

Antiobesity Effect of *Benincasa hispida* Fruit Extract in High Fat Diet Fed Wistar Albino Rats

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Available Online: 25th December, 2016

ABSTRACT

Aim: The study was designed to evaluate the efficacy of ethanolic extract of *Benincasa hispida* (EEBH) and its active fraction (AFBH) on high fat diet fed rats. Methods: Male wistar rats (200±10g) were divided into five groups of 6 rats. Group I (Normal control), Group II (High fat diet(HFD)), Group III (HFD+300mg/kg ethanolic fruit extract), Group IV (HFD+100mg/kg active fraction from ethanolic fruit extract) and Group V (HFD+25mg/kg Orlistat (drug control)). After induction of High fat diet, Physical parameters such as body weight, organ weight, fat pad weight, anthropometric parameters were measured. Biochemical parameters such as serum lipid profile, Glucose, insulin, Homeostatic insulin resistance (HOMOIR), Free fatty acids, Phospholipids, homocysteine, apolipoprotein-B(Apo-B), leptin and adiponectin levels were analysed. Enzyme parameters such as Aspartate transaminase (AST), Alanine transaminase (ALT), Alkaline phosphatase (ALP), Lactate dehydrogenase (LDH), Creatinine kinase (CK) and lipase were analysed. Histopathological changes in the adipose tissue were identified using haematoxylin eosin stain and similarly histopathological changes in the liver were identified using haematoxylin eosin stain and oil red O staining. Results: Active fraction of *Benincasa hispida* showed better result in reducing lipid levels such as cholesterol and Triglycerides (TG), Low density lipoprotein (LDL), free fatty acids, Phospholipids, Insulin, HOMO-IR, leptin and enzyme levels. Homocysteine and apolipoprotein B (Apo-B) was an important cardioprotective marker. These parameters also were reduced in AFBH and EEBH treated groups, EEBH and AFBH increases HDL and adiponectin levels, when compared with HFD fed groups. Antilipidemic activity was observed with AFBH.

Keywords: *Benincasa hispida*, High fat diet, Anti-obesity, Orlistat.

INTRODUCTION

Obesity is a most common health problem that results from the inequity occurring when energy intake exceeds expenditure. It is associated with metabolic disorders such as hyperlipidemia, type 2 diabetes mellitus, hypertension, stroke, coronary heart disease, gallbladder disease, osteoarthritis and certain types of cancer. Obesity treatment includes lifestyle changes, diet control, regular exercise, and pharmacotherapy. Pharmacotherapy for obesity is not an immense success because most of the antiobesity drugs have serious adverse effects. Orlistat is a drug that reduces fat absorption by inhibiting pancreatic lipase activity and is currently used for the treatment of Obesity¹. Pancreatic lipase is the foremost enzyme involved in triglyceride absorption in the intestine by inhibiting fat absorption from the diet and it is the target for treating Obesity². This drug has many limitations due to the adverse side effects such as stomach pain, steatorrhea and headaches³. So there is a growing interest in herbal remedies which contain many bioactive compounds that play a crucial role in the treatment of numerous diseases with fewer side effects. *Benincasa hispida*(Thunb) Cogn. or *Benincasa cerifera* belongs to the family Cucurbitaceae and is otherwise called as

petha(Hindi), white pumpkin (English) and Kushmanda (Sanskrit), winter melon, ash gourd, winter gourd, white pumpkin, wax gourd, white gourd, tallow gourd, gourd melon and Chinese water melon belongs to the cucurbitaceae family. It was cultivated more or less throughout India and in warm countries. The fruits were traditionally used as a laxative, diuretic, aphrodisiac, cardiogenic and used to treat urinary calculi, blood disease, insanity, epilepsy, schizophrenia and other psychologic disorders, jaundice, dyspepsia, fever, and menstrual disorder^{4,5}. The major constituents of *Benincasa hispida* fruits are volatile oils, flavonoids, glycosides, saccharides, proteins, carotenes, vitamins, minerals, β -sitosterin and uronic acid^{6,7}. The pharmacological studies revealed that the plant exerted many pharmacological activities and so it was used for the treatment of nervous system (anxiolytic, muscle relaxant, antidepressant in the treatment of Alzheimer's disease, antioxidant, anti-inflammatory, analgesic, antiasthmatic, diuretic, nephroprotective, antidiabetic and antimicrobial effects. Hence the present study was aimed to investigate the antihyperlipidemic and antiobesity effect of EEBH and AFBH in high fat diet induced obese rats.

MATERIALS AND METHODS

Collection of plant material

Benincasa hispida fruit specimen was collected from medicinal plant vendor and was authenticated by Dr. P. Jayaraman, Director of National Institute of Herbal science, Plant Anatomy Research Centre, Chennai.

Preparation of extract

The *Benincasa hispida* fruit skin was peeled off and seeds were removed. The fruit pulp were taken, cut into pieces, dried under shade, segregated, pulverized by mechanical grinder and passed through a 40 mesh sieve. The powder was extracted with solvent ethanol using soxhlet apparatus and concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained. The extract was stored in a glass bottle in refrigerated condition throughout the period of experiment.

Active fraction preparation

Ethanol extract was subjected to silica gel column and based on the polarity solvent was used. Totally 84 fractions were obtained and was subjected to TLC analysis. Based on the R_f values the fractions were pooled into five fractions. The resulting five fractions were dried using rotary evaporator and it was subjected to MTT assay. The ethanolic fraction had highest cell viability was used for the *in vivo* studies and it was labelled as AFBH.

Animals and experimental diets

The present study was carried out in accordance with the regulations of Institutional animal Ethical committee (IAEC) approved by committee for the purpose of control and supervision of experiments on animals (CPCSEA) with IAEC approval number 135/PHARMA/SCRI.2013. Male Adult albino wistar rats (200-206g) were used for the study. The animals were housed and were provided with standard commercially available food and water *ad libitum*.

High fat diet

Rats were housed in polypropylene cages with free access to drinking water and food. They were fed standard rat chow, that was procured from Tamil Nadu Veterinary and animal Sciences University, Kattupakkam, Chennai and animals were fed with HFD. HFD composition is as follows, the energy given by the normal diet is 3.43 Kcal/g and the HFD is 5.24 Kcal/g. High fat diet contain 34.9 % fat.

Induction of Experimental Animal and treatment protocol

The animals were grouped into five groups of six rats in each group.

Group I: Control -rats fed with standard diet.

Group II: High fat diet fed rats for 8 weeks.

Group III: HFD fed rats were treated with 300mg/kg of ethanolic extract of *Benincasa hispida* fruit extract (EEBH) orally for 8 weeks.

Group IV: HFD rats were treated with 100mg/kg of active fraction IV of ethanolic extract of *Benincasa hispida* fruit extract (AFBH) orally for 8 weeks.

Group V: HFD rats were treated with 25mg/kg of Orlistat for 8 weeks.

Standard

Orlistat used as standard anti-obesity drug and was purchased from Sigma Aldrich. The weight of the each animal was noted. The amount of food and water intake was measured every day. Then the end of the treatment anthropometrical parameters was measured and blood sample was used for biochemical analysis.

Anthropometrical determination

The abdominal circumference (AC) was anterior to the forefoot, Thoracic circumference (TC) was behind the foreleg, body length from nose to anus and weight of animals was determined. Body weight and length of the rats were used to determine the body mass index⁸. Body Mass Index (BMI) = Body weight (g)/Length²(cm²). The blood samples were collected at the end of experimental period from retro-orbital plexus and allowed to coagulate at room temperature and then the samples were centrifuged at 3000rpm for 10minutes and used for biochemical analysis except homocysteine. Plasma sample was also collected in tube with EDTA as anticoagulant and used for homocysteine analysis.

Biochemical analysis

Biochemical analysis such as cholesterol⁹, Triglycerides(TG)¹⁰, High density lipoprotein(HDL)¹¹, Low density lipoprotein (LDL), Very low density lipoprotein(VLDL), free fatty acids^{12,13}, phospholipids¹⁴, Serum glucose¹⁵, Insulin was measured using Mercodia rat Insulin ELISA enzyme assay kit method, Serum glutamate oxaloacetate transaminase(SGOT or AST)¹⁶, Serum Glutamate pyruvate transaminase(SGPT or ALT)¹⁷, Alkaline phosphatase(ALP)¹⁶ Lactate dehydrogenase(LDH)¹⁸, Creatinine kinase(CK) and lipase were measured by using kit method of agappe diagnostic Ltd. Leptin¹⁹, Adiponectin²⁰ and apo-lipoprotein (Apo-B)²¹ was done in serum sample. Homocysteine level²² was measured using plasma sample. The homeostatic model assessment (HOMA) is a method used to quantify insulin resistance. It was measured by using the formula, HOMA-IR= Glucose (mg/dl) × Insulin (μU/ml)/ 405

Statistical analysis

The data were expressed as mean ± SD. All statistical analysis was performed using SPSS 20.0 statistical software (IBM, USA). Significant differences among the treatment groups were analysed by variance (One way ANOVA) followed by least significant difference (LSD) test. Results were considered to be statistically significant at P values < 0.05.

Adipose tissue weight and Histopathological studies

After sacrifice, the white adipose tissues (Subcutaneous, Gonadal, epididymal and retroperitoneal) were removed from rats and weight of the fat pad was weighed immediately after washing with saline. For adipocyte staining, it was fixed in 10% buffered formalin and was embedding in paraffin. Sections (5-6μm) were cut and stained with haematoxylin and eosin and subjected to microscopic examination using 40X magnification and viewed under light microscopy for identification of adipocyte changes and also changes in the liver was stained by haematoxylin and eosin stain, and lipid accumulation in the liver was identified using Oil red O staining.

RESULTS

Physical parameters such as body weight changes, Thoracic circumference (TC) and abdominal circumference (AC) changes were observed during the experimental period.

Body weight changes

Table 1 showed the body weight changes during the experimental period and this final body weight in the obesity induced Group II animals is about 356 ± 2.64 and weight reduction was shown in group III (HFD+EEBH treated) is about 325 ± 3.00 and Group IV has 303.33 ± 2.08 (HFD+AFBH treated). After the 8 weeks of experimental period Group II rats showed significant ($P < 0.001$) increase in body weight when compared with that of normal control. AFBH treated animals showed better weight reduction after the experimental period. Group I and Group II animals were compared with Group III, Group IV and Group V animals. EEBH and AFBH treated rats do not exert any side effects during the experimental periods and no death was observed in experimental animals and also no abnormal changes were observed in experimental animal groups. This shows that the extract and active fractions does not contain any toxic substances.

Anthropometrical Determinations

Thoracic Circumference (TC), Abdominal Circumference (AC) and BMI were significantly increased in HFD fed animals. These results indicated that there was an increase in fat accumulation in the region of thoracic and abdomen.

Organ weight

Organ weight such as heart, liver, spleen, kidney, pancreas and brain weight was noted. Organ weight of heart, liver, spleen, kidney and pancreas of Group II animals were compared with Group I normal control (Table 2). Group II animals showed significantly increased organ weight ($P < 0.001$) than high fat diet treated AFBH Group IV and Group III animals showed significantly decreased weight of organs $P < 0.001$ and also standard Orlistat treated animals (Group V). These results are compared with group I and group II animals. AFBH treated animals showed better result than other groups. No significant changes were observed in the organ weight of brain.

Fat pad weight

Fat pad weight such as perirenal, mesenteric, epididymal and gonadal fat pad weight was assessed. This was shown in Table 2. Fat pad like perirenal, mesenteric, epididymal and gonadal fat pad weight increased in Group II high fat diet fed rats ($P < 0.001$) when compared with Group III, Group IV and Group V animals. Group III and Group IV animals significantly decreased the weight of fat pad. When comparing Group IV (AFBH treated) and Group III (EEBH treated) animals Group IV (active fraction) treated animals showed significant decrease in fat pad weight. Thus fat pad weight comparison was compared to other groups and from the experimental results it can be proved that AFBH showed the decreased fat pad weight of the animals was decreased than the other groups.

Lipid profile in serum of experimental animals

High fat diet

Ingredients	gm%	Kcal%
Protein	26.2	20
Carbohydrate	26.3	20
Fat	34.9	60
Total(Kcal/gm)		100

Ingredients of High fat diet

Ingredients	gm%	Kcal%
Casein	200	800
L-Cystine	3	12
Corn starch	0	0
Maltodextrin	125	500
Sucrose	68.8	275.2
Cellulose	50	0
Soybean oil	25	225
Lard	245	2205
Mineral mix	10	0
Dicalcium Phosphate	13	0
Calcium Carbonate	5.5	0
Potassium citrate	16.5	0
Vitamin Mix	10	40
Choline Bitartrate	2	0
dye	0.05	0
Total	773.85	4057

The feeding of high fat diet results in excess hepatic triglycerides accumulation due to increased synthesis and decreased secretion of triglycerides and increased de novo lipogenesis²³. After 8 weeks the changes in the lipid profile of experimental animals was observed. The levels of total cholesterol, triglycerides, LDL cholesterol was significantly increased ($p < 0.001$) in Group II (HFD fed rats) and was compared with Group I animals. This is shown in the Table 3. Group III (EEBH treated rats), Group IV (AFBH treated rats) and Group V animals were found to have significantly decreased ($P < 0.001$) levels of total cholesterol, triglycerides and Low density lipoprotein (LDL). This result was compared with Group I (normal animals) and Group II animals. The levels of HDL cholesterol was significantly decreased ($P < 0.001$) in Group II animals when compared to Group I animals. In case of EEBH treated (Group III) and AFBH treated (Group IV) showed significant increased levels ($P < 0.001$), when compared with Group I and Group II. Comparing Group III and Group IV animals, Group IV animals showed significant results than group III animals.

Free fatty acids and Phospholipids

There was elevation of serum free fatty acids and phospholipids in HFD fed rats (Table 3). There was a significant increase ($P < 0.001$) in Group II rats which was caused due to hyperlipidemia, when compared to the corresponding control group (Group I). Group III and Group IV animals showed significant decrease ($P < 0.001$) in free fatty acids and phospholipids when compared with Group I and Group II animals. From the result Group IV animals showed significant reduction of free fatty acids and phospholipids (Table 3).

Coronary risk index and atherogenic index

Table 1: Effect of AFBH and EEBH on Body weight and anthropometrical changes in experimental animals.

S. No	Parameter	Group I	Group II	Group III	Group IV	Group V
1.	Initial body weight(g)	200.0± 1.00	200.33 ±2.08	202.33 ± 1.52	206.34 ± 6.80	202.00± 2.64
2.	Final body weight(g)	288.30± 7.37	356.00±2.64 a*	325 ± 3.00 a*b*	303.33 ± 2.08 a*b*	316±1.00 a*b*
3.	Weight gain(g)	88.33± 6.65	155.66 ± 3.51 a*	122.66 ± 2.88 a*b*	97.00 ± 8.88 aNSb*	114±3.60 a*b*
Anthropometrical determination						
4.	Thoracic circumference(TC in cm)	13.20± 0.10	14.20 ± 0.10 a*	13.80 ± 0.10 a*b*	13.4± 0.57a#b*	13.6 ± 0.57 a*b*
5.	Abdominal circumference (AC in cm)	6.9 ± 0.10	9.10 ± 0.30 a*	7.85 ± 0.05 a*b*	7.10± 0.10aNSb*	7.50 ± 0.10 a*b*
6.	BMI	0.67 ± 0.01	1.00 ± 0.02 a*	0.82 ± 0.06 a*b*	0.74± 0.02 a@b*	0.77 ± 0.01 a#b*

Each Value is expressed as mean ± SD for six animals in each group.

Group I - Control animals, Group II - HFD fed animals (Obesity induced animals), Group III - Obesity induced animals treated with EEBH (300mg/kg body wt), Group IV - Obesity induced animals treated with AFBH (100mg/kg body wt), Group V - Obesity induced animals treated with orlistat (25mg/kg body wt). Statistical significance: * - P < 0.001; # - P < 0.01; @ - P < 0.05, NS - Non significant. Comparison: a-as Compared with Group I; b-as Compared with Group II.

Table 2: Effect of AFBH and EEBH on Organ weight and Fat pad weight in HFD fed rats.

Parameter	Group I	Group II	Group III	Group IV	Group V
Pancreas(g)	1.32± 0.08	1.61 ± 0.02 a*	1.24 ± 0.01 a#b*	0.92 ± 0.02 a*b*	0.90 ± 0.06 a*b*
Liver(g)	7.45± 0.45	11.91± 1.08 a*	9.34 ± 0.34 a*b*	8.41 ± 0.11 a#b*	8.75 ± 0.05 a*b*
Kidney(g)	1.68± 0.02	2.24± 0.10 a*	2.15 ± 0.04 a*b@	1.92 ± 0.07 a*b*	1.83 ± 0.02 a*b*
Spleen(g)	0.81± 0.03	1.27± 0.09 a*	0.98 ± 0.02 a*b*	0.87 ± 0.04 a@b*	0.84 ± 0.03 aNSb*
Heart(g)	0.83± 0.01	1.16± 0.07 a*	1.04 ± 0.01 a*b*	0.93 ± 0.08 a#b*	1.01 ± 0.04 a*b*
Brain(g)	1.51± 0.01	1.55 ± 0.05 a@	1.53± 0.03 aNSbNS	1.52± 0.06 aNSbNS	1.54± 0.02 aNSbNS
Fat pad weight					
Perirenal(g)	0.43 ± 0.02	2.22 ± 0.02 a*	1.84 ± 0.01 a*b*	1.52 ± 0.02 a*b*	1.12 ± 0.02 a*b*
Mesentric(g)	0.61 ± 0.01	3.14 ± 0.03 a*	1.44 ± 0.02 a*b*	0.95 ± 0.02 a*b*	0.78 ± 0.01 a*b*
Epididymal(g)	0.43 ± 0.02	2.23 ± 0.02 a*	1.51 ± 0.01 a*b*	1.16 ± 0.02 a*b*	0.86 ± 0.03 a*b*
Gonadal(g)	1.51 ± 0.01	3.82 ± 0.02 a*	2.32 ± 0.02 a*b*	1.92 ± 0.03 a*b*	0.91 ± 0.01 a*b*

Each Value is expressed as mean ± SD for six animals in each group.

Group I - Control animals, Group II - HFD fed animals (Obesity induced animals), Group III - Obesity induced animals treated with EEBH (300mg/kg body wt), Group IV - Obesity induced animals treated with AFBH (100mg/kg body wt), Group V - Obesity induced animals treated with orlistat (25mg/kg body wt). Statistical significance: * - P < 0.001; # - P < 0.01; @ - P < 0.05, NS - Non significant. Comparison: a-as Compared with Group I; b-as Compared with Group II.

Coronary risk index (CRI) and atherogenic index (AI) have been used as a marker for atherosclerosis. The CRI and AI are increased during coronary condition (Table 3). The atherogenic index and coronary risk index was significantly decreased (P<0.001) in EEBH treated (Group III) and AFBH treated (Group IV) animals. This was compared with Group II and Group I animals. Comparing all the other groups AFBH treated animals showed best result in reducing the AI and CRI.

Apolipoprotein B

Apo-B is synthesized in liver and indicates the amount of atherogenic lipoproteins in plasma or hepatic tissue, and it is considered as a better predictor of coronary heart disease²⁸. Obesity is an independent risk factor for cardiovascular disease (CVD), via its influence on other known risk factors such as dyslipidemia and hypertension.

Apo-B is the lipoprotein responsible for transport of lipids. Apolipoprotein B level was significantly decreased (P<0.001) in EEBH and AFBH treated HFD fed rats (Table 3). High fat diet fed rats showed increased apolipoprotein B level (P<0.001). Group III and Group IV animals showed decreased level of apolipoprotein B and this result was compared with Group I and Group II. AFBH treated animals showed better result in reducing the Apo B than EEBH treated animals. Apo-B levels in Group IV animals are similar to that of Group I animals. So AFBH showed better result and is found to reduce Apo-B.

Glucose, insulin level and HOMO IR

The consumption of HFD leads to obesity, fat accumulation in body and disturbance in glucose and lipid homeostasis. High fat diet lowers the fat uptake but also suppresses hepatic glucose production stimulated by

Table 3: Effect of AFBH and EEBH on Lipid profile of experimental animals.

Parameter	Group I	Group II	Group III	Group IV	Group V
Total cholesterol(mg/dl)	111 ± 3.60	218 ± 6.08 ^{a*}	170 ± 5.00 ^{a*b*}	134 ± 3.51 ^{a*b*}	150.33 ± 5.03 ^{a*b*}
Triglycerides	93.33 ± 2.08	187.66 ± 7.23 ^{a*}	149.33 ± 5.03 ^{a*b*}	118.00 ± 2.00 ^{a*b*}	135 ± 4.04 ^{a*b*}
HDL cholesterol(mg/dl)	56.66 ± 1.15	30.00 ± 2.00 ^{a*}	36.00 ± 2.00 ^{a*b*}	46.00 ± 1.00 ^{a*b*}	41.00 ± 1.00 ^{a*b*}
LDL cholesterol(mg/dl)	63.33 ± 1.52	150.46 ± 7.80 ^{a*}	104.13 ± 6.66 ^{a*b*}	65.60 ± 2.94 ^{aNSb*}	82.26 ± 5.19 ^{a*b*}
VLDL cholesterol(mg/dl)	18.60 ± 0.41	37.53 ± 1.44 ^{a*}	29.86 ± 1.00 ^{a*b*}	23.6 ± 0.40 ^{a*b*}	27.06 ± 0.61 ^{a*b*}
Atherogenic index(A.I)	0.95 ± 0.02	6.28 ± 0.52 ^{a*}	3.73 ± 0.40 ^{a*b*}	1.92 ± 0.11 ^{a#b*}	2.66 ± 0.18 ^{a*b*}
Coronary risk index(CRI)	1.95 ± 0.02	7.28 ± 0.52 ^{a*}	4.73 ± 0.40 ^{a*b*}	2.92 ± 0.11 ^{a#b*}	3.66 ± 0.18 ^{a*b*}
HDL/LDL	0.93 ± 0.008	0.20 ± 0.023 ^{a*}	0.34 ± 0.041 ^{a*b*}	0.70 ± 0.03 ^{a*b*}	0.50 ± 0.04 ^{a*b*}
Free fatty acid(mg/dl)	15.23 ± 1.11	29.48 ± 1.26 ^{a*}	25.16 ± 1.69 ^{a*b*}	17.67 ± 1.29 ^{a#b*}	21.54 ± 1.29 ^{a*b*}
Phospholipids(mg/dl)	110.33 ± 8.59	250 ± 8.94 ^{a*}	211.33 ± 11.67 ^{a*b*}	128.66 ± 7.22 ^{a*b*}	188.00 ± 5.58 ^{a*b*}
ApolipoproteinB(mg/dl)	30.33 ± 1.36	64.66 ± 3.14 ^{a*}	56.00 ± 2.36 ^{a*b*}	41.33 ± 1.36 ^{a*b*}	47.00 ± 0.89 ^{a*b*}

Each Value is expressed as mean ± SD for six animals in each group.

Group I - Control animals, Group II - HFD fed animals (Obesity induced animals), Group III - Obesity induced animals treated with EEBH (300mg/kg body wt), Group IV - Obesity induced animals treated with AFBH (100mg/kg body wt), Group V - Obesity induced animals treated with orlistat (25mg/kg body wt).

Statistical significance: * - P < 0.001; # - P < 0.01; @ - P < 0.05, NS - Non significant. Comparison: a-as Compared with Group I; b-as Compared with Group II.

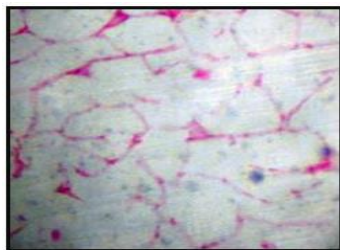


Plate A1: GROUP I

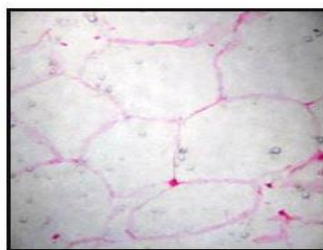


Plate A2: GROUP II

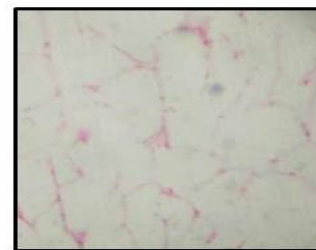


Plate A3: GROUP III

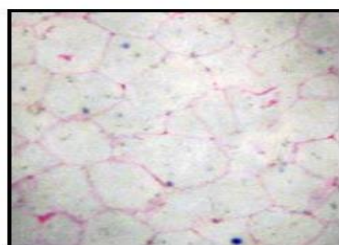


Plate A4: GROUP IV

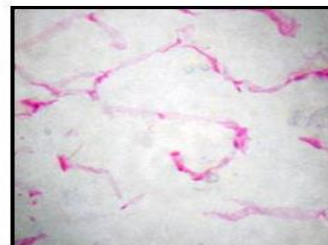


Plate A5: GROUP V

Figure 1: Histopathological changes in adipocyte of experimental animals.

The images are shown at 40X magnification.

Plate A1 shows normal morphology of adipose tissue.

Plate A2 shows enlargement of adipocyte. (HFD fed rats)

Plate A3 shows slightly enlarged adipocyte. (HFD treated with EEBH (300mg/kg body wt))

Plate A4 shows very slightly enlarged adipocyte. (HFD treated with AFBH (100mg/kg body wt))

Plate A5 shows moderately enlarged adipocyte. (HFD treated with Orlistat(25mg/kg body wt))

insulin leading to insulin resistance as well as hyperglycaemia²⁴. The concentration of plasma insulin and glucose present in the HFD fed rats is shown in Table 4. The levels of glucose, insulin and homo IR were significantly increased ($P<0.001$) in Group II animals when compared with Group I animals. The drug treated EEBH and AFBH was significantly less ($P<0.001$) when compared to Group II animals. AFBH treated groups showed better result in decreasing the insulin and glucose compared to the EEBH treated HFD fed animals.

Homocysteine

Homocysteine is a non-essential amino acid. Homocysteine is formed from demethylation of methionine. Homocysteine can be remethylated into methionine by vitamin B₁₂ dependent methionine synthase and methyl tetra hydrofolate. Homocysteine and hypercholesterolemia are associated with risk factor of cardio vascular disease²⁵. The level of homocysteine increases in the blood and causes cholesterol to be oxidized and increase LDL, which can damage the arteries by causing plaque inside artery wall²⁶. So levels of homocysteine were observed. This study showed that homocysteine level is significantly increased ($P<0.001$) in Group II animals. This result was compared with Group I animals. These results are shown in the Table 4. Homocysteine level of high fat diet fed rats treated with EEBH and AFBH showed significant decrease ($P<0.001$).

Orlistat treated animals showed significant decrease ($P<0.001$). These results were compared with group I and group II animals. Comparing all the groups AFBH showed better result in reducing the homocysteine level, which was near normal when compared to control animals.

Leptin and Adiponectin

Adipocytes secrete various adipocytokines such as tumor necrosis factor- α , leptin, adiponectin, and resistin. Adiponectin plays an important role in insulin sensitivity and fatty acid oxidation, and adiponectin levels are negatively correlated with body fat mass, and serum glucose, insulin and triglyceride levels²⁷. Table 4 showed that changes in the leptin and adiponectin levels in serum and control experimental animals. The levels of leptin was significantly increased ($P<0.001$) in Group II. Group III and Group IV animals showed significant decreased ($P<0.001$) level of leptin. This results were compared to the Group II and Group I animals. AFBH treated animals showed best result in reducing leptin than EEBH treated animals. The level of adiponectin level (Table 4) was significantly decreased ($P<0.001$) in Group II animals. The results were compared with Group I (normal) control animals. The adiponectin level was significantly increased in Group III (EEBH) treated and Group IV (AFBH) treated groups. These results were compared with Group I and Group II animals. The current study reported that AFBH treated animals showed significantly increased level of adiponectin but EEBH treated animals showed slight

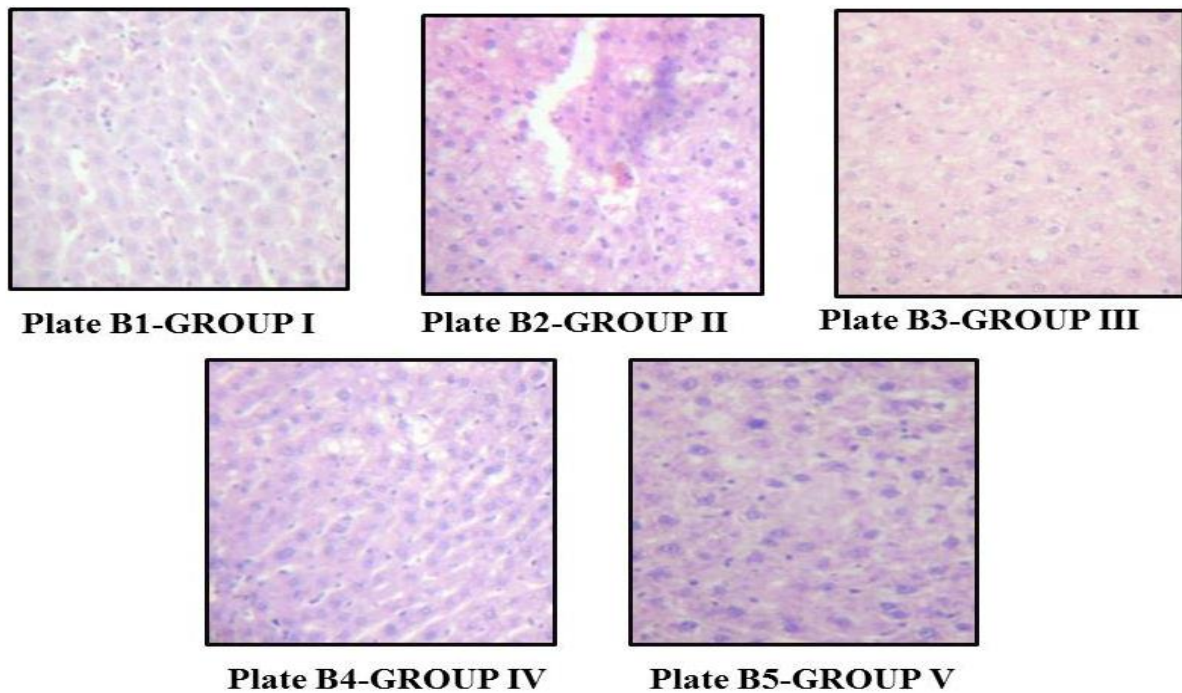


Figure 2: Histopathology of Liver of experimental animals using haematoxylin eosin stain.

The images are shown at 40X magnification.

Plate B1 shows normal morphology of liver.

Plate B2 shows steatosis of liver (HFD fed rats).

Plate B3 shows less degree of steatosis of liver. (HFD treated with EEBH (300mg/kg body wt))

Plate B4 shows less degree of steatosis of liver. (HFD treated with AFBH (100mg/kg body wt))

Plate B5 shows moderate steatosis of liver. (HFD treated with Orlistat (25mg/kg body wt))

Table 4: Effect of AFBH and EEBH on Biochemical parameters of experimental animals.

Parameter	Group I	Group II	Group III	Group IV	Group V
Serum Glucose(mg/dl)	83.33 ± 4.93	152.00 ± 6.08a*	105.33 ± 2.08a*b*	99 ± 2.64a*b*	91.00 ± 1.73a@b*
Insulin(μU/ml)	11.12 ± 0.29	19.10 ± 0.10a*	12.72 ± 0.10a*b*	12.22 ± 0.15a*b*	11.98 ± 0.12a*b*
Homo IR	2.28 ± 0.13	7.16 ± 0.20a*	3.17 ± 0.08a* b*	3.11 ± 0.12a* b*	2.69 ± 0.03a#b*
Homocysteine(μmol/L)	9.7±0.24	13.40±0.40 a*	12.10±0.25 a*b*	10.0±0.10 aNSb*	11.50±0.26 a*b*
Leptin(ng/ml)	1.60±0.20	9.93±0.49 a*	5.73±0.19 a*b*	4.20±0.10 a*b*	2.77±0.06 a#b*
Adiponectin(ng/ml)	7.44±0.37	3.30±0.10 a*	3.78±0.07 a* b@	6.62 ±0.42 a#b*	4.70±0.10 a*b*

Each Value is expressed as mean ± SD for six animals in each group.

Group I - Control animals, Group II - HFD fed animals (Obesity induced animals),

Group III- Obesity induced animals treated with EEBH (300mg/kg body wt),

Group IV- Obesity induced animals treated with AFBH (100mg/kg body wt),

Group V- Obesity induced animals treated with orlistat (25mg/kg body wt).

Statistical significance: * - P < 0.001; # - P < 0.01; @ - P < 0.05, NS - Non significant.

Comparison: a-as Compared with Group I; b-as Compared with Group II.

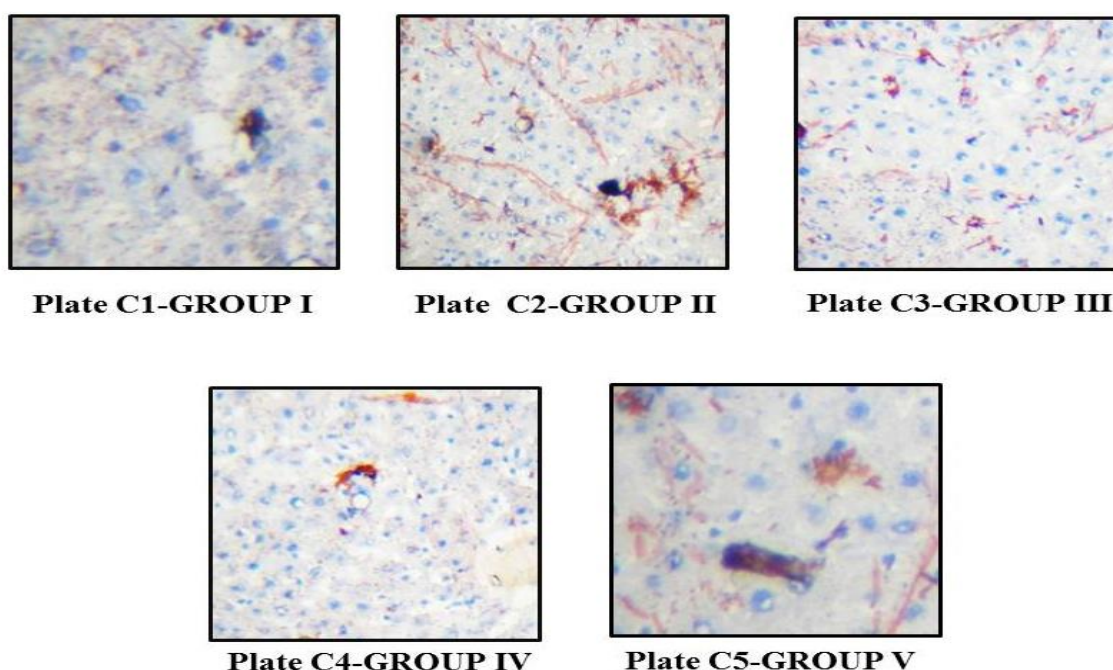


Figure 3: The histopathology of liver using oil red O staining of experimental animals.

The images are shown at 40X magnification.

Plate C1 shows normal lipid accumulation of liver.

Plate C2 shows more lipid accumulation of liver. (HFD fed rats)

Plate C3 shows slight increase in lipid accumulation of liver. (HFD treated with EEBH (300mg/kg body wt))

Plate C4 shows normal lipid accumulation of liver. (HFD treated with AFBH (100mg/kg body wt))

Plate C5 shows slight lipid accumulation of liver. (HFD treated with Orlistat (25mg/kg body wt))

elevation of adiponectin level. So AFBH treated animals showed better good result than EEBH treated animals.

Enzymatic markers in serum

The animals fed with high fat diet resulted in elevated serum and tissue cholesterol level. This causes metabolic disturbances like hypercholesterolemia which causes the fat deposition in the liver and decreased hepatocyte production. This causes steatosis of liver due to the intracellular accumulation of lipids. High fat diet fed rats induces the production of free radicals. This in turns leads to the oxidative stress. Table 5 represents the activities of marker enzyme such as SGOT (AST), SGPT (ALT), ALP,

LDH, CK and lipase in serum samples of control and experimental animals. The activities of SGOT, SGPT, ALP, CK, lipase and LDH showed significant increase (P<0.001) in Group II animals when compared to that of Group I animals. These marker activities are significantly decreased (P<0.001) in both EEBH and AFBH treated (Group III and IV) animals as compared to Group II animals. Liver enzyme marker level is also significantly decreased in Group IV animals. AFBH treated animals showed better result than EEBH treated animals.

Histopathological analysis

Histopathology of adipose tissue

Table 5: Effect of AFBH and EEBH on Enzyme parameters of experimental animals.

Parameter	Group I	Group II	Group III	Group IV	Group V
AST(IU/L)	120.33 ± 0.57	179.66 ± 9.07a*	114.66 ± 3.51a ^{NS} b*	115.66 ± 2.51a ^{NS} b*	128 ± 2.64a ^{NS} b*
ALT(IU/L)	55 ± 1.00	91.66 ± 2.08 a*	63.06 ± 1.40a*b*	57.66 ± 0.5751a##b*	69.66 ± 1.52a*b*
ALP(IU/L)	161.33 ± 1.52	251.66 ± 2.08a*	182.66 ± 2.51 a*b*	173.66 ± 3.21 a*b*	216.66 ± 3.05 a*b*
LDH(IU/L)	426.66 ± 16.64	673 ± 8.80a*	570 ± 19.49a*b*	445 ± 11.83a#b*	516 ± 6.83a*b*
CK(IU/L)	411 ± 9.30	643.33 ± 13.66a*	569.66 ± 8.96 a*b*	436.66 ± 10.32 a*b*	528.33 ± 9.30 a*b*
Lipase(IU/L)	649 ± 13.05	765.16 ± 13.51a*	716.83 ± 6.96 a*b*	676.33 ± 4.45 a*b*	688.33 ± 5.16 a*b*

Each Value is expressed as mean ± SD for six animals in each group

Group I - Control animals,

Group II- HFD fed animals (Obesity induced animals),

Group III- Obesity induced animals treated with EEBH (300mg/kg body wt),

Group IV- Obesity induced animals treated with AFBH (100mg/kg body wt),

Group V- Obesity induced animals treated with orlistat (25mg/kg body wt).

Statistical significance: * - P < 0.001; # - P < 0.01; @ - P < 0.05, NS - Non significant.

Comparison: a-as Compared with Group I; b-as Compared with Group II.

Fig. 1: Plate A1-A5 shows the histopathological changes in adipose tissue of control and experimental animals. The adipose tissue of control animals reveals normal histology (Group I). In Group II (HFD fed animals) showed enlarged adipocytes. Group III and Group IV animals showed reduced size of adipocyte. Group V Orlistat treated animals showed moderate size of adipocyte.

Histopathology of Liver

Fig.2: Plate B1-B5 showed the histological analysis of liver of control and experimental groups. The control group showed the normal morphology of liver. Hepatocytes are clear broad with central vein at portal layer. Group II animals showed the steatosis of liver and also lipid accumulation was assessed by oil red O staining. This result was shown in Fig.3: Plate C1-C5. Group III animals and level of lipid accumulation, Group IV animals showed reduced lipid content. Group II animals showed steatosis of liver when compared with Group III and IV. Group V showed moderate steatosis of liver.

DISCUSSION

Obesity and overweight are mainly caused by a chronic imbalance between energy intake and energy expenditure. Overeating and physical inactivity are main causes of obesity. Orlistat is a drug, which can reduce dietary fat absorption. Natural plant products are used as therapeutic drug, due to fewer side effects. In the present study, we have evaluated the effect of *Benincasa hispida* fruit extract on high fat diet fed rats. The present study revealed that consumption of High calorific diet treated with *Benincasa hispida* extract and active fraction showed a significant reduction in body weight and Body mass index (BMI). BMI is mostly used to measure the body fat and was highly correlated with body fat stores²⁹. Body weight was increased by the excess of energy intake of adipose tissue caused by fat deposition³⁰. The major risk factor of obesity is dyslipidaemia and hypertension³¹. In this study triglycerides and total cholesterol level was reduced in AFBH treated rats than EEBH treated rats. The high fat diet is resulting in excess of hepatic triglyceride accumulation due to increased or decreased secretion of triglycerides and also due to increased lipogenesis. This study proved that HFD group showed fat accumulation in

adipose tissue and treatment with AFBH and EEBH reduce the same. Treatment of plant drug will reduce total cholesterol in serum of liver enzyme 3-hydroxy 3-methyl glutaryl coenzyme A (HMG-COA). This is a rate limiting enzyme in cholesterol biosynthesis. The increased level of HDL is involved in the transport of cholesterol from the serum to the liver, where it is catabolized and excreted³². Cholesterol is catabolized and forms bile acids. The syntheses of bile acids in small intestine are increased due to their fecal excretion. Cholesterol oxidation to bile acids resulted in fecal loss of steroids. Reduction of cholesterol and triglycerides are mainly due to the oxidation of cholesterol by the action of AFBH and EEBH on HFD treated rats. Apolipoprotein B is a better predictor of coronary heart disease²⁸. It is synthesized in liver and indicates the amount of atherogenic lipoproteins in plasma or hepatic tissue. Apolipoprotein B was also significantly reduced in group III and IV than group II. Apo-B secretion by the liver is regulated by factors such as rate of cholesterol biosynthesis, availability of triglycerides and cholesterol ester^{33,34}. The level of homocysteine is a possible risk factor for cardiovascular diseases³⁵. 5-7% of general population has mild to moderate hyperhomocysteinemia. Homocysteine level was also increased in HFD control and significant decrease in treatment group and drug control. High homocysteine levels in the blood may cause cholesterol to convert oxidized LDL which damages the arteries by creation of plaque inside artery walls²⁶. Homocysteine level was also decreased in AFBH treated animals. Lipase plays a major role in hydrolysis of lipids before absorption in intestinal lining³⁶. In this study it was noted that serum lipase level was significantly higher in HFD group than treatment group. HFD diet induces pancreatitis, so lipase level is increased in HFD group. Atherogenic index (AI) and Coronary risk index (CRI) are the powerful indicators for the risk of heart disease³⁷. HFD group showed increased AI and CRI values. High fat diet treated with AFBH and EEBH decreases the lipid absorption and weight gain and also decreases the AI and CRI value. Saturated fats increase blood cholesterol and thereby increase the risk of atherosclerosis and coronary heart diseases, abnormal lipoprotein metabolism, obesity, insulin resistance and

diabetes mellitus^{38,39}. Histopathological studies also showed the reduced lipid accumulation in liver and adipose tissue on treatment of AFBH and EEBH. Comparing Group III and Group IV, Group IV (AFBH) treated animals showed better result than Group III animals. From the results AFBH treated rats (Group IV) shown better antilipidemic and cardioprotective drug.

CONCLUSION

Ethanol extract of *Benincasa hispida* and active fraction of *Benincasa hispida* showed the reduced body weight gain, BMI, lipid accumulation in adipose tissue and reduced serum lipid profiles when compared with HFD control. While comparing extract and active fraction of *Benincasa hispida*, AFBH showed better result in reducing body weight, fat pad weight, lipids, homocysteine, ALT, AST, ALP, Lipase, CK, LDH, phospholipid, Apo-B, glucose, Insulin and leptin. AFBH increases the level of adiponectin. This has shown that it may possess cardioprotective effect and these findings may potentially be useful for the management of obesity, hyperlipidemia and atherosclerosis.

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