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ORIGINAL ARTICLE

Antidyslipidemic and Antioxidant Effects of Novel Lupeol-Derived Chalcones

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Abstract A series of Lupeol-based chalcones have been synthesized aiming to enhance the therapeutic efficacy of parent compound, the novel compounds were evaluated for their antidyslipidemic activity in triton-WR 1339 induced hyperlipidemic rats. Among the ten synthesized chalcones, the most active K4, K8, and K9 reversed the plasma levels of TC by (24, 25, 27 %), phospholipid by (25, 26, 25 %) and triacylglycerol by (27, 24, 24 %) respectively. In addition, the compounds showed significant in vitro antioxidant activity. The lipid lowering activity of these compounds were mediated through lipoprotein lipase activation (12–21 %) and enhanced post-heparin lipolytic activity (15–16 %). The compounds also displayed noteworthy inhibitory effect on 3-hydroxy-3-methyl-glutaryl reductase activity (in vitro). The in vitro effect of the most active compounds on MDI-induced adipogenesis using 3T3-L1 preadipocytes at 10 and 20 μM concentrations showed significant inhibition (20–32 %) of adipogenesis.

Keywords Chalcones · Lupeol · Lupenone · Lipoprotein lipase (LPL) · Antihyperlipidemic activity

Abbreviations

PHLA	Post-heparin lipolytic activity
LPL	Lipoprotein lipase
TC	Total cholesterol
TAG	Triacylglycerol
PL	Phospholipids
HDL	High density lipoprotein
LDL	Low density lipoprotein
VLDL	Very low density lipoprotein
CVD	Cardiovascular diseases
DCM	Dichloromethane
PCC	Pyridinium chlorochromate
MHz	Mega hertz
NMR	Nuclear magnetic resonance
ESI	Electrospray ionization
MS	Mass spectrometry

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Introduction

Hypercholesterolemia is the main cause and concern for development of atherosclerosis and other related fallouts like myocardial ischemia and cardiac events [1]. Elevation of blood cholesterol followed by the generation of reactive oxygen species lead to endothelial dysfunction [2]. Cellular damage to the endothelium is the basic cause for initiation and maintenance of atherosclerosis. The cumulative effect of hyperlipidemia and free radical species has a detrimental effect on the body and begins a chain of events leading to

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coronary heart disease (CHD). Among the drug therapy approaches for the disease, Statins have significantly reduced the risk of CHD related deaths over the past two decades [3]. Statins [4] generally focus on reducing the low density lipoproteins (LDL). Although statins are effective as a treatment but their drawback is that they induce certain adverse coronary events as well as not possessing antioxidant properties. Fibrates represent another class of compounds used for the treatment of hyperlipidemia but requires higher doses for significant effects [5]. The combination therapy is also used for these two classes resulting in serious safety concerns. Therefore, there is an emergent need for different classes of compounds to combat this dreaded metabolic disorder. Natural products are the most promising and consistent source of drug leads. As a part of our drug discovery program on Indian medicinal plants actively pursued by CDRI, we have been working on the indigenous plant, *Crataeva nurvala* Buch–Ham (Capparidaceae). The bark of the plant is a major source of lupeol, a molecule of interest, a pentacyclic triterpene with diverse biological activities like antiurolithic [6], anti-inflammatory [7], cytoprotective [8], antidiabetic, and antidyslipidemic [9].

The present study deals with (1) the synthesis of various lupeol derived chalcones to enhance the therapeutic efficacy of the parent compound against dyslipidemia, (2) estimation of the plasma lipid profile and determination of the possible mechanism of action, (3) evaluation of the antioxidant potential of the derivatives, and (4) effects of the most active compounds on 3-hydroxy, 3-methyl-glutaryl CoA (HMG-CoA) reductase activity and adipogenesis (3T3L-1 cell line) in a dose-dependent manner.

Materials and Methods

Chemistry

Plant Material

The stem bark of *C. nurvala* was purchased from a local market and identified by the Botany Department of CDRI, Lucknow, India. A voucher specimen (no. 3664) has been preserved for future reference in our institution.

Isolation of Lupeol

Lupeol was isolated from the stem bark of *C. nurvala* [5]. *C. nurvala* stem bark powder (1 kg) was extracted with petroleum ether by cold percolation and the resulting extract was subjected to column chromatography on silica gel (60–120 mesh), eluting successively with hexane, hexane–benzene, and benzene. The fraction collected with benzene gave a single component, lupeol (yield 0.7 %), mp 215 °C, which was identified by spectroscopic techniques [10].

Preparation of Lupenone (I) from Lupeol (S)

Lupeol (1) (10 g, 23.37 mmol) dissolved in dichloromethane (50 ml) was stirred at room temperature with pyridinium chlorochromate (35.2 mmol, 1.5 equivalent) for 24 h. The mixture was filtered through Celite with ether, concentrated on a rotavapor to give the product **2** (8 g, 80 %), mp 166–168 °C [11].

Representative Procedure for the Synthesis of Target Compounds (K1–K10)

To a magnetically stirred solution of lupenone (**I**) (0.424 g, 1.0 mmol) in ethanol (20 ml), different substituted benzaldehydes (1.0 mmol) and NaOH (1.0 mmol) were added. The reaction mixture was stirred for 24 h at room temperature. After completion of the reaction (TLC monitoring) ethanol was evaporated under reduced pressure. The compound was extracted with chloroform. The combined organic extract was washed with water, brine solution, dried (Na_2SO_4) and solvent was removed under reduced pressure. The crude product obtained was column chromatographed (60–120 mesh, SiO_2) using hexane and chloroform in different concentrations (ranging from 10 to 80 % chloroform in hexane) to give the target compounds. (Scheme 1). These chalcone derivatives were synthesized in moderate to good yield (Table 1).

Biology

Animals

Male adult rats of the Charles Forest (CF) strain (body weight 100–150 g) were kept in animal houses with controlled temperature (25–26 °C), humidity (60–80 %) and 12/12 h light/dark cycle, under hygienic conditions. Before starting the experiment, the animals were acclimatized for 1 week and had free access to the normal diet and water ad libitum. Experimental procedures were approved by ethical committee of our institute.

Lipid Lowering Activity in Triton-Induced Hyperlipidemic Rats

The rats were divided into 15 groups [group 1: control, group 2: triton-induced hyperlipidemic rats, group 3–14: triton-induced hyperlipidemic rats with compounds

Scheme 1 Synthesis of lupeol derived chalcone. Reagents and conditions: (*a*) pyridinium chlorochromate (pcc), DCM, 24 h. (*b*) Different aromatic aldehyde (RCHO), NaOH, ethanol, 24 h

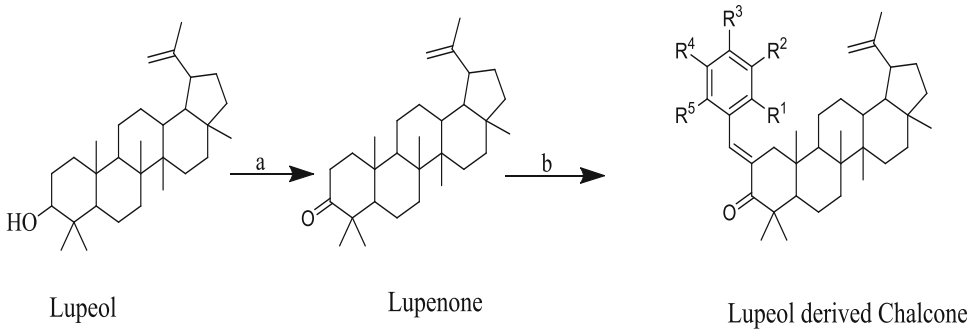


Table 1 Percentage yield of differently substituted chalcone derivatives of lupeol

Compound no.	R^1	R^2	R^3	R^4	R^5	Yield %
K1	H	OCH_3	OCH_3	OCH_3	H	63
K2	H	OCH_3	OCH_3	H	H	61
K3	H	H	C_2H_6N	H	H	67
K4	H	H	CH_3O	H	H	62
K5	H	OCH_3	$C_6H_5OCH_2$	H	H	54
K6	H	NO_2	H	H	H	55
K7	H	H	Cl	H	H	60
K8	H	H	Br	H	H	49
K9	H	H	NO_2	H	H	53
K10	H	H	F	H	H	46

(S = lupeol, I = lupenone, K1–K10), and group 15: triton-induced hyperlipidemic rats treated with the standard drug Gemfibrozil (100 mg/kg)] containing six rats in each group. In this 18-h acute experiment, hyperlipidemia was induced by the administration of triton WR-1339 [12–15] (Sigma chemical company, St. Louis, MO, USA) intraperitoneally at a dose of 400 mg/kg body weight to rats of all the groups except the control group. The compounds were macerated with gum acacia suspended in water (0.2 % w/v) and fed orally simultaneously with triton at a dose of 100 mg/kg [16, 17] and the diet was withdrawn. Rats of the control and triton-treated groups were given the same amount of the gum acacia suspension (vehicle). After 18 h of treatment, the rats were anaesthetized with thiopentone solution (50 mg/kg body weight) prepared in normal saline and then 1.0 ml blood was withdrawn from the retro to orbital plexus using a glass capillary and put into EDTA coated tubes (3.0 mg/ml blood). The blood was centrifuged at 2,500×*g* for 10 min at 4 °C and the plasma was separated and used for analysis of total cholesterol (TC), phospholipids (PL), triacylglycerol (TAG) by standard enzymatic methods [18–20]. Lipoprotein lipase (LPL) and post-heparin lipolytic activity (PHLA) were determined as per the method reported earlier [21, 22]. Total cholesterol (TC), phospholipid (PL), triacylglycerol (TAG), and apo-lipo-protein contents of VLDL, LDL and HDL were analyzed by standard procedures [23]. The free radical scavenging potential of these compounds against formation of superoxide anions (O_2^-) and hydroxyl free radicals ($OH^·$) as well as their effect on microsomal lipid peroxidation were measured (in vitro) in the absence or presence of the compounds (100 µg and 200 µg/ml) using the method reported earlier [24].

In-Vitro HMG-CoA Reductase Inhibitory Activity

The HMG-CoA reductase assay was performed using the HMG-CoA reductase assay kit from Sigma-Aldrich (St. Louis, MO, USA). HMG-CoA (substrate), NADPH, assay buffer and enzyme HMGR were supplied with the assay kit.

Anti-Adipogenic Assay

The 3T3-L1 mouse embryo fibroblast cells were obtained from the American Type Culture Collection. Cells were seeded in 24-well plates (50,000 cells/well) and incubated at 37 °C and 5 % CO_2 in Dulbecco's Modified Eagle Medium (DMEM) containing 10 % heat-inactivated FBS (v/v) and antibiotic amphotericin B and gentamycin, after 48 h of incubation, the culture media was replaced with adipogenesis media, i.e., MDI + ve (insulin 5 µg/ml, dexamethasone 1 µM and isobutylmethylxanthine (IBMX) 0.5 mM). Two days after the induction medium treatment, the cells were treated with insulin alone (5 µg/ml in DMEM containing 10 % FBS). The culture medium was replaced every 48 h until the preadipocytes differentiated into mature adipocytes. The most active compounds (K4, K8, and K9) were assessed for their effect on 3T3-L1 adipocyte differentiation. These compounds were added at concentrations of 10 and 20 µM throughout the differentiation. The differentiated cells were fixed in 4 % paraformaldehyde w/v for 20 min, washed with phosphate

buffered saline (PBS) and stained with 0.34 % Oil Red O in 60 % isopropanol for 20 min. Then they were washed with PBS two times and the stain was extracted with 100 % isopropanol by keeping it at room temperature for 10 min on an orbital shaker. An OD of the extracted dye was taken at 520 nm [25].

Statistical Analysis:

All groups were compared by one way analysis of variance (ANOVA) and the significance of the mean difference between different groups was done by Tukey's post hoc test. A two-tailed ($\alpha = 2$) probability $p < 0.05$ was considered statistically significant ($*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $p > 0.05$ (ns = not significant).

Results

The acute administration of triton WR-1339 caused a marked increase in plasma levels of TC (2.00 fold), PL (1.59 fold), and TAG (2.83 fold) and inhibition of plasma PHLA (19 %). A significant decrease was noticed in TC, PL, and TAG after treatment with lupeol derivatives in comparison to the parent compound. However, gemfibrozil exerted a greater lipid lowering effect than these compounds The results shows that lupeol derivatives K4, K8, and K9 significantly decreased the levels of TC by 24, 25, and 27 %; PL by (25, 26, and 25 % and TAG by 27, 24, and 24 % respectively. Other compounds were mildly active. The lupeol derivatives partially reactivated PHLA activity in hyperlipidemic rats. K4, K8, and K9 were found to be more active and enhanced the PHLA by 15, 15, and 16 % respectively (Table 2). Other compounds were moderately active.

The most active compounds were further analyzed for their effect on the lipid profile of lipoproteins. The triton administration in rats significantly increased the TC, PL, TAG and apo-protein level of VLDL by 2.8, 1.8, 22, and 2.3 folds, LDL by 2.4, 3.1, 3.0 and 2.0 folds and followed by decrease in HDL by 23, 26, 25, and 23 % respectively. The results shows that lupeol derivatives K4, K8, and K9 significantly decreased the levels of VLDL–TC by 22, 24, and 24 %; VLDL–PL by 20, 23, and 25 %; VLDL–TAG by 20, 16, and 27 % and VLDL–apoprotein by 23, 26, and 25 % respectively. Similarly LDL–TC decreased by 25, 26, and 23 %; LDL–PL by 27, 25, and 26 %; LDL–TAG by 23, 27, and 25 % and LDL–apoprotein by 26, 23, and 25 % followed by increase in HDL–TC by 25, 26, and 22 %, HDL–PL by 23, 22, and 24 %, HDL–TAG by 22, 24, and 25 % and HDL–apoprotein by 25, 23, and 24 % respectively (Fig. 1).

Table 2 Antihyperlipidemic activity of lupeol based chalcone derivatives (**K1–K10**) in Triton-induced hyperlipidemic model at a dose of 100 mg/kg

S. no.	Compound	TC[a]	PL[a]	TAG[a]	PHLA[b]
1	S (Lupeol)	−11*	−12*	−11*	+3
2	I (Lupenone)	−13*	−14*	−13*	+4
3	K1	−13*	−13*	−11*	+8
4	K2	−15*	−16*	−14*	+10
5	K3	−15*	−11*	−11*	+7
6	K4	−24***	−25***	−27***	+15
7	K5	−10*	-8^{ns}	-9^{ns}	+5
8	K6	-6^{ns}	-9^{ns}	-5^{ns}	+3
9	K7	-9^{ns}	−10*	−12*	+5
10	K8	−25***	−26***	−24***	+15
11	K9	−27***	−25***	−24***	+16
12	K10	-9^{ns}	-6^{ns}	-8^{ns}	+4
13	Gemfibrozil (Standard)	−31***	−33***	−33***	+19

Values are means ± SD of six rats

Units of chalcone series compounds: [a]mg/dl, [b]*n* mol of free fatty acids formed/h/ml of plasma

TC Total cholesterol, *PL* phospholipid, *TAG* triacylglycerol, *ns* insignificant starting, *I* intermediate

*** $p < 0.001$; ** $p < 0.01$; *$p < 0.05$

Triton administration inhibited the LPL activity by 35 % while treatment with K4, K8, and K9 reactivated the LPL activity by 12.6 %, 16.1, and 20.8 % respectively (Fig. 2). The results are significant and explain the possible mechanism of action.

The inhibitory activity of K4, K8, and K9 were evaluated at five different concentrations ranging from 5 to 80 μM for HMG-CoA reductase activity. Compounds exhibited maximum inhibition at a dose of 80 μM (69.5 64.44, and 70.30 %) (Fig. 3), results are comparable to those of lovastatin which showed an inhibition of 73.13 % at this dose.

It is well established that an excess of reactive oxygen species induces lipid peroxidation as well as protein modification, which in turn results in cardiovascular complications. In view of the above facts, we evaluated antioxidant activity of the lupeol derivatives in vitro. Compounds K2, K4, K8, and K9 significantly decreased superoxide anions (17, 28, 23, and 23 % at 100 μg/ml and 27, 39, 39, and 25 % at 200 μg/ml), hydroxyl radicals (18, 15, 17, and 18 % at 100 μg/ml and 25, 24, 26, and 20 % at 200 μg/ml) and microsomal lipid peroxidation (17, 16, 18, and 12 % at 100 μg/ml and 28, 25, 26, and 20 % at 200 μg/ml) respectively. Other compounds were moderately active. The standard drug Allopurinol, Mannitol and

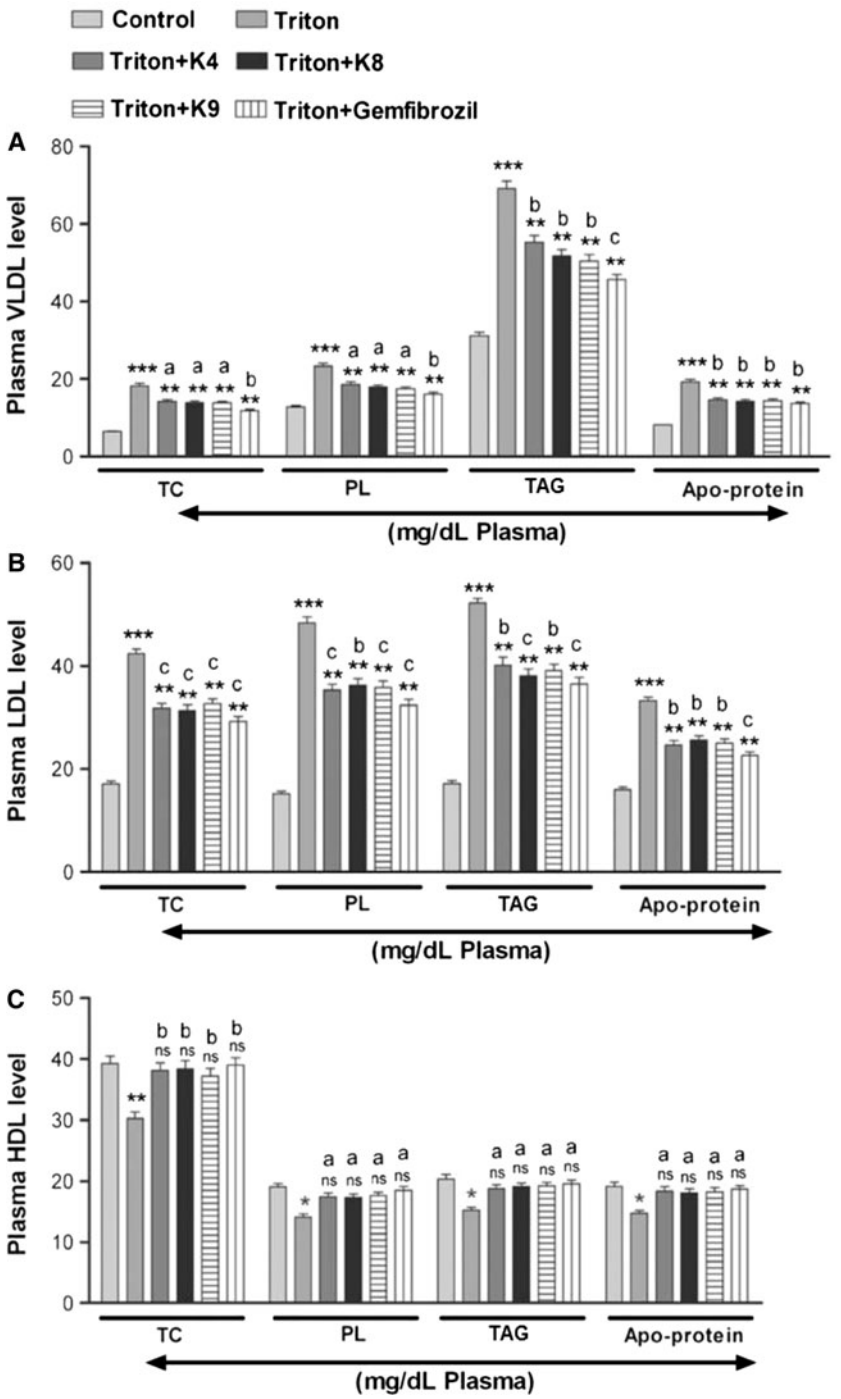


Fig. 1 Effect of compounds K4, K8, and K9 on lipoprotein metabolism of triton-induced hyperlipidemic rats. Compounds K4, K8, and K9 (100 mg/kg) improve the lipoprotein (VLDL, LDL, and HDL) lipid levels in triton-induced hyperlipidemic rats. Each parameter represents pooled data from six rats/group and values are expressed as means ± SD ***$p < 0.001$; **$p < 0.01$; ns (non significant) comparison of the control group with the triton and triton plus compounds treated rat groups, $^{c}p < 0.001$; $^{b}p < 0.01$; $^{a}p < 0.05$ comparison between the triton and the triton plus compounds treated groups. Gemfibrozil was taken as the standard drug

α-Tocopherol at 200 μg/ml concentrations showed 41, 40, and 48 % inhibition of superoxide anions, hydroxyl free radicals and microsomal lipid peroxidation, respectively. (Table 3).

The result of the anti-adipogenic assay show that MDI-induced 3T3-L1 preadipocytes cells exhibited greater lipid accumulation as compared to MDI-ve cells, treatment with compounds K4, K8, and K9 significantly inhibited MDI-induced adipogenesis in 3T3-L1 preadipocytes at concentrations 10 and 20 μM each (Fig. 4).

Physicochemical Data of Compounds K1–K10

Melting points were recorded on a Buch-530 capillary melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer AC-1 spectrometer.

^{1}H-NMR spectra were run on a Bruker Avance DPX 300 at 200 MHz in $CDCl_3$. ^{13}C-NMR spectra were recorded at 75 MHz and 50 MHz in $CDCl_3$. Chemical shifts are reported as values in ppm relative to $CHCl_3$ (7.26) in $CDCl_3$ and TMS was used as internal standard. ESI mass spectra were recorded on a JEOL SX 102/DA-6000 instrument. Chromatography was carried out with silica gel (60–120 mesh) using mixtures of chloroform and hexane as eluents in varying ratios.

Compound K1 was prepared from lupenone and 3,4,5-trimethoxy benzaldehyde using a representative procedure to afford the pure compound with 70 % chloroform in hexane as the eluent. Yield: 63 %; mp: 140–142 °C; IR (KBr cm^{-1}): 3,452, 2,938, 2,367, 1,659, 1,440, 1,128, 1,027.^{1}H NMR: (300 MHz, $CDCl_3$) δ 7.4 (s, 1H), 6.64 (s, 2H),4.69 (s, 1H),4.58 (s, 1H), 3.88 (s, 3H), 3.85 (s, 6H), 2.48–2.37 (m, 1H), 1.90–2.02 (m, 1H), 1.75 (s, 3H), 1.49–1.25 (m, 24H), 1.17 (s, 3H), 1.13 (s, 3H), 1.10 (s, 3H), 1.02 (s, 3H), 0.82 (s, 3H). ^{13}C NMR:(75 MHz, $CDCl_3$) δ 208.13, 152.92, 150.73, 137.23, 133.94, 131.54, 109.27, 107.68, 60.97, 56.19, 52.73, 48.47, 48.30, 47.91, 43.07, 42.94, 40.70, 40.00, 38.20, 36.40, 35.51, 33.06, 29.97, 29.61, 27.44, 25.36, 22.36, 21.80, 20.45, 19.49, 18.01, 15.86, 15.45, 14.49. HRMS (EI) calc. for $[M + H]^+$: $C_{40}H_{59}O_4$: 603.4413, found 603.4369.

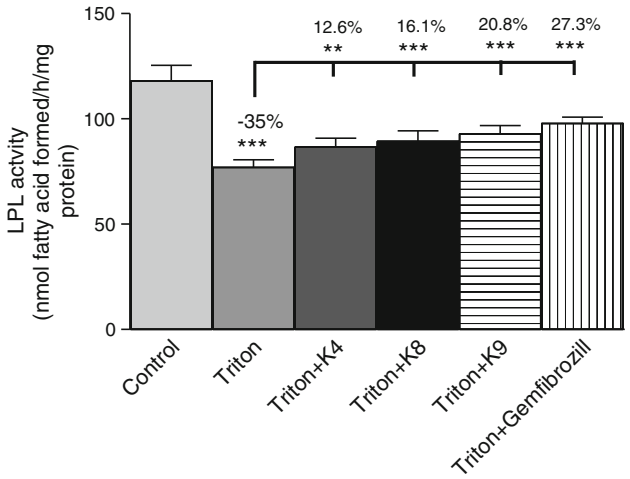
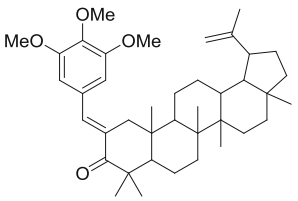


Fig. 2 Effect of compounds K4, K8, and K9 on lipoprotein lipase (LPL) activity. Compound K4, K8, and K9 at different doses 100 mg/kg re-activate hepatic LPL activity (+12.6, +16.1, and 20.8 %) in triton-induced hyperlipidemic rats. Each parameter represents pooled data from 6 rats/group and values are expressed as means ± SD. ***$p < 0.001$; **$p < 0.01$ between the control and the triton, and the triton plus compound treated rats groups, Gemfibrozil (100 mg/kg) was taken as the standard drug

K1



(*E*)-3a,5a,5b,8,8,11a-hexamethyl-1-(prop-1-en-2-yl)-10-(3,4,5-trimethoxybenzylidene)octadecahydro-1*H*-cyclopenta[*a*]chrysen-9(5b*H*)-one

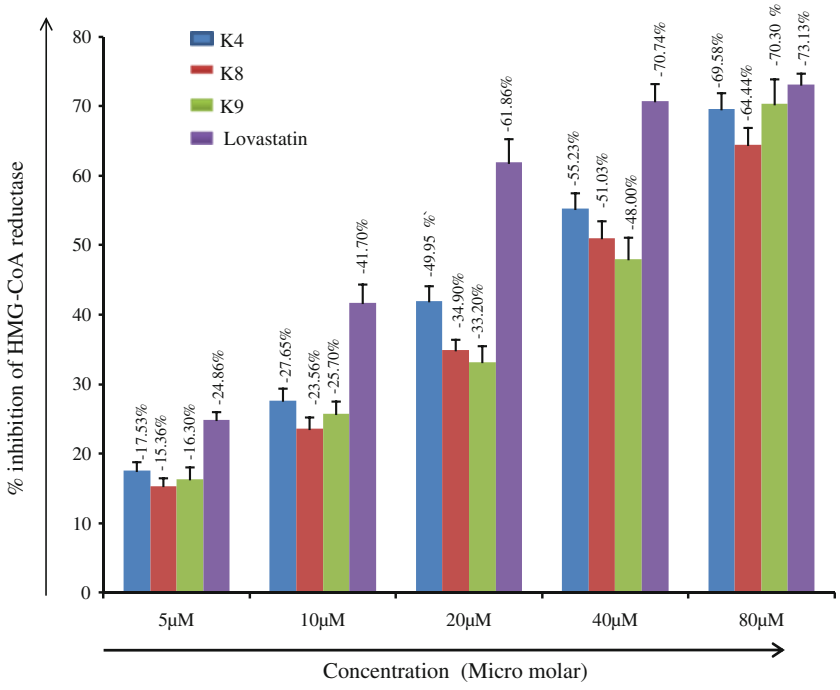


Fig. 3 Effect of compounds K4, K8, and K9 on HMG-CoA reductase activity in a dose-dependent manner. The inhibitory effect of compounds K4, K8, and K9 on HMG-CoA reductase activity (in vitro) in a dose-dependent manner

Table 3 Antioxidant activity of lupeol, lupenone, and lupeol derivatives

S. no	Compound	Dose (μg/ml)	Superoxide[a] anions (O_2^-)	Hydroxyl ions[b](OH^-)	Microsomes lipid peroxidation[b]
1	S (Lupeol)	100	−20**	−17*	−11*
		200	−24***	−20**	−18*
2	I (Lupenone)	100	−20**	−18*	−14*
		200	−25***	−22***	−20**
3	K2	100	−17*	−18*	−17*
		200	−27***	−25***	−28***
4	K4	100	−28***	−15*	−16*
		200	−39***	−24***	−25***
5	K8	100	−23***	−17*	−18*
		200	−39***	−26***	−26***
6	K9	100	−23***	−18*	−12*
		200	−25***	−20**	−20**
7	Standard	200	−41***	−40***	−48***
			Allopurinol	Mannitol	α-Tocopherol

Values are means ± SD of six rats

Units: [a] *n* mol formazone formed/minute. [b] *n* mole MDA formed/h

*** $p < 0.001$; ** $p < 0.01$; *$p < 0.05$

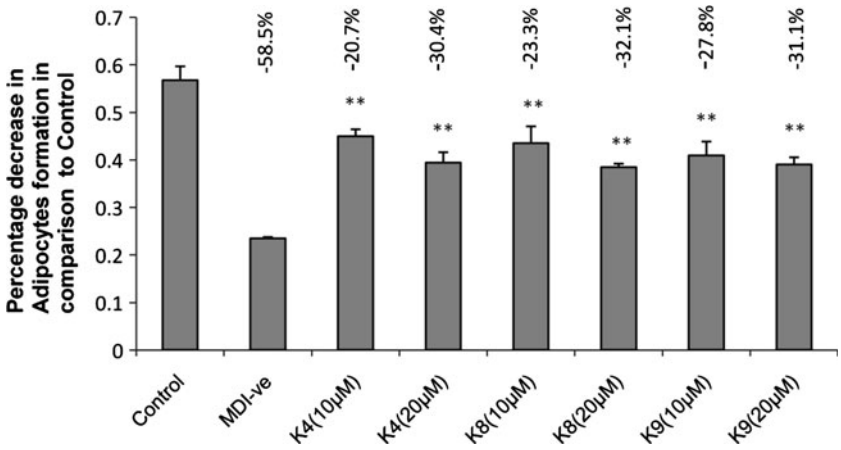
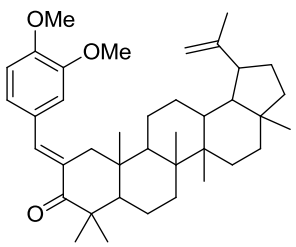


Fig. 4 Effect of compounds K4, K8, and K9 on MDI induced adipogenesis of 3T3-L1 cells. Effects of K4, K8, and K9 on adipogenesis of 3T3-L1 cells. Data are presented as means ± SD of triplicate experiments ($n = 3$). Values are means ± SD ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$. All groups were compared with the control. *Control* MDI +ve, MDI *3-isobutyl 1-methylxanthine*, dexamethasone and insulin

K2

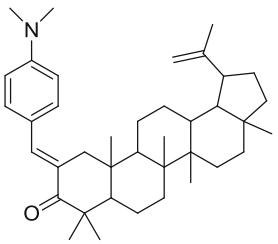


(*E*)-10-(3,4-dimethoxybenzylidene)-3a,5a,5b,8,8,11a-hexamethyl-1-(prop-1-en-2-yl)octadecahydro-1*H*-cyclopenta[*a*]chrysen-9(5b*H*)-one

Compound K2 was prepared from lupenone and 3,4-dimethoxy benzaldehyde using the representative procedure to afford the pure compound with 60 % chloroform in hexane as the eluent. Yield: 61 %; mp: 160–162 °C; IR (KBr cm^{-1}): 3,397, 2,947, 2,368, 1,668, 1,456, 1,254, 1,143, 1,026. ^{1}H NMR: (300 MHz, $CDCl_3$) δ 7.5 (s, 1H), 7.08 (d, $J = 8.49$ Hz, 2H), 6.95 (s, 1H) 4.72 (s, 1H), 4.62 (s, 1H), 3.93 (s, 3H), 3.87 (s, 3H), 2.48–2.37 (m, 1H), 1.90–2.02 (m, 1H), 1.75 (s, 3H), 1.49–1.25 (m, 24H), 1.17 (s, 3H), 1.13 (s, 3H), 1.10 (s, 3H), 1.02 (s, 3H), 0.82 (s, 3H)^{13}C NMR: (75 MHz, $CDCl_3$) δ 208.13, 151.05, 149.42, 148.63, 137.25, 132.65, 128.98, 123.03, 114.38, 111.03,

109.29,55.91, 55.87, 52.73, 48.47, 48.30, 47.91, 43.07, 42.94, 40.70, 40.00, 38.20, 36.40, 35.51, 33.06, 29.97, 29.61, 27.44, 25.36, 22.36, 21.80, 20.45, 19.49, 18.01, 15.86, 15.45, 14.49;HRMS (EI) calc. for $[M + H]^+$: $C_{39}H_{57}O_3$: 573.4308, found 573.4293.

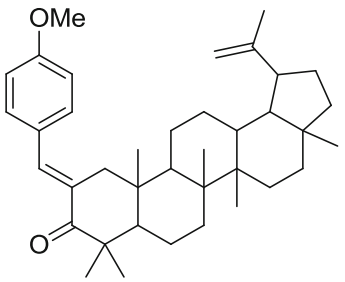
K3



(*E*)-10-(4-(dimethylamino)benzylidene)-3a,5a,5b,8,8,11a-hexamethyl-1-(prop-1-en-2-yl)octadecahydro-1*H*-cyclopenta[*a*]chrysen-9(5b*H*)-one

Compound K3 was prepared from lupenone and 4-(dimethylamino) benzaldehyde using the representative procedure to afford the pure compound with 80 % chloroform in hexane as the eluent. Yield: 67 %; mp: 240–242 °C; IR (KBr cm^{-1}): 3,445, 2,961, 2,376, 1,610, 1,371, 1,164. ^1H NMR: (300 MHz, $CDCl_3$) δ 7.50 (s, 1H), 7.41 (d, $J = 8.49$ Hz, 2H), 6.74 (d, $J = 8.46$ Hz, 2H) 4.71 (s, 1H), 4.61 (s, 1H), 3.03 (s, 6H), 2.48-2.37 (m, 1H), 1.90–2.02(m, 1H), 1.75(s, 3H), 1.49–1.25(m, 24H), 1.17 (s, 3H), 1.13 (s, 3H), 1.10 (s, 3H), 1.02 (s, 3H), 0.82(s, 3H) ^{13}C NMR: (75 MHz, $CDCl_3$) δ 207.93, 151.26, 138.40, 132.55, 129.48, 124.04, 111.82, 109.21, 52.57, 48.47, 48.30, 47.91, 43.07, 42.94, 40.72, 40.31, 40.14, 40.00, 38.20, 36.40, 35.51, 33.06, 29.97, 29.61, 27.44, 25.36, 22.36, 21.80, 20.45, 19.49, 18.01, 15.86, 15.45, 14.49. HRMS (EI) calcd for $[M + H]^+$: $C_{39}H_{58}NO$: 556.4518, found 556.4503.

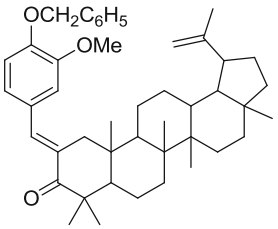
K4



(*E*)-10-(4-methoxybenzylidene)-3a,5a,5b,8,8,11a-hexamethyl-1-(prop-1-en-2-yl)octadecahydro-1*H*-cyclopenta[*a*]chrysen-9(5b*H*)-one

Compound K4 was prepared from lupenone and 4-methoxy benzaldehyde using the representative procedure to afford the pure compound with 50 % chloroform in hexane as the eluent. Yield: 62 %; mp: 176–178 °C; IR (KBr cm^{-1}):3,477, 2,957, 2,365, 1,598, 1,251, 1,029. ^1H NMR: (300 MHz, $CDCl_3$) δ 7.48 (s, 1H), 7.42 (d, $J = 8.52$ Hz, 2H), 6.96 (d, $J = 8.52$ Hz, 2H) 4.74 (s, 1H), 4.64 (s, 1H), 3.86 (s, 3H), 2.48–2.37 (m, 1H), 1.90–2.02 (m, 1H), 1.75 (s, 3H), 1.49–1.25 (m, 24H), 1.17 (s, 3H), 1.13 (s, 3H), 1.10 (s, 3H), 1.02 (s, 3H), 0.82 (s, 3H) ^{13}C NMR: (75 MHz, $CDCl_3$) δ 208.13, 159.81, 151.13, 137.17, 132.18, 132.12, 128.72, 114.00, 109.27, 55.30, 52.73, 48.47, 48.30, 47.91, 43.07, 42.94, 40.70, 40.00, 38.20, 36.40, 35.51, 33.06, 29.97, 29.61, 27.44, 25.36 22.36, 21.80, 20.45, 19.49, 18.01, 15.86, 15.45, 14.49 HRMS (EI) calcd for $[M + H]^+$: $C_{38}H_{55}O_2$: 543.4202, found 543.4186.

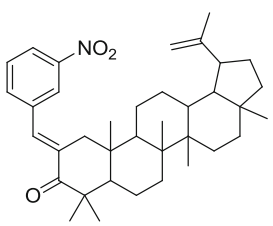
K5



(*E*)-10-(4-(benzyloxy)-3-methoxybenzylidene)-3a,5a,5b,8,8,11a-hexamethyl-1-(prop-1-en-2-yl)octadecahydro-1*H*-cyclopenta[*a*]chrysen-9(5b*H*)-one

Compound K5 was prepared from lupenone and 4 benzyloxy-3-methoxy benzaldehyde using representative procedure to afford the pure compound with 50 % chloroform in hexane as the eluent. Yield: 54 %; mp: 190–192 °C; IR (KBr cm^{-1}): 3,329, 2,944, 2,365, 1,665, 1,586, 1,456, 1,142, 1,025. ^1H NMR (300 MHz, $CDCl_3$): δ 7.40–7.45 (m, 6H),6.98 (m,3H) 4.73 (s, 1H), 4.63 (s, 1H), 3.91 (s, 3H), 2.48–2.37 (m, 1H), 1.90–2.02 (m, 1H), 1.75 (s, 3H), 1.49–1.25 (m, 24H), 1.17 (s, 3H), 1.13 (s, 3H), 1.10(s, 3H), 1.02 (s, 3H),0.82 (s, 3H) ^{13}C NMR:(75 MHz, $CDCl_3$) δ 208.28, 151.07, 149.28, 148.61, 137.24, 136.83, 132.75, 129.42, 128.62, 127.96, 127.22, 122.99, 115.02, 113.53, 109.33, 70.86, 56.02, 52.57, 48.47, 48.30, 47.91, 43.07, 42.94 40.70,40.00, 38.20, 36.40, 35.51, 33.06, 29.97, 29.61, 27.44, 25.36, 22.36, 21.80, 20.45, 19.49, 18.01, 15.86, 15.45, 14.49. HRMS (EI) calcd for $[M + H]^+$: $C_{45}H_{61}O_3$: 649.4621, found 649.4600.

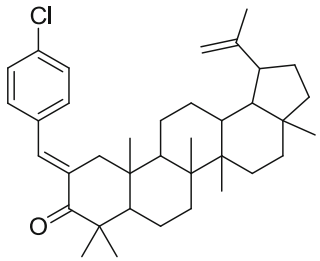
K6



(*E*)-3a,5a,5b,8,8,11a-hexamethyl-10-(3-nitrobenzylidene)-1-(prop-1-en-2-yl)octadecahydro-1*H*-cyclopenta[*a*]chrysen-9(5b*H*)-one

Compound K6 was prepared from lupenone and 3-NO_2 benzaldehyde using representative procedure to afford the pure compound with 40 % chloroform in hexane as the eluent. Yield: 53 %; mp: 188–190 °C; IR (KBr cm^{-1}): 3,227, 2,942, 2,370, 1,592, 1,459, 1,350. ^{1}H NMR (300 MHz, $CDCl_3$):δ 8.18–8.25 (m,2H),7.70 (d, $J = 9$ Hz, 1H), 7.62 (d, $J = 9$ Hz, 1H), 7.58–7.49 (m,1H), 4.71 (s, 1H), 4.61 (s, 1H), 3.91(s, 3H), 2.48–2.37 (m, 1H), 1.90–2.02 (m, 1H), 1.75 (s, 3H), 1.49–1.25 (m, 24H), 1.17 (s, 3H), 1.13 (s, 3H), 1.10 (s, 3H), 1.02 (s, 3H), 0.82 (s, 3H) ^{13}C NMR:(75 MHz, $CDCl_3$) δ 207.81, 150.87, 137.16, 135.39, 134.29, 129.50, 124.64, 122.86, 109.43, 52.88, 48.47, 48.30, 47.91, 43.07, 42.94, 40.70, 40.00, 38.20, 36.40, 35.51, 33.06, 29.97, 29.61, 27.44, 25.36, 22.36, 21.80, 20.45, 19.49, 18.01, 15.86, 15.45, 14.49. HRMS (EI) calcd for $[M + H]^+$: $C_{37}H_{52}NO_3$: 558.3947, found 558.3936.

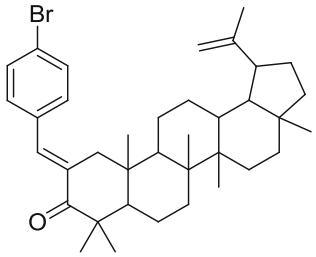
K7



(*E*)-10-(4-chlorobenzylidene)-3a,5a,5b,8,8,11a-hexamethyl-1-(prop-1-en-2-yl)octadecahydro-1*H*-cyclopenta[*a*]chrysen-9(5b*H*)-one

Compound K7 was prepared from lupenone and 4-chloro benzaldehyde using representative procedure to afford the pure compound with 10 % chloroform in hexane as the eluent. Yield: 60 %; mp: 164–166 °C; IR (KBr cm^{-1}): 3,288, 2,942, 2,363, 1,669, 1,457.^{1}H NMR (300 MHz, $CDCl_3$):δ 7.43 (d, $J = 6$ Hz, 2H), 7.38 (d, $J = 6$ Hz, 2H), 7.32(s, 1H), 4.73 (s, 1H), 4.63 (s, 1H), 3.91 (s, 3H), 2.48–2.37 (m, 1H), 1.90-2.02 (m, 1H), 1.75 (s, 3H), 1.49–1.25 (m, 24H), 1.17 (s, 3H), 1.13 (s, 3H), 1.10 (s, 3H), 1.02 (s, 3H), 0.82 (s, 3H) ^{13}C NMR:(75 MHz, $CDCl_3$) δ 208.04, 151.03, 135.89,134.90, 134.29, 134.53, 131.53, 109.34, 52.88, 48.47, 48.30, 47.91, 43.07, 42.94, 40.70, 40.00, 38.20, 36.40, 35.51, 33.06, 29.97, 29.61, 27.44, 25.36, 22.36, 21.80, 20.45, 19.49, 18.01, 15.86, 15.45, 14.49. HRMS (EI) calcd for $[M + H]^+$: $C_{37}H_{52}ClO$: 547.3707, found 547.3738.

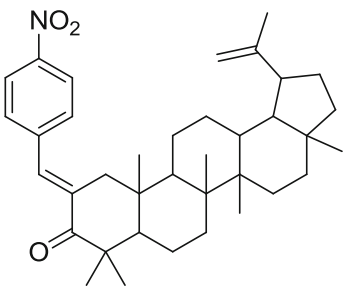
K8



(*E*)-10-(4-bromobenzylidene)-3a,5a,5b,8,8,11a-hexamethyl-1-(prop-1-en-2-yl)octadecahydro-1*H*-cyclopenta[*a*]chrysen-9(5b*H*)-one

Compound K8 was prepared from lupenone and 4-bromo benzaldehyde using representative procedure to afford the pure compound with 25 % chloroform in hexane as the eluent. Yield: 49 %; mp: 170–172 °C; IR (KBr cm^{-1}): 3,279, 2,936, 2,365, 1,656, 1,473, 1,380; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.53 (d, $J = 9$ Hz, 2H), 7.40 (s, 1H), 7.26 (d, $J = 9$ Hz, 2H), 4.71 (s, 1H), 4.61 (s, 1H), 3.91 (s, 3H), 2.48–2.37 (m, 1H), 1.90–2.02 (m, 1H), 1.75 (s, 3H),1.49–1.25 (m, 24H), 1.17 (s, 3H),1.13 (s, 3H), 1.10 (s, 3H), 1.02 (s, 3H), 0.82 (s, 3H); ^{13}C NMR:(75 MHz, $CDCl_3$) δ 207.80, 150.94, 135.91, 135.02, 134.87, 131.69, 131.66, 122.71, 109.43, 52.88, 48.47, 48.30, 47.91, 43.07, 42.94, 40.70, 40.00, 38.20, 36.40, 35.51, 33.06, 29.97, 29.61, 27.44, 25.36, 22.36, 21.80, 20.45, 19.49, 18.01, 15.86, 15.45, 14.49. HRMS (EI) calcd for $[M + H]^+$: $C_{37}H_{52}BrO$: 591.3202, found 591.3185.

K9

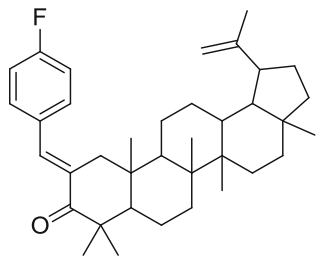


(*E*)-3a,5a,5b,8,8,11a-hexamethyl-10-(4-nitrobenzylidene)-1-(prop-1-en-2-yl)octadecahydro-1*H*-cyclopenta[*a*]chrysen-9(5b*H*)-one

Compound K9 was prepared from lupenone and 4-NO_2 benzaldehyde using representative procedure to afford the pure compound with 50 % chloroform in hexane as the eluent. Yield: 55 %; mp: 186–188 °C; IR (KBr cm^{-1}): 3,260, 2,957, 2,369, 1,660, 1,462, 1,115; ^{1}H NMR

(300 MHz, $CDCl_3$): δ 8.28 (d, $J = 9$ Hz, 2H),7.5(d, J = 9 Hz, 2H),7.49 (s, 1H), 4.73 (s, 1H), 4.63(s, 1H), 3.91 (s, 3H), 2.48–2.37 (m, 1H), 1.90–2.02 (m, 1H), 1.75 (s, 3H), 1.49–1.25 (m, 24H), 1.17 (s, 3H), 1.13 (s, 3H), 1.10 (s, 3H),1.02 (s, 3H), 0.82 (s, 3H) ^{13}C NMR: (75 MHz, $CDCl_3$) δ 207.80, 151.07, 137.24, 135.73, 130.87, 130.46, 125.85, 125.44, 114.99, 109.33, 70.86, 56.02, 52.57, 48.47, 48.30, 47.91, 43.07, 42.94, 40.70, 40.00, 38.20, 36.40, 35.51, 33.06, 29.97, 29.61, 27.44, 25.36, 22.36, 21.80, 20.45, 19.49, 18.01, 15.86, 15.45, 14.49. HRMS (EI) calcd for $[M + H]^+$: $C_{37}H_{52}NO_3$: 558.3947, found 558.3928.

K10



(*E*)-10-(4-fluorobenzylidene)-3a,5a,5b,8,8,11a-hexamethyl-1-(prop-1-en-2-yl)octadecahydro-1*H*-cyclopenta[*a*]chrysen-9(5b*H*)-one

Compound K10 was prepared from lupenone and 4-fluoro benzaldehyde using the representative procedure to afford the pure compound with 35 % chloroform in hexane as the eluent. Yield: 46 %; mp: 152–154 °C; IR (KBr cm^{-1}): 3,273, 2,946, 2,363, 1,673, 1,456. ^{1}H NMR: (300 MHz, $CDCl_3$) δ 7.40 (m, 2H),7.10 (m,3H), 4.71 (s, 1H), 4.61 (s, 1H), 2.48–2.37(m, 1H), 1.90–2.02 (m, 1H), 1.75 (s, 3H), 1.49–1.25 (m, 24H), 1.17(s, 3H), 1.13 (s, 3H), 1.10 (s, 3H), 1.02 (s, 3H), 0.82 (s, 3H) ^{13}C NMR: (75 MHz, $CDCl_3$) δ 208.13, 151.06, 136.09, 134.00, 132.20, 132.10, 115.71,115.42, 109.33, 52.73, 48.47, 48.30, 47.91, 43.07, 42.94, 40.70, 40.00, 38.20, 36.40, 35.51, 33.06, 29.97, 29.61, 27.44, 25.36, 22.36, 21.80, 20.45, 19.49, 18.01, 15.86, 15.45, 14.49. HRMS (EI) calcd for $[M + H]^+$: $C_{37}H_{52}FO$: 531.4002, found 531.3991.

Discussion

The triton-induced hyperlipidemic rat model has been successfully employed to evaluate antidyslipidemic activity [23, 26]. Triton WR-1339 (tyloxapol) is a non-ionic surfactant which elevates the plasma lipids on administration, cause structural changes in circulatory lipoproteins, hinders the uptake of circulating lipids by extra hepatic tissues, increases the HMG-CoA reductase activity and alleviates the activity of LPL resulting in increased blood lipid concentrations, hence hyperlipidemia.

In the present study, lupeol derivatives, particularly K4, K8, and K9, significantly improved the blood lipid profile in tyloxapol-induced hyperlipidemic rats. Tyloxapol induction suppresses LPL activity, which is responsible for the breakdown of lipoproteins (VLDL, LDL, and HDL) [27]. LPL is synthesized by parenchymal cells in a variety of tissues, including adipose tissue, skeletal muscle, and heart and is subsequently moved to its site of action, the endothelium. Fatty acids synthesized by the liver are converted to triglycerides and transported to the blood as VLDL. In peripheral tissues, LPL hydrolyzes triglyceride in VLDL making it triglyceride-depleted, cholesterol-enriched lipoprotein particles now called LDL which are absorbed via LDL receptors The liver controls the concentration of cholesterol in the blood by removing LDL. Another type of lipoprotein known as high-density lipoprotein, or HDL collects cholesterol, glycerol and fatty acids from the blood and transports them to the liver. The derivatives K4, K8 and K9 significantly reactivated the LPL activity thus contributing to the normalization of the lipoprotein levels. Furthermore HMG-CoA reductase, a key enzyme, involved in cholesterol biosynthesis catalyzes the conversion of HMG-CoA to mevalonate, inhibition of this enzyme results in decreased level of cholesterol. K4, K8, and K9 displayed inhibitory effect on HMG-CoA reductase in a dose dependent manner (in vitro) which may be responsible for reduced levels of cholesterol.

Moreover, Increased oxidative stress and excessive production of reactive oxygen species (ROS) followed by inadequate antioxidant defense is directly related to adversely affecting the biologically active molecules such as lipids, proteins and nucleic acid. Lipid peroxidation increases in the case of elevated levels of ROS and this hastens the chain of events leading to the hyperlipidemic condition and indirectly atherosclerosis [28, 29]. Results of antioxidant activity reveal that chalcone derivatives (K2, K4, K8, K9) of lupeol have significant antioxidant activity and may have acted like super oxide dismutase or xanthine oxidase, scavenging the free radicals thus preventing lipid peroxidation, protecting the endothelium and avoiding atherosclerosis, further reducing the risk of cardiovascular diseases.

Based on the above facts, it can be stated that the lupeol derived chalcones have promising lipid lowering and antioxidant potentials. The lipid lowering activity is possibly a multi-prong effect that the compounds exert through significant antioxidant activity, increased lipase activity, and reduced HMG-CoA reductase activity.

Regarding the activity of the compounds, the follows observations have been made. (1) As the number of methoxy groups in the chalcone derivatives of lupeol decrease, the activity increases. (2)Among the NO_2

derivatives, para-substituted chalcones were found to be more active than ortho-substituted. (3)Among the halogen-substituted chalcones, the bromo-substituted one was found to be more active. (4) No specific pattern was observed on the basis of the electron donating/withdrawing nature of the groups.

The inference which can be drawn on consideration of the above facts is that para-substituted derivatives increase the activity of lupeol based chalcones.

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Conflict of interest The authors declare that there are no conflicts of interest.

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