

ANTI-OBESITY EFFECT OF AQUEOUS FRUIT EXTRACT OF *CARICA PAPAYA* L. IN RATS FED ON HIGH FAT CAFETERIA DIET

ATHESH K¹, KARTHIGA D², BRINDHA P³

¹Dept. of Biochemistry, Srimad Andavan Arts & Science College, Trichy-5, Tamil Nadu, India. ²Dept. of Biochemistry, Srimad Andavan Arts & Science College, Trichy-5, Tamil Nadu, India, ³carism, Sastra University, Thanjavur, Tamil Nadu, India. Email: atesh_rahul@yahoo.com

Received: 26 Aug, 2012, Revised and Accepted: 09 Oct, 2012

ABSTRACT

Obesity is a chronic disease of increasing prevalence in most countries, which leads to substantial increase in morbidity and mortality in association with insulin resistance, type 2 diabetes, hyperlipidaemia, hypertension and other cardiovascular diseases. A variety of plant species have been found to have beneficial effects on health. This study was designed to determine the anti-obesity potential of aqueous fruit extract of *Carica papaya* L. on high fat cafeteria diet (HFD) fed obese rats. The rats were divided into six groups, each comprising of six animals: Normal Diet (ND), High Fat Diet (HFD), HFD plus fruit extract (200mg/kg b.w.), HFD plus fruit extract (400mg/kg b.w.), HFD plus fruit extract (600mg/kg b.w.) and HFD plus standard drug, Orlistat (50 mg/kg b.w.). After the treatment period of 45 days, the animals were sacrificed, blood and tissues were collected and various biochemical parameters were analyzed. The results showed that the BMI, body weight, organ weight of the liver, kidney and spleen were significantly decreased in the treated groups than in the HFD group animals. Serum Glucose, Triglycerides, Total cholesterol, LDL-Cholesterol and VLDL-Cholesterol were significantly decreased while HDL-Cholesterol was elevated in the treated groups in a dose-dependent manner as compared to the HFD group. Elevated hepatic triglycerides, total cholesterol, free fatty acid and phospholipid levels were also significantly reduced in the treated groups. Furthermore, administration of fruit extract of *Carica papaya* L. produced a significant inhibition of MDA production and a significant increase in GSH and activity of SOD. In conclusion, these results suggest that aqueous fruit extract of *Carica papaya* L. could be useful in the treatment of obesity and related disorders.

Keywords: Anti-obesity, *Carica papaya* L., Cafeteria Diet, Orlistat.

INTRODUCTION

Obesity is a medical condition in which excess body fat gets accumulated to the extent that it may produce an adverse effect on health, leading to reduced life span and/or increased health problems. It is one of the leading causes of death worldwide, with increasing prevalence especially among adults and children, and is considered as one of the most serious public health problems of the 21st century¹. Obesity occurs mainly due to chronic imbalance between energy intake and energy expenditure. High intake of dietary fat, poor exercise, sedentary lifestyle, genetic susceptibility and metabolic errors in energy utilization are some of the major causes of obesity. Obesity increases the likelihood of various diseases, particularly heart disease, type 2 diabetes, breathing difficulties during sleep (Sleep Apnea), gallbladder disease, gallstones, Gout, osteoarthritis and certain types of cancer².

Obesity is a challenging clinical condition to treat, because of their complex environmental components. Efforts to develop innovative anti-obesity drugs with benefits for metabolic syndrome have been recently intensified. Moreover, due to absolute etiology of obesity, non-availability of drugs for its treatment, short-term efficacy and limiting contraindication and side effects of available drugs, the treatment is not satisfactory and thus there is a demand for search of new safer ones for combating this epidemic.

Traditional medicine/herbal medicine has been with us for ages but there has been a renewed interest in the subject in the recent past due to its low cost, efficacy and safety. Also, the World Health Organization (WHO) has recommended that this area warrants attention. *Carica papaya* L. (papaya) is one of the most commonly consumed fruit by the people throughout world. It has various health benefits due to its rich antioxidant substances. However, little is known about its effect on obesity. Therefore this study was designed with the aim to evaluate the anti-obesity potential of aqueous fruit extract of *Carica papaya* L. on high fat cafeteria diet induced obese rats.

MATERIALS AND METHODS

Plant Material and Extraction Procedures

Fresh fruits of *Carica papaya* L. were purchased from local market and used for this study. The skin was peeled off, seeds were removed

and the pulp was cut into pieces. 500g of the fruits was weighed and blended into a beaker and 1.5 L of water was used to soak the peeled and diced *Carica papaya* L. overnight. The juice was filtered using a Whatman filter paper 125 mm and concentrated using a rotary evaporator.

Animals

Healthy adult Wistar strain of albino rats of both sexes weighing around 200g, obtained from Tamil Nadu Veterinary and Animal Sciences University, Chennai were used for the present study. They were housed in polypropylene cages under controlled environment (inverted 12hr daylight cycles) with free access to standard rat chow pellet (obtained from Sai Durga Foods and Feeds, Bangalore, India) and water *ad libitum*. The animals were acclimatized to the laboratory conditions for a week before experiments.

All animal experimental procedures have been approved by the animal ethical committee (Approval No: 790/03/ac/CPCSEA).

Cafeteria Diets (High Fat Diet)

The cafeteria diet³ consisted of 3 diets (condensed milk, 40g + bread, 40g), (chocolate, 15g + biscuits, 30g + dried coconut, 30g), (cheese, 40g + boiled potatoes, 50g). The three diets were presented to group of 6 rats on day 1, 2 and 3 respectively and then repeated in same succession. These diets were provided in addition to normal pellet chow.

Experimental Design

The rats were divided into six groups with six rats each:

Group I: Normal Diet (ND) Fed or Normal Control

Group II: High Fat Cafeteria Diet (HFD) Fed or Obesity control

Group III: HFD + Aqueous fruit extract of *Carica papaya* L. (200mg/kg b.w.)

Group IV: HFD + Aqueous fruit extract of *Carica papaya* L. (400mg/kg b.w.)

Group V: HFD + Aqueous fruit extract of *Carica papaya* L. (600mg/kg b.w.)

Group VI: HFD + Standard Drug, Orlistat (50mg/kg b.w.)

All the drugs were given orally for a period of 45 days. At the end of experimental period the animals were sacrificed by cervical decapitation. The blood was collected and the serum was separated and used for various biochemical analyses. The liver, kidney and spleen were dissected out, washed in ice cold saline and weighed. Liver tissues were homogenized in 0.1M phosphate buffer (pH 7.4) and used for the analysis of hepatic lipids and antioxidants.

Biochemical Analysis

Blood glucose content was estimated by Folin- Wu⁴ method. Tissue lipids were extracted by the method of Folch *et al.*,⁵. Total cholesterol⁶, Triglycerides⁷, Phospholipids^{8,9}, Free fatty acids¹⁰ and Serum HDL-Cholesterol¹¹ were determined using their standard procedures. The LDL¹¹ and VLDL-Cholesterols were determined using the following formulas:

$$\text{LDL-Cholesterol} = \text{Total Cholesterol} - \{ \text{HDL-C} - (\text{Triglyceride}/5) \}$$

$$\text{VLDL-Cholesterol} = \text{Total Serum Triglyceride}/5$$

Lipid peroxide content was measured by the method of Ohkawa¹². Reduced Glutathione was estimated using the method of Sedlak *et*

al.,¹³. The assay of SOD was carried out using the method of Misra and Fridovich¹⁴.

In addition to these, physical parameters such as daily food intake and body weight (weekly) were also determined.

Statistical Analysis

All the data reported are expressed as mean ± S.E.M. Statistical analysis was performed by one-way analysis of variance (ANOVA). The values were considered to be significantly different when the P-value was less than 0.05 compared to baseline or control values.

RESULTS AND DISCUSSION

Effect on Body Weight

After 45 days of feeding, body weights in the high fat cafeteria diet (HFD) group were heavier than those of the normal diet (ND) group. Moreover, the body weights of the HFD+AFCEP (Aqueous Fruit Extract of *Carica papaya* L.) treated groups were significantly decreased in a dose dependent manner as compared to the HFD group (Figure:1).

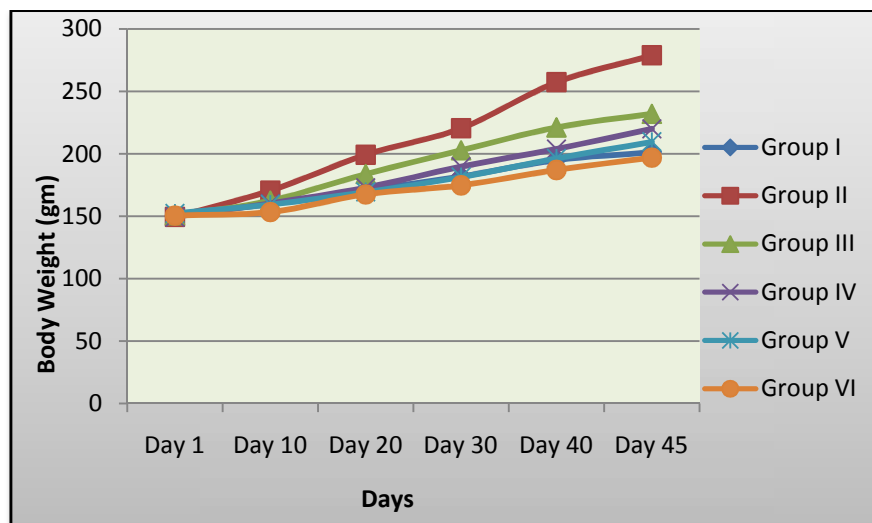


Fig. 1: Effect of AFCEP on Body Weight in HFD Induced Obese Rats

Values are mean ± S.E.M., (n = 6)

These inhibitions did not depend on decreased food or energy intake because there was no difference was observed in the food consumption between the HFD group and the treated groups, which were monitored daily. This observation indicated that dietary factors other than energy intake play important role in body weight regulation. Reduction in body weight may most likely be mediated via the activation of thermogenesis through the stimulation of the β-adrenergic receptors¹⁵.

Effect on Organ Weight

The absolute weights of liver, kidney and spleen of HFD group were significantly higher when compared to ND group animals. This could be

related to an accumulation of TG and cholesterol in these organs. There were no abnormalities in growth performance; however, the relative weights of the liver, kidney and spleen were significantly lower in the AFCEP treated groups as compared to the HFD group. This is probably because of the lower fat content in those tissues (Table:1).

Effect on Body Mass Index (BMI)

There was a significant increase in the BMI of HFD group animals as compared to the ND group. The increased BMI demonstrated the efficiency of cafeteria diet to induce obesity in these animals. Moreover, the treatment with AFCEP maintained the BMI compared to that of ND group (Table:1).

Table 1: Effect of AFCEP on Organ Weight and BMI in HFD Induced Obese Rats

Groups	Liver (g)	Kidney (g)	Spleen (g)	BMI (g/cm ²)
Group I	6.678 ± 1.48*	1.249 ± 0.61*	0.587 ± 0.40*	0.691 ± 0.28*
Group II	9.529 ± 1.30**	1.652 ± 0.05**	0.852 ± 0.32**	0.733 ± 1.43**
Group III	7.849 ± 1.15	1.561 ± 0.91	0.683 ± 1.01	0.698 ± 1.98
Group IV	7.338 ± 0.94	1.501 ± 1.01	0.613 ± 0.98	0.693 ± 1.08
Group V	6.772 ± 0.50**	1.4 ± 0.79**	0.598 ± 0.17**	0.692 ± 0.14**
Group VI	6.52 ± 1.55	1.125 ± 0.07	0.565 ± 0.07	0.662 ± 1.09

Values are mean ± S.E.M., (n = 6)

* - Compared between Normal and Disease Control (p<0.05).

** - Compared between Disease Control and High Dose Drug Treated Group (p<0.05).

Effect on Blood Glucose

There was a significant increase in serum glucose level in HFD group when compared to ND group. The significant increase in glucose in high fat cafeteria diet fed animal can be attributed to defective insulin signaling and a decreased insulin efficiency to induce glucose transport from the blood into key target cells such as muscle and fat (adipocyte) cells¹⁶. Treatment with AFCEP almost normalized the glucose levels in dose dependent manner. This could be probably mediated through enhanced secretion of insulin from the β -cells of the islets of pancreas or through an extra pancreatic mechanism¹⁷.

Effect on Serum Lipid Profile

The levels of serum lipid profile such as total cholesterol (TC), Triglycerides (TG) and lipoproteins such as Low Density Lipoprotein Cholesterol (LDL-C), Very Low Density Lipoprotein Cholesterol (VLDL-C) were significantly elevated in the HFD group, while High Density Lipoprotein Cholesterol (HDL-C) was significantly decreased as compared to the ND group. However, administration of AFCEP significantly improved these changes in a dose dependent manner (Table: 2).

The significant increase in total cholesterol and triglyceride level in HFD animals can be attributed to increase in both *de novo* synthesis and intestinal absorption of cholesterol. The increased blood levels of triglycerides, total cholesterol, LDL, VLDL as well as lowered levels of HDL in HFD rats have been identified in the development of hypercholesteremia, which is one of the risk factors for CAD¹⁸.

A substantial reduction of total cholesterol in serum by AFCEP could be attributed to a reduction in the activities of the liver enzyme 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) reductase, which is a rate-limiting enzyme in cholesterol biosynthesis. Also, a substantial reduction in LDL-cholesterol and total cholesterol level in serum could be achieved by decreased production of total cholesterol by liver tissue and/or efficient removal of the LDL-cholesterol by various tissues without subsequent renewal¹⁹. A higher content of HDL-C is very important 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 decrease of TG in AFCEP treated group may be related to increase in endothelium bound lipoprotein lipase activity that hydrolyses the triglycerides into fatty acids.

Effect on Hepatic Lipid

The levels of hepatic lipid profile such as total cholesterol (TC), Triglycerides (TG) Phospholipids (PL) and Free Fatty Acid (FFA) were significantly elevated in the HFD group, however, administration of AFCEP significantly restored these changes in a dose dependent manner (Table: 3). This indicated AFCEP efficiently regulated triglyceride and cholesterol metabolism. This may be accomplished through the decreased expression of genes for fatty acid synthesis and enhanced activation of expression of genes for β -oxidation.

Table 2: Effect of AFCEP on Blood Glucose and Serum Lipid Profiles in HFD Induced Obese Rats

Group	Glucose (mg/dl)	Serum Lipid Profiles				
		Total Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)
Group I	74.16 ± 0.62*	110.76 ± 0.75*	55.77 ± 0.44*	70.36 ± 0.99*	33.35 ± 0.65*	12.4 ± 0.97*
Group II	148.3 ± 1.66***	182.14 ± 1.23***	103.13 ± 2.13***	47.42 ± 1.41***	110.09 ± 1.01***	20.63 ± 0.28***
Group III	120.96 ± 1.63	169.38 ± 0.87	81.6 ± 1.14	49.95 ± 0.87	102.11 ± 1.17	17.22 ± 1.20
Group IV	98.06 ± 1.80	130.8 ± 1.62	68.23 ± 1.86	62.71 ± 1.38	54.44 ± 0.82	13.65 ± 1.11
Group V	80.7 ± 0.16**	109.48 ± 1.47**	59.92 ± 0.16**	67.73 ± 1.39**	35.32 ± 0.37**	11.98 ± 0.43**
Group VI	71.64 ± 1.70	107.8 ± 0.75	57.54 ± 0.70	75.366 ± 1.92	29.74 ± 0.02	11.51 ± 0.21

Values are mean ± S.E.M., (n = 6)

* - Compared between Normal and Disease Control (p<0.05).

** - Compared between Disease Control and High Dose Drug Treated Group (p<0.05).

Table 3: Effect of AFCEP on Hepatic Lipid Profiles in HFD Induced Obese Rats

Group	Total Cholesterol (mg/g tissue)	Triglycerides (mg/g tissue)	Phospholipids (mg/g tissue)	Free Fatty Acid (μ Eq/g tissue)
Group I	4.62 ± 0.93*	34 ± 0.95*	26.74 ± 1.06*	5.38 ± 1.09*
Group II	9.4 ± 0.84***	78 ± 0.88***	35.54 ± 1.82***	11.47 ± 0.95***
Group III	8.82 ± 1.57	72.34 ± 2.07	32.85 ± 2.15	11.21 ± 1.28
Group IV	6.93 ± 1.09	58.5 ± 1.52	30.72 ± 2.06	9.83 ± 2.07
Group V	5.38 ± 0.85**	49.12 ± 1.08**	28.07 ± 1.02**	7.06 ± 0.99**
Group VI	4.89 ± 0.09	37.18 ± 0.93	26.49 ± 0.95	5.8 ± 0.56

Values are mean ± S.E.M., (n = 6)

* - Compared between Normal and Disease Control (p<0.05).

** - Compared between Disease Control and High Dose Drug Treated Group (p<0.05).

Table 4: Effect of AFCEP on Hepatic Antioxidants in HFD Induced Obese Rats

Group	LPO (n moles of MDA formed/g tissue)	GSH (mg/g tissue)	SOD Activity (Unit/mg protein)
Group I	97.33 ± 1.09*	4.402 ± 1.07*	3.93 ± 1.85*
Group II	142.22 ± 0.95***	2.17 ± 0.64***	1.85 ± 0.44***
Group III	138.84 ± 1.19	3.12 ± 1.29	2.25 ± 1.32
Group IV	132.28 ± 1.07	3.63 ± 0.57	2.88 ± 0.71
Group V	126.81 ± 0.63**	4.182 ± 0.62**	3.41 ± 0.69**
Group VI	111.18 ± 0.56	4.28 ± 0.38	3.89 ± 0.06

Values are mean ± S.E.M., (n = 6)

* - Compared between Normal and Disease Control (p<0.05).

** - Compared between Disease Control and High Dose Drug Treated Group (p<0.05).

Effect on Antioxidants

HFD generates oxidative stress in obese rats as shown by a marked increase in the levels of MDA and a distinct diminution in hepatic GSH, as well as activities of the antioxidant enzyme SOD. All showed reduced activity in obese or hyperlipidemic rats (Table: 4).

Generally obesity is associated with a decrease in tissue or plasma antioxidant capacity. Several studies have shown increased lipid peroxidation in clinical and experimental hyperlipidemia. It has been established that hyperlipidemia leads to increased production of oxygen free radicals²⁰ which exert their cytotoxic effect by causing lipid peroxidation.

GSH constitutes the first line of defense against free radicals in the liver, and it is also responsible for the maintenance of protein thiols and acting as a substrate for GPx. Hyperglycemia in the HFD group activates different pathways leading to increased oxidative stress. Increased activity of the polyol pathway, inhibition of the pentose phosphate pathway as a result of hyperglycemia resulted in decreased intracellular levels of NADPH, which is required for regeneration of GSH from its oxidized form GSSG²¹. The net result was non-enzymatic disruption of H₂O₂ and increased levels of cellular superoxides, hydroperoxides, hydroxyl radicals as well as other radicals.

SODs are a family of antioxidant enzyme which are important in the catalytic decomposition of the superoxide radical which are formed during aerobic metabolism. Feeding a HFD to animals depresses the SOD activity due to increased lipid peroxidation and formation of excessive free radicals.

In addition, oxidative stress may be increased in metabolic syndrome due to dyslipidemia resulting from increased levels of FFA and TGs that led to increased formation of foam cells, rendering LDL less dense and more vulnerable to oxidation and uptake by macrophages²².

On treatment with AFCEP produced a significant inhibition of MDA production and a significant increase in GSH and activity of SOD. The test drug might have been inhibiting the lipase activity which resulted in decreased lipid content in hepatic tissue which in turn decreased the lipid peroxidation. This potent antioxidant effect of aqueous fruit extract of *C. papaya* L. might be contributed by its rich antioxidant contents such as vitamin C, β -carotene and other flavonoid compounds.

CONCLUSION

In conclusion, these data indicates that aqueous fruit extract of *C. papaya* L. appears to be a promising health drink capable of modulating weight control without inducing any adverse side effects. Further studies can be undertaken to establish the possible mechanism of action of *C. papaya* L. fruit extract. This could include measuring serum leptin levels, serum insulin levels, serum lipoprotein lipase activity, pancreatic lipase activity, brown adipose tissue mass and other neurotransmitters involved in control of appetite.

REFERENCES

- Barness LA, Opitz JM and Gilbert-Barness E. "Obesity: genetic, molecular, and environmental aspects". *Am J Med Genet* 2007;143A (24): 3016–3034.
- Haslam DW, and James WP. "Obesity". *Lancet* 2005;366 (9492): 1197–1209.
- Harris RB. The impact of high- or low-fat cafeteria foods on nutrient intake and growth of rats consuming a diet containing 30% energy as fat. *Int J Obes* 1993;17: 307–315.
- Folin O and Wu H. Determination of Glucose. *J Biol Chem* 1999; 81.
- Folch J, Lees M and Stanley GHS. A simple method for isolation and purification of total lipids from animal tissues. *J Biol Chem* 1970;226: 497-509.
- Zak B, Zlatkins A and Boyle. A new method for the determination of serum cholesterol. *J Lab Clin Med* 1953; 14: 486.
- Foster LB and Dunn RT. "Stable reagents for determination of Serum triglycerides by a colorimetric Hantzsch condensation method". *Clin Chem* 1973;196: 338-340.
- Bartlette GR, "Phosphorus assay in column chromatography". *J Biol Chem* 1959; 234: 466-468.
- Fiske CH, Subbarow Y. "The colorimetric determination of inorganic phosphorus". *J Biol Chem* 1925;66: 375-400.
- Falholt K, Falholt W, Lund B. "An easy colorimetric method for routine determination of free fatty acids in plasma". *Clin Chim Acta* 1997;46: 105-111.
- Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low density lipoprotein cholesterol in plasma without the use of preparative centrifuge. *J Clin Chem* 1972;18: 499.
- Ohkawa H, Ohishi N, Yagi K. "Assay of lipid peroxides in animal tissues for thiobarbituric acid reaction". *Annual Biochem* 1979;95: 351-358.
- Sedlak J, Lindsay RH. Estimation of total protein bound and non- protein sulfhydryl groups in tissue with Ellman's reagent. *Anal Bio Chem* 1968;25: 192-205.
- Misra HP, Fridovich I. "The role of superoxide anion in the autooxidation of epinephrine and a simple assay for SOD". *J Biol Chem* 1972;247: 3170-3175.
- Preuss HG, DiFerdinando D, Bagchi M, Bagchi D. Citrus aurantium as a thermogenic, weight-reduction replacement for ephedra: an overview. *J Med* 2002; 33: 247–264.
- Kahn BB, Flier JS. Obesity and insulin resistance. *J Clin Invest* 2000;106: 473–481.
- Kamal A Amin, Mohamed A Nagy. Effect of Carnitine and herbal mixture extract on obesity induced by high fat diet in rats. *Diabetology and Metabolic Syndrome* 2009;1: 17.
- Ouwens DM, Boer C, Fodor M, de Galan P, Heine RJ, Maassen JA, Diamant M. Cardiac dysfunction induced by high-fat diet is associated with altered myocardial insulin signalling in rats. *Diabetologia* 2005;48: 1229-1237.
- Moonkyu Kang, Jung-Wan OH, Hee-Kyung LEE *et al.*, Anti-obesity Effect of PM-F2-OB, an Anti-obesity Herbal Formulation, on Rats Fed a High-Fat Diet. *Biol Pharm Bull* 2004;27(8): 1251-1256.
- Prasad K, Kalra J. Oxygen free radicals and hypercholesterolemic atherosclerosis. *Am Heart J* 1993;125: 958–971.
- Brownlee M. Biochemistry and molecular cell biology of diabetic complications. *Nature* 2001;13: 813-820.
- Holvoet P. Relations between metabolic syndrome, oxidative stress inflammation cardiovascular disease. *Verh K Acad Geneesk Belg* 2008;70(3): 193-219.