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Original Article

Hypolipidemic activity of friedelin isolated from *Azima tetracantha* in hyperlipidemic rats

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ABSTRACT

The hypolipidemic activity of friedelin isolated from *Azima tetracantha* Lam., Salvadoraceae, was studied in Triton WR-1339 and high-fat diet-induced hyperlipidemic rats. In Triton WR-1339 induced hyperlipidemic rats, treatment with friedelin (50 and 70 mg/kg) showed a significant ($p < 0.01$) lipid-lowering effect as assessed by reversal of plasma levels of total cholesterol (TC), triacylglycerides (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C). In high-fat diet fed hyperlipidemic rats, treatment with friedelin (50 and 70 mg/kg) caused lowering of lipid levels in plasma and liver. The hypolipidemic activity of friedelin was compared with fenofibrate, a known lipid-lowering drug, in both models.

Introduction

Hyperlipidemia is a disorder characterized by increase in blood lipoprotein or cholesterol levels. It is the major cause of cardiovascular diseases, such as coronary heart disease, ischemic cerebrovascular disease, and peripheral vascular disease (Baby and Anuradha, 2013; Li et al., 2010). These diseases are the major causes of mortality and morbidity worldwide. Approximately 12 million people reportedly die of cardiovascular disease each year worldwide (Sunil et al., 2012). Experimental and epidemiological studies have demonstrated that excess low-density lipoprotein (LDL) deposited in the artery wall is an important risk factor in the initiation and progression of atherosclerotic impasse (Basuny et al., 2012). The known lipid-lowering drugs (fibrates, statins, bile acid sequestrants, etc.) regulate the lipid metabolism by different mechanisms, but they also have many side effects like hyperuricemia, diarrhea, nausea, severe muscle damage (myopathy), gastric irritation, flushing, dry skin and abnormal liver function (Sagar et al., 2012). Therefore, the development of lipid-lowering drugs from natural sources is the best option and is in great demand. A number of plants have been used traditionally for the treatment of various cardiovascular diseases. World ethanobotanical information reported that a number of herbal medicines from plants and vegetables are used for controlling hyperlipidemia (Stephen Irudayaraj et al., 2013). Therefore, efforts to develop effective hypolipidaemic drugs have led to discovery of natural products for the regulation of altered lipids.

Azima tetracantha Lam., Salvadoraceae, is known as 'Mulsangu' in Tamil and 'Kundali' in Sanskrit. Its root, root bark and leaves are used with food as a remedy for rheumatism (Kirtikar et al., 1984; Duraipandiyan et al., 2010). It is a powerful diuretic; it is used to treat dropsy, dyspepsia, chronic diarrhea, cough, phthisis, asthma, and small pox (Nargis Begum et al., 2009). The leaf extracts of *A. tetracantha* showed *in vitro* antioxidant and free radical scavenging activities (Thendral Hepsibha et al., 2011). Azimine, azecarpin, carpine, narcissoside, friedelin, lupeol, glutinol and β-sitosterol (Natarajan et al., 2014) are the isolated compounds from the leaves. It showed good anti-inflammatory, analgesic and antipyretic properties on various animal models (Antonisamy et al., 2011). Jiao et al. (2007) reported the antihyperlipidemic effect of friedelin from bamboo shavings. This study aimed to investigate the hypolipidemic activity of friedelin isolated from the leaves of *A. tetracantha* in Triton WR-1339 and high-fat diet-induced hyperlipidemic rats.

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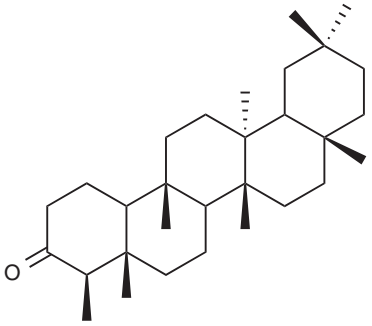
Materials and methods

Drugs and chemicals

Triton WR-1339 was obtained from Sigma Chemical Co. (St Louis, MO, USA). Fenofibrate was purchased from Ranbaxy, India. Biochemical kits and all other chemicals were of analytical grade.

Friedelin identification and characterization

Isolation and characterization of friedelin (**1**) from the leaves of *Azima tetracantha* has been previously reported (Antonisamy et al., 2011).



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Animals

Male Wistar rats (180–200 g) were reared at Entomology Research Institute. The animals were kept in polypropylene cages, under controlled temperature, humidity and 12/12 light/dark cycles. The animals were fed pellet diet (Pranav Agro Industries Ltd., Maharashtra) and water *ad libitum*. This study was carried out with prior approval from Institutional Animal Ethical Committee (IAEC-ERI-LC-28).

Acute toxicity study

The doses for the study were fixed based on Irwin test (Roux et al., 2004). Five groups of fasted healthy rats (six per group) were orally administered friedelin at a dose of 300, 400, 500, 600 and 700 mg/kg; the control group was given distilled water. The rats were observed for 1 h continuously and then hourly for 4 h and finally after every 24 h up to 14 days for any physical signs of toxicity, such as writhing, gasping, palpitation and decreased respiratory rate or mortality. On the 15^{th} day the animals were sacrificed and all the organs were observed for gross pathological lesions. Friedelin at these doses did not show any abnormal behavior. No treatment related gross pathological lesions were observed (data not shown). $1/10^{th}$–$1/20^{th}$ of the dose, in which no behavioral alterations were observed, was considered safe for further assays. Hence, doses of 30, 50 and 70 mg/kg b wt. were preferred for subsequent experiments based on Oliveira et al. (2008).

Effect of friedelin on Triton WR-1339-induced hyperlipidemic rats

Induction of hyperlipidemia

To investigate the short-term effects of friedelin (**1**) on triton-induced hyperlipidemic rats, male Wistar rats weighing 160–180 g were used in the study. The hyperlipidemia was induced by intraperitoneal injection of Triton WR-1339 (200 mg/kg) dissolved in phosphate buffered saline (pH 7.4) (Levine and Saltzman, 2007).

Overnight fasted rats were divided into six groups of six each. Group I served as normal control rats treated with vehicle; Groups II–VI were given intraperitoneal injection of Triton WR 1339. Group II hyperlipidemic rats were treated with vehicle alone; Group III–V hyperlipidemic rats were treated with friedelin 30, 50 and 70 mg/kg, respectively; Group VI hyperlipidemic rats were treated with fenofibrate (65 mg/kg) (Harnafi et al., 2007). Friedelin (dissolved in 1% tween 80) and fenofibrate were given orally, immediately after triton injection. In the following period of study (18 h), rats had access only to water. After 18 h from treatment, rats were euthanized and blood was collected. The blood samples were immediately centrifuged (2500 rpm/10 min) and plasma was used for lipid analysis.

Biochemical analysis of plasma

The plasma total cholesterol, triacylglycerides and HDL-C were quantified using enzymatic kits. LDL-C level was calculated using the formula of Friedewald et al. (1972): LDL-C = TC – (HDL-C – TG/5).

Effect of friedelin on high-fat diet-induced hyperlipidemic rats

Induction of hyperlipidemia

The hypolipidemic effect of friedelin was studied in high-fat diet-induced hyperlipidemic rats. Male Wistar rats weighing 180–200 g were used in this study. The rats were fed with a high-fat diet composed of standard rat chow-68%, dalda (saturated fat)-30% and cholesterol-2% for 15 days (Guido and Joseph, 1992).

The rats were divided into five groups of six rats. Group I and Groups II–V were fed continuously with normal pellet and high-fat diet, respectively for 28 days.

Group I normal control rats were treated with vehicle alone;

Group II hyperlipidemic control rats were treated with vehicle alone;

Group III and IV hyperlipidemic rats were treated with friedelin at 50 and 70 mg/kg, respectively.

Group V hyperlipidemic rats were treated with fenofibrate (65 mg/kg).

Friedelin (**1**) and fenofibrate were given once daily continuously for 28 days, orally. On days 0, 7, 14, 21 and 28, the blood samples were collected by retro-orbital sinus and the TC, TG, HDL-C and LDL-C levels were measured.

At the end of the study the rats were sacrificed, and blood and liver samples were collected. The liver samples were stored at −70° C for the analysis of TC and TG levels.

Biochemical analysis on serum and liver

The TC, TG and HDL-C were quantified using enzymatic kits. LDL-C level was calculated using the formula of Friedewald et al. (1972): LDL-C = TC – (HDL-C – TG/5).

Statistical analysis

The results were presented as mean ± SEM. Statistical analysis of all the data obtained was evaluated using one-way ANOVA followed by Dunnett's test (Graph Pad Instat; Version 3.10). The differences were considered significant at $p \leq 0.05$.

Results

Acute toxicity study

Acute toxicity study revealed the non-toxic nature of friedelin at the doses of 300–700 mg/kg. No mortality and behavioral alternations were observed in friedelin treated rats. There was no lethality

Table 1
Effect of friedelin on triton-induced hyperlipidemic model.

Groups	Total cholesterol (mg/dl)	Triacylglycerides (mg/dl)	LDL-C (mg/dl)	HDL-C (mg/dl)
Normal control	75.34 ± 7.63**	124.83 ± 5.48**	60.37 ± 6.38**	0.65 ± 0.01**
Triton	301.35 ± 9.38	734.38 ± 2.38	150.73 ± 14.29	0.30 ± 0.03
Triton + friedelin (30 mg/kg)	333.47 ± 22.39	702.37 ± 6.48	130.26 ± 11.28	0.35 ± 0.03
Triton + friedelin (50 mg/kg)	233.12 ± 2.38**	443.83 ± 7.38**	90.12 ± 5.60**	0.50 ± 0.02**
Triton + friedelin (70 mg/kg)	204.73 ± 4.10**	403.04 ± 9.39**	72.85 ± 3.94**	0.57 ± 0.06**
Triton + fenofibrate (65 mg/kg)	154.51 ± 9.32**	209.52 ± 4.10**	65.92 ± 4.34**	0.61 ± 0.02**

Values are mean ± SEM ($n = 6$).
** $p < 0.01$, compared with hyperlipidemic control values.

or toxic reaction found at any selected dose until the end of the study.

Effect of friedelin on triton-induced hyperlipidemia

The plasma TC, TG, LDL-C and HDL-C levels of friedelin (50 and 70 mg/kg) treated groups are shown in Table 1. In comparison with the normal control group, triton caused a marked increase on plasma TC, TG and LDL-C, and decrease in HDL-C levels. After treatment with friedelin (50 and 70 mg/kg) there was a significant reduction in the levels of TC ($p < 0.01$), TG ($P < 0.01$), and LDL-C ($p < 0.01$) and increase in the level of HDL-C ($p < 0.01$). Friedelin (30 mg/kg) showed less effect.

Effect of friedelin on high-fat diet-induced hyperlipidemia

The plasma TC, TG, LDL-C and HDL-C levels of friedelin (50 and 70 mg/kg) treated groups on day 0, 7, 14, 21 and 28 are shown in Table 2. The normal rats fed with high-fat diet for 15 days showed increased plasma TC, TG and LDL-C, and decreased HDL-C levels. After treatment with friedelin for 28 days the plasma levels of TC ($p < 0.01$), TG ($p < 0.01$), and LDL-C ($P < 0.01$) were significantly reduced and the levels of HDL-C ($p < 0.01$) were significantly increased.

The levels of liver TC and TG of normal and hyperlipidemic rats are shown in Fig. 1. The normal rats fed with fat for 15 days showed a significant increase ($p < 0.01$) in TC and TG levels of liver. After treatment with friedelin (50 and 70 mg/kg) the TC and TG levels of liver reduced to normal.

Discussion

In the present study, we investigated the hypolipidemic effect of friedelin on Triton WR-1339- and high-fat diet-induced models. Triton WR-1339, a non-ionic detergent (oxyethylated tertiary octylphenol formaldehyde polymer), provokes acute hyperlipidemia, and thus it has been widely used as a hyperlipidemia inducing agent to study lipid metabolism and the metabolic interrelationship between plasma lipoproteins (Zarzecki et al., 2014; Ghatak and Panchal, 2012). It induces hyperlipidemia by increasing hepatic cholesterol biosynthesis through its interference with the tissue uptake of plasma lipids and its inhibition of cholesterol excretion, and by preventing or delaying the plasma clearance (Jo et al., 2014). Inhibitory effects on cholesterol biosynthesis occurred in an earlier study within the first 24 h of triton treatment, and the inhibition of cholesterol metabolism and excretion was exhibited after 24 h of triton treatment (Harnafi et al., 2008). In our study, we evaluated the hypolipidemic activity of friedelin (30, 50 and 70 mg/kg) on triton-induced hyperlipidemic rats. Among the three doses, 50 and 70 mg/kg showed significant effects by decreased serum lipid levels and increased HDL-C levels in Triton WR-1339-induced hyperlipidemic rats. This indicates that friedelin altered

Table 2
Effect of friedelin on serum total cholesterol, triacylglycerides, LDL-C and HDL-C of control and treated rats on 0, 7th, 14th, 21st and 28th day of high-fat diet-induced model.

Parameter	Groups	0 day	7th day	14th day	21st day	28th day
TC	NC	80.83 ± 3.24	84.73 ± 3.28**	90.74 ± 4.95**	93.43 ± 3.28**	90.45 ± 5.49**
	HC	140.82 ± 2.38	147.86 ± 5.74	167.83 ± 6.58	175.48 ± 9.58	190.74 ± 8.47
	HC + friedelin (50 mg/kg)	152.75 ± 4.58	147.84 ± 7.49	134.53 ± 4.55	124.03 ± 8.41	120.08 ± 6.52**
	HC + friedelin (70 mg/kg)	149.64 ± 3.80	132.54 ± 5.38	123.93 ± 8.47**	120.38 ± 0.33**	110.34 ± 5.49**
	HC + fenofibrate (65 mg/kg)	145.74 ± 7.39	124.40 ± 3.74*	110.30 ± 3.21**	105.12 ± 6.32**	96.26 ± 5.35**
TG	NC	107.53 ± 5.41	103.83 ± 3.24**	100.73 ± 9.42**	96.48 ± 3.23**	102.12 ± 5.46**
	HC	206.30 ± 4.02	353.37 ± 12.72	362.37 ± 3.63	364.74 ± 6.83	370.93 ± 5.39
	HC + friedelin (50 mg/kg)	350.83 ± 3.20	332.93 ± 6.48	321.30 ± 4.32**	283.43 ± 10.21**	262.37 ± 4.31**
	HC + friedelin (70 mg/kg)	340.18 ± 12.68	310.93 ± 8.28**	300.23 ± 5.49**	270.54 ± 11.83**	254.83 ± 0.62**
	HC + fenofibrate (65 mg/kg)	336.73 ± 5.93	315.83 ± 27.37**	301.82 ± 3.12**	262.73 ± 12.56**	221.34 ± 9.74**
LDL-C	NC	50.74 ± 1.41	52.52 ± 3.84**	54.83 ± 6.38**	55.49 ± 7.48**	53.64 ± 2.37**
	HC	105.26 ± 4.73	110.33 ± 5.48	122.38 ± 8.49	130.48 ± 7.39	1132.12 ± 2.93
	HC + friedelin (50 mg/kg)	126.38 ± 3.72	111.73 ± 3.12	108.27 ± 5.48	102.64 ± 5.99**	100.33 ± 7.49**
	HC + friedelin (70 mg/kg)	132.85 ± 3.84	120.85 ± 6.51	110.32 ± 5.46*	100.39 ± 3.42**	97.58 ± 6.50**
	HC + fenofibrate (65 mg/kg)	125.04 ± 3.48	98.59 ± 2.94**	90.49 ± 4.39**	84.39 ± 3.24**	85.11 ± 9.45**
HDL-C	NC	40.36 ± 2.84	42.93 ± 3.28**	40.92 ± 5.93**	41.88 ± 3.64**	38.93 ± 4.39**
	HC	30.63 ± 5.48	25.47 ± 9.48	19.38 ± 6.48	15.84 ± 0.39	13.38 ± 1.39
	HC + friedelin (50 mg/kg)	32.37 ± 3.48	37.39 ± 6.59*	40.95 ± 5.49*	43.58 ± 9.68**	45.45 ± 0.23*,**
	HC + friedelin (70 mg/kg)	35.67 ± 3.47	41.84 ± 2.83**	43.11 ± 2.11**	45.83 ± 4.38**	46.38 ± 5.23**
	HC+ Fenofibrate (65 mg/kg)	36.23 ± 1.02	39.48 ± 5.23**	40.37 ± 7.12**	42.73 ± 6.84**	48.66 ± 3.12**

Values are mean ± SEM ($n = 6$).
* $p < 0.05$, compared with hyperlipidemic control values.
** $p < 0.01$, compared with hyperlipidemic control values.
NC: normal control, HC: hyperlipidemic control.

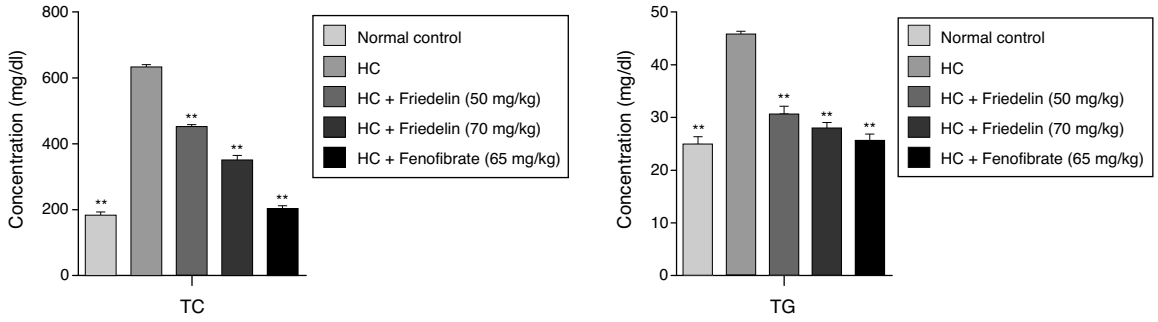

Fig. 1. Effect of friedelin on liver total cholesterol and triacylglyceride levels in rats fed with high-fat after 28 days of treatment. Values are mean ± SEM ($n = 6$). $^{*}p < 0.05$, $^{**}p < 0.01$, compared with hyperlipidemic control values. NC: normal control, HC: hyperlipidemic control.

the cholesterol biosynthesis in the liver in the favor of "good" HDL-C.

A number of studies in the past have also shown that a diet with high-fat content caused a considerable increase in the serum cholesterol and triacylglyceride concentrations in rats and mice (Ban et al., 2012). High levels of TC and more importantly LDL cholesterol are major coronary risk factors (Huang et al., 2008). LDL carries cholesterol from the liver to the peripheral cells and smooth muscle cells of the arteries; a rise in LDL may cause deposition of cholesterol in the arteries and aorta and hence is bad for health and a direct risk factor for coronary heart disease (De Graat et al., 2002). Administration of friedelin (50 and 70 mg/kg) lowered both TC and LDL cholesterol in experimental rats. The lowering of TC and LDL-C in serum would reduce the incidence of coronary events (Verma et al., 2012).

Friedelin showed protective action on high-fat diet-induced hyperlipidemic rats, as it significantly increased the serum HDL-cholesterol levels. The possible mechanism of this activity may be due to enhancement of the activity of lecithin–cholesterol acyltransferase and inhibition of the action of hepatic TG-lipase on HDL, which may contribute for a rapid catabolism of blood lipids through extra-hepatic tissues. The increased HDL facilitates the transport of triacylglyceride or cholesterol from serum to liver by a pathway termed 'reverse cholesterol transport' where it is catabolised and excreted out of the body (Srinivasa Rao and Saileela, 2013).

Triacylglycerides play a key role in the regulation of lipoprotein interactions to maintain normal lipid metabolism. Recent studies also showed that triacylglycerides are independently related to coronary heart disease (Noorani et al., 2011) and most of the antihypercholesterolemic drugs do not decrease triacylglycerides levels, but friedelin lowered it significantly and this effect might be related to increase in the endothelium bound lipoprotein lipase which hydrolyses the triacylglycerides into fatty acids (Vembu et al., 2012). Previous study by Jiao et al., 2007 reported that friedelin isolated from bamboo shavings reduced the serum TC, TG, and LDL-C levels but there was no effect in increasing the HDL-C levels in diet-induced hyperlipidemic Sprague–Dawley rats. But in both our studies friedelin decreased the TC, TG, and LDL-C and increased the HDL-C levels significantly.

In conclusion, the present study demonstrated that friedelin possessed maximum efficacy in lowering the elevated lipid levels in Triton WR-1339 and high-fat diet-induced hyperlipidemic rats. This investigation revealed that friedelin isolated from *A. tetracantha* leaves could be probed further to be used as a drug to treat hyperlipidemia.

Authors' contributions

VD contributed to the isolation and characterization of friedelin. CS evaluated the hypolipidemic activity of friedelin and wrote the manuscript. SSI contributed to laboratory work with CS. NAAD and SI evaluated the data and corrected the manuscript. All the authors have read the final manuscript and approved submission.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgement

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