

Hypolipidemic effect of methanol fraction of *Aconitum heterophyllum* wall ex Royle and the mechanism of action in diet-induced obese rats

Arun Koorappally Subash,
Anu Augustine

Department of Biotechnology and
Microbiology, Thalassery Campus,
Kannur University, India

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ABSTRACT

Aconitum heterophyllum is an endangered Himalayan plant included in "lekhaneyagana," a pharmacological classification mentioned by Charaka in "Charakasamhita" which means reduce excess fat. The subterranean part of the plant is used for the treatment of diseases like nervous system disorders, fever, diarrhea, obesity, etc. In the present study, we are reporting the hypolipidemic effect of methanol fraction of *A. heterophyllum*. The methanol extract of *A. heterophyllum* was orally administered in diet-induced obese rats. After four weeks treatment, blood samples were collected for the estimation of serum lipids and lecithin-cholesterol acyltransferase (LCAT). Liver was collected for the assay of HMG-CoA reductase (HMGR). The fecal samples were also collected to estimate the fecal fat content. The *A. heterophyllum* treatment markedly lowered total cholesterol, triglycerides and apolipoprotein B concentrations in blood serum. It also showed positive effects (increase) on serum high-density lipoprotein cholesterol (HDL-c) and apolipoprotein A1 concentrations. On the other hand, *A. heterophyllum* treatment lowered HMGR activity, which helps to reduce endogenous cholesterol synthesis and also activated LCAT, helping increase in HDL-c. An increase in fecal fat content is also an indication of the hypolipidemic effect of *A. heterophyllum*. The significant hypolipidemic effect of *A. heterophyllum* may be linked to its ability to inhibit HMGR activity and block intestinal fat absorption. The increase in HDL-c may be linked to its ability to activate LCAT enzyme.

Key Words: Apolipoproteins, diet-induced obesity, HMG-CoA reductase, lecithin-cholesterol acyltransferase

INTRODUCTION

Obesity is the overabundance of body weight for a particular age, sex, and height due to the imbalance between energy intake and its expenditure. It remains a major global public

health issue because of its increasing prevalence, cutting across all sex, age-groups, ethnicity, or race.^[1] *Aconitum heterophyllum* Wall ex Royle (Family: Ranunculaceae) is an endangered plant species which is commonly known as "Ativisha" in Ayurveda and used in Indian System of Medicines.^[2] The subterranean part (root) of this plant is used in various ayurvedic preparations for treating digestive disorders, nervous system disorders, fever, diarrhea, rheumatism, dyspepsia, cough, and also as astringent and antidiabetic.^[2-4] The plant is rich in compounds like diterpene alkaloids, flavonoids, tannins, saponins, and sugars.^[5,6] The plant has been reported to possess antifungal,^[7] cytotoxic, antiviral, anti-inflammatory,^[8] and immune-stimulant properties.^[9-11] *A. heterophyllum* is traditionally used to control obesity and included in "lekhaneyagana," a pharmacological classification mentioned in Charakasamhita.^[12] Mechanism behind the hypolipidemic effect of *A. heterophyllum* was unknown. In this study, we are reporting the possible mechanism of

Address for correspondence:

Dr. Anu Augustine,
Department of Biotechnology and Microbiology,
Thalassery Campus, Kannur University, 670661, India.
E-mail: anuaugus@rediffmail.com

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hypolipidemic effect of *A. heterophyllum*, using diet-induced obese rats as model. The diet-induced obese rats were orally administered with the methanol extract of *A. heterophyllum* for a period of 4 weeks and the lipid level in blood serum and feces were monitored.

MATERIALS AND METHODS

Chemicals

Mevinolin and Sodium iodoacetic acid were obtained from Sigma Aldrich (St. Louis, U.S.A). The diagnostic kits for total cholesterol (T-c), triglycerides (TG), high-density lipoprotein cholesterol (HDL-c), and immunoturbidimetric assay kits for ApoA1 and B were obtained from Agappe diagnostics, Switzerland GmbH. Apo A1 and B standards were purchased from Denka Seiken Co Ltd., Japan. All other chemicals were obtained from Merck (Germany).

Animal Models

Sprague dawley (SD) strain rats, of body weight 160 ± 10 g, were used for experiments. The animals were housed in polypropylene cages at controlled temperature ($22 \pm 2^\circ\text{C}$) and humidity ($50 \pm 10\%$) and were kept in 12-hour light cycle.^[13] The experiment was approved by the Institutional animal ethics committee (IAEC No-KULS/IAEC 2011-04) and performed based on CPCSEA accepted guidelines for care and use of laboratory animals.

Preparation of the Root Extract of *A. heterophyllum*

Root samples of *A. heterophyllum* were purchased from local market. The plant part was identified and authenticated by Centre for Medicinal Plants Research (CMPR), Kottakkal, India. Voucher specimen was processed and deposited (Voucher No: CMPR 1486). 2.3 kg of the fine powdered sample was used for Soxhlet extraction using methanol. The homogenate was then filtered using Millipore filtration system 2 (Millipore, USA) and dried using a rotary evaporator at 40°C to get 273 g extract. The methanol fraction was loaded to a silica gel column (18×500 mm column) and then eluted with methanol: water solvent, in different proportions. The 30% methanol soluble fraction was used for treating diet-induced obese rats.^[11]

Establishment of Experimental Model and Drug Treatment

Rats were randomly divided into 2 groups: normal control group (10 rats) and high-fat group (40 rats). The normal control rats were fed with standard diet and high-fat group rats were fed with high-fat diet^[14,15] for 4 weeks [Table 1]. All high-fat group rats met the hyperlipidemic criteria and were randomly divided into four groups with ten rats per group: high-fat model control group, *A. heterophyllum* I group (rats fed with high-fat diet and orally given *A. heterophyllum* extract at dosage of 200 mg/kg body weight (BW)), *A. heterophyllum* II group (rats fed with high-fat

diet and orally given *A. heterophyllum* extract at dosage of 400 mg/kg BW), and mevinolin (3.0 mg/kg BW)-treated positive control group.^[14] The acute toxicity of the extract was performed and the doses were chosen according to the acute toxicological study results divided by security factor 10.^[16-18] After 4 weeks treatment, overnight fasted rats were sacrificed and blood samples and liver were taken for the estimation of serum T-c, TG, HDL-c, LDL-c,^[19,20] HMG-CoA reductase (HMGR),^[21] Lecithin-cholesterol acyltransferase (LCAT),^[22] and apolipoproteins.^[23]

Statistical Analysis

All values are presented as mean \pm s.d. Statistical comparisons of the groups were made by ANOVA, and each group was compared with the others by Posthoc Fisher's PLSD test (SPSS Inc-IBM, USA). Statistical significance was defined as $P < 0.05$.

RESULTS

Effect of *A. heterophyllum* on blood serum and fecal lipid levels in diet-induced obese rats

The estimated lipids levels in normal control, high fat model control, extract-treated groups, and positive control are given in Table 2. The T-c and TG levels in *A. heterophyllum* extract-treated groups were lower than model control group. In extract-treated groups, HDL-c level was found to be increased. The mevinolin-treated group had also shown a significant hypocholesterolemic effect compared with the model control. Detailed fecal analysis of *A. heterophyllum* extract-treated group showed remarkable increase in fecal T-c and TG, compared to model control. The body weight of *A. heterophyllum* extract-treated groups was considerably reduced, when compared to control groups.

Effect of *A. heterophyllum* HMGR and LCAT activity in diet-induced obese rats

The activities of HMGR and LCAT enzymes in control and test groups are given in Figures 1 and 2 respectively. Results showed that the enzymatic activities of HMGR were significantly reduced and that of LCAT was significantly enhanced.

Table 1: High fat diet ingredients

S/N	Ingredients	HF diet (g/100 g diet)
1	Basic diet	82.8
2	Lard	10
3	Cholesterol	2
4	Bile salt	0.5
5	Propylthiouracil	0.2
6	AIN ^a -76 Vitamin mix	3.5
7	AIN ^a -76 Mineral mix	1

^a AIN-76-American Institute of Nutrition formulation 76. Control rats were fed freely with the standard diet. Diet induced obese rat models received high fat diet for 4 weeks

Effect of *A. heterophyllum* on Serum Apolipoproteins in Diet-Induced Obese Rats

The serum apolipoproteins (apo A1 and B) levels in *A. heterophyllum* extract-treated groups are given in Table 2. ApoA1 level was found to be increased considerably by the administration of *A. heterophyllum* extract and the apo B level was decreased.

DISCUSSION

The elevated level of T-c, TG, and LDL-c results in three-fold increased risk of obesity and cardiovascular diseases.^[24,25] High triglyceride levels increase the atherogenicity of HDL-c and LDL-c. Moreover, recent studies showed that triglycerides are independently related to coronary heart disease.^[26] The LDL-c transport TG and cholesterol to the tissues, but excess LDL-c may permeate the inner arterial wall and result in development of atherosclerotic lesions.^[24,27] High levels of HDL-c can lower an individual's risk of developing heart disease.

A. heterophyllum is traditionally used for the treatment of obesity, which is included in "lekhaneyagana," a

pharmacological classification mentioned by Charaka in "Charakasamhita" which means reduce excess fat.^[12] The mechanism of anti-obesity activity of this plant was unknown. In our study, the oral administration of *A. heterophyllum* methanol fraction reduced the body weight significantly and was able to reduce serum T-c, TG, LDL-c, and VLDL-c levels compared to high-fat model control rats. The reduction in TG level is a promising result as most of the anti-hypercholesterolemic drugs were not able to reduce triglycerides levels.^[26] HDL-c level in *A. heterophyllum*-treated groups was enhanced which can lower a risk of developing heart diseases. HDL-c transports cholesterol from the tissues to the liver for removal from the body. Similar results were observed in *Monascus*-fermented soybean extracts in rats fed with a high fat and cholesterol diet.^[28] A significant increase in fecal fat content in *A. heterophyllum* extract-treated groups indicates that the treatment can block intestinal fat absorption and reduce blood cholesterol level.^[29]

HMGR is the rate-limiting enzyme in the cholesterol biosynthesis pathway. Inhibiting HMGR enzyme leads to a block in the formation of mevalonate and thereby cholesterol synthesis. The *A. heterophyllum* treatment

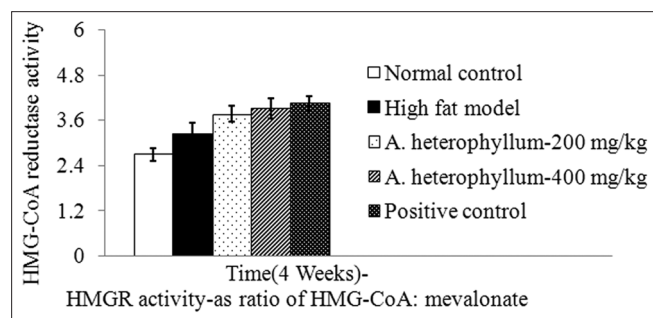


Figure 1: Graph showing the effect on *A. heterophyllum* on HMGR activity in diet-induced obese rats. HMG-CoA reductase activity is expressed in term of HMG-CoA/mevalonate ratio

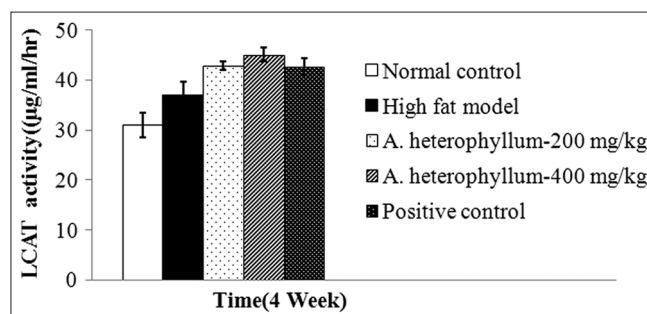


Figure 2: Graph showing the effect on *A. heterophyllum* on LCAT activity in diet-induced obese rats

Table 2: Effect of *A. heterophyllum* on serum lipids, fecal lipids, and apolipoproteins in diet-induced obese rats

	Normal control	High fat model	<i>A. heterophyllum</i> I group (200 mg/kg)	<i>A. heterophyllum</i> II group (400 mg/kg)	Positive control
Serum lipids (mg/dl)					
T-c	72.34±7.64 ^a	111.81±6.67	95.55±1.35 ^b	88.36±1.34 ^b	83.7±1.93
TG	78.10±4.14 ^a	107.1±6.62	97.84±1.52 ^b	88.81±1.85 ^b	81.81±1.77
HDL-c	35.1±3.49	22.43±4.08 ^a	27.03±1.63	29.22±1.71 ^b	31.6±1.65 ^b
LDL-c	21.62±8.02	67.96±9.88 ^a	48.97±3.11 ^b	41.37±2.46 ^b	35.75±1.29 ^b
Fecal lipids (mg/dl)					
T-c	3.87±0.32	4.61±0.28 ^a	5.47±0.21 ^b	5.74±0.34 ^b	5.31±0.29
TG	2.82±0.29	6.24±0.37 ^a	6.51±0.23	6.95±0.28 ^b	4.5±0.32
Apolipoproteins (mg/dl)					
ApoA1	6.28±0.37	4.26±0.12 ^a	4.69±0.09	5.11±0.18 ^b	5.31±0.29
ApoB	2.82±0.29	8.11±0.41 ^a	7.68±0.21 ^b	6.57±0.36 ^b	4.5±0.32

Normal control, rat fed with normal diet; High fat model, rat fed with high fat diet; *A. heterophyllum* I group – 200 mg/kg and *A. heterophyllum* II group – 400 mg/kg, high-fat diet rats treated with 200 and 400 mg/kg body weight of *A. heterophyllum* extract, respectively; Positive control-mevinolin, high-fat diet rats treated with mevinolin (3.0 mg/kg body weight). The values are mean ± s.d. for ten rats. ^a p < 0.05 compared with control group. ^b p < 0.05 compared with untreated model group

shows a substantial decrease in HMGR activity, which blocks the cholesterol biosynthesis. Furthermore, no potential toxic precursors are formed when pathway is blocked. These make HMGR a promising target to develop drugs to reduce cholesterol levels.^[25,30] LCAT is an enzyme that catalyzes the formation of cholesteryl esters on HDL and by that promotes maturation of HDL particles in plasma and facilitates reverse cholesterol transport. Several studies show that an increase in HDL-c is associated with a decrease in coronary risk. Lack of normal cholesterol esterification impairs the formation of mature HDL particles and leads to rapid catabolism of circulating apoA1. LCAT promotes maturation of HDL particles in plasma and facilitates reverse cholesterol transport by maintaining a concentration gradient for the diffusion of cellular unesterified cholesterol to HDL-c.^[31] *A. heterophyllum* treatment could activate LCAT and thereby increased the HDL-c levels.

Apolipoproteins serve to activate enzymes important in lipoprotein metabolism and mediate the binding of lipoproteins to cell-surface receptors. Apo A1 is the main protein component of HDL-c, which helps in the removal of excess cholesterol from extra-hepatic tissues. Apo B, present in LDL-c, is the ligand concerned with the uptake of cholesterol. Elevated levels of apo B and low apo A1 levels indicate an increased risk of cardiovascular disease even when T-c and LDL-c levels are normal.^[32] The *A. heterophyllum* treatment resulted in notable increase in apo A1 and decrease in apo B levels. The decrease in apoB/apo A1 ratio shows the anti-atherogenic potential of *A. heterophyllum* extract.

To the best of our knowledge, the mechanism of hypolipidemic activity of *A. heterophyllum* was not studied till date. In the present study, we suggest that the higher hypolipidemic effect of *A. heterophyllum* might be due to the combined effect of HMGR inhibition, which results in suppression of endogenous cholesterol biosynthesis and blocking the intestinal fat absorption. The increase in apo A1 level and LCAT activity support for the increment in HDL-c levels. On the other hand, drop down of Apo B level is responsible for the reduction of LDL-c.

CONCLUSION

The administration of *A. heterophyllum* extract was able to reduce serum T-c, TG, and LDL-c levels. Furthermore, *A. heterophyllum* helps to improve lipid HDL-c level. From the results, we can presume that the change in lipid profile by *A. heterophyllum* is due to the inhibition of HMGR and the activation of LCAT enzymes. The extract was also able to block intestinal fat absorption which helps to reduce cholesterol level. Based on this observation, it can be comprehended that the *A. heterophyllum* methanol fraction exhibits potential hypolipidemic activity. These results

constitute a valid scientific groundwork for the medicinal application of *A. heterophyllum* and a valid support for "lekhaneya" action of extract mentioned in Charaka samhitha.

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