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Research Article

PHARMACOLOGICAL SCREENING OF ANTI-OBESITY POTENTIAL OF *ACORUS CALAMUS* LINN. IN HIGH FAT CAFETERIA DIET FED OBESE RATS

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ABSTRACT

Objective: To study the anti-obesity potential of aqueous rhizome extract of *Acorus calamus* Linn. (AREAC) in high-fat diet (HFD) fed obese rats.

Methods: Adult strain male Wistar rats used in this study were fed with HFD for 60 days. For the treatment groups, AREAC was administered in a dose levels of 100, 200, and 300 mg/kg bw, orally once a day along with HFD. Rats fed with normal pellet chow were served as normal control. The effect of AREAC on physical parameters such as body weight, organ weight, fat pad weights, and various biochemical parameters such as serum glucose, insulin, leptin, lipid profile, liver markers, kidney markers, and oxidative stress markers were analyzed. *In vitro* pancreatic lipase inhibition assay of AREAC was also studied.

Results: Data of *in vivo* studies revealed significant ($p<0.05$) reduction in percentage body weight gain, organ weights, fat pad weights and levels of serum glucose, insulin, and leptin after treatment with AREAC in a dose-dependent manner. Furthermore, administration of AREAC significantly inhibited the increases in the concentrations of triglycerides, total cholesterol, low-density lipoprotein-cholesterol, very low-density lipoprotein-cholesterol, free fatty acid, and phospholipids in a dose dependent manner whereas, the level of high-density lipoprotein-cholesterol was found to be elevated on treatment. Moreover, on treatment with a test drug, the elevated levels of serum liver and kidney markers such as aspartate transaminase, alanine transaminase, alkaline phosphatase, urea, and creatinine were also brought back to near normalcy. Antioxidant status was found to be enhanced in liver tissues after treatment. *In vitro* studies showed significant inhibition in the activity of pancreatic lipase by AREAC.

Conclusion: The data of the results obtained clearly depicted that AREAC was found to have pronounced anti-obesity activity particularly at the dose levels of 300 mg/kg bw.

Keywords: Obesity, High fat diet, Leptin, *Acorus calamus* Linn., Orlistat.

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INTRODUCTION

Obesity is a medical condition in which excess fat has been accumulated in the body. This metabolic disorder is resulting from a chronic imbalance between energy intake and expenditure [1]. A net excess of energy, caused by either, greater intake or lesser expenditure, results in exaggeration of the adipose tissue, and weight gain. Body mass index (BMI), a measurement which compares weight and height, defines people as overweight (pre-obese) when their BMI is between 25 and 30 kg/m^2, and obese when it is >30 kg/m^2.

Obesity is a serious health problem. It is associated with increased risk for numerous medical problems and health hazards, including hypertension, dyslipidemia, coronary heart disease, type 2 diabetes, gallbladder disease, sleep apnea, osteoarthritis, and various forms of cancer [2]. For example, the prevalence of hypertension is 2.9 times greater among overweight adults compared to their nonoverweight peers. Overweight women experience substantially greater risk for all-cause and some cause-specific mortality than leaner women [3]. Obesity also is associated with increased health and socioeconomic costs (e.g., hospital care, physician's services, and medications, productivity changes related to illness, and death). Yearly, an estimated 300,000 deaths are directly due to being overweight or obese. In one study, it was estimated that overweight individuals have a decreased life expectancy of 3 years, while obese individuals have a life expectancy deficit of 7.1 years for females and 5.8 years for males [4].

An alarming rate of obesity prevalence has been observed globally over the last 30 years independent of age, sex, race, socioeconomic status, profession, etc., creating a serious health concern and also becoming an epidemic of the 21st century. It is estimated that there were over one billion of overweight individuals and more than 300 million who are obese. Childhood obesity is also rapidly emerging as one of the greatest global challenges of the 21st century. Worldwide, the prevalence of childhood overweight and obesity increased from 4.2% in 1990 to 6.7% in 2010. This trend is expected to reach 9.1%, in 2020 [5].

Obesity is mainly associated with modern lifestyle, for this, the modern medicine has little to offer. Routine medical treatment of obesity consisting of nutrition education, behavior modification, exercise therapy, and activity training was reported to have a failure rate of about 85% over 5-10 year when treatment was not continued [6]. Pharmacological interventions for obesity have produced modest and temporary reductions in weight, but have been associated all too frequently with aversive side effects and health risks [7]. Massively obese patients who meet the criteria for obesity surgery achieve long-term weight loss in a good percentage of cases, but post-operative complications are the worrying concern.

The obesity epidemic, along with its associated comorbidities and the discouraging results of the current treatment methods points out the absolute necessity of safe and effective alternative therapy. Ayurvedic system of medicines is one of the oldest systems of medicine having a history of more than 5000 years. Several prototypes derived from these herbal medicines are in use for various kind of disease and disorders including obesity. More than 50% of modern drugs existing in clinical

use for various ailments today are derived from natural products such as plants [8,9].

In this work also attempts have been made to develop an eco-friendly and efficacious plant drug for managing obesity through scientific evaluation of the anti-obesity potential of aqueous rhizome extract of a common, well known medicinal herb *Acorus calamus* L. belonging to the family *Araceae* through *in vitro* and *in vivo* approaches.

METHODS

Collection, identification, and authentication of plant material

Plant source selected for this study (rhizome of *Acorus calamus* Linn.) was collected freshly from farmland at Nagapattinam and identified with the help of Flora of Presidency of Madras [10] and was authenticated with the help of herbarium specimen deposited at Rapinat Herbarium, St. Joseph's College, Trichy, Tamil Nadu, India.

Preparation of plant extract

After proper identification, the selected plant source was cleaned, shade dried, and coarsely powdered. The granulated powder of the selected herb was mixed thoroughly with six times the volume of water, boiled, and stirred continuously until the volume reduced to 1/3rd. The extract was then filtered using a muslin cloth, and the filterate was evaporated in a water bath until it reaches a thick paste consistency. A paste form of the extracts obtained was stored in an airtight container at 4°C for further use.

Experimental animals

Healthy adult Wistar strain male albino rats weighing around 150-200 g obtained from Tamil Nadu Veterinary and Animal Sciences University, Chennai were used for this study. The animals were housed in polypropylene cages under controlled environment (12:12 - hrs light/dark cycle; an ambient temperature of 23±2°C with 65±5% humidity) with free access to standard rat chow pellet (obtained from Sai Durga Foods and Feeds, Bengaluru, India) and water *ad-libitum*. The animals were acclimatized to the laboratory conditions for a week before experiments.

All the studies were conducted according to the ethical guidelines of CPCSEA after obtaining necessary clearance from the committee (Approval No: 790/03/ac/CPCSEA).

Induction of obesity

Obesity was induced in albino rats of normal weight by feeding them a high-fat cafeteria diet. The cafeteria diet [11] contain three diets - (a) condensed milk (48 g)+bread (48 g), (b) chocolate (18 g)+biscuits (36 g)+dried coconut (36 g), and (c) cheese (48 g)+boiled potato (60 g). The three diets were presented to the individual rats on days one, two, and three, respectively, and then repeated for 60 days in the same succession.

Experimental design

Adult Wistar strain albino rats were divided into six groups of six rats each:

Group I: Normal control - Rats fed with normal rat chow.

Group II: Obese control - Rats fed with high-fat diet (HFD) for 60 days.

Group III: Rats fed with HFD+treated with aqueous rhizome extract of *Acorus calamus* (AREAC) at a dose level of 100 mg/kg body weight, once a day orally for 60 days.

Group IV: Rats fed with HFD+treated with AREAC at a dose level of 200 mg/kg body weight, once a day orally for 60 days.

Group V: Rats fed with HFD+treated with AREAC at a dose level of 300 mg/kg body weight, once a day orally for 60 days.

Group VI: Rats fed with HFD+treated with the reference drug orlistat at a dose level of 50 mg/kg body weight, once a day orally for 60 days.

At the end of the experimental period of 60 days, the animals were sacrificed by cervical decapitation after a 12 hr fast. Blood was collected and used for various biochemical estimations. Heart, liver, kidney, spleen, and the fat pads were excised, weighed and processed for biochemical analysis. Histopathological studies of liver, heart, and kidney tissues were carried out. Liver tissues were homogenized, in 0.1 M phosphate buffer, pH 7.4 and were used for analyzing various biochemical parameters.

In vitro pancreatic lipase inhibition assay [12] of AREAC was also analyzed in addition to this *in vivo* study.

Statistical analysis

All the results were expressed as mean±standard error of the mean. The data were statistically analyzed by one-way analysis of variance.

RESULTS

Pancreatic lipase inhibitory activity of AREAC

The anti-lipase activity of AREAC was tested, and the obtained results are shown in Fig. 1.

Effect on daily food intake

The daily food intake of experimental rats treated with AREAC was measured, and the obtained results are shown in Fig. 2. The data reveals slight decrease in food intake in AREAC treated group rats than obese control rats, but not much difference was observed between Group III, IV, and V animals. This result suggested that AREAC treatment does not influence the daily food intake.

Effect on body weight

The changes in the body weight of the experimental animals were recorded on day 1 and at a regular interval of 10 days, and the data were graphically represented in Fig. 3. The obtained results clearly shows a significant reduction in the body weight of the obese rats in a dose dependent manner when treated with different dose levels (100, 200, and 300 mg/kg bw) of AREAC.

Effect on fat pad and organ weights

The effect of treatment of obese rats with AREAC on weight of fat pad (such as mesenteric, retroperitoneal, and perirenal) and organs (such as liver, kidney, and spleen) are presented in Figs. 4 and 5, respectively. The weights of fat pads and organs were increased in HFD fed obese control animals while treatment with plant extract showed decrease in the weights of fat pads and organs.

Effect on glucose, insulin, and leptin levels

The effect of treatment of obese rats with AREAC on blood glucose, serum insulin, and leptin levels in the experimental animals are given in Table 1. The elevated levels of blood glucose, insulin, and leptin in HFD fed obese rats were restored to near normalcy on treatment with plant extract.

Effect on serum and hepatic lipid profile

The effect of AREAC on serum lipid profile was shown in Table 2. Administration of HFD to the experimental animals resulted in the

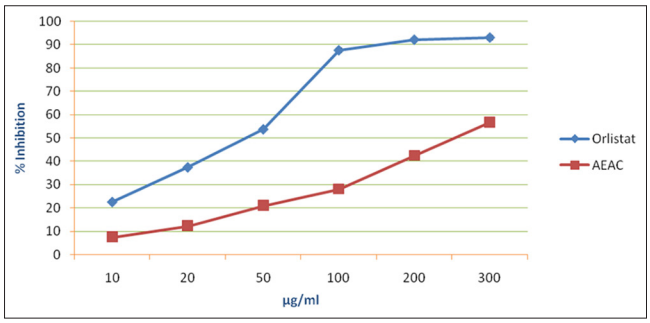

Fig. 1: The inhibitory effect of aqueous rhizome extract of *Acorus calamus* on pancreatic lipase activity

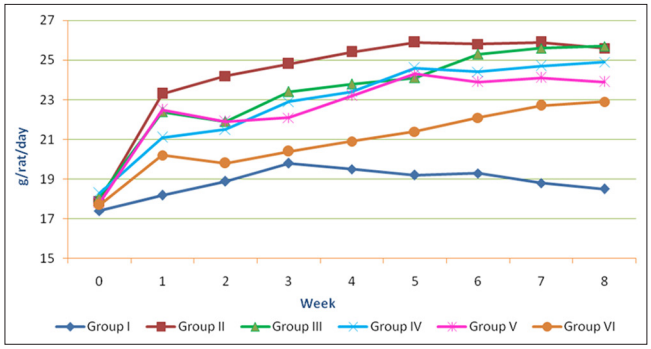

Fig. 2: Effect of aqueous rhizome extract of *Acorus calamus* on food intake in experimental rats

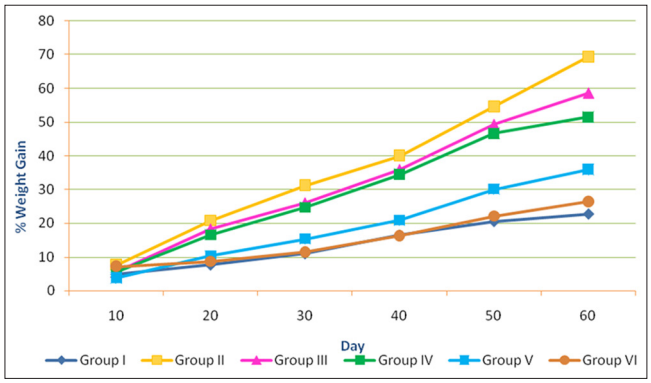

Fig. 3: Effect of aqueous rhizome extract of *Acorus calamus* on percentage body weight gain in experimental rats

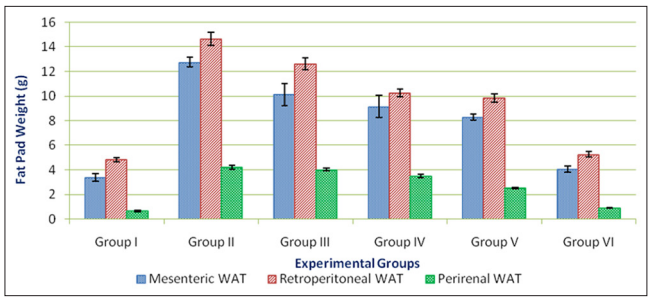

Fig. 4: Effect of aqueous rhizome extract of *Acorus calamus* on fat pad weights in experimental rats. Values are mean±standard error of the mean of 6 rats

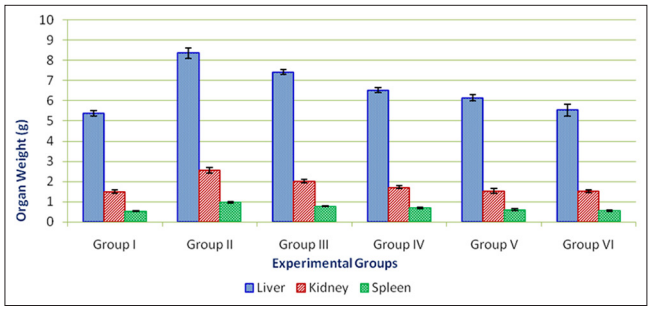

Fig. 5: Effect of aqueous rhizome extract of *Acorus calamus* on organ weights in experimental rats. Values are mean±standard error of the mean of 6 rats

elevated levels of serum triglyceride (TG), total cholesterol (TC), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL),

Table 1: Effect of AREAC on glucose, insulin, and leptin levels in experimental rats

Experimental Groups	Blood glucose (mg/dl)	Insulin (µIU/ml)	Leptin (ng/ml)
Group I	74.83±0.88	3.34±0.11	2.97±0.08
Group II	146.96±1.14#	7.23±0.18#	6.86±0.1#
Group III	130.07±1.13	6.97±0.18	5.91±0.19
Group IV	99.93±1.4	4.78±0.22	4.25±0.13
Group V	86.85±0.98*	3.98±0.13*	3.60±0.2*
Group VI	74.15±1.15*	3.87±0.15*	3.11±0.12*

Values are mean±SEM of 6 rats. #p<0.01 versus normal control; *p<0.05 versus obese control. SEM: Standard error of the mean, AREAC: Aqueous rhizome extract of *Acorus calamus*

atherogenic index (AI), phospholipids (PL), and free fatty acids (FFAs) while high-density lipoprotein-cholesterol (HDL-C) was found to be decreased. Hepatic TG and TC levels were also elevated in HFD fed rats. Oral administration of different dose levels of aqueous extracts of selected plant drug to the obese rats efficiently restored these altered lipid profiles to normal. The effect was statistically significant ($p<0.05$) and dose dependent.

Effect on liver and kidney markers

The levels of liver marker such as alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) were elevated while protein level was decreased in serum of HFD fed rats when compared to normal rats. Upon treatment with AREAC these alterations were normalized in a dose-dependent manner. Elevated levels of kidney markers such as urea and creatinine in HFD fed rats were also brought back to normal in a dose dependent manner on treatment with AREAC (Table 3).

Effect on enzymatic and nonenzymatic antioxidant status in liver

The effect of AREAC on enzymatic and nonenzymatic antioxidant status in hepatic tissues is given in Table 4.

DISCUSSION

Obesity is a health problem of epidemic proportions in the industrialized world. It is associated with an increased risk of life-threatening pathologies such as diabetes, hypertension, and heart diseases. The obesity epidemic in the world today, warrants increasing awareness and need to improve the quality and effectiveness of available treatments. The long-term effectiveness of the existing lines of treatment for obesity such as lifestyle modification or pharmacotherapy is not satisfactory. Moreover, the side effects associated with the modern pharmacotherapy forced the suffering mankind to resort to better, long lasting and safe therapy. Herbal supplements and diet-based therapies for the management of obesity are preferable among the most common complementary and alternative medicine. Hence in this study, the antiobesity potential of AREAC was evaluated in HFD induced obese rats through *in vitro* and *in vivo* analysis.

Pancreatic lipase is the most important enzyme for the digestion of dietary triacylglycerols (TAGs). It is well known that dietary fat is not directly absorbed from the intestine unless it has been subjected to the action of pancreatic lipase [13]. The application of pancreatic lipase inhibitor was examined earlier as a treatment for diet-induced obesity in humans. It has been clinically reported that a pancreatic lipase inhibitor orlistat prevented obesity and hyperlipidemia through the increment of fat excretion into faeces and the inhibition of pancreatic lipase. In this study, it was found that AREAC inhibited the pancreatic lipase activity significantly and the effect was comparable to that of the positive control, orlistat. IC_{50} value of the extract was found to be 248.7 µg/ml.

A gain in body weight is a common index of obesity development. HFD feeding resulted in approximately 69.37% increase in body weight of

Table 2: Effect of AREAC on levels of serum and hepatic lipid profiles in experimental rats

Experimental groups	TG (mg/dL)	TC (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	VLDL-C (mg/dL)	Atherogenic index	Phospholipids (mg/dL)	Free fatty acid (mg/dL)	Hepatic TG (mg/g tissue)	Hepatic TC (mg/g tissue)
Group I	66.11±1.12	94.31±1.51	58.8±0.75	20.37±1.48	13.22±0.22	1.55±0.03	111.36±1.88	21.91±0.91	9.81±0.24	7.68±0.32
Group II	159.42±2.24#	189.69±1.8#	37.52±0.49#	120.28±1.77#	31.88±0.45#	5.06±0.07#	207.46±1.96#	40.96±0.93#	16.99±0.24#	14.17±0.61#
Group III	142.77±2.24	172.22±1.57	40.48±0.67	103.18±1.62	28.55±0.41	4.26±0.08	180.21±2.11	36.31±1.24	14.89±0.38	13.42±0.42
Group IV	101.34±1.98	129.17±1.37	50.02±1.16	58.88±2.46	20.27±0.40	2.59±0.08	144.19±2.84	29.60±0.97	11.89±0.24	10.38±0.29
Group V	89.27±1.92*	110.7±1.82*	54.73±0.65*	38.11±2.49*	17.85±0.38*	2.02±0.05*	131.52±1.76*	26.61±0.79*	11.01±0.14*	9.44±0.32*
Group VI	72.90±0.4*	100.96±1.01*	59.75±1.27*	26.63±1.22*	14.58±0.08*	1.69±0.03*	124.47±1.3*	23.34±0.54*	9.97±0.11*	8.02±0.4*

Values are mean±SEM of 6 rats. #p<0.01 versus normal control; *p<0.05 versus obese control. SEM: Standard error of the mean, TG: Triglyceride, TC: Total cholesterol, LDL-C: Low-density lipoprotein cholesterol, VLDL-C: Very low-density lipoprotein cholesterol, HDL-C: High-density lipoprotein cholesterol, AREAC: Aqueous rhizome extract of *Acorus calamus*

the rats in a period of 60-day. This weight gain was around 47% more as compared to normal control animals. Consumption of HFD led to obesity because it facilitated the development of a positive energy balance leading to an increase in visceral fat deposition. In this study, it was found that the administration of AREAC along with HFD displayed significant reduction ($p<0.05$) in weight gain in a dose-dependent manner when compared to the HFD alone fed rats. These inhibitions in weight are not due to decreased food or energy intake as there was no significant difference in the amount of food consumed between the high-fat diet and plant extract treated groups (Fig. 2). This observation also indicated that dietary factors other than energy intake play an important role in body weight regulation. Reduction in body weight may partially be mediated via the inhibition of pancreatic lipase and partially may via the activation of thermogenesis by phytoconstituents through the stimulation of the β-adrenergic receptors. Yun, 2010 reported that some flavonoids activate β-adrenergic receptors which are involved in the burning of fats [14].

Adipose tissue is vital for maintaining whole body energy homeostasis and it consists of adipocytes, which store TAG as a fuel for the body. Excessive adipose tissue deposition is attributed to an imbalance between energy intake and energy expenditure [15]. Adipose tissue is accumulated as fat pads. Therefore, measuring the weight of these fat pads provided an insight about the severity of obesity. It was found that the weights of the mesenteric, retroperitoneal, and perirenal adipose tissue deposits were significantly higher in the HFD fed rats than the normal diet fed rats. While, treatment with AREAC significantly ($p<0.05$) reduced the weights of fatty deposits at the mesenteric, intraperitoneal, and perirenal regions as compared to that of HFD group.

The gain in organ weight such as liver, kidney, and spleen can be also used as an index of obesity because intake of excess energy led to accumulation of extra energy in adipose tissues followed by accumulation of the same in organs such as liver, kidney, and spleen [16]. HFD feeding resulted in an increase in the weight of all these organs, i.e., liver, kidney, and spleen by approximately 56%, 71%, and 80%, respectively. However, the plant extract reduced the observed gains in organ weights in a dose-dependent manner.

Insulin is a hypoglycemic hormone which mediates the uptake of glucose by cells which are used for energy. In obesity, however, insulin signaling is defective due to insulin resistance and is the major cause of type 2 diabetes mellitus. Insulin resistance or glucose intolerance in obesity condition may be attributed partly for the concomitant increases in circulating FFAs in the blood stream [17].

Due to defective insulin signaling glucose cannot be transported into the muscle or liver cells hence leads to hyperglycemia. In responsive to hyperglycemia, pancreatic β-cells continually synthesize insulin which in turn leads to hyperinsulinemia. Hyperinsulinemia, hyperglycemia and insulin resistance are frequently associated with human obesity [18]. Our results also showed significant ($p<0.05$) increase in the levels of serum glucose and insulin in HFD fed rats when compared to normal diet fed rats. However, rats administered AREAC along with HFD showed significant ($p<0.05$) decrement in the levels of serum glucose and insulin. The hypoglycemic and hypoinsulinemic effect was dose dependent and this hypoglycemic and hypoinsulinemic effect may be mediated by the active phytoconstituents present in the test drug through increased adiponectin secretion, suppressed tumor necrosis factor-alpha secretion in white adipose tissue, and increased GLUT4 expression in skeletal muscle.

Leptin is synthesized and secreted mainly by adipose tissue in approximate proportion to fat stores. Circulating leptin communicates the level of energy reserves in the periphery to the central nervous system to suppress food intake and to permit energy expenditure. However, 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. Several authors have

Table 3: Effect of AREAC on levels of liver and kidney markers in experimental rats

Experimental groups	ALT (IU/dL)	AST (IU/dL)	ALP (IU/dL)	Protein (g/dL)	Urea (mg/ml)	Creatinine (mg/ml)
Group I	69.03±1.29	40.89±1.34	69.75±1.45	6.59±0.12	16.98±0.36	0.63±0.01
Group II	145.99±1.74#	74.33±1.58#	148.11±1.8#	4.24±0.07#	40.39±0.84#	1.10±0.05#
Group III	133.46±1.3	62.36±1.57	128.06±1.87	4.42±0.1	31.94±0.51	1.02±0.02
Group IV	95.53±1.86	51.33±1.4	93.8±1.37	4.74±0.06	24.24±0.49	0.86±0.02
Group V	82.13±1.47*	47.72±0.84*	88.03±1.8*	4.91±0.05*	20.07±0.45*	0.78±0.02*
Group VI	74.72±1.34*	44.1±1.18*	75.09±1.36*	6.37±0.1*	18.14±0.51*	0.67±0.02*

Values are mean±SEM of 6 rats. #p<0.01 versus normal control; *p<0.05 versus obese control. SEM: Standard error of the mean, AREAC: Aqueous rhizome extract of *Acorus calamus*, ALT: Alanine transaminase, AST: Aspartate transaminase, ALP: Alkaline phosphatase

Table 4: Effect of AREAC on LPO and antioxidant levels in experimental rats

Experimental groups	LPO (nM of MDA formed/g tissue)	Reduced glutathione (mg/g tissue)	Glutathione reductase (µM of GSH produced/minute/mg protein)	Glutathione peroxidase (µM of GSH utilized/minute/mg protein)	Superoxide dismutase (µMoles of epinephrine oxidized/mg protein)	Catalase (µM of H_2O_2 utilized/ minute/mg protein)
Group I	86.01±0.92	4.52±0.15	157.88±1.94	3.61±0.09	4.89±0.15	29.15±1.09
Group II	147.01±1.43#	2.35±0.08#	88.26±1.56#	1.13±0.05#	1.85±0.06#	13.89±0.73#
Group III	129.91±1.42	2.71±0.07	101.09±1.74	1.36±0.04	2.41±0.05	17.8±0.36
Group IV	103.42±1.69	3.88±0.11	131.18±2.35	2.82±0.08	3.63±0.08	23.28±1.03
Group V	97.59±1.56*	4.17±0.09*	143.22±2.63*	3.11±0.1*	4.28±0.07*	25.03±0.44*
Group VI	92.67±0.74*	4.47±0.07*	156.56±2.27*	3.42±0.09*	4.61±0.15*	28.04±0.62*

Values are mean±SEM of 6 rats. #p<0.05 versus normal control; *p<0.05 versus obese control. LPO: Lipid peroxidation, SEM: Standard error of the mean, AREAC: Aqueous rhizome extract of *Acorus calamus*, MDA: Malondialdehyde, GSH: Glutathione

reported that the consumption of a HFD results in the development of leptin resistance in rodents [19,20]. This is measured as a failure of leptin either to inhibit food intake or to induce weight loss as observed in this study (Table 1). Increased circulating leptin, a marker of leptin resistance, is common in obese condition and is independently associated with insulin resistance and cardio vascular diseases in humans [21].

In general, all lipids are absorbed into the blood in the gastrointestinal tract in the form of chylomicrons, composed of TGs, PL, cholesterol, and apolipoprotein B. The TGs in these chylomicrons are then digested as fatty acids and glycerol by lipoprotein lipase. These fatty acids are transported and stored in liver and adipose tissues in the form of TGs. The remnants of the chylomicrons are mainly taken up by liver, and then transformed into several lipoproteins containing TGs, PL, cholesterol, and apolipoproteins. As a result, increased intake of lipids in food causes not only an accumulation of body fat, but also increases cholesterols, PL, and FFAs in the bloodstream. Furthermore, excess carbohydrates and proteins in the body are converted to TGs, which are the main fats stored in adipose and liver tissues [22]. All of these cause obesity and obesity-derived cardiovascular diseases, such as hyperlipidemia and atherosclerosis.

Increased levels of TGs, TC, LDL-cholesterol (LDL-C), VLDL-cholesterol (VLDL-C), FFA, PL and, decreased levels of HDL-C obtained in HFD fed rats confirmed that HFD intake induced obesity experimentally. In contrast, administration of AREAC along with HFD significantly ($p<0.05$) inhibited the increases in the concentrations of TG, TC, LDL-C, VLDL-C, FFA, and PL in a dose-dependent manner. The level of HDL-C was found to be elevated after treatment (Table 2).

A substantial reduction of TC in serum by AREAC could be attributed by reduction in the activities of the liver enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase, which is a rate-limiting enzyme in cholesterol biosynthesis. Furthermore, a significant reduction in LDL-C and TC level in serum could be achieved by decreased production of TC by liver tissue and/or efficient removal of the LDL-C by various tissues without subsequent renewal [16]. Reductions in FFA, TG, and PL in serum could be attributed to the inhibition of lipid absorption in the gastrointestinal tract, through the inhibition of lipid digestive enzymes like pancreatic lipase in the GI tract. *In vitro* studies revealed significant inhibition ($p<0.05$) in the activity of pancreatic lipase by AREAC (Fig. 1). Studies have illustrated the beneficial effects of saponins on blood cholesterol levels. Saponins cause a depletion of body cholesterol by preventing its reabsorption, thus increasing its excretion, in a similar way as other cholesterol – lowering drugs, such as cholestyramine [23]. Therefore, the significant decrease ($p<0.05$) in serum cholesterol may also be attributed to the effect of the phytochemicals such as saponins present in the AREAC. A higher content of HDL-C is very important in humans because it is correlated with a reduced risk of coronary heart disease. The increased HDL facilitates the transport of cholesterol from the serum to the liver, where it is catabolized and excreted from the body.

The AI, defined as the ratio of TC and HDL-C, is believed to be an important risk factor of atherosclerosis. The data of this study clearly demonstrated that administration of AREAC significantly decreased the ratio. The effect was dose dependent and showed a maximum reduction at 300 mg/kg bw. It has been proven that abnormally high serum levels of TC and low serum levels of HDL-C are associated with an increased atherosclerosis risk. Increasing the HDL-C concentrations and decreasing the TC concentrations in HFD-fed rats indicates the antiatherogenic property of AREAC.

The liver is the central organ for cholesterol, phospholipid, TAG, and lipoprotein metabolism. In obesity, the liver is the receiver of large amounts of fatty acids, which cause its steatosis. Although the exact mechanism remains unclear, the pathogenesis of fatty liver (steatosis) is multifactorial, and it has been suggested that the presence of insulin resistance is an essential requirement for the accumulation of hepatocellular fat. It is postulated that insulin resistance results in combination of elevated plasma concentrations of glucose and fatty acids which leads to increased hepatic fatty acid synthesis, impaired β-oxidation, and hepatic steatosis [24]. The data of this study indicated that intake of HFD for 9 weeks elevated the levels of hepatic TGs and cholesterol indicating fatty liver. While administration of AREAC along with HFD suppressed the increased levels of hepatic TGs and cholesterol induced by HFD in a dose-dependent manner. This shows the amelioration of fatty liver. The lipid lowering actions of test drug may be due to increased expression of energy expenditure-related

genes in liver and decreased fatty acid synthesis and fat intake in the liver.

Obesity-related liver dysfunction falls within the abnormalities pertaining to the complex spectrum of non-alcoholic fatty liver disease (NAFLD). In the two-hit model of NAFLD pathogenesis, insulin resistance plays a pivotal role in determining the first hit: Steatosis. This increases the sensitivity of the liver to the second hits responsible for progression of liver disease and to hepatic necroinflammatory damage. Second hits involve oxidative stress resulting from an imbalance between pro-oxidant and antioxidant processes in the liver [25]. In this study, the levels of hepatic marker enzymes such as ALT, AST, and ALP were found to be elevated in the serum of HFD fed rats as compared to normal control. Such increases in enzymes may attribute to fatty liver and hepatic necroinflammatory injury induced by a high-fat diet. Whereas, the elevated levels of hepatic marker enzymes were significantly ($p<0.05$) diminished in the AREAC treated animals in a dose-dependent manner (Table 3). The decreased levels of ALP, AST, and ALT in the test drug-treated rats suggested the amelioration of fatty liver. The improvement of liver functions might be the outcome of the improved obesity condition due to the inhibitory effect of AREAC on pancreatic lipase activity. On the other hand, it is known that tannins (polyphenols) possess antioxidative activity, and proven antioxidants, such as β-carotene, vitamin E, and astaxanthin, might cause decrease in plasma transaminase levels as a result of prevention against oxidative damage [26]. Thus, there is also a possibility that the antioxidant efficacy of selected plant drug (AREAC) contributed to the decrement of elevated plasma transaminase levels.

The protein content was decreased significantly in rats administered with HFD as compared to the normal control animals. A healthy liver is so crucial for protein metabolism since liver disease is frequently associated with alterations in proteins and disturbances of protein metabolism [27]. The decrement in protein content may be due to their inadequate synthesis by liver in disease condition. However, after treatment with aqueous plant extract, the levels of protein raised to normal in a dose-dependent manner.

The obese rats showed a highly significant ($p<0.05$) increase in the concentration of serum urea and creatinine as compared to the normal group which is in agreement with the results of Cindik *et al*., 2005 [28]. These increases are attributed to damaged kidneys induced by excess accumulation of fat. Several mechanisms may contribute to the onset and or toward the progression of kidney damage/lesions in patients or animals suffering from obesity. Among them, lipid peroxidation and oxidative stress, high glucose levels, and glycated products, have been frequently proposed [29]. HFD induces alteration of renal lipid metabolism by causing an imbalance between lipogenesis and lipolysis in the kidney, as well as systemic metabolic abnormalities and subsequent renal lipid accumulation leading to renal injury [30]. Rats treated with AREAC showed decreased levels of urea and creatinine in serum in a dose-dependent manner. This decrement in the level of kidney markers indicated the amelioration of kidney damage and this protective effect might be mediated by the antioxidant molecules in the test drug by decreasing the oxidative stress through reduced LPO and improved blood lipid profiles and glucose levels.

An elevated metabolism during obesity results in increased production of free radicals or reactive oxygen species (ROS). These free radicals have an unpaired electron, which causes them to attack and capture electrons from other substances such as membrane lipids, nucleic acids, proteins, enzymes, and other small molecules to neutralize themselves. Although the initial attack causes the free radical to become neutralized, another free radical is formed in the process, causing a chain reaction to occur. Until subsequent free radicals are deactivated, thousands of free radical reactions can occur within seconds of the initial reaction leading to cellular damage. Thus, cells under aerobic condition are always threatened with the insult of ROS, which however are efficiently taken care of by the highly powerful antioxidant systems of the cell. Whenever, the balance between ROS production and antioxidant defence is lost, "oxidative stress" results which through a series of events deregulate the cellular functions leading to various pathological conditions including atherosclerosis, cancer, and hypertension. This oxidative stress is one of the key mechanism underlying obesity-related morbidities [31].

In liver cells, increased rate of metabolism and accumulation of fat (fatty liver) during obesity leads to the generation of large amount of ROS which in turn is vulnerable to LPO and oxidative stress. LPO is a free radical-generating process which occurs on every membranous structure of the cell [32]. Malondialdehyde (MDA), the product of LPO, is an index of the level of oxygen free radicals. The content of MDA in rats fed with HFD was significantly increased in liver tissues as compared to rats fed with standard laboratory diet, suggesting that obesity could enhance the process of LPO. Administration of AREAC prevented the HFD induced elevation of MDA and resulted in a significantly decreased content of MDA in liver.

Glutathione (GSH), a powerful antioxidant present within the cytosol, directly quenches ROS such as lipid peroxides and plays a major role against inflammatory response and oxidative stress. GSH also acts as substrate for glutathione peroxidase (GPx) and glutathione-S-transferase (GST). The level of GSH in liver was significantly depleted in HFD fed animals when compared to normal control animals. This depletion of GSH in HFD fed animals may be due to its utilization in large amounts to combat the HFD induced oxidative damage, as GSH is a major non-protein thiol in living organisms playing a crucial role in coordinating the body's antioxidant defence processes [33]. In the AREAC treated animals the level of GSH was significantly ($p<0.05$) restored.

Glutathione reductase (GR) is a flavoprotein enzyme, regenerates GSH from oxidized GSH in the presence of NADPH. It was found that the activity of GR was decreased in liver tissue of the HFD fed obese rats when compared to normal control rats. This might be due to decreased intracellular levels of NADPH-mediated by hyperglycemia. Hyperglycemia in the HFD group activates polylol pathway and inhibits pentose phosphate pathway resulting in decreased intracellular levels of NADPH, which is required for regeneration of GSH from its oxidized form GSSG. The net result was nonenzymatic disruption of H_2O_2 and increased levels of cellular superoxides, hydroperoxides, hydroxyl radicals as well as other radicals.

GPx is a major peroxide scavenging enzyme. In HFD fed obese rats, a fall in the activity of GPx was observed and this might be due to the depletion of GSH as GSH is directly involving in quenching the ROS in addition to, acts as a substrate for the detoxification of H_2O_2 by GPx. Administration of test drug effectively restored the activity of GPx and thereby decreased the production of hydroxyl radicals.

During oxidative damage of liver, superoxide radicals are generated at the site of damage and modulate superoxide dismutase (SOD) and catalase (CAT) resulting in the loss of activity and, accumulation of superoxide radical, which damages organs further. SOD and CAT are the most important enzymes in ameliorating the effects of oxygen metabolism. SODs are a family of endogenous antioxidant enzymes which act as the first line of defence system against ROS and are important in the catalytic decomposition of the superoxide radical into oxygen and hydrogen peroxide while CAT are enzymes that catalyze the conversion of hydrogen peroxide to water and oxygen. In the present study, the activities of SOD and CAT are decreased in HFD fed obese control rats as compared to normal control rats and this decrement might be due to generation of excessive free radicals and increased LPO. The activities of SOD and CAT were restored in the test drug-treated group rats. Overall, AREAC administration showed better effect on antioxidant levels or activity and this might be due to the involvement of natural antioxidant such as flavones present in the plant extract, in free radical scavenging process.

CONCLUSION

Overall, the data of the results obtained clearly depicted that AREAC possesses pronounced anti-obesity activity particularly at the dose levels of 300 mg/kg bw. Furthermore, the data of the results observed in AREAC treated groups were in par with that of standard drug orlistat. As observed in this study, the anti-obesity potentials of the selected plant drug might probably be mediated through delayed intestinal absorption of dietary fat due to the inhibition of pancreatic lipase activity, enhancement of antioxidant status mediated by the antioxidants such as flavones and activation of the leptin signaling pathway.

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