INFLUENCE OF *ALPINIA GALANGA* RHIZOMES ON CAFETERIA DIET INDUCED OBESITY IN RATS

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Summary

In the present work, the antiobesity effects of ethanol extract of *Alpinia galanga* rhizomes was evaluated in cafeteria diet fed obese rats. Obesity was induced in albino rats by feeding cafeteria diet daily for 6 weeks in addition to normal diet. The extract was administered at a daily dose of 400 mg/kg orally for 6 weeks. Body weight was measured initially and then every week thereafter. Individual food intake was measured during last 5 consecutive days. On day 42, serum glucose and lipid levels were estimated and then the weight of liver and parametrial adipose tissues was determined. The liver triglyceride content was estimated. The *in vitro* pancreatic lipase inhibitory activity of the extract was also determined. The extract produced inhibition of increase in body weight and parametrial adipose tissue weight induced by cafeteria diet, without producing significant change in food intake. The extract significantly reduced serum lipid levels, which were elevated by feeding cafeteria diet. In addition, the extract significantly inhibited the increase in liver weight and accumulation of hepatic triglycerides. The extract also produced dose dependent inhibition of *in vitro* pancreatic lipase activity. We conclude that ethanol extract of *Alpinia galanga* rhizomes is useful for treatment of cafeteria diet induced obesity in rats.

Keywords: *Alpinia galanga*, Body weight, Cafeteria diet, Antiobesity, Pancreatic lipase, Sibutramine

Introduction

Obesity is a medical condition involving an excess accumulation of body fat. It has increased at an alarming rate and is now a worldwide health problem. It is known that obesity results from disequilibrium between energy intake and expenditure, and obesity is known to be strong risk factor for coronary heart diseases including dyslipidemia, glucose intolerance, insulin resistance, and hypertension (1).

The drug treatment for obesity includes reducing nutrient absorption, anorectic drugs, thermogenic drugs or drugs that affect lipid metabolism. At present only two drugs, Sibutramine and Orlistat are approved for long-term use in treatment of obesity and each of these typically promotes 5% to 10% loss of body weight and has their own limitations and

side effects. An endocannabinoid receptor antagonist, Rimonabant was withdrawn from market due to concerns about its safety, including risk of seizures and suicidal tendencies (2).

Plants have formed the basis for traditional medicine systems that have been in existence for thousands of years. It has been estimated by WHO that approximately 80% of world's population relies mainly on traditional medicines for their primary health care. Numerous preclinical and clinical studies with various herbal medicines have been performed and some studies reported significant improvement in controlling body weight without any noticeable adverse effects (3, 4, 5, and 6).

Alpinia galanga Willd (Zingiberaceae) is a rhizomatous herb widely cultivated in shady situations of Malaysia, India, Indochina, and Indonesia. It is a reputed drug in the indigenous system of medicine and used in southern India as a domestic remedy for treatment of rheumatoid arthritis, inflammations, cough, asthma, obesity, diabetes, etc. (7). The hypoglycemic (8), hypolipidemic (9), antioxidant (10), antiulcer (11), and immunostimulating activity (12) of *Alpinia galanga* rhizomes is scientifically documented. However, its antiobesity profile is not reported.

Hence, the objective of the present study was to investigate the effect of ethanol extract of *Alpinia galanga* rhizomes on cafeteria diet induced obesity in rats. Sibutramine was used as standard control drug. In addition, the possible mechanism of antiobese action of the extract was investigated by measuring its influence on *in vitro* pancreatic lipase activity.

Materials and Methods

Preparation of *Alpinia galanga* extract (AGE)

The rhizomes of *Alpinia galanga* were collected from the fields surrounding Belgaum, Karnataka, India during September 2008 and were positively identified by Dr. S.S. Sasalatti, Head, Department of Botany, R.L. Science College, Belgaum, Karnataka, India, where a voucher specimen has been deposited. The rhizomes were air dried, powdered, and then extracted with 70% ethanol by Soxhlet method. The extract was filtered with Whatman No. 1 filter paper and then solvent evaporated at reduced pressure by using Rotavapor apparatus to get a viscous mass, which was then stored at 4°C until used. The % yield of the extract obtained was 4.2%.

Phytochemical study

The extract was subjected to standard phytochemical screening tests for determination of presence of various phytoconstituents (13).

Acute toxicity studies

The oral acute toxicity test of the AGE was determined as per OECD guidelines No. 420. Female wistar rats (150-200 g) were used for this study. The starting dose of 2000 mg/kg p.o. of the AGE was selected and given to group of five animals. The treated animals were monitored for 14 days, for mortality and general behaviour. No toxic symptoms and mortality was observed till the end of the study. The lethal dose (LD_{50}) selected was 2 g/kg body weight. Hence, $1/5^{th}$ of LD_{50} (400 mg/kg) was selected as experimental dose for further study.

Experimental animals

Albino wistar rats (180-200 g) of either sex were obtained from Central Animal House, J.N. Medical College, Belgaum and housed in a group of six animals for one week in a 12:12 h light and dark cycle in a temperature and humidity controlled room. The animals

were given free access to food and water. After one-week adaptation period, the healthy animals were used for the study. The Institutional Animal Ethics Committee, KLE University, Belgaum approved the experimental protocol.

Composition of Cafeteria diet

The cafeteria diet consisted of 3 diets – a) condensed milk (8 g) + bread (8 g); b) chocolate (3 g) + biscuits (6 g) + dried coconut (6 g); and c) cheese (8 g) + boiled potato (10 g). The 3 diets were presented to individual rats on day 1, 2, and 3, respectively, and then repeated for 42 days in same succession (14). The calorie value of the cafeteria diet is given in Table 1.

Table 1. Composition and calorie value of cafeteria diet.

Ingredients	Calorie value (kcal/100 g)
Condensed milk	335
Bread	230
Chocolate	550
Biscuit	360
Dried coconut	660
Cheese	320
Boiled potato	80

Treatment protocol

Animals were divided into following four groups of six animals each and individually housed in cages.

Group I: Normal control group fed with normal laboratory pellet chow *ad libitum* (calorie value = 280 kcal/100 g) and treated with 10% Tween 80 (5 ml/kg p.o.).

Group II: Cafeteria diet control group received cafeteria diet in addition to normal diet and received 10% Tween 80 (5 ml/kg p.o.).

Group III: AGE control group received cafeteria diet in addition to normal diet and AGE as a suspension in 10% Tween 80 (400 mg/kg p.o.)

Group IV: Standard control group received cafeteria diet in addition to normal diet and sibutramine (5 mg/kg p.o.).

The treatment was continued for 6 weeks. The animals were weighed at the start of the experiment and then every week thereafter.

Measurement of food intake

Food intake of each animal was determined by measuring the difference between the preweighed chows and weight of the food that remained every 24 h, during the last 5 consecutive days of the 6-week diet period and the results were expressed as mean energy intake for 5 days in kcal/day/kg.

Serum biochemical analysis

On day 42, blood was collected by retro-orbital puncture in ether-anaesthetized rats and subjected to centrifugation to obtain serum. The serum levels of glucose, total-cholesterol, HDL, LDL and triglycerides (TGs) were estimated using the biochemical kits (Beacon Diagnostics). The atherogenic index of plasma (AIP) was calculated by using: AIP = log (TGs/HDL)

Estimation of liver weight, parametrial adipose tissue weight and liver triglyceride content

Animals were then killed with an overdose of diethyl ether. The liver and parametrial adipose tissues were quickly removed and weighed. The liver tissues were stored at -80°C until analysis was performed. The liver triglyceride content was estimated as follows; a portion (0.5 g) of liver tissue was homogenized in Krebs Ringer Phosphate buffer (pH 7.4, 4.5 ml), and the homogenate (0.2 ml) was extracted with chloroform-methanol mixture (2:1, v/v, 4 ml). The extract was concentrated and the residue was analyzed using a Triglyceride E-test kit.

Measurement of in vitro pancreatic lipase activity

Lipase activity was determined by measuring the rate of release of oleic acid from triolein. A suspension of triolein (80 mg), phosphatidylcholine (10 mg), and taurocholic acid (5 mg) in 9 ml of 0.1 M N-Tris (hydroxymethyl) methyl-2-aminoethanesulfonic acid (TES) buffer (pH 7.0) containing 0.1 M NaCl was sonicated for 5 min. This sonicated substrate suspension (0.1 ml) was incubated with 0.05 ml (final concentration 5 units per tube) pancreatic lipase and 0.1 ml of various concentrations (1000, 2000, and 4000 mg/ml) of AGE for 30 min at 37°C in a final volume of 0.25 ml and the released oleic acid was measured (15).

Statistical analysis

The results were expressed as mean ± standard error (SEM). Data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test and were considered significantly different at $p < 0.05$.

Results

Phytochemical study

The preliminary phytochemical screening of AGE revealed the presence of saponin glycosides, sterols, flavonoids, triterpenes, and carbohydrates as phytoconstituents.

Effect of AGE on body weight

Table 2 shows the changes in body weight of the different group of animals during the experiment. Consumption of cafeteria diet for six weeks produced significant increase in body weight compared with consumption of normal pellet chow (normal control group). Treatment with AGE at the dose of 400 mg/kg significantly reduced the increase in body weight measured at 2-6 weeks as compared to cafeteria diet control group. However, treatment with sibutramine also resulted in significant reduction of body weight of cafeteria diet fed rats.

Table 2: Effect of *Alpinia galanga* extract (AGE) and sibutramine on body weight (g) in cafeteria diet fed rats.

Weeks	Normal control	Cafeteria diet control	AGE control	Standard control
0	196.5 ± 4.45	198.8 ± 2.14	200.2 ± 2.64	196.2 ± 3.44
1	225.6 ± 3.18*	246.4 ± 7.84	228.4 ± 4.12	230.4 ± 3.16
2	257.3 ± 2.16**	296.6 ± 7.46	266.8 ± 3.68**	262.8 ± 4.62**
3	280.2 ± 4.18**	320.4 ± 2.48	280.6 ± 4.26**	274.6 ± 3.46**
4	292.5 ± 4.08**	338.2 ± 4.26	302.8 ± 3.18**	298.8 ± 4.68**
5	298.6 ± 2.14**	352.8 ± 3.64	312.4 ± 2.64**	308.4 ± 3.24**
6	302.4 ± 4.56**	376.2 ± 3.16	332.6 ± 3.56**	326.6 ± 4.26**

Values are mean ± SEM (n = 6 in each group), * $p < 0.05$, ** $p < 0.01$ significant as compared to respective cafeteria diet control value.

Effect of AGE on food intake

There was significant ($p < 0.001$) increase in food consumption among the cafeteria diet fed rats as compared to normal diet fed rats. The mean energy intake measured for five days being 270.6 ± 2.46 kcal/day/kg and 439.2 ± 4.26 kcal/day/kg for normal control and cafeteria diet control group of rats, respectively. There was no significant difference observed in energy intake between the cafeteria diet control group and AGE treated group of rats. The mean energy intake measured for five days being 470.3 ± 4.26 kcal/day/kg for AGE treated rats. However, sibutramine control group of rats showed significant ($p < 0.01$) reduction in energy intake when compared to cafeteria diet control group of rats. The mean energy intake measured for five days being 328.6 ± 5.64 kcal/day/kg for sibutramine treated rats

Effect on AGE on serum biochemical parameters

Rats fed with cafeteria diet showed significant increase in serum levels of total-cholesterol, LDL and triglycerides as compared to normal diet fed rats. In contrast, AGE treated and standard control group of rats significantly inhibited the increase in serum levels of total-cholesterol, LDL and triglycerides, which were induced by cafeteria diet and significantly increased serum HDL levels. There was no significant change in the glucose concentration among normal control, cafeteria diet control, AGE treated group, and standard control group of rats (Table 3). The atherogenic index was calculated to be 0.135, 0.203, -0.024, and -0.061 for normal control, cafeteria diet control, AGE treated, and standard control group of rats, respectively.

Table 3: Effect of *Alpinia galanga* extract (AGE) and sibutramine on serum biochemical parameters in cafeteria diet fed rats.

	Normal control	Cafeteria diet control	AGE control	Standard control
Glucose	138.45 ± 2.48	142.28 ± 3.12	143.54 ± 2.76	133.24 ± 1.24
Total cholesterol	98.28 ± 3.24**	124.56 ± 2.16	110.42 ± 1.86**	98.24 ± 2.46**
HDL	24.22 ± 1.06	26.86 ± 1.28	35.12 ± 1.32**	37.26 ± 1.42**
LDL	52.34 ± 1.12**	98.64 ± 1.64	64.28 ± 1.25**	62.42 ± 1.62**
Triglycerides	65.64 ± 2.08**	98.26 ± 2.82	76.18 ± 1.72**	74.24 ± 1.84**

Values are mean ± SEM (n = 6 in each group), ** $p < 0.01$ significant as compared to respective cafeteria diet control value.

Effect of AGE on liver weight, parametrial adipose tissue weight and liver triglyceride content

Feeding a high fat cafeteria diet for six weeks produced significant increase in parametrial adipose tissue weight as compared to normal diet fed rats. Furthermore, the cafeteria diet also induced fatty liver, with the accumulation of triglycerides when compared to normal control group. The AGE treated and standard control group of rats showed significant inhibition of increases in weight of liver, parametrial adipose tissue, and the accumulation of liver triglyceride, which were induced by cafeteria diet (Table 4).

Table 4: Effect of *Alpinia galanga* extract (AGE) and sibutramine on parametrial adipose tissue (PAT) weight, liver weight, and liver triglyceride content in cafeteria diet fed rats.

	Normal control	Cafeteria diet control	AGE control	Standard control
PAT weight (g)	4.26 ± 0.98**	10.42 ± 1.06	6.12 ± 0.84*	6.18 ± 0.82*
Liver weight (g)	6.54 ± 1.22*	12.28 ± 1.64	7.48 ± 1.22*	7.26 ± 0.46*
Liver triglycerides (mg/g)	68.56 ± 4.28**	94.64 ± 3.12	79.74 ± 2.64**	76.2 ± 2.62**

Values are mean ± SEM (n = 6 in each group), * $p < 0.05$, ** $p < 0.01$ significant as compared to respective cafeteria diet control value.

Effect of AGE on pancreatic lipase activity

The amount of free fatty acids released from triolein was found to be 0.19, 0.14, and 0.10 µmol/ml/h by 1000, 2000, and 4000 mg/ml of AGE, respectively.

Discussion

Obesity, which affects up to 30% of adult population in developed countries, is associated with serious mortalities including a high incidence of type 2 diabetes, hyperlipidemia, hypercholesterolemia, cardiovascular diseases, osteoarthritis, and increased risk of many forms of cancer (16). When body weight increases to 20% above the average, the likelihood of mortality rises by 20% for men and 10% for women (17). Because, mortality risk through development of various deadly diseases is dramatically increased in obese patients, a quick and effective treatment is required.

Various animal models of obesity have been used to emulate obesity like condition in humans in order to develop effective antiobesity treatments. Among the animal models of obesity, rats that are fed a high fat diet are considered useful; a high % of fat in their diet is considered to be an important factor in the development of obesity, leading to accumulation of body fat even in the absence of an increase in calorie intake (18).

In the present study, the antiobesity effect of *Alpinia galanga* rhizomes in rats fed with high fat cafeteria diet for 6 weeks was investigated by analyzing the changes in body weight, food intake, serum biochemicals, liver weight, parametrial adipose tissue weight, and liver triglyceride content.

The present study showed that, the administration of cafeteria diet for 6 weeks caused obesity with increase in body weight, parametrial adipose tissue weight, and serum lipid levels. Furthermore, it also induced fatty liver with the accumulation of hepatic triglycerides. Treatment with AGE at the dose of 400 mg/kg/day, significantly reduced the increase in body weight induced by cafeteria diet, a clear sign of an antiobesity effect. Individual food intake was measured per day during last five consecutive days to study whether or not AGE influenced food intake in cafeteria diet fed rats. There was no significant difference in energy intake between cafeteria diet control and cafeteria diet plus AGE treated rats. This result suggests that, body weight reducing effect of AGE in cafeteria diet fed rats was not caused by a refusal to ingestion of food. However, sibutramine treated group of rats showed significant weight reducing and hypophagic effects.

Significant increase in serum lipids, such as total-cholesterol, LDL, and triglycerides is typically observed in obese animals and people. In addition, a decrease in HDL/LDL ratio is also detected in obese human and animal subjects. Thus, alteration of these lipid profiles can be used as an index of obesity. Treatment with AGE and sibutramine caused significant changes in serum biochemical parameters, including decreased level of total cholesterol,

LDL, and triglycerides, but an increased level of HDL-cholesterol. These results indicated a significant improvement in AIP by the treatment with AGE and sibutramine. AIP correlates with the size of pro- and antiatherogenic lipoprotein particles and is known to predict cardiovascular risk (19). An AIP value of less than 0.10 predicts low cardiovascular risk, which was observed with animals treated with AGE and sibutramine. A significant increase in glucose concentration in obesity is known to be an indication of obesity-induced diabetes. However, since there were no significant changes in glucose concentration among normal and cafeteria diet control group of rats, the obesity induced by cafeteria diet did not cause diabetes. The AGE and sibutramine also did not produce any significant changes in serum glucose levels.

The extract and sibutramine significantly reduced the weight of liver and parametrial adipose tissue. The accumulation of liver triglycerides was also significantly inhibited by the treatment with extract and sibutramine. The rate of reduction of body weight corresponded with that in parametrial adipose tissue weight.

It is well known that, dietary lipid is not directly absorbed from the intestine unless it has been subjected to the action of pancreatic lipase enzyme. The two products formed by the hydrolysis of fat in the presence of pancreatic lipase enzyme are fatty acids and 2-monoacylglycerol, which are absorbed (20). Thus the inhibition of this enzyme is beneficial in treatment of obesity. Orlistat, an approved antiobese drug is clinically reported to prevent obesity and hyperlipidemia through the increment of fat excretion into feces and the inhibition of pancreatic lipase enzyme (21). In the present study, the influence of AGE at different concentrations on pancreatic lipase activity was studied to ascertain its mechanism of antiobese action. The AGE inhibited the action of pancreatic lipase enzyme *in vitro* at all the concentrations studied, as indicated by reduction in amount of free fatty acids released in dose dependent manner.

It has been reported that, the saponins in *Platycodon grandiflorum* Jacq (Campanulaceae), and *Panax japonicus* C.A. Mey (Araliaceae) rhizomes, both belonging to the family of triterpenoid family of saponins, showed strong inhibitory effects on pancreatic lipase *in vitro* and suppressed the increase in body weight induced by high fat diet *in vivo* (22, 23). In the present study, the saponin glycosides and triterpenes were found to be present in phytochemical screening of the AGE, which might be responsible for inhibition of *in vitro* pancreatic lipase activity and consequently, the reduction of dietary fat absorption and body weight in cafeteria diet fed rats.

In conclusion, AGE may modulate obesity and obesity derived cardiovascular diseases by reducing the excess accumulation of body fat and changing the lipid profile by inhibiting the intestinal absorption of dietary fat via inhibition of pancreatic lipase activity. The results obtained were comparable with that of sibutramine. The present study confirms the rational basis for its use in traditional medicine for the treatment of obesity. However, further studies are required to elucidate exact molecular mechanism of antiobese action and to isolate and characterize the phytoconstituents responsible for the antiobese activity.

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