
Hypolipidemic Effect of Methanol Fraction of *Acorus calamus* Linn. in Diet-Induced Obese Rats

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Abstract

Acorus calamus Linn. is a traditional medicinal plant included in “lekhaneyagana” (which means “reduce excess fat”), a pharmacological classification mentioned by Charaka in Charakasamhita. Traditionally, this plant has been prescribed as a first-line treatment for many ailments such as digestive problems, diabetes, obesity, and related problems. The purpose of the present study was to check the effect of methanolic fraction of *A. calamus* on serum lipids, Lecithin-cholesterol acyltransferase (LCAT) enzyme, and apolipoproteins. We found that treatment of *A. calamus* at concentrations of 200 and 400 mg/kg body weight was able to reduce total cholesterol (T-c), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-c) levels and increase high-density lipoprotein cholesterol (HDL-c) levels compared to model control group. *A. calamus* treatment results in activation of LCAT enzyme, which helps in the maturation of HDL-c. ApoA1 level was found to be increased considerably by the administration of *A. calamus* extract, and the Apo B level was decreased. GCMS analysis of the *A. calamus* extract showed the presence of compound, Friedelan-3-one. The result suggests the use of methanol fraction of *A. calamus*, as a potential drug for controlling the cholesterol level.

Keywords

A. calamus • LCAT • GCMS • Apolipoproteins

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Abbreviations

Apo A1	Apolipoprotein A1
Apo B	Apolipoprotein B
GCMS	Gas chromatography mass spectrometry
HDL-c	High-density lipoprotein cholesterol
LCAT	Lecithin-cholesterol acyltransferase
LDL-c	Low-density lipoprotein cholesterol
MSMS	Tandem mass spectrometry
RT	Retention time
T-c	Total cholesterol
TG	Triglycerides

Introduction

Acorus calamus Linn. (Family: Araceae), also known as sweet flag, is a semiaquatic perennial herb with creeping and branched aromatic rhizome. The roots and rhizomes of this plant have been used in the Indian and Chinese systems of medicine for at least 2,000 years. The essential oils isolated from *A. calamus* showed anti-hypercholesterolemia, anti-inflammatory, and antioxidative activity, respectively [1–3]. Furthermore, *A. calamus* extract showed antifungal activity and insecticidal properties [4].

Obesity is an excess proportion of total body fat and is reaching epidemic proportions. Direct health costs attributable to obesity have been estimated at \$52 billion in 1995 and \$75 billion in 2003 and increasing in alarming rate [5]. *A. calamus* is a plant included in Iekhaneyagana, a pharmacological classification mentioned in “Charakasamhita” which means “reduce excess fat” [6]. In the present study, we screened the methanolic fraction of *A. calamus* for hypolipidemic effects in diet-induced rat models. The fraction which shows hypolipidemic effect was used to perform GCMS analysis.

Materials and Methods

Preparation of the Root Extract of *A. calamus*

Root samples of *A. calamus* were purchased from local market and authenticated by the Centre for Medicinal Plants Research (CMPR), Kottakkal,

India. Voucher specimen was processed and deposited (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 (273 g). The methanol fraction was loaded to a silica gel column eluted with MeOH: water (18×500 mm column), increasing water gradually to supply five fractions, since the 30% MeOH-soluble fraction exhibited considerable hypolipidemic activity [1, 4]. The fourth fraction was used to feed the diet-induced obese rats, and the same was used for GCMS analysis.

Establishment of Experimental Model and Drug Treatment

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±3°C) and humidity (50±10%) and were kept in 12-h light cycle. 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.

Rats were randomly divided into two groups: normal control group ($n=10$) and high-fat group ($n=40$). The normal control rats were fed with standard diet, and hyperlipidemic rats were fed with high-fat diet [7, 8]. The rats in high-fat model group were randomly divided into four groups with ten rats per group: high-fat model control group, *A. calamus I* group (200 mg/kg body weight (BW)), *A. calamus II* group (400 mg/kg BW), and mevinolin (3.0 mg/kg body weight)-treated positive control group [7]. The acute toxicity of the extract was performed, and the doses were chosen according to the acute toxicological study [9, 10]. At the end of the experiment, the overnight fasted rats were sacrificed and blood samples were taken for the estimation of serum T-c, TG, HDL-c, LDL-c [11–14], Lecithin-cholesterol acyltransferase [15], and apolipoproteins [16].

GCMS Analysis of Methanolic Extract of *A. calamus*

GCMS and MSMS analysis of the methanolic extract of *A. calamus* was performed using Perkin Elmer Clarus 600 GCMS and Turbomatrix 40 trap GC-HS controlled by Turbo mass software version 5.4.2. One microliter of the sample was injected by direct liquid injection mode into Perkin Elmer Clarus 600 Gas Chromatogram equipped with Elite-5MS, 30 m×0.25-mm ID×0.25-um film thickness. The GC conditions were, injection temperature 275°C, helium as carrier gas with flow of 15 psi, and a split ratio of 10. GC effluent was introduced to the mass spectrometer for EI mass spectrometry analysis where data were recorded in full scan or linear mode in the mass range of 10–600 *m/z*.

Statistical Analysis

All values are presented as mean ± 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 and Discussion

Effect of *A. calamus* on Serum Lipid Levels in Diet-Induced Obese Rats

The estimated lipid levels in normal control, high-fat model control, extract-treated groups, and positive control are given in Table 47.1. The T-c and TG levels of *A. calamus* extract-treated groups were lower than the high-fat group; the dose response was also obvious. Moreover, *A. calamus* facilitates enhancement of cardio-protective lipid HDL-c, and this effect may be due to the increase in activity of lecithin: cholesterol acyl transferase (LCAT) enzyme, which may contribute to the regulation of blood lipids. The effect of *A. calamus* on HDL-c may be largely attributed to its central function in the reverse cholesterol transport, a process whereby excess cell cholesterol is taken up and processed by HDL particles for further delivery to the liver for metabolism [17, 18].

Treatment of hyperlipidemic rats with *A. calamus* extract at a dose of 200 and 400 mg/kg body weight lowered the serum LDL-c levels. The mevinolin-treated group had also showed a significant hypocholesterolemic effect in serum as compared to model control group [19, 20].

Table 47.1 Effect of *A. calamus* on serum lipids, apolipoproteins, and LCAT activity in diet-induced obese rats

	Normal control	High-fat model	<i>A. calamus</i> I 200 mg/kg	<i>A. calamus</i> II 400 mg/kg	Positive control mevinolin
<i>Serum lipids (mgdL⁻¹)</i>					
T-c	72.34 ± 7.64 ^a	111.81 ± 6.67	97.58 ± 2.32 ^b	87.03 ± 2.07 ^b	83.7 ± 1.93
TG	78.10 ± 4.14 ^a	107.1 ± 6.62	95.03 ± 2.18 ^b	85.11 ± 1.81 ^b	81.81 ± 1.77
HDL-c	35.1 ± 3.49	22.43 ± 4.08 ^a	24.1 ± 1.01	28.21 ± 0.94 ^b	31.6 ± 1.65 ^b
LDL-c	21.62 ± 8.02	67.96 ± 9.88 ^a	54.47 ± 1.79 ^b	41.79 ± 2.71 ^b	35.75 ± 1.29 ^b
<i>Apolipoproteins (mgdL⁻¹)</i>					
ApoA1	6.28 ± 0.37	4.26 ± 0.12 ^a	4.83 ± 0.17 ^b	5.62 ± 0.21 ^b	5.31 ± 0.29
ApoB	2.82 ± 0.29	8.11 ± 0.41 ^a	7.48 ± 0.27 ^b	6.61 ± 0.5 ^b	4.5 ± 0.32
LCAT	30.98 ± 2.53	37.01 ± 2.67	39.25 ± 1.44	41.53 ± 0.94 ^b	42.66 ± 1.60

LCAT Lecithin-cholesterol acyltransferase, normal control, rat fed with normal diet; high-fat model, rat fed with high-fat diet; *A calamus* I-200 mg/kg and *A calamus* II-400 mg/kg, high-fat diet rats treated with 200 and 400 mg/kg body weight of *A calamus* extract, respectively; positive control mevinolin, high-fat diet rats treated with mevinolin (3.0 mg/kg). 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

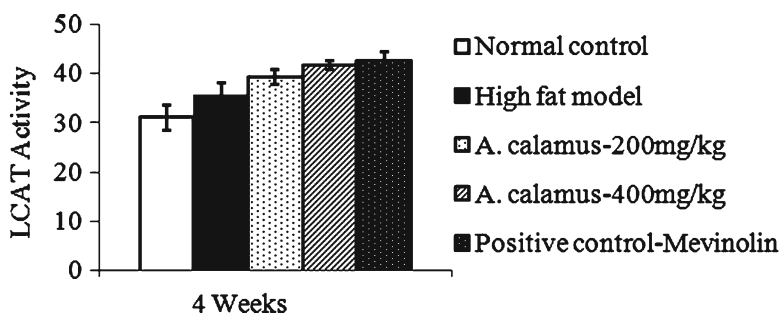


Fig. 47.1 Graph showing the effect on *A. calamus* on LCAT activity in diet-induced obese rats

Effect of *A. calamus* on LCAT Activity in Diet-Induced Obese Rats

Oral administration of *A. calamus* extracts that result in significant increase in LCAT activity supports the above result (Fig. 47.1). 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 by maintaining a concentration gradient for the diffusion of cellular unesterified cholesterol to HDL. It helps to improve cholesterol exportation in biliary acids [18, 19]. Similar results were observed for lipid profiles treating *Curcuma kwangsiensis* polysaccharides [7] and LCAT activity in case of *Gymnema sylvestre* and *Monascus*-fermented soybean extracts [21, 22] (Fig. 47.1).

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

The administration of *A. calamus* extract significantly increases the apoA1 and drops down Apo B levels. Both lipid-poor apoAI and HDL act as acceptors for cellular lipids, and lipid efflux from cells can be mediated via the cell surface proteins. Apo A-I and Apo A-II are the main protein components of HDL cholesterol, which helps in removing excess cholesterol from the tissues for removal by the liver [23]. On the other hand, Apo B, which is a component of LDL-c, enables tissue cells to take up cholesterol [24].

GCMS Analysis of Methanolic Extract of *A. calamus*

The GCMS and MSMS analysis done using *A. calamus* extract shows the presence of Friedelan-3-one (Fig. 47.2). The spectrum and library search of 34.41 min RT peak represents the compound, Friedelan-3-one.

Conclusions

The administration of *A. calamus* extract reduced serum T-c, TG, and LDL-c levels. Furthermore, *A. calamus* helps to improve cardio-protective lipid HDL-c. The mechanism of hypolipidemic activity of *A. calamus* was not studied till date. Our study addresses the shortfall; it is presumed that a higher hypolipidemic effect of *A. calamus* might be due to the activation of LCAT enzyme. GCMS analysis data of same fraction of *A. calamus* extract shows that major peak present is Friedelan-3-one. The decrease of serum TG level is also a promising result, since triglycerides are independently related to cardiovascular disease and most of hypercholesterolemic drugs were not able to reduce TG levels. Based on this observation, it can be comprehended that the *A. calamus* fraction containing compound Friedelan-3-one is able to activate LCAT and exhibit potential hypolipidemic activity. The results constitute a valid scientific groundwork for its medicinal application and valid support for “lekhaneya” action of *A. calamus*.

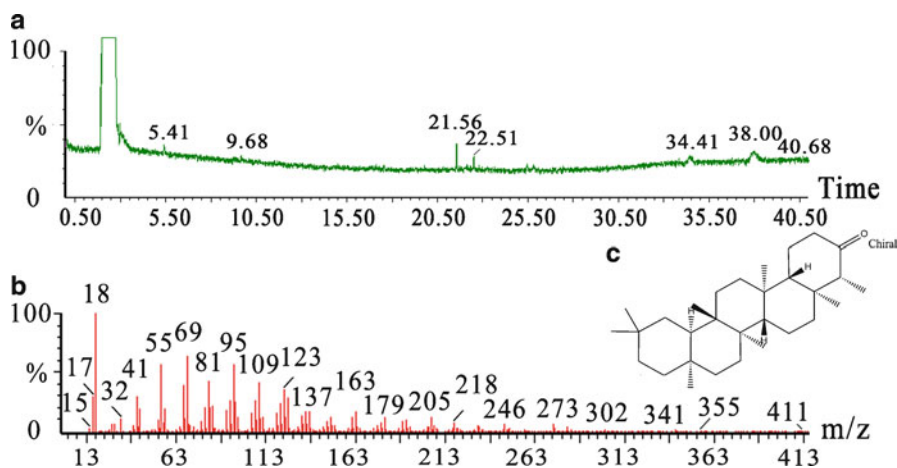


Fig. 47.2 (a) The total ion chromatogram of *A. calamus* extract. (b) The spectrum and library search of 34.41 min RT peak of Friedelan-3-one. (c) Structure of Friedelan-3-one

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