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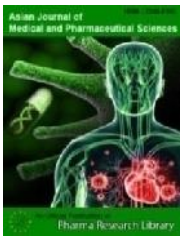

Research Article **Open Access**

Evaluation of Antihyperlipidemic Activity of Methanolic Extract of *Acorus Calamus* in fat diet Induced Rats

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A B S T R A C T

The present research work is involved with the evaluation of Acorus Calamus Extracts on plasma lipid levels in Triton -X-100 induced hyperlipidemic rats. In conclusion our results suggest that the post treatment with phenolic and triterpenoidal extract of A.calamus (AC) showed dose dependent antihyperlipidemic activity against Triton-X & high fat diet induced hyperlipidemia indicating that naturally occurring plant components phenolics and triterpenoids of this plant may be used as starting structures for the potential development of antihyperlipidemic agents.

Keywords: Acorus Calamus, antihyperlipidemic, Triton -X-100.

A R T I C L E I N F O

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1. Introduction

Obesity is a medical condition in which excess body fat has accumulated to the extent that it may have a negative effect on health. People are generally considered obese when their body mass index (BMI), a measurement obtained by dividing a person's weight by the square of the person's height, is over 30 kg/m^2, with the range 25–30 kg/m^2 defined as overweight. Some East Asian countries use lower values. Obesity increases the likelihood of various

diseases, particularly heart disease, type 2 diabetes, obstructive sleep apnea, certain types of cancer, and osteoarthritis. Obesity is a medical condition in which excess body fat has accumulated to the extent that it may have a negative effect on health. People are generally considered obese when their body mass index (BMI), a measurement obtained by dividing a person's weight by the square of the person's height, is over 30 kg/m2, with the range 25–30 kg/m2 defined as overweight. Some East Asian countries use lower values. Obesity increases the likelihood of various diseases, particularly heart disease, type 2 diabetes, obstructive sleep apnea, certain types of cancer and osteoarthritis. A growing public health concern is that the prevalence of obesity among children aged 6–19 is up to 16.5% in the USA and has also increased in Europe, Asia, Africa and South American countries. Despite increased attention given to overweight and obesity by virtually every major body concerned with public health, including the National Institutes of Health (NIH) (National Task Force, 1994) the Centers for Disease Control, the United States Department of Agriculture and the World Health Organization (World Health Organization, 2000)

Other Specific Syndromes Associated with Obesity:

Insulinoma Patients with insulinoma often gain weight as a result of overeating to avoid hypoglycemia symptoms. The increased substrate plus high insulin levels promote energy storage in fat. This can be marked in some individuals but is modest in most.

Cushing's syndrome

Although obese patients commonly have central obesity, hypertension, and glucose intolerance, they lack other specific stigmata of Cushing's syndrome. Nonetheless, a potential diagnosis of Cushing's syndrome is often entertained. Cortisol production and urinary metabolites (17OH steroids) may be increased in simple obesity. Unlike in Cushing's syndrome, however, cortisol levels in blood and urine in the basal state and in response to corticotropin-releasing hormone (CRH) or ACTH are normal; the overnight 1-mg dexamethasone suppression test is normal in 90%, with the remainder being normal on a standard 2-day low-dose dexamethasone suppression test. Obesity may be associated with local reactivation of cortisol in fat by 11 ß hydroxysteroid dehydrogenase 1, an enzyme that converts cortisone to cortisol.

Hypothyroidism: The possibility of hypothyroidism should be considered, but it is an uncommon cause of obesity; hypothyroidism is easily ruled out by measuring thyroid-stimulating hormone (TSH). Much of the weight gain that occurs in hypothyroidism is due to myxedema.

Hyperlipidemia, hyperlipoproteinemia

Hyperlipidaemia (British English) is abnormally elevated levels of any or all lipids and/or lipoproteinsin the blood. It is the most common form of dyslipidemia (which includes any abnormal lipid levels).

Complications of Hyperlipidemia with obesity: The medical problems caused by obesity begin at the head and end at the toes and involve almost every organ in between.

Obesity Comorbidity:

From the top of the head to the tip of the toes and almost every organ in-between.

2. Experimental

Atorvastatin, Normal saline, chloroform, Diethyl ether, Triton X-100, Shimadzu Electronic Balance , U.V spectrophotometer, Ultra homogenizer, Centrifuge RM-12C, Rotary Evaporator, Total cholesterol kits, Triglycerides Kits, HDL-Cholesterol Kits

Methodology

Collection and Authentification of Plant Material: The Aerial Parts of *A. calamus* were collected and authenticated

Extraction of Plant Material

The plant is grinded in to a coarse powder with the help of suitable grinder.

Cold Extraction (Methanol Extraction)[38]

In this work the cold extraction process was done with the help of methanol. About 200gms of powdered material was taken in a clean, flat bottomed glass container and soaked in 750 ml of methanol. The container with its contents were sealed and kept for period of 7 days accompanied by continuous shaking with the shaker. The whole mixture then went under a coarse filtration by a piece of a clean, white cotton wool.

Evaporation of Solvent

The filtrates (methanol extract) obtained were evaporated using Rotary evaporator in a porcelain dish. They rendered a gummy concentrate of greenish black. The extract was kept in vaccum dissecator for 7 days.

% Yield value of Methanol Extract from Aerial Parts of *A.Calamus* Plant.

Powder taken for extraction = 200gm
Weight of the empty china dish = 53.70gm
Weight of the china dish with extract = 73.24gm
Weight of the extract obtained = (73.24-53.7)gm =18.54 gm
% yield of methanol extract = (weight of extract)/(powder taken for extraction) × 100 = 18.54/200 ×100 = 9.27 %.

Preliminary Phytochemical Screening

Preliminary phytochemical screening of the *A. calamus* extract was carried out for the analysis of Alkaloids, Carbohydrates, Tannins, Saponins, Steroids, Phenols, Flavonoids as per the standard methods

- Detection of Alkaloids
- Detection of Carbohydrates
- Detection of saponins
- Detection of steroids
- Detection of Phenols
- Detection of Tannins
- Detection of Flavonoids

Animals

Healthy Adult Male wistar rats of 8-10 weeks old with Average weight in the range of 150-180gms were selected. Animals are housed 4 per cage in temperature controlled (27 ^{0}C ±3 ^{0}c) room with light/dark cycle in a ratio of 12:12 hrs is to be maintained. The Animals are allowed to acclimatize to the environment for seven days and are supplied with a standard diet and water *adlibitum*. The prior permission was sought from the Institutional Animal Ethics Committee (IAEC) for conducting the study.

Acute toxicity studies

The Acute oral toxicity test of the extracts was determined prior to the experimentation on animals according to the

OECD (Organization for Economic Co-operation and Development) guidelines no 423. Female Albino wistar rats (130-200 g) were taken for the study and dosed once with 2000 mg/kg of the extract. The treated animals were monitored for 14 days to observe general clinical signs and symptoms as well as mortality. No mortality was observed till the end of the study revealing the 2000 mg/kg dose to be safe. Thus, ¼ and 1/8 doses of 2000 mg/kg i.e. 500 mg/kg and 250mg/kg were chosen for subsequent experimentation.

Method of Induction

The systemic administration of the surfactant Triton X-100 to rats and supportive high fat diet results in elevation of plasma cholesterol and triglycerides. Hyperlipidemia was induced in Wistar albino rats by single *i.p* injection of freshly prepared solution of Triton-X-100 (100 mg/kg) in physiological saline solution after overnight fasting for 18 h. And it is supplied with high fat diet **Experimental Animal Protocol**

Experimental rats, straved for 18 hr, were provided water *ad libitum*. The rats were divided in to five groups containing four animals in each group.

Group-I: Normal Control (Normal saline 10ml/kg orally) for 7 days

Group-II: Hyperlipidemic control, three i.v. injection of Triton-X-100 on consecutive days with high fat diet

Group-III: Hyperlipidemic Rats treated with Atorvastatin (Standard drug) at 10 mg /kg orally for 7 days with high fat diet

Group-IV: Hyperlipidemic rats treated with Ethanolic extract of *A.calamus* (AC) (Low Dose) at a daily dose of 250mg/kg orally for 7 days with high fat diet

Group-V: Hyperlipidemic Rats treated with Ethanolic extract of *A.calamus* (AC) (High Dose) at a daily dose of 500mg/kg orally for 7 days with high fat diet

The rats were divided into five groups containing six animals in each group. All the groups receives three i.v. injection of Triton-X-100 (100 mg/kg) on consecutive days with supportive high fat diet , simultaneously with Group-II, Group – III, Group – IV, Group – V, expect Group – I (Normal control) to induce hyperlipidemia.

The Group – III receives atorvastatin at dose of 10 mg/kg, was prepared by suspending bulk atorvastatin in aqueous 5% methyl cellulose for 7 days. The Group– IV, receive AC at a dose of 250mg/kg for 7 days and Group – V, receives ACat a dose of 500mg/kg for 7 days 99

Blood Sample Collection and Analysis

The rats are Anesthetised by ether and then Blood samples were collected on 0^{th} and 8^{th} day from retro-orbital plexus of rat using micro capillary technique from rats of all the groups, and centrifuged at 3000 rpm for 15 min so as to get serum. The serum is analyzed for total cholesterol, triglycerides and HDL levels using biochemical kits (diagnostic kit.).

VLDL, and LDL- Cholesterol were calculated by the below formula

Serum LDL- Cholesterol concentration was calculated Accordingto the equation of Fried and wald.

LDL-Cholesterol=Total Cholesterol – (HDL- Cholesterol +TG/5)

VLDL-C = TG/5

Estimation of cholesterol (Total cholesterol) CHOD/ POD Method.

Clinical Significance

Heart disease is often the result of cholesterol deposits on the arteries. While not the only factor for heart disease, serum cholesterol levels are often checked to determine the risk of heart disease on patient.

Estimation of HDL cholesterol

Procedure: It includes two steps.

Step: 1- precipitation

Table 1

Serum	0.2 ml
HDL precipitating reagent	0.3 ml

Step: 2 – color development

Take 3 clean glass tubes labelled as blank (B), standard (S), and test (T). Mix well and stand at room temperature for 10min, centrifuge at 3000 rpm for 10 min.

Table 2

	Blank	**Standard**	**Test**
Enzyme reagent	1 ml	1ml	1 ml
Cholesterol (Standard)	-	0.01 ml	-
Supernatant serum Step-1	-	-	0.1 ml
Distilled water	0.1 ml	0.1 ml	-

LDL Calculation: It is calculated using formula: LDL = TC-HDL-TG/5.0 (mg/dl).

VLDL is calculated using formula:

VLDL = Triglycerides (mg/dl) / 5,

According to these guidelines, the normal range of lipid profile

LDL Calculation: It is calculated using formula: LDL = TC-HDL-TG/5.0 (mg/dl).

VLDL is calculated using formula:

VLDL = Triglycerides (mg/dl) / 5, According to these guidelines, the normal range of lipid profile

Table 3

Total cholesterol	**< 200 mg/dl**
Triglycerides	< 200 mg/dl
HDL	> 40 mg/dl
LDL	< 150 mg/dl
VLDL	5-30 mg/dl

LDL/HDL and TC/HDL ≤ 5 mg/dl are the favourable risk factor

Statistical Analysis:

Results are expressed as Mean ± S.D .all the results were compared with control subject one-way analysis of variance (ANOVA), followed by the dunnet t-test using Graph Pad Prism Software 6 version. P Values<0.05 were as considered statistically significant.

3. Results and Discussion

%Yield value of Ethanolic Extract from Aerial Parts *A.calamus* (AC) was found to be **12.27%**

Preliminary Phytochemical Screening: Investigation revealed the presence of steroid, Alkaloid, Carbohydrate, Phenol& Flavonoid in Ethanolic Extract of *A.calamus* (AC)

Table 4: Preliminary Phytochemical Screening

Phytochemical	**Results**
Steroid	+
Alkaloid	+
Tannin	-
Carbohydrate	+
Phenol	+
Flavonoid	+
Saponin	-

Acute toxicity studies: As per (OECD) draft guidelines 423 Female albino rats were administered *A.calamus* (AC) and doses was be selected in the sequence (1.75- 5000) using the default dose progression factor, for the purpose of toxicity study. Animals are observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours and daily thereafter, for a total of 14 days,. In all the cases, no death was observed within 14 days. Attention was also given to observation of tremors and convulsions, salivation, diarrhoea, lethargy, sleep and coma. Overall results suggested the LD_{50} value as 2000 mg/kg. Hence therapeutic dose was calculated as $1/4^{th}$ and $1/8^{th}$ i.e. 500mg/kg and 250 mg/kg of the lethal dose for the purpose of antihyperlipidemic investigations.

Table 5: Effect of *A.calamus* (AC) Extracton Serum Total Cholesterol levels

Groups	**TC**	
	0^{th} day	**8^{th} day**
Normal	62.1±2.1	63.1±2.0
Hyperlipidemic	159.16±4.37	158.93±3.0#
Std. Atorvostatin 10mg/kg	166.24± 2.2	115.2±2.03⋆
AC 250mg/kg.	152.26 ±2.35	135.28±3.6⋆
AC500mg/kg.	163.06± 6.3	118.7±3.3⋆

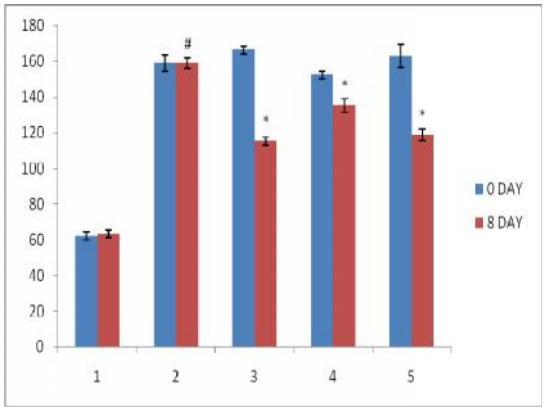

Figure 1: Effect of *A.calamus* (AC) Extracton Serum Total Cholesterol levels# P<0.01 vs Normal Control, ⋆p<0.01, vs Hyperlipidemic control.

Table 4: Effect of *A.calamus* (AC) Extracton Serum triglyceride levels

Groups	**TG**	
	0^{th} day	**8^{th} day**
Normal	78.58±3.2	82.42±2.8
Hyperlipidemic	177.20±3.2	175.21±5.5#
Std. Atorvostatin 10mg/kg	176.57± 2.3	94.49±2.5⋆
AC 250mg/kg.	163.27± 3.6	139.65±6.9⋆
AC 500mg/kg.	172.4± 4.3	109.37±4.1⋆

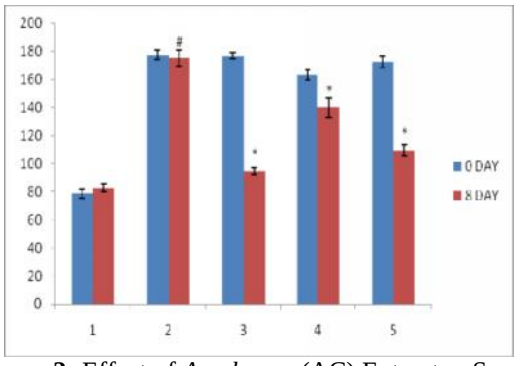

Figure 2: Effect of *A.calamus* (AC) Extracton Serum Triglyceride Levels #P<0.01 vs Normal Control, ⋆ p<0.01, vs Hyperlipidemic control.

Table 6: Effect of *A.calamus* (AC) Extracton Serum LDL levels

Groups	**LDL**	
	0^{th} day	**8^{th} day**
Normal	20.24±2.7	21.84±2.6
Hyperlipidemic	97.52±3.5	98.74±3.1#
Std. Atorvostatin 10mg/kg	101.16±1.8	54.9±1.5⋆
AC 250mg/kg.	88.87± 2.7	70.86±4.1⋆
AC 500mg/kg.	98.36± 6.1	56.09±4.6⋆

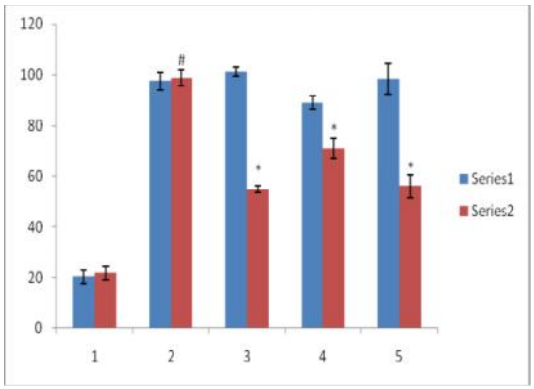

Figure 3: Effect of A.*calamus (AC)* Extracton Serum LDL-C levels #P <0.01 vs Normal Control, *p<0.01, vs Hyperlipidemic control.

Table 7: Effect of *A.calamus* (AC) Extracton Serum VLDL levels

Groups	VLDL	
	0th day	**8 th day**
Normal	14.69±1.8	14.78±2.3
Hyperlipidemic	35.43±0.61	35.03±1.1#
Std. Atorvostatin 10mg/kg	35.31± 0.45	18.89±0.5⋆
AC 250mg/kg.	32.65± 0.7	27.92±1.3⋆
AC 500mg/kg.	34.48± 0.8	21.77±0.75⋆

Table 8: Effect of *A.calamus* (AC) Extracton Serum HDL levels

Groups	HDL	
	0th day	**8th day**
Normal	39.8±2.0	38.89±2.7
Hyperlipidemic	26.20±1.0	24.90±1.85#
Std. Atorvostatin 10mg/kg	29.77 ± 1.1	41.45±0.45⋆
AC 250mg/kg.	30.78 ± 0.95	36.52±0.8⋆
AC500mg/kg.	30.41± 0.95	40.75±1.72⋆

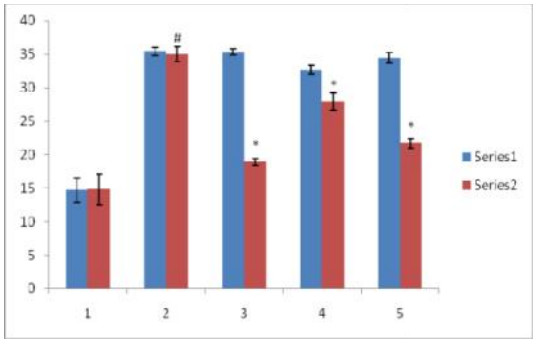

Figure 4: Effect of *A.calamus* (AC) Extracton Serum VLDL-C levels#

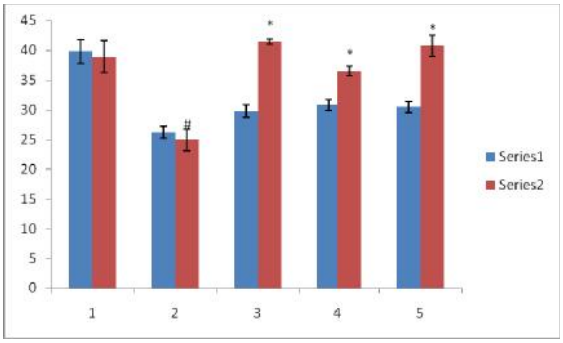

Figure 5: Effect of *A.calamus* (AC) Extracton Serum HDL levels

Table 1: Lipid profiles obtained on 0th day (Before treatment) and 8th day (After treatment)

Groups	TC		TG		HDL		LDL		VLDL	
	0th day	**8th day**	**0th day**	**8th day**	**0th day**	**8th day**	**0th day**	**8th day**	**0th day**	**8th day**
Normal	62.1±2.2	63.13±2.3	78.58±3.2	82.42±2.8	39.8±2.0	38.89±2.7	20.24±2.7	21.84±2.6	14.69±1.8	14.78±2.3
Hyperlipidemic	159.16±4.37	158.93±3.0#	177.2±3.2	175.21±5.5#	26.20±1.0	24.90±1.85#	97.52±3.5	98.74±3.1#	35.43±0.61	35.03±1.1#
Std. Atorvostatin 10mg/kg	166.24±2.2	115.2±2.03⋆	176.57±2.3	94.49±2.5⋆	29.77 ± 1.1	41.45±0.45⋆	101.16±1.8	54.9±1.5⋆	35.31±0.45	18.89±0.5⋆
AC 250mg/kg.	152.26±2.35	135.28±3.6⋆	163.27 ± 3.6	139.65±6.9⋆	30.78 ± 0.95	36.52±0.8⋆	88.87±2.7	70.86±4.1⋆	32.65±0.7	27.92±1.3⋆
AC 500mg/kg.	163.06±6.3	118.7±3.3⋆	172.4±4.3	109.37±4.1⋆	30.41±0.95	40.75±1.72⋆	98.36±6.1	56.09±4.6⋆	34.48±0.8	21.77±0.75⋆

4. Conclusion

In conclusion our results suggest that the post treatment with phenolic and triterpenoidal extract of *A.calamus* (AC) showed dose dependent antihyperlipidemic activity against Triton-X & high fat diet induced hyperlipidemia indicating that naturally occurring plant components phenolics and triterpenoids of this plant may be used as starting structures for the potential development of antihyperlipidemic agents.

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