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Influence of *Alpinia galanga* rhizomes on cafeteria diet induced obesity in rats

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Abstract

The present study was aimed to screen the possible antiobesity effects of ethanol extract of *Alpinia galanga* rhizomes 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 ethanol extract of rhizomes was administered at a daily dose of 500 mg/kg orally for 6 weeks. Body weight and food intake was measured initially and then every week thereafter. On day 42, serum glucose, lipids and leptin 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, energy intake and parametrial adipose tissue weight induced by cafeteria diet. The extract significantly reduced serum lipid and leptin 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. The present study concludes that, ethanol extract of *Alpinia galanga* rhizomes is useful for treatment of cafeteria diet induced obesity in rats.

Key words : *Alpinia galanga*, Body weight, Cafeteria diet, Antiobesity, Pancreatic lipase, Sibutramine, Leptin.

1. 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 An endocannabinoid receptor antagonist, Rimonabant was withdrawn from

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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 [3-6]

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.

2. Materials and methods

2.1 Preparation of Alpinia galanga extract (AGE)

The rhizomes of *Alpinia galanga* Willd were collected from the agricultural fields around Belgaum in August 2007 and were positively identified. 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 15.8%.

The crude extract obtained was subjected to preliminary phytochemical investigation [13], which showed the presence of carbohydrates, amino acids, alkaloids, glycosides, flavonoids**,** phytosterols, and volatile oils in the extract.

2.2 Experimental animals

Albino wistar rats (150-200 g) of either sex were selected and housed in a group of six animals for one week in a 12:12 h light and dark cycle at 23 ± 2^{0}C temperature and 55-60% relative humidity. 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.

2.3 Oral toxicity study

The oral toxicity test of the AGE was determined as per OECD guidelines No. 423, using albino rats of either sex weighing between 150 -200 g. It involved a stepwise procedure, each step using 03 animals. The study was started at 300 mg/kg body weight and proceeded further at lower dose (50 and 5 mg/kg) or higher dose (2000 and 5000 mg/kg) depending on the presence or absence of compound related mortality of the animals, to determine LD_{50} value. No toxic symptoms and mortality was observed even at the highest dose tested. The LD_{50} selected was 5000 mg/kg body weight. Hence, $1/10^{th}$ of LD_{50} (500 mg/kg) was selected as experimental dose for further study.

2.4 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

2.5. Treatment protocol

Animals were divided into following four groups of six animals each-

Group I: Normal control group fed with normal laboratory pellet chow ad libitum (calorie value = 280 kcal/100 g) and treated with 1% Tween 80 (5 ml/kg p.o.).

Group II: Cafeteria diet control group received cafeteria diet in addition to normal diet and received 1% 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 1% Tween 80 (500 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 above treatment was continued for 6 weeks. The animals were weighed at the start of the experiment and then every week thereafter. Food intake of each group of animal was determined by measuring the difference between the preweighed chows and weight of the food that remained every 24 h initially and then every week thereafter and the results were expressed as mean food intake in g/day for group of 6 rats and mean energy intake in Kcal/day for group of 6 rats.

2.5.1 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 standard biochemical kits. The atherogenic index of plasma (AIP) was calculated by using: AIP = log (TGs/HDL). The concentration of serum leptin was also determined using Rat Leptin Elisa kit.

2.5.2 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 -20°C until analysis was performed. The liver triglycerides content was determined directly by using modified method of Van Handel and Zilversmit [15].

2.6 Measurement of 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 [16].

2.7 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 $p < 0.05$.

3. Results

3.1 Effect of AGE on body weight

Feeding of cafeteria diet for 06 weeks produced significant ($p<0.001$) increase in body weight as compared to normal control animals fed with normal pellet chow *ad libitum*. The mean difference in body weight between day 1 and day 42 observed was 159.4 ± 2.56 and 77.9 ± 2.06 g respectively in CD control and normal control rats. The body weight readings of different groups at weekly intervals are shown in table no. 2. However, treatment with sibutramine and AGE produced significant inhibition of increase in body weight of animals fed with CD. The % reduction of body weight produced by AGE and sibutramine is 43.78% and 52.58% respectively compared to CD control rats (Table no. 3).

3.2 Effect of AGE on food intake

There was no significant change in amount of food intake between normal and CD control rats. However, there was significant increase ($p<0.001$) in calorie intake in CD control rats as compared to normal control rats. The mean energy intake observed for 6 week intervals was 386.10 ± 2.31 and 474.00 ± 3.28 Kcal/day for normal control and CD control group of rats respectively. However, treatment with sibutramine and AGE produced significant ($p<0.001$) decrease in energy intake of animals fed with CD. The mean food/energy intake values observed with various treatments is depicted in table no. 4.

3.3 Effect on AGE on serum biochemical parameters

The cafeteria fed rats showed slight but significant ($p<0.05$) increase in serum glucose concentration as compared to normal control rats. Treatment with AGE resulted in significant ($p<0.01$) decrease in serum glucose levels as compared to CD control rats. There was significant increase ($p<0.001$) in serum concentration of total-cholesterol, LDL-cholesterol and triglycerides in CD control rats in comparison to normal diet fed rats. However there was no significant difference in serum HDL-cholesterol levels among CD control and normal control group of rats. Sibutramine and AGE produced significant decrease in serum concentrations of total-cholesterol, LDL-cholesterol and triglycerides. The extract and sibutramine also produced an increase in serum HDL-cholesterol levels compared to CD control rats. These results produced significant improvement in AIP values with extract treated rats in comparison with CD control rats. The serum glucose, lipid levels and AIP values of different group of animals is tabulated in table no. 5.

3.4 Effect on serum leptin concentration

There was significant increase ($p<0.001$) in serum concentration of leptin in CD control rats in comparison to normal diet fed rats. The serum concentration of leptin observed was 230. 30 ± 3.11 and 619.3 ± 5.40 ng/L respectively in normal control and CD control rats. The serum leptin concentrations of

different groups are shown in table no. 5. AGE and sibutramine produced highly significant ($p<0.001$) decrease in serum leptin concentration in comparison to CD control rats.

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

The rats fed with CD for 6 weeks resulted in highly significant ($p<0.001$) increase in weight of liver tissue and parametrial adipose tissue. Treatment with AGE or sibutramine produced significant ($p<0.001$) decrease in liver and PAT weight as compared to that observed in CD control rats. The liver triglyceride concentration was significantly ($p<0.001$) increased in CD control rats (12.45 ± 0.44 mg/g) as compared to normal control group of rats (5.71 ± 0.25 mg/g). However, the rats treated with sibutramine or AGE showed significant decrease in liver triglyceride concentrations as compared to CD control rats. (Table no. 6).

3.6 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 ìmol/ml/h by 1000, 2000, and 4000 mg/ml of AGE, respectively.

Table 2: Effect of *Alpinia galanga* extract (AGE) and Sibutramine on change in body weight (g) for 06 weeks in cafeteria diet fed rats.

Weeks	Normal control	Cafeteria diet control	AGE	Sibutramine
0	164.5 ± 4.45	166.8 ± 2.14	164.6 ± 2.64	166.6 ± 2.46
1	172.6 ± 3.18*	202.4 ± 7.84	176.2 ± 3.12	178.6 ± 2.42*
2	194.3 ± 2.16***	246.6 ± 7.46	196.2 ± 3.28***	188.4 ± 3.24***
3	210.2 ± 4.18***	270.4 ± 2.48	210.6 ± 3.16***	205.2 ± 2.48***
4	222.5 ± 4.08***	288.2 ± 4.26	222.2 ± 3.14***	227.8 ± 1.48***
5	238.6 ± 2.14***	302.8 ± 3.64	242.2 ± 2.42***	235.6 ± 3.26***
6	242.4 ± 4.56***	326.2 ± 3.16	254.2 ± 3.26***	242.2 ± 2.62***

Values are mean ± SEM (n = 6);
* $p < 0.05$, *** $p < 0.001$ significant as compared to respective cafeteria diet control value.

Table 3: % Reduction in body weight produced by *Alpinia galanga* extract (AGE) and Sibutramine in cafeteria diet fed rats.

	Mean difference in body weight in g between day 1 and 42	% reduction in body weight compared to CD control group
Normal control	77.9 ± 2.06	-
Cafeteria diet control	159.4 ± 2.56 (104.62%)	-
AGE	89.6 ± 2.24 (15.01%)	43.78%
Sibutramine	75.6 ± 1.86 (-2.95%)	52.58%

Values in parenthesis indicate % change in body weight as compared to normal control group.

Table 4: Effect of *Alpinia galanga* extract (AGE) and Sibutramine on food/energy in take in cafeteria diet fed rats.

	Food intake in g/day	Energy intake in Kcal/day
Normal control	137.89 ± 2.22	386.10 ± 2.31***
Cafeteria diet control	143.63 ± 2.45	474.00 ± 3.28
AGE	109.06 ± 2.35***	359.90 ± 3.93***
Sibutramine	105.18 ± 1.84***	347.10 ± 4.00***

Values are mean ± SEM (n = 6).
*** $p < 0.001$ significant as compared to cafeteria diet control value.

Table 5: Effect of *Alpinia galanga* extract (AGE) and Sibutramine on serum biochemical parameters in cafeteria diet fed rats.

	Normal control	Cafeteria diet control	AGE	Sibutramine
Glucose	141.00 ± 2.96*	153.28 ± 2.56	138.04 ± 3.15**	134.24 ± 2.76***
Total cholesterol	96.83 ± 3.01***	125.20 ± 2.83	109.72 ± 2.40**	103.92 ± 2.69***
HDL	23.50 ± 1.17	25.50 ± 1.17	36.67 ± 1.14***	35.83 ± 1.24***
LDL	50.67 ± 1.54***	100.20 ± 1.40	62.67 ± 2.60***	69.67 ± 1.43***
Triglycerides	64.17 ± 3.37***	100.3 ± 2.84	73.83 ± 2.73***	76.50 ± 3.48***
AIP value	0.076	0.235	-0.056	-0.031
Leptin (ng/L)	230.30 ± 3.11***	619.3 ± 5.40	364.0 ± 4.71***	291.3 ± 3.28***

Values are mean ± SEM (n = 6) expressed as mg/dl, except for AIP value and leptin levels.
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ significant as compared to cafeteria diet control value.

Table 6: 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	Sibutramine
PAT weight (g)	4.02 ± 0.23***	10.65 ± 0.48	6.24 ± 0.29***	6.56 ± 0.16***
Liver weight (g)	5.99 ± 0.18***	12.46 ± 0.41	7.04 ± 0.25***	5.92 ± 0.19***
Liver triglycerides (mg/g)	5.71± 0.25***	12.45 ± 0.44	7.90 ± 0.32***	7.14 ± 0.27***

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

4. 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 [17]. When body weight increases to 20% above the average, the likelihood of mortality rises by 20% for men and 10% for women [18]. 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 [19].

In the present study, the antiobesity effect of ethanol extract 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 and leptin levels. Furthermore, it also induced fatty liver with the accumulation of hepatic triglycerides. Treatment with AGE at the dose of 500 mg/kg/day, significantly reduced the increase in body weight induced by cafeteria diet, a clear sign of an antiobesity effect.

Though there was a significant difference in body weight between the CD control and normal control group, no significant difference in quantity of food intake between them was observed. This shows that differences of the diet content do not affect to the amount of food consumed by the animals. The CD group of rats continuously consumed similar quantities of food regardless to the calorie content in the diet. As a result, the calorie intake was significantly higher in CD group than the normal group of rats. The high calorie intake was proportional to the increment of body weight, hence resulted in obese state. There was significant decrease in calorie intake of AGE treated group of animals as compared to CD control rats, implicating for the hypophagic property of the extract.

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 or 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 [20]. 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, in the present study there was little increase in serum glucose levels in rats fed with CD for six weeks in comparison with normal diet fed rats. Treatment with AGE resulted in little but significant reduction of serum glucose concentration in CD fed rats. 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.

Leptin, a 167-amino acid protein, is synthesized and secreted mainly by adipose tissue in approximate proportion to the fat stores. Circulating leptin communicates the level of energy reserves the periphery to the CNS in order to suppress further food intake and enhance energy expenditure. But, elevated concentrations of endogenous leptin do not appear to be capable of preventing, or reversing, the accumulation of adipose tissue during, or after, the development of obesity [21, 22]. Several authors have reported that consumption of high fat diet results in the development of leptin resistance in rodents, marked by increased circulating leptin level and measured as a failure of leptin either to inhibit food intake or to induce weight loss. Increased circulating leptin, a marker of leptin resistance, is common in obesity and this increase is not a direct effect of diet, but rather to the secondary increase in body fat content [23]

In our study, serum leptin levels in CD fed rats was significantly raised in comparison to normal diet fed rats, which reflects the increased lipid contents in high fat diet rats. Rats treated with AGE produced highly significant decrease in serum leptin concentrations as compared to CD control rats, suggesting the improvement of leptin resistance induced by obesity.

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 [24]. 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 [25]. 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.

In conclusion, our study demonstrates that ethanol extract of *Alpinia galanga* rhizomes roots may modulate over weight, obesity and obesity derived cardiovascular complications by reducing the excess accumulation of body fat due to inhibition of pancreatic lipase activity, altering the lipid profile, decreasing the calorie intake and overcoming the leptin resistance. 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 under progress to elucidate exact molecular mechanism of antiobese action and to isolate and characterize the phytoconstituents responsible for the antiobese activity.

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