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## The influence of *Alpinia calcarata* extract on the serum lipid and leptin levels of rats with hyperlipidemia induced by high-fat diet

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### ABSTRACT

**Objective:** To study the effect of ethanolic extract from *Alpinia calcarata* rhizomes on the serum lipid and leptin levels of rats with hyperlipidemia induced by high-fat diet. **Methods:** Male wistar rats were divided into six groups: normal, high-fat diet control (HFD) and ethanolic extract of *Alpinia calcarata* rhizomes (ACRE) (100, 200 and 300 mg/kg; *p.o.*) was administered to the high fat-diet-induced hyperlipidemic rats for 30 days to evaluate its antihyperlipidemic activity. Atorvastatin (10 mg/kg; *p.o.*) was used as a standard drug. **Results:** The results demonstrated that in HFD-induced hyperlipidemic rat, ACRE reduced rat weight gain, triglyceride (TG), total cholesterol levels (TC), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), total protein (TP) and leptin level (pg/mL). **Conclusions:** These encouraging findings suggest that ACRE has excellent pharmacological potential to prevent hyperlipidemia.

## 1. Introduction

Hyperlipidemia, mainly increase level of total cholesterol (TC), triglycerides (TG) and low-density lipoprotein (LDL) cholesterol along with decrease in high-density lipoprotein (HDL) cholesterol, is the predictor of coronary artery disease (CAD). Hyperlipidemia is an important risk factor in the initiation and progression of atherosclerotic impasse<sup>[1,2]</sup>. The protein leptin, a satiety hormone, regulates appetite, energy homeostasis and glucose/lipid metabolism<sup>[3,4]</sup>.

Many medicinal plants have been found to be useful to successfully manage hyperlipidemia; these include *Persea americana* Mill<sup>[5]</sup>, *Allium porrum*<sup>[6]</sup>, *Purslane*<sup>[7]</sup>, *Eclipta prostrata*<sup>[8]</sup>, *Scoparia dulcis*<sup>[9]</sup>, *Trigonella foenum-graecum* and red yeast rice<sup>[10]</sup>. However, only a limited amount of clinical research exists to support their efficacy. *Alpinia calcarata* Roscoe belongs to the family of Zingiberaceae. In India, Kulanjan in Hindi, Heen-araththa in Sinhala,

Amkolinji in Tamil and Kattuchena in Malayalam is a rhizomatous perennial herb. The mature rhizomes are branched and dense with a light to dark brown color. The leaf of the plant is simple, alternative, 25.0–32.0 cm long, 2.5–5.0 cm broad. The flowers are irregular, bisexual and pendunculate. Terminal densed flowers are found in panicles 8.5 cm long<sup>[11,12]</sup>. *Alpinia calcarata* is cultivated in tropical countries including China, India, Sri Lanka and Malaysia. Especially the rhizomes of *Alpinia calcarata* are used for the medicinal purposes<sup>[12]</sup>.

Kong *et al*<sup>[13,14]</sup> have isolated some diterpenes such as calcaratarins A–E, sesquiterpenes such as shyobunone and coumarins such as herniarin from the rhizomes of *Alpinia calcarata* grown in china. On the other hand, some benzenoids such as protocatechuic acid, vanillic acid and syringic acid, terpenoids, phenolic compounds, flavonoids and alkaloids were isolated from the leaves of *Alpinia calcarata* grown in India<sup>[15]</sup>.

*Alpinia* is a large, widespread and taxonomically complex genus in the Zingiberaceae with 230 species occurring throughout tropical and subtropical Asia<sup>[16]</sup>. *Alpinia calcarata* is a slender aromatic herb belonging to this genus and in India; it is used in the traditional systems of medicine to treat diabetes, rheumatism, fever and stomachache<sup>[17]</sup>. In

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siddha system of medicine, decoction of the plant rhizome is used in the treatment of respiratory disorder, expectorant, diabetes and obesity<sup>[18]</sup>. Some researchers have shown anti-inflammatory effect<sup>[19]</sup>, antioxidant, antifungal<sup>[20]</sup> and anticancer activity<sup>[21]</sup> in extract of *Alpinia calcarata*. Screening plants with such ethnomedical uses is believed to increase the odds in discovering new medicines<sup>[22]</sup>.

However, antihyperlipidemic activity of ethanolic extract of *Alpinia calcarata* rhizomes (ACRE) on serum lipid and leptin levels has not been investigated using scientifically controlled experiments. Therefore, an attempt has been made to investigate the effects of ACRE in high-fat diet-induced hyperlipidemic rats.

## 2. Materials and methods

### 2.1. Plant materials and extraction

The rhizomes of *Alpinia calcarata* were collected in the month of July 2009 from ABS Botanical garden Kari Patti, Salem (T.N.). The rhizomes of *Alpinia calcarata* were authenticated by Mr. A. Balsubramanian, Department of Botany, Consultant Central Siddha Research, Salem, (T.N.) and voucher specimen (DOBS/10/03) was deposited in our research laboratory for the future reference. Fresh *Alpinia calcarata* rhizomes were cut into small pieces and air dried for 12–15 days in the shade. Five hundred grams of powdered rhizomes were extracted with 1.5 L of ethanol using soxhlet extraction apparatus for 4 h. The miscella was filtered and the filtrate was evaporated to dryness under reduced pressure at 55 °C (yield 19.5%, w/w, dry weight basis) and stored at 4 °C until use. The extract (1%, w/v) was dissolved in carboxy methyl cellulose (CMC) for oral administration to experimental animals.

### 2.2. Phytochemical analysis

ACRE was subjected to phytochemical screening<sup>[23]</sup> for the detection of various phyto-constituents.

### 2.3. Chemicals

Biochemical kits for total TG (GPO-POD), TC (CHOD-POD), high density lipoprotein (PEG) and total protein (Biuret) were purchased from Crest Biosystems Kits (India). Leptin levels measurements the R&D Systems ELISA kit was used. Normal diet and high fat diet were obtained from Technocrats Institute of Technology Pharmacy, Bhopal.

### 2.4. Animals

Male wistar rats weighing (200±20) g were provided by the animal house of TIT Pharmacy, Bhopal. All animals were maintained in standard polypropylene cages and maintained at

(23±20 °C under 12:12 h light/dark cycle with free access to rodent chow and tap water. All animal experimentation was carried out after approval of the protocol by the Institutional Ethical Committee of RGPV University. The guidelines of CPCSEA, India, were strictly followed (Reg no. TIT/IAEC/831/2010/01).

### 2.5. Determination of effective dose

The ACRE was administered at different doses of 50, 100, 250, 500, 750 and 1000 mg/kg/day orally for 4 days of six groups of rats (six in each group) and the animals were observed for mortality during the course of treatment or on the fifth day were tested again at lower dose levels and dose showing no mortality in rats was selected as effective dose.

### 2.6. Antihyperlipidemic activity in high-fat diet-induced hyperlipidemic rats

The method described by Sampathkumar *et al.* <sup>[24]</sup> was employed in the study. Male wistar rats were divided into six groups each comprising six rats. Initially all the animals were given the normal diet for 1 week. This was a period of acclimatization. Group I was served as normal and fed with normal diet throughout the course of study. Animals of Group II to VI were fed with high-fat diet for 30 days. Subsequently, the high-fat diet fed animals were replaced by normal diet. Group I received normal diet only. Group II, served as HFD control received high fat diet only, Group III was treated with standard drug Atorvastatin suspension prepared with Tween 80 (10 mg/kg; *p.o.*) and the groups IV, V and VI received ACRE in divided doses of 100, 200 and 300 mg/kg; *p.o.*, respectively in addition for next 30 days.

### 2.7. Determination of body weight, serum lipid and hormonal profile

Throughout the study change in body weight of each rat was measured once in a week. Lipid profiles of all the rats were determined on 30th day (post-treatment). The blood samples were collected from each rat by retro-orbital venepuncture of the overnight fasted rats into micro centrifuge tubes containing heparin (10 µL, 1000 IU/mL). Serum TC, TG, HDL, VLDL, LDL, TP and Leptin were estimated using commercially available kits.

### 2.8. Statistical analysis

Statistical analysis was carried out by using Graph-Pad InStat statistical package (Graphpad Software Inc.). Values are expressed as mean±SEM. For multiple comparisons, one way ANOVA was used followed by Tukey test. *P* value < 0.05 was considered to be significant.

### 3. Results

#### 3.1. Phytochemical screening of ACRE

Phytochemical screening revealed the presence of alkaloids, steroids, terpenoids, phenolic compounds, coumarins, vanillic acid, protocatechuic acid, syringic acid, reducing sugars and flavonoids in alcoholic extract.

#### 3.2. Antihyperlipidemic effects

##### 3.2.1. Effect of ACRE on body weight

**Table 1.**

Effect of ACRE on weight gain in rats fed a high-fat diet.

Group	Treatment	Body weight (g)	
		Onset of study	End of study
I	Normal	172.17±2.88	178.83±4.26
II	HFD Control	245.83±3.57	298.17±6.68 <sup>#</sup>
III	ACRE (100 mg/kg)	238.14±4.51	219.33±5.97 <sup>*</sup>
IV	ACRE (200 mg/kg)	246.12±2.46	213.00±3.30 <sup>**</sup>
V	ACRE (300 mg/kg)	239.16±1.36	204.00±4.90 <sup>***</sup>
VI	Atorvastatin (10 mg/kg)	241.33±2.12	195.50±3.21 <sup>***</sup>

Values are expressed as mean±SEM,  $n = 6$ . Values are statistically significant at  $\# P < 0.001$  vs. normal group;  $*** P < 0.001$ ,  $** P < 0.01$ ,  $* P < 0.05$  vs. HFD control group, respectively (One-way ANOVA followed by Tukey's post hoc test).

The rats when fed high-fat diet showed marked increase in weight gain. Further, post hoc test revealed that at the 30th day, most significant ( $P < 0.001$ ) reduction in weight gain was evidenced in the ACRE-treated (300mg/kg; *p.o.*) groups (204.00±4.90) as compared with HFD control (298.17±6.68). However, body weight of obese rats was restored by treating with Atorvastatin (10mg/kg; *p.o.*) and treatment with ACRE

(300 mg/kg; *p.o.*) groups show most significant ( $P < 0.001$ ) reduction in weight gain as compared to HFD control group (Table 1). The effect of ACRE at a dose of 300 mg/kg body weight was more significant than at 100 and 200 mg/kg (219.33 ± 5.97, 213.00 ± 3.30).

##### 3.2.2. Effect of ACRE on serum lipid and hormonal profile

The rats when fed high-fat diet showed marked hyperlipidemia. The level of serum TC was increased in all the hyperlipidemia groups. One-way ANOVA indicated that treatment with ACRE (89.9 ± 3.89 mg/dL) and Atorvastatin (82.3 ± 1.12 mg/dL) significantly ( $P < 0.001$ ) decreased the elevated TC level from the end of the study (Table 2). Similarly there were also a raise in the level of serum TG with hyperlipidemia animals. ACRE (193.7 ± 0.30 mg/dL) had significantly ( $P < 0.001$ ) decreased the elevated serum TG after 30 days of the treatment as compared to the HFD control animals (243.2 ± 2.65 mg/dL). There were also a raise in the serum VLDL level observed in the HFD control group (48.0 ± 0.83 mg/dL), which was effectively reduced by treatment with ACRE (35.1 ± 0.74 mg/dl) after 30 day of the treatment. There was an elevated serum LDL level observed with HFD control animals (70.4±0.56 mg/dL), which was significantly ( $P < 0.001$ ) decreased by treatment with ACRE (53.9 ± 1.01 mg/dl) at the 30 day of treatment. The level of serum HDL decreased in all HFD control animals (31.5±0.43 mg/dL). This level was brought to increase near to normal level by treatment with ACRE (46.9 ± 0.24 mg/dl) after 30 days of treatment. Similarly, total protein (TP) level was raise in HFD control animals (10.15 ± 0.14 g/dl), which was significantly ( $P < 0.001$ ) decreased by treatment with ACRE (6.15 ± 0.10 g/dl) and Atorvastatin (5.88 ± 0.13 g/dl) at the 30th day of treatment (Table 2).

**Table 2.**

Effect of ACRE on serum lipid and leptin levels in rats fed a high-fat diet.

Parameter	Days	Group I	Group II	Group III	Group IV	Group V	Group VI
TC (mg/dL)	0	74.20±2.40	115.00±3.30	129.30±1.41	125.10±0.87	121.30±0.60	116.80±2.7
	30	78.30±1.05	150.30±2.00 <sup>#</sup>	100.30±2.46 <sup>*</sup>	94.00±4.21 <sup>**</sup>	89.90±3.89 <sup>***</sup>	82.30±1.12 <sup>***</sup>
TG (mg/dL)	0	158.40±1.54	220.20±1.04	237.70±0.15	242.20±0.25	239.30±0.37	239.70±0.90
	30	164.20±1.23	243.20±2.65 <sup>#</sup>	219.20±0.23 <sup>*</sup>	218.30±0.30 <sup>*</sup>	193.70±0.30 <sup>***</sup>	186.20±0.11 <sup>***</sup>
HDL (mg/dL)	0	31.20±0.10	36.00±0.11	37.00±0.37	35.00±0.17	36.70±0.67	37.40±0.32
	30	40.30±0.44	31.50±0.43	43.10±0.11 <sup>*</sup>	43.70±0.13 <sup>*</sup>	46.90±0.24 <sup>***</sup>	47.00±0.26 <sup>***</sup>
LDL (mg/dL)	0	50.40±0.13	64.00±0.72	63.90±0.37	62.70±0.97	65.90±0.97	62.00±0.90
	30	57.60±0.60	70.40±0.56 <sup>#</sup>	57.00±0.16 <sup>**</sup>	58.00±0.06 <sup>**</sup>	53.90±1.01 <sup>***</sup>	50.40±0.71 <sup>***</sup>
VLDL (mg/dL)	0	30.80±0.61	43.70±0.67	45.70±0.45	46.20±0.35	46.50±0.11	45.40±0.16
	30	34.00±0.23	48.00±0.83 <sup>#</sup>	39.50±0.74 <sup>*</sup>	36.80±0.17 <sup>***</sup>	35.10±0.74 <sup>***</sup>	34.00±0.60 <sup>***</sup>
TP (g/dL)	0	3.13±0.38	7.95±0.24	7.21±0.23	8.98±0.49	7.70±0.17	7.87±0.20
	30	5.00 ± 0.11	10.15±0.14 <sup>#</sup>	7.48±0.42 <sup>*</sup>	7.16 ± 0.36 <sup>*</sup>	6.15±0.10 <sup>***</sup>	5.88±0.13 <sup>***</sup>
Leptin (pg/mL)	0	67.13±4.07	210.03±19.13	222.12±50.12	219.46±14.23	214.35±23.07	232.43±19.03
	30	83.14±2.23	241.10±14.16 <sup>#</sup>	134.56±31.29 <sup>*</sup>	129.24±15.38 <sup>*</sup>	93.36±48.57 <sup>***</sup>	89.24±27.38 <sup>***</sup>

Values are expressed as mean±SEM,  $n = 6$ . Values are statistically significant at  $\# P < 0.001$  vs. normal group;

$*** P < 0.001$ ,  $** P < 0.01$ ,  $* P < 0.05$  vs. HFD control group respectively (One-way ANOVA followed by Tukey's post hoc test). Group-I served as normal, Group-II served as HFD control, Group III received 100 mg/kg (*p.o.*) of ACRE, Group IV received 200 mg/kg (*p.o.*) of ACRE, Group V received 300 mg/kg (*p.o.*) of ACRE, and Group VI received 10 mg/kg (*p.o.*) of Atorvastatin. Parameters-TC-total cholesterol, TG-triglycerides, LDL-low-density lipoprotein, VLDL-very low-density lipoprotein, HDL-high-density lipoprotein and TP-total protein, HFD-High fat diet.

Fasting serum leptin levels was elevated in HFD control animals ( $241.10 \pm 14.16$  pg/ml), which was significantly ( $P < 0.001$ ) decreased by treatment with ACRE ( $93.36 \pm 48.57$  pg/ml) and Atorvastatin ( $89.24 \pm 27.38$  pg/ml) at the 30th day of treatment (Table 2).

#### 4. Discussion

The main causative factor for atherothrombotic diseases is the disturbances occurring in lipid metabolism. Though there is a large class of hypolipidemic drugs used in the treatment, none of the existing ones available worldwide is fully effective, absolutely safe and free from side effects[25]. Hence efforts are being made to find out safe and effective agents that may be beneficial in correcting the lipid metabolism and preventing cardiac diseases. High level of serum cholesterol, triglyceride, LDL and VLDL along with low level of serum HDL in high-fat diet-induced hyperlipidemia state focused lipid lowering activity of ACRE or due to its influence on various lipid regulation systems[26]. Many herbs and plant products have been shown to have hypolipidemic properties[5–10]. The present study yielded several novel findings. ACRE (100–300mg/kg/day; *p.o.*) when administered initially to the hyperlipidemic rats causes a sharper and more significant decrease in the serum TC, TG and leptin level. Interestingly, ACRE showed significant ( $P < 0.001$ ) increase in the HDL cholesterol level of the experimental groups at 30 day of treatment. Atorvastatin (10mg/kg; *p.o.*) has also been included in the study in order to understand how far ACRE activity is comparable to that of a standard drug.

In the present study, we examined whether the ACRE treatment might improve the lipid and hormonal profile as well as in body weight resulting from a high-fat diet induced in rats. The results of the study reveal that the ACRE (100–300 mg/kg/day; *p.o.*) when administered for 30 days showed beneficial effects in hyperlipidemic rats. ACRE significantly reduced the weight gain in high-fat diet induced hyperlipidemic rats. ACRE inhibited the incremental increase in body weight in these animal models compared with that of control group. Diet-induced hyperlipidemia is the most relevant stimulus for the induction of atherosclerosis lesions in human. Diet-induced hypercholesterolemia is almost always useful for the assessment of agents that interfere with absorption, degradation and excretion of cholesterol with minimal effects on cholesterol biosynthesis[27]. In our study, significant decrease of cholesterol in the ACRE treated groups is manifested in all the lipoprotein fractions. These effects might be due to high plasma lipoprotein lipase activity, enzyme involved in hydrolysis of plasma VLDL triacylglycerols. High level of TC and most importantly, LDL cholesterol are the predictors of atherosclerosis[28]. Plasma leptin levels correlate with body fat content[3,4,29],

the increase of fat cells in number and in size is coupled with an increase in leptin secretion[29–31]. ACRE significantly ( $P < 0.001$ ) reduced TC, VLDL, LDL and leptin levels.

Several possible mechanisms may be proposed to prevention of hyperlipidemia may be that rhizome extract ingestion decreased the intestinal absorption of exogenous cholesterol. This effect may be involved based on the observed decrease in lipid digestibility. Various phytochemical studies have allowed the screening revealed the presence of protocatechuic acid, vanillic acid, syringic acid, terpenoids, phenolic compounds, flavonoids and alkaloids in the ACRE.

However, the supplementation of ACRE itself contributed to the decreased body weight gain when compared to the HFD group, which suggests that other functional factor(s) could be present in the ACRE, for instance, steroidal compounds, phenolic compounds and alkaloids. Generally, a high-fat diet significantly increases the total cholesterol, triglyceride and leptin levels in rats. Supplementation of ACRE significantly lowered serum total cholesterol, triglyceride and leptin levels, when compared to the HFD group. This could be related to the high contents of alkaloids and flavonoids in *Alpinia calcarata*. Flavonoids and alkaloids have also suggested for their hypocholesterolemic and hypolipidemic effects[32,33]. During the experimentation, rats did not show any mortality or any other adverse effects when the rats fed orally with ACRE the doses of 100–300 mg/kg/day. It is indicating that the ACRE have a good margin of safety.

Conclusively, the observed cholesterol-reducing action of ACRE in high-fat diet-induced hyperlipidemic rats possesses some potential medicinal value and could validate and explain its ethnomedical use on the obese and heart patients in India. Finally, we propose that ACRE may be effective as hyperlipidemic agents. We plan to conduct further studies to better understand the mechanisms of action of this medicinal plant.

#### Conflict of interest statement

We declare that we have no conflict of interest.

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