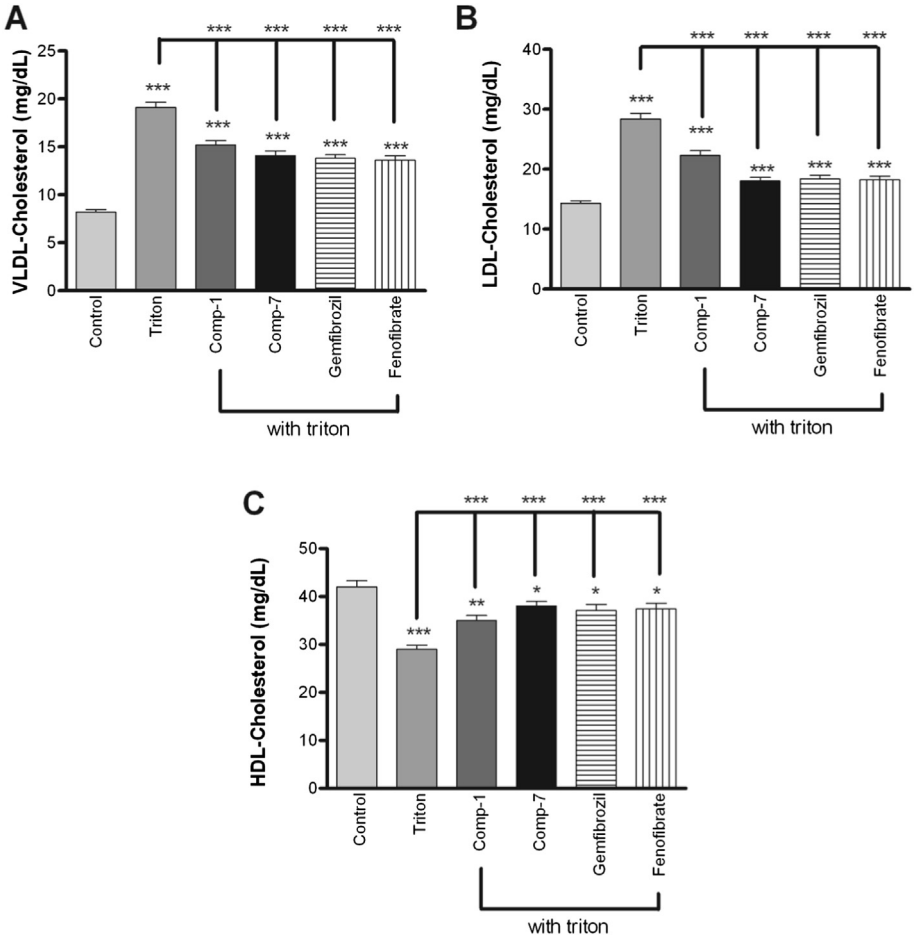


Fig. 2. Effect of compounds **1** and **7** on lipoprotein metabolism in triton induced hyperlipidemic rats. Blood was drawn after 18 h of hyperlipidemia was developed by administration of triton WR-1339 with and without compounds **1** and **7**. Gemfibrozil and fenofibrate were taken as reference drug (100 mg/kg). (A) Very low density lipoprotein (VLDL). (B) Low density lipoprotein. (C) High density lipoprotein. Values are expressed as the mean ± SD ($n = 6$). $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; NS (non significant).

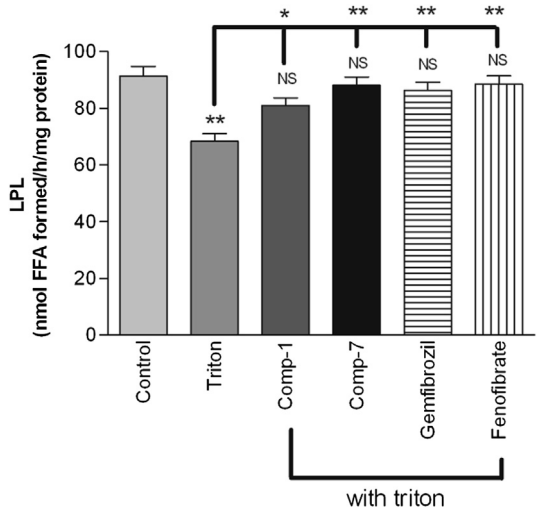


Fig. 3. Compounds **1** and **7** (100 mg/kg) re-activates LPL activity significantly in Triton induced hyperlipidemic rats. Each parameter represents pooled data from 6 rats/group and values are expressed as mean ± S.D. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; NS (non significant). Gemfibrozil and Fenofibrate are taken as reference drug.

2.2.4. Effect of compounds **1** and **7** LPL activities in triton induced hyperlipidemic rats

Further the compounds **1** and **7** were studied for LPL activity in triton induced hyperlipidemic rats. Administration of triton in rats markedly decreases in LPL activity in liver. After treatment with compounds **1** and **7** LPL activity was significantly increased similar to standard drug gemfibrozil and fenofibrate (Fig. 3).

2.2.5. Antioxidant activity

Recent studies have demonstrated that the generation of large quantities of reactive oxygen species can cause activation of lipid peroxidation, protein modification, which leads to cardiovascular diseases (CVD) [33–35] therefore we have screened alcoholic for their antioxidant activity using earlier reported method [36]. The scavenging potential of compounds **1** and **7** at 200 μg/ml against formation of O^{2-} and $OH^{\bullet}$ in nonenzymatic systems were studied (Table 3).

Table 3
Effect of andographolide (**1**) and its derivatives (**2-16**) on superoxide anions, hydroxyl radicals and lipid-peroxidation at 200 μg/ml dose.

Dose (200 μg/ml)	Superoxide anions (%) (*n* mol formazone formed/minute)	Hydroxyl radicals (%) (*n* mol MDA formed/h/mg protein)	Lipid-peroxidation (%) (*n* mol MDA formed/h/mg protein)
1	−33***	−36***	−38***
2	−15*	−11*	−17*
3	−23***	−25***	−26***
4	−19**	−17**	−19**
5	−27***	−30***	−29***
6	−31***	−28***	−26***
7	−41***	−46***	−40***
8	−23***	−25***	−26***
9	−14*	−11*	−10*
10	−11*	−16*	−13*
11	−16*	−18*	−13*
12	−17*	−15*	−14*
13	−16*	−12*	−14*
14	−19*	−15*	−17*
15	−13*	−17*	−14*
16	−12*	−16*	−11*
Standards (200 μg/ml)	−54*** (Allopurinol)	−50*** (Manitol)	−55*** (α-tocopherol)

Each value is mean ± sd of six values, $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; ns (non significant) experimental values compared with control values.

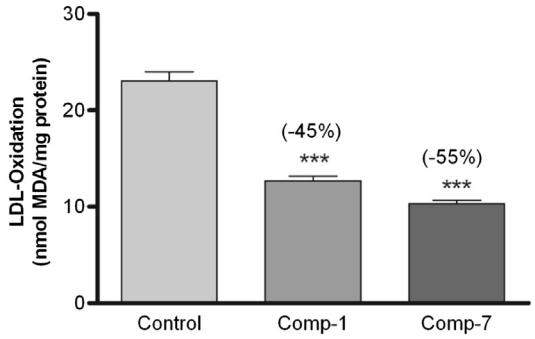


Fig. 4. Compounds **1** and **7** reduced the LDL-oxidation at 200 μg/ml concentration in normal donor blood sample. Data expressed as mean ± S.D. $^{***}P < 0.001$; compared to treated values with untreated (control).

A significant decrease in superoxide anions (33% and 41%) and hydroxyl radicals (36%–46%) were observed by compounds **1** and 7. Furthermore, compounds **1** and **7** at 200 μg/ml reduced the microsomal lipid per-oxidation (38%–40%).

2.2.6. Effect of compounds **1** and **7** on LDL oxidation

Aerobic oxidation of LDL even in the absence of metal ions caused formation of TBARS (n mol MDA/mg protein), which were greatly increased by 10–15 folds in presence of Cu^{+2}. Addition of compounds **1** and **7** at 200 μg/ml concentrations in above reaction mixture protected LDL against oxidative changes (Fig. 4).

3. Conclusion

In conclusion we have prepared few new derivatives of Andrographolide (**1**) and evaluated their antidyslipidemic, LDL oxidation and antioxidant activity. The tosylated derivative **7** has turned out to be more potent than the parent compound **1** and the activity profile is comparable to the marketed antidyslipidemic drugs.

4. Experimental section

4.1. General chemistry

IR spectra were recorded on Perkin–Elmer RX-1 spectrometer using either KBr pellets (or) in neat. ^{1}H NMR, ^{13}C NMR, DEPT-90 and DEPT-135 spectra were run on Bruker Advance DPX 300 MHz and 200 MHz in $CDCl_3$.Chemical shifts are reported as values in ppm relative to $CHCl_3$/DMSO with TMS as internal standard. ESI mass spectra were recorded on JEOL SX 102/DA-6000. Thin layer chromatography (TLC) plates of silica gel 60 GF254 of Merck Company were used for identification and purity check of compounds. Chromatography was executed with silica gel (60–120 mesh) using mixtures of chloroform, methanol and hexane as eluants. Visualization was obtained under UV light and spraying with 10% sulfuricsulfuric acid in methanol.

4.2. Collection of medicinal plant

A. paniculata .Nees (Whole plant) was collected from India and the authentification was done by the Botany Division of Central Drug Research Institute, Lucknow.

4.3. Extraction

Powdered *A. paniculata* .Nees (Whole plant) (4 kg) were placed in glass percolator with 95% ethanol (10 L) and allowed to stand for 24 h at room temperature. The percolate was collected and these

processes were repeated for four times. The combined percolate of 40 L was evaporated under reduced pressure at 50 °C to afford ethanol extract. The weight of extract was found to be 300 g.

4.4. Fractionation

The ethanol extract was macerated with hexane. The hexane soluble fraction was separated and evaporated under reduced pressure to afford hexane fraction (F001, 80 g). Distilled water was added to hexane insoluble portion, which was fractionated with chloroform and the resultant solution was evaporated under reduced pressure to afford chloroform fraction (F002, 120 g). The water soluble fraction was evaporated under reduced pressure at 60 °C to afford the aqueous fraction (F003, 70 g).

4.5. Isolation of diterpenoid

Chloroform fraction (120 g) was chromatographed on a column of silica gel (60–120 mesh) and eluted with chloroform – methanol (96:4) to afford compound 1 (2 g) as colorless needles. The compound's visualization was done under UV light and by spraying with 10% sulfuric acid in methanol, which also gives positive Liebermann–Burchard test for terpenoids.

Andrographolide (1): IR (KBr) 3435, 2956, 2917, 2849, 2143, 1639, 1465, 1377, 1218, 1073, 1018, 771, 655 cm^{-1}; ^1H NMR (DMSO, 300 MHz) δ 6.62 (t, $J = 6.73$ Hz, 1H), 5.72 (d, $J = 6.14$ Hz, 1H), 5.04 (d, $J = 4.92$ Hz, 1H), 4.81 (s, 1H), 4.63 (s, 1H), 4.37 (t, $J = 6.37$ Hz, 1H), 4.02 (d, $J = 9.41$ Hz, 1H), 3.82 (d, $J = 10.24$ Hz, 1H) 1.09 & 0.66 (each s, 3H); ^{13}C NMR (DMSO, 75 MHz) 170.41, 148.04, 146.76, 129.43, 108.71, 78.94, 74.78, 64.99, 63.13, 55.95, 54.84, 42.73, 40.79, 37.96, 36.97, 28.35, 24.43, 24.40, 23.52, 15.21; ESI - MS 350 $[M + H]^+$.

4.6. Preparation of andrographolide derivatives

4.6.1. Synthesis of (S, E)-4-hydroxy-3-(2-((4aR, 6aS, 7R, 10aS, 10bR)-3, 3, 6a, 10b-tetramethyl-8-methylenedecahydro-1H-naphtho[2,1-d][1,3]dioxin-7-yl)ethylidene)dihydrofuran-2(3H)-one (**2**)

Iodine (30 mg) was dissolved in acetone (5 mL) and compound 1 (100 mg, 0.00028 mol) was added and stirred at the ambient temperature (28 °C). The reaction was monitored by TLC and was found to be complete when the compound 1 went into solution. Iodine was destroyed by the addition of dilute aqueous sodium hydroxide solution. The resulting solution would be colorless. The isopropylidene derivative was extracted with ethyl acetate (3 × 25 mL), the organic layer was washed with water, dried over anhyd Na_2SO_4 and evaporated under reduced pressure. Then the crude product was chromatographed on silica gel to afford the desired compound 2. Yield: 90%; IR (KBr) 3854, 3400, 3016, 2946, 2834, 2515, 2402, 2219, 2039, 1642, 1449, 1415, 1216, 1111, 1023, 928, 878, 769, 668 cm^{-1}; ^1H NMR (DMSO, 300 MHz) δ 6.62 (t, $J = 6.75$ Hz, 1H), 5.14 (d, $J = 6.21$ Hz, 1H), 4.91 (d, $J = 4.87$ Hz, 1H), 4.81 (s,1H), 4.63 (s, 1H), 4.35 (t, $J = 6.42$ Hz, 1H), 4.02 (d, $J = 9.40$ Hz, 1H), 3.83 (d, $J = 10.13$ Hz, 1H), 1.36 (s, 6H), 1.08 & 0.66 (each s, 3H); ESI - MS: 391 $[M + H]^+$.

4.6.2. Synthesis of (S, E)-5-oxo-4-(2-((4aR, 6aS, 7R, 10aS, 10bR)-3, 3,6a, 10b-tetramethyl-8-methylenedecahydro-1H-naphtho[2,1-d][1,3]dioxin-7-yl)ethylidene)tetrahydrofuran-3-yl acetate (**3**)

Compound 2 (100 mg, 0.00025 mol) magnetically stirred in a solution of pyridine (2 mL) and acetic anhydride (0.00128 mol) at 60-70 °C for 4 h. The reaction mixture was put into cold water for crystallization, then filtered and dried to get desired compound 3.Yield: 98%; IR (KBr) 3413, 3018, 2948, 2837, 2400, 2129, 1638, 1449, 1412, 1216, 1019, 927, 770, 669 cm^{-1}; ^1H NMR (DMSO, 300 MHz) δ 6.61 (t, $J = 6.69$ Hz, 1H), 5.67 (d, $J = 6.24$ Hz, 1H), 4.92 (d, $J = 4.73$ Hz, 1H), 4.81 (s,1H), 4.62 (s, 1H), 4.58 (t, $J = 6.46$ Hz, 1H), 4.05 (d, $J = 9.73$ Hz, 1H), 3.86 (d, $J = 10.33$ Hz, 1H), 2.18 (s, 3H), 1.31 (s, 6H), 1.08 & 0.65 (each s, 3H); ESI - MS: 433 $[M + H]^+$.

4.6.3. Synthesis of (S, E)-4-(2-((1R, 4aS, 5R, 6R, 8aS)-6-hydroxy-5-(hydroxymethyl)-5, 8a-dimethyl-2-methylene decahydronaphthalen-1-yl)ethylidene)-5-oxotetrahydrofuran-3-yl acetate (**4**)

To a solution of compound 3 (100 mg, 0.00023 mol) in THF (5 mL) was added 1 N HCl (5 mL). The mixture was stirred at 20 °C for 2 h, and then extracted with ethyl acetate (3 × 25 mL), the organic layer was washed with water, dried over anhyd Na_2SO_4 and evaporated under reduced pressure. Then the crude product was chromatographed on silica gel to afford the desired compound 4.Yield: 95%; IR (KBr) 3683, 3620, 3436, 3019, 2975, 2400, 1602, 1522, 1475, 1423, 1216, 1045, 928, 876, 849, 768, 758, 669, 627 cm^{-1}; ^1H NMR (DMSO, 300 MHz) δ 6.60 (t, $J = 6.83$ Hz, 1H), 5.68 (d, $J = 6.23$ Hz, 1H), 4.91 (d, $J = 4.93$ Hz, 1H), 4.79 (s,1H), 4.60 (s, 1H), 4.52 (t, $J = 6.53$ Hz, 1H), 3.81 (d, $J = 9.83$ Hz, 1H), 3.62 (d, $J = 10.13$ Hz, 1H), 2.30 (s, 3H), 1.06 & 0.63 (each s, 3H); ESI - MS: 393 $[M + H]^+$.

4.6.4. General procedure for synthesis of sulfonyl compounds (**5**, **6**)

Compound **4** (100 mg, 0.00025 mol) magnetically stirred in a solution of pyridine (2 mL) and tosyl chloride/2-Mesitylene sulfonyl chloride at 60-70 °C for 4 h. Solvents were removed by vacuum, and then extracted with ethyl acetate (3 × 25 mL), the organic layer was washed with water, dried over anhyd Na_2SO_4 and evaporated under reduced pressure. Then the crude product was chromatographed on silica gel to afford the desired compounds **5, 6**.

4.6.4.1. (S, E)-4-(2-((1R, 4aS, 5R, 6R, 8aS)-6-hydroxy-5, 8a-dimethyl-2-methylene-5-tosyloxymethyl) decahydronaphthalen-1-yl) ethylidene)-5-oxotetrahydrofuran-3-yl acetate **5**.

Yield: 70%; IR (KBr) 3664, 3425, 3073, 2986, 2471, 2252, 2125, 1751, 1639, 1508, 1418, 1242, 1221, 1031, 881, 819, 758, 665, 621, 568 cm^{-1}; ^1H NMR (DMSO, 300 MHz) δ 7.51 (d, $J = 8.02$ Hz, 2H), 7.18 (d, $J = 7.93$ Hz, 2H), 6.64 (t, $J = 6.53$ Hz, 1H), 4.94 (d, $J = 6.73$ Hz, 2H), 4.83 (s,1H), 4.64 (s, 1H), 4.50 (t, $J = 6.42$ Hz, 1H), 4.04 (d, $J = 9.59$ Hz, 1H), 3.85 (d, $J = 10.13$ Hz, 1H), 2.32 (s, 3H), 2.18 (s, 3H), 1.10 & 0.67 (each s, 3H); ESI - MS: 547 $[M + H]^+$.

4.6.4.2. (S, E)-4-(2-((1R, 4aS, 5R, 6R, 8aS)-6-hydroxyl-5-((mesitylsulfonyloxy) methyl)-5, 8a-dimethyl-2-methylene decahydronaphthalen-1-yl) ethylidene)-5-oxotetrahydrofuran-3-yl acetate **6**.

Yield: 70%; IR (KBr) 3424, 3019, 2979, 2400, 1743, 1637, 1452, 1383, 1215, 1088, 1013, 851, 758, 683, 669, 582, 548 cm^{-1}; ^1H NMR (DMSO, 300 MHz) δ 6.79 (s, 2H), 6.62 (t, $J = 6.63$ Hz, 1H), 5.81 (d, $J = 6.47$ Hz, 1H), 4.92 (d, $J = 4.83$ Hz, 1H), 4.81 (s,1H), 4.62 (s, 1H), 4.52 (t, $J = 6.42$ Hz, 1H), 4.03 (d, $J = 9.83$ Hz, 1H), 3.83 (d, $J = 10.33$ Hz, 1H), 2.65 (s, 6H), 2.33 (s, 3H), 2.18 (s, 3H), 1.08 & 0.65 (each s, 3H); ESI - MS: 575 $[M + H]^+$.

4.6.5. General procedure for synthesis of deacetylated sulfonyl compounds (**7**, **8**)

To a solution of compound **5** (100 mg, 0.00018 mol) in methanol (5 mL) was added sodium methoxide (0.00025 mol). The mixture was stirred at room temperature for 1 h, and then extracted with ethyl acetate (3 × 25 mL), the organic layer was washed with water, dried over anhyd Na_2SO_4 and evaporated under reduced pressure. Then the crude product was chromatographed on silica gel to afford the desired compound.

4.6.5.1. ((1R, 2R, 4aS, 5R, 8aS)-2-hydroxyl-5-((E)-2-((S)-4-hydroxyl-2-oxodihydrofuran-3(2H)-ylidene) ethyl)-1, 4a-dimethyl-6-methylenedecahydronaphthalen-1-yl) methyl 4-methylbenzenesulfonate **7**.

Yield: 85%; IR (KBr) 3375, 3020, 2980, 2941, 2402, 1747, 1605, 1452, 1216, 1084, 1013, 927, 854, 761, 674, 582, 546 cm^{-1}; ^1H NMR

(DMSO, 300 MHz) δ 7.51 (d, J = 8.10 Hz, 2H), 7.15 (d, J = 7.83 Hz, 2H), 6.60 (t, J = 6.68 Hz, 1H), 4.92 (d, J = 6.54 Hz, 2H), 4.80 (s, 1H), 4.61 (s, 1H), 4.37 (t, J = 6.37 Hz, 1H), 4.04 (d, J = 9.54 Hz, 1H), 3.85 (d, J = 10.14 Hz, 1H), 2.29 (s, 3H), 1.07 & 0.64 (each s, 3H); ^{13}C NMR (DMSO, 75 MHz) 170.46, 148.03, 146.76, 144.80, 138.96, 129.40, 128.80, 125.95, 108.72, 78.90, 74.81, 72.94, 63.09, 55.93, 54.81, 42.70, 38.91, 37.94, 36.94, 28.33, 24.43, 24.37, 23.52, 21.26, 15.21; ESI - MS 505 [M + H].$^+$

*4.6.5.2. ((1R, 2R, 4aS, 5R, 8aS)-2-hydroxyl-5-((E)-2-((S)-4-hydroxyl-2-oxodihydrofuran-3(2H)-ylidene) ethyl)-1, 4a-dimethyl-6-methylenedecahydronaphthalen-1-yl) methyl 2, 4, 6-trimethyl benzene sulfonate **8**.* Yield: 85%; IR (KBr) 3411, 2940, 1743, 1668, 1452, 1213, 1086, 1013, 852, 763, 681, 582, 547 cm^{-1}; ^{1}H NMR (DMSO, 300 MHz) δ 6.92 (s, 2H), 6.75 (t, J = 6.86 Hz, 1H), 5.58 (d, J = 6.28 Hz, 1H), 5.04 (d, J = 4.88 Hz, 1H), 4.94 (s, 1H), 4.75 (s, 1H), 4.51 (t, J = 6.46 Hz, 1H), 4.16 (d, J = 9.73 Hz, 1H), 3.99 (d, J = 10.52 Hz, 1H), 2.64 (s, 6H), 2.31 (s, 3H), 1.21 & 0.78 (each s, 3H); ^{13}C NMR (DMSO, 75 MHz) 170.44, 148.05, 146.78, 141.93, 137.56, 136.53, 130.54, 129.42, 108.71, 78.91, 74.81, 73.05, 63.11, 55.94, 54.83, 42.72, 37.95, 36.96, 28.34, 24.43, 23.52, 23.09, 20.75, 15.21; ESI - MS 533 [M + H].$^+$

*4.6.6. Synthesis of (4S)-4-hydroxyl-3-(2-((1R, 4aS, 5R, 6R, 8aS)-6-hydroxyl-5-(hydroxymethyl)-5, 8a-dimethyl-2 -methylenedecahydronaphthalen-1-yl) ethyl) dihydrofuran −2(3H)-one (**9**)*

To a magnetically stirred solution of compound **1** (100 mg, 0.00028 mol) in methanol (10 mL) was added gradually $NiCl_2.6H_2O$ (0.00042 mol) at rt. When the clear solution acquired a greenish color, the whole reaction mixture was brought to 0 °C and $NaBH_4$ (0.00036 mol) was added portion wise. After addition of $NaBH_4$, the whole solution was stirred for 30 min at 0 °C to rt. Methanol was removed by vacuum, and then extracted with ethyl acetate (3 × 25 mL), the organic layer was washed with water, dried over anhyd Na_2SO_4 and evaporated under reduced pressure. Then the crude product was chromatographed on silica gel to afford the desired compound **9**. Yield: 90%; IR (KBr) 3684, 3614, 3405, 3021, 2401, 1640, 1522, 1424, 1215, 1039, 928, 761, 671, 627 cm^{-1}; ^{1}H NMR (DMSO, 300 MHz) δ 5.71 (d, J = 6.14 Hz, 1H), 5.05 (d, J = 4.87 Hz, 1H), 4.82 (s, 1H), 4.63 (s, 1H), 4.39 (t, J = 6.56 Hz, 1H), 4.13 (d, J = 9.73 Hz, 1H), 4.03 (d, J = 10.49 Hz, 1H), 1.09 (s, 3H), 0.66 (s, 3H); ^{13}C NMR (DMSO, 75 MHz) 170.42, 146.77, 108.71, 78.92, 74.79, 68.99, 63.12, 55.96, 54.84, 48.74, 42.80, 37.97, 36.98, 28.35, 24.43, 23.53, 15.22; ESI − MS: 353 [M + H].$^+$

*4.6.7. Synthesis of 3-(2-((1R, 4aS, 5R, 6R, 8aS)-6-hydroxyl-5-(hydroxy methyl)-5, 8a-dimethyl-2 -methylenedecahydronaphthalen-1-yl) ethyl) furan-2(5H)-one (**10**)*

To a solution of compound **9** (100 mg, 0.00028 mol) in methanol (5 mL) was added sodium carbonate (0.00025 mol). The mixture was stirred at 50 °C for 2 h, and then extracted with ethyl acetate (3 × 25 mL), the organic layer was washed with water, dried over anhyd Na_2SO_4 and evaporated under reduced pressure. Then the crude product was chromatographed on silica gel to afford the desired compound **10**. Yield: 80%; IR (KBr) 3435, 3018, 2990, 2937, 1794, 1751, 1644, 1452, 1383, 1345, 1217, 1148, 1078, 1028, 894, 845, 756, 668 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.08 (s, 1H), 4.86 (s, 1H), 4.76 (s, 2H), 4.58 (s, 1H), 3.45 (d, J = 9.51 Hz, 1H), 3.31 (d, J = 10.26 Hz, 1H), 1.22 & 0.62 (each s, 3H); ^{13}C NMR($CDCl_3$, 75 MHz) 174.77, 147.20, 144.49, 135.00, 107.68, 80.83, 70.51, 64.54, 56.42, 55.66, 43.17, 39.39, 38.54, 37.24, 28.55, 24.88, 24.35, 23.10, 22.30, 15.58; ESI-MS: 335 [M + H].$^+$

*4.6.8. Synthesis of (E)-3-(2-((1R, 4aS, 5R, 6R, 8aS)-6-hydroxy-5-(hydroxymethyl)-5, 8a-dimethyl-2 -methylenedecahydronaphthalen-1-yl) ethylidene) furan-2(3H)-one (**11**)*

$NaBH_4$ (0.00033 mol) was added over 5 min to a stirred mixture of compound **1** (100 mg, 0.00028 mol) and Amberlyst-15 (0.00142 mol) in THF (5 mL) and the reaction was stirred at room temperature for 2 h, and then extracted with ethyl acetate (3 × 25 mL), the organic layer was washed with water, dried over anhyd Na_2SO_4 and evaporated under reduced pressure. Then the crude product was chromatographed on silica gel to afford the desired compound **11** and **9**. Yield: 75%; IR (KBr) 3424, 2925, 2855, 1746, 1643, 1454, 1378, 1218, 1037, 893, 767, 668 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.16 (s, 1H), 6.84 (t, J = 6.56 Hz, 1H), 6.13 (d, J = 15.79 Hz, 1H), 4.81 (d, J = 6.12 Hz, 2H), 4.18 (d, J = 9.79 Hz, 1H), 3.47 (d, J = 10.36 Hz, 1H), 1.25 & 0.80 (each s, 3H); ^{13}C NMR($CDCl_3$, 75 MHz) 170.43, 148.12, 143.09, 129.21, 113.98, 111.07, 109.15, 80.72, 61.19, 55.64, 54.63, 42.87, 38.55, 38.23, 36.56, 28.04, 22.95, 22.68, 15.91; ESI-MS: 333 [M + H].$^+$

*4.6.9. Synthesis of (S, E)-4-hydroxy-3-(2-((1S, 2S, 4aS, 5R, 6R, 8aR)-6-hydroxy-5-(hydroxymethyl)-5, 8a-dimethyloctahydro-1H-spiro [naphthalene-2, 2′-oxirane]-1-yl) ethylidene) dihydrofuran-2(3H)-one (**12**)*

To a solution of compound **1** (100 mg, 0.00028 mol) in methanol (5 mL) was added m-CPBA (0.00033 mol). The mixture was stirred at room temperature for 12 h, and then extracted with ethyl acetate (3 × 25 mL), the organic layer was washed with water, dried over anhyd Na_2SO_4 and evaporated under reduced pressure. Then the crude product was chromatographed on silica gel to afford the desired compound **12**. Yield: 80%; IR (KBr) 3427, 2987, 2471, 2252, 2126, 1647, 1508, 1416, 1242, 1223, 1154, 1028, 820, 758, 664, 622 cm^{-1}; ^{1}H NMR (DMSO, 300 MHz) δ 6.71 (t, J = 6.68 Hz, 1H), 4.83 (s, 1H), 4.36 (t, J = 6.38 Hz, 1H), 4.13 (s, 1H), 3.99 (d, J = 9.56 Hz, 1H), 3.87 (d, J = 10.16 Hz, 1H), 2.76 (d, J = 2.68 Hz, 1H), 2.70 (d, J = 2.56 Hz, 1H), 1.09 & 0.80 (each s, 3H); ^{13}C NMR(DMSO, 75 MHz) 170.89, 148.74, 128.23, 80.33, 79.90, 74.86, 65.44, 63.50, 59.40, 54.76, 53.94, 50.19, 42.93, 37.52, 36.64, 29.83, 28.10, 23.82, 23.15, 15.67; ESI-MS: 367 [M + H].$^+$

*4.6.10. Synthesis of (1R, 4aS, 5R, 8aS)-5-((E)-2-((S)-4 -hydroxy-2-oxodihydrofuran-3(2H)-ylidene) ethyl)-1, 4a -dimethyl-6-methylene-2-oxodecahydronaphthalene-1-carbaldehyde (**13**)*

To a magnetically stirred solution of compound **1** (100 mg, 0.00028 mol) in DCM (5 mL), the whole reaction mixture was brought to 0 °C and DMP (0.00084 mol) was added. After addition of DMP, the whole solution was stirred for 30 min at 0 °C to rt. DCM was removed by vacuum, and neutralized with saturated $NaHCO_3$ then extracted with ethyl acetate (3 × 25 mL), the organic layer was washed with water, dried over anhyd Na_2SO_4 and evaporated under reduced pressure. Then the crude product was chromatographed on silica gel to afford the desired compound **13**. Yield: 70%; IR (KBr) 3624, 3405, 3015, 2947, 2835, 2403, 2037, 1638, 1450, 1415, 1217, 1022, 927, 767, 669 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.76 (s, 1H), 6.91 (t, J = 6.68 Hz, 1H), 5.01 (d, J = 6.23 Hz, 2H), 4.95 (s, 1H), 4.64 (s, 1H), 4.46 (t, J = 6.38 Hz, 1H), 0.87 & 0.68 (each s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) 209.08, 201.04, 173.27, 148.98, 143.87, 130.08, 121.93, 110.04, 70.56, 65.01, 56.52, 55.50, 43.71, 39.46, 39.09, 37.43, 28.89, 23.82, 23.54, 16.75; ESI-MS: 347 [M + H].$^+$

*4.6.11. Synthesis of (4S, E)-4-hydroxy-3-(2-((1R, 4aS, 5R, 6R, 8aS)-6-hydroxy-5-(hydroxymethyl)-2, 5, 8a -trimethyldecahydronaphthalen-1-yl) ethylidene) dihydrofuran-2(3H)-one (**14**)*

Compound **1** (100 mg, 0.00028 mol) was dissolved in MeOH and added Pd (10% on carbon) in a bottle under atmosphere of nitrogen. Then nitrogen was completely replaced by hydrogen in parr assembly. The reaction was allowed to run for 1 h under pressure of 50 psi of H_2. After completion of reaction (1 h, TLC monitoring), the catalyst was removed by filtration through celite, solvent was removed under vacuum, to afford the desired compound **14**. Yield: 90%; IR (KBr) 3435, 3019, 1639, 1427, 1216, 1026, 758, 669 cm^{-1}; ^{1}H

NMR (DMSO, 300 MHz) δ 6.61 (t, J = 6.71 Hz, 1H), 4.81 (s, 1H), 4.62 (s, 1H), 4.38 (t,J = 6.32 Hz, 1H), 4.01 (d, J = 9.56 Hz, 1H), 3.82 (d, J = 10.23 Hz, 1H), 1.17 (s, 3H), 0.87 (d, J = 6.80 Hz, 3H), 0.65 (s, 3H); ^{13}C NMR(DMSO, 75 MHz) 170.37, 148.02, 129.41, 78.90, 74.75, 64.96, 63.09, 55.93, 54.82, 42.71, 40.78, 37.94, 36.95, 28.32, 24.40, 23.49, 15.82, 15.18; ESI-MS: 353 [M + H].$^+$

4.6.12. Synthesis of (4S)-4-hydroxy-3-(2-((1R, 4aS, 5R, 6R, 8aS)-6-hydroxy-5-(hydroxymethyl)-2, 5, 8a -trimethyldecahydronaphthalen-1-yl) ethyl) dihydrofuran-2(3H)-one ***15***

Yield: 90%; IR (KBr) 3436, 3019, 1637, 1215, 1035, 928, 757, 669, 484 cm^{-1};; ^{1}H NMR (DMSO, 300 MHz) δ 4.86 (s, 1H), 4.67 (s, 1H), 4.44 (t, J = 6.28 Hz, 1H), 4.06 (d, J = 9.76 Hz, 1H), 3.86 (d, J = 10.34 Hz, 1H), 1.22 (s, 3H), 0.80 (d, J = 6.86 Hz, 3H), 0.70 (s, 3H); ESI-MS: 355 [M + H]. $^+$

4.6.13. Synthesis of (1R, 2R, 4aS, 5R, 8aS)-5-((E)-2-((S)-4- acetoxy-2-oxodihydrofuran − 3 (2H)-ylidene) ethyl)-1-(acetoxymethyl)-1, 4a-dimethyl-6-methylenedecahydronaphthalen-2-yl acetate ***16***

Yield: 98%; IR (KBr) 3684, 3618, 3436, 3019, 2976, 2400, 1602, 1521, 1476, 1422, 1215, 1045, 928, 877, 849, 770, 669, 627, 497 cm^{-1}; ^{1}H NMR (DMSO, 300 MHz) δ 6.90 (t, J = 6.71 Hz, 1H), 5.70 (d,J = 6.16 Hz, 1H), 5.20 (d, J = 4.98 Hz, 1H), 5.09(s,1H), 4.90 (s, 1H), 4.67 (t, J = 6.42 Hz, 1H), 4.32 (d, J = 9.76 Hz, 1H), 4.11 (d, J = 10.56 Hz, 1H), 2.23 (s, 9H), 1.36 & 0.94 (each s, 3H); ESI-MS: 477 [M + H].$^+$

4.7. Biological experiments

4.7.1. Animals

Rats (Charles Foster strain, male, adult, body wt 200–225 g) were kept in a room with controlled temperature at 25–26 °C, humidity 60–80% and 12/12 h light/dark cycle (light on from 8.00 AM to 8 PM) under hygienic conditions. Animals were acclimatized for one week before starting the experiment. The animals had free access to the normal diet and water.

4.7.2. Antidyslipidemic activity in triton induced hyperlipidemic rat model

Rats were divided into different groups- control, triton treated and triton plus extraction, fractionation and derivatives of compound **1** treated containing six animals in each group. In the acute experiment of 18 h, hyperlipidemia was developed by administration of triton WR-1339 (Sigma Chemical Company, St. Louis, MO, USA) at a dose of 400 mg/kg, b.w. intraperitoneally (i.p) to animals of all the treated groups with extraction, fractionation and derivatives of compound1 were macerated with gum acacia, suspended in water and fed simultaneously with triton at a dose of 100 mg/kg p.o. to the animals of treated groups, diet was withdrawn. Animals of control and triton group without treatment with extraction, fractionation and derivatives of compound **1** were given same amount of gum acacia suspension (vehicle). After 18 h of treatment the animals were anaesthetized with thiopentone solution (50 mg/kg b.w.) prepared in normal saline and 1 mL blood was withdrawn from retro-orbital sinus using glass capillary in EDTA coated tubes (3.0 mg/ml blood). The blood was centrifuged at 2500 × g for 10 min at 4 °C and plasma was separated. Plasma was diluted with normal saline (ratio of 1:3) and used for analysis of total cholesterol (TC), Phospholipid (PL) and triglyceride (TG) by standard enzymatic methods. Using Beckmann auto-analyzer and standard kit purchase from Merck Company and post-heparin lipolytic activity (PHLA) was assayed using spectrophotometer [37]. After the confirmation of most active compounds in primary screening we further, evaluate the activity of compound **1** and compound **7** in same model at different doses (50, 100 & 150 mg/kg b.w).

4.7.3. Lipoprotein measurement

Plasma was fractionated into very low density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL) by poly anionic precipitation methods. Plasma and lipoproteins were analyzed for their total cholesterol (TC), phospholipid (PL), and triglyceride (TG) by standard procedures reported earlier [38].

4.7.4. Lipoprotein lipase activity in liver of triton induced hyperlipidemic rats

Liver was homogenized (10%, w/v) in cold 100 mM phosphate buffer pH 7.2 and used for the assay of total lipolytic activity of lipoprotein lipase (LPL) [39].

4.7.5. Antioxidant activity

Superoxide anions were generated enzymatically by xanthine (160 mM), xanthine oxidase (0.04 U), and nitroblue tetrazolium (320 μM) in absence or presence of compound **1** and **7** at concentrations 200 μg/mL in 100 mM phosphate buffer (pH 8.2). Compounds were sonicated well in phosphate buffer before use. The reaction mixtures were incubated at 37 °C and after 30 min the reaction was stopped by adding 0.5 mL glacial acetic acid. The amount of formazone formed was calculated spectrophotometrically. In another set of experiment effect of compounds on the generation of hydroxyl radical was also studied by non-enzymatic reactants. Briefly, $^{\bullet}$OH were generated in a non-enzymatic system comprising deoxy ribose (2.8 mM), $FeSO_4.7H_2O$ (2 mM), sodium ascorbate (2.0 mM) and H_2O_2 (2.8 mM) in 50 mM KH_2PO_4 buffer (pH 7.4) to a final volume of 2.5 mL. The above reaction mixtures in the absence or presence of test compounds were incubated at 37 °C for 90 min. The test compounds were also studied for their inhibitory action against microsomal lipid peroxidation in vitro by nonenzymatic inducer. Reference tubes and reagents blanks were also run simultaneously. Malondialdehyde (MDA) contents in both experimental and reference tubes were estimated spectrophotometrically by thiobarbituric acid [40]. Alloprinol, Mannitol and α-tocopherol were used as standard drugs for superoxide, hydroxylations and microsomal lipid peroxidation.

4.7.6. LDL oxidation

Serum was separated from the blood of normolipemic donors who were fasted overnight and fractionated into very low-density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL) by ultracentrifugation [41]. The lipoproteins preparations were dialyzed against 150 mM NaCl containing EDTA (0.02% w/v) in presence of N_2 gas in cold. The purity of LDL was checked on polyacrylamide gel electrophoresis. LDL (0.71 mg) and $CuCl_2 \cdot 2H_2O$ (10 μM) in the absence or presence of compound 2 in 50 mM phosphate buffer saline (pH 7.4) to a final volume of 1.5 mL, was incubated at 37 °C for 16 h. The level of lipid peroxides in unoxidized LDL, oxidized LDL with Cu^{++} in the absence or presence of compounds **1** and **7** at dose of 200 μg/mL were assayed as thiobarbuteric acid reactive substances (TBARS). Briefly the reaction mixture contained 0.5 mL SDS (8% w/v), 0.5 ml glacial acetic acid, 1.5 ml TBA (0.8% w/v) was heated in a boiling water bath for 1 h. After cooling up to room temperature optical density of reaction mixture was read at 532 nm with respective reagent blank. The level of lipid peroxide as nmol of Malondialdehyde formed was calculated by taking absorption Coefficient of MDA as 1.78 × 10^5 cm^{-1} M^{-1} mg protein.

4.7.7. Statistical evaluation

All results are presented as the means ± S.D. of results from three independent experiments. Groups were analyzed via *t*-tests (two-sided) or ANOVA for experiments with more than two subgroups. Probability values of $p < 0.05$ were considered to be statistically significant.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.09.002.

References

[1] A.J. Lusis, Atherosclerosis review, Nature 407 (2000) 233–241.
[2] S.M. Grundy, Cholesterol and coronary heart disease a new era, J. Am. Med. 256 (1986) 2849–2858.
[3] G. Davey Smith, Cholesterol lowering and mortality, the importance of considering initial level of risk, Int. J. Med. 306 (1993) 1367–1373.
[4] R. Chattopadhyaya, D. Pathak, D.P. Jindal, Antihyperlipidemic agents, a review, Ind. Drugs 33 (1996) 85–98.
[5] G. Davey Smith, J. Pekkanen, Should there be a moratorium on the use of cholesterol lowering drugs, Br. J. Med. 304 (1992) 431–440.
[6] S. Parthasrthy, D. Steinbert, J.L. Switztum, The role of oxidized low-density lipoproteins in the pathogenesis of atherosclerosis, Ann. Rev. Med. 43 (1992) 219–225.
[7] C.D.A. Stehouwer, J. Lambert, A.J. Donker, V.W.M. Mand Vanhinsbergh, Endothelial dysfunction and pathogenesis of diabetic angiopathy, Cardiovasc. Res. 34 (1997) 55–61.
[8] S.L. Brown, Lowered serum cholesterol and low mood, Br. J. Med. 313 (1996) 637–638.
[9] T.M. Speight, Avery's, Drug Treatment Principles and Practice of Clinical Pharmacology and Therapeutics, third ed., ADIS Press Ltd, 1987, p. 599.
[10] E.A. Werner, J. Bell, Antihyperglycemic and antidyslipidemic agent from *Aegle marmelos*, J. Chem. Soc. 221 (1922) 1790–1794;
(a) S.L. Shapiro, V.A. Parrino, L. Freedman, Hypoglycemic agents. III. N-Alkyl- and aralkyl biguanides, J. Am. Chem. Soc. 81 (1959) 3728–3736.
[11] C.T. Bailey, Traditional plant medicines as treatments for diabetes, Diabetes Care 12 (1989) 553–564.
[12] J.P. Deslypere, The role of HMG-Co.A reductase inhibitors in the treatment of hyperlipidemia: a review of fluvastatin, Curr. Ther. Res. 56 (1995) 111–128.
[13] K.J. McClellan, R. Lynda, Wiseman, Donna McTavish, Cerivastatin, Drugs 55 (1998) 415–420.
[14] A. Yamamoto, T. Yamamura, S. Yokoyama, H. Sudo, Y. Matsuzara, Combined drug therapy cholestyramine and compactin for familial hypercholesterolemia, Int. J. Clin. Pharmacol. Ther. Toxicol. 22 (1984) 493–497.
[15] H.O. Bang, J. Dyerberg, A.B. Nielsen, Plasma lipid and lipoprotein pattern in Greenlandic west-coast Eskimos, Lancet 1 (1971) 1143–1146;
(a) H.O. Bang, J. Dyerberg, Plasma lipids and lipoproteins in Greenlandic west coast Eskimos, Acta Med. Scand. 192 (1972) 85–94;
(b) H.O. Bang, J. Dyerberg, N. Hjorne, The composition of food consumed by Greenland Eskimos, Acta Med. Scand. 200 (1976) 69–73.
[16] T. Narender, Shweta, P. Tiwari, K. Papi Reddy, T. Khaliq, P. Prathipati, A. Puri, A.K. Srivastava, R. Chander, S.C. Agarwal, K. Raj, Anti hyperglycemic and antidyslipidemic agent from *Aegle marmelos*, Bio-org. Med. Chem. Lett. 17 (2007) 1808–1811.
[17] T. Narender, P. Anju, Shweta, K. Tanvir, S. Rashmi, G. Bhatia, R. Chander, 4-Hydroxy isoleucine an unusual amino acid as antidyslipidemic and anti-hyperglycemic agent, Bio-org. Med. Chem. Lett. 16 (2006) 293–296.
[18] M. Roxas, J. Jurenka, Colds and influenza: a review of diagnosis and conventional, botanical and nutritional considerations, Altern. Med. Rev. 12 (2007) 25–48.
[19] W.F. Chiou, J.J. Lin, C.F. Chen, Andrographolide suppresses the expression of inducible nitric oxide synthase in macrophage and restores the vasoconstriction in rat aorta treated with lipopolysaccharide, Br. J. Pharmacol. 125 (1998) 327–334.
[20] H.R. Tsai, L.M. Yang, W.J. Tsai, W.F. Chiou, Andrographolide acts through inhibition of ERK1/2 and Akt phosphorylation to suppress chemotactic migration, Eur. J. Pharmacol. 498 (2004) 45–52.
[21] L. Chen, H. Zhu, R. Wang, K. Zhou, Y. Jing, F. Qiu, ent-Labdane diterpenoid lactone stereoisomers from *Andrographis paniculata*, J. Nat. Prod. 71 (2008) 852–855.
[22] C. Satyanarayana, D.S. Deevi, R. Rajagopalan, N. Srinivas, S. Rajagopal, DRF3188 a novel semi-synthetic analog of andrographolide: cellular response to MCF 7 breast cancer cells, BMC Cancer 4 (2004) 26–33.
[23] Y.F. Xia, B.Q. Ye, Y.D. Li, J.G. Wang, X.J. He, X. Lin, Andrographolide attenuates inflammation by inhibition of NF-κB activation through covalent modification of reduced cysteine 62 of p50, J. Immunol. 173 (2004) 4207–4217.
[24] V.L. Reddy, S.M. Reddy, V. Ravikanth, P. Krishnaiah, T.V. Goud, T.P. Rao, T.S. Ram, R.G. Gonnade, M. Bhadbhade, Y. Venkateswarlu, New bisandrographolide ether from *Andrographis paniculata* nees and evaluation of anti-HIV activity, Nat. Prod. Res. 19 (2005) 223–230.
[25] A. Jaruchotikamol, K. Jarukamiorn, W. Sirisangtrakul, T. Sakuma, Y. Kawasaki, N. Nemoto, Strong synergistic induction of CYP1A1 expression by andrographolide plus typical CYP1A inducers in mouse hepatocytes, Toxicol. Appl. Pharmacol. 224 (2007) 156–162.
[26] J.H. Chen, G. Hsiao, A.R. Lee, C.C. Wu, M.H. Yen, Andrographolide suppresses endothelial cell apoptosis via activation of phosphatidyl inositol-3- kinase/Akt pathway, Biochem. Pharmacol. 67 (2004) 1337–1345.
[27] A. Kapil, I.B. Koul, S.K. Banerjee, B.D. Gupta, Antihepatotoxic effects of major diterpenoid constituents of *Andrographis paniculata*, Biochem. Pharmacol. 46 (1993) 182–185.
[28] S. Das, S. Neogy, N. Gautam, S. Roy, *In vitro* nitotine induced superoxide mediated DNA fragmentation in lymphocytes: protective role of *Andrographis paniculata* Nees, Toxicol. In vitro 23 (2009) 90–98.
[29] B.C. Yu, C.R. Hung, W.C. Chen, J.T. Cheng, Antihyperglycemic effect of andrographolide in streptozotocin induced diabetic rats, Planta Med. 69 (2003) 1075–1079.
[30] C.J. Huang, M.C. Wu, Differential effects of foods traditionally regarded as 'heating' and 'cooling' on prostaglandin E2 production by a macrophage cell line, J. Biomed. Sci. 9 (2002) 596–606.
[31] W.W. Chao, Y.H. Kuo, W.C. Li, B.F. Lin, The production of nitric oxide and prostaglandin E2 in peritoneal macrophages is inhibited by *Andrographis paniculata, Angelica sinensis* and *Morus alba* ethyl acetate fractions, J. Ethnopharmacol. 122 (2009) 68–75.
[32] S.C. Mandal, A.K. Dhara, B.C. Maiti, Studies on psychopharmacological activity of *Andrographis paniculata* extract, Phytother. Res. 15 (2001) 253–256.
[33] A.C. Kaliora, G.V.Z. Dedoussis, H. Schmidt, Dietary antioxidants in preventing atherogenesis, Atherosclerosis 187 (2006) 1–17.
[34] S.K. Wattanapitayakul, J.A. Bauer, Oxidative pathways in cardiovascular disease: roles, mechanisms and therapeutic implications, Pharmacol. Ther. 89 (2001) 187–206.
[35] K.K. Griendling, M. Ushio-Fukai, Redox control of vascular smooth muscle proliferation, J. Lab. Clin. Med. 132 (1998) 9–15.
[36] A. Shrivastava, U. Chaturvedi, R. Sonkar, A.K. Khanna, J.K. Saxena, G. Bhatia, Antioxidant effect of *Azadirachta indica* on high fat diet induced diabetic Charles foster rats, Appl. Biochem. Biotechnol. 167 (2012) 229–236.
[37] D.R. Wing, D.S. Robinson, Clearing factor lipase in adipose tissue, Biochem. J. 109 (1968) 841–849.
[38] A.K. Khanna, R. Chander, C. Singh, A.K. Srivastava, N.K. Kapoor, Hypolipidemic activity of *Terminalia chebula* in rats, Fitoterpia 64 (1993) 351–356.
[39] D.R. Wing, D.F. Robinson, J. Biochem. 109 (1968) 841–848.
[40] H. Okhawa, Qohishi, assay for lipid peroxides in animal tissues by thiobarbituric acid reaction, Anal. Biochem. 95 (1978) 351–358.
[41] R.J. Havel, H.A. Edor, J.A. Bingdon, The distribution and chemical composition of ultracentrifugically separated lipoproteins in human serum, J. Clin. Invest 34 (1955) 1345–1353.