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Retardation of hepatic lipid accumulation and insulin resistance by ethanol extract of *Garcinia cambogia* in high fructose fed rats

Short title: *Garcinia cambogia* and metabolic complications in rats

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Abstract

The study investigated the effects of *Garcinia cambogia* extract (GE) on hepatic lipid accumulation and insulin resistance in high fructose fed rats. Forty male Wistar rats were divided into four groups (N=10). Two groups received a standard rodent diet, two groups received a 60% fructose diet and 400 mg/kg body weight of GE was given to each of the two diet groups for an experimental duration of ten weeks. GE administration decreased relative organ mass (liver, adipose tissue, epididymis), lipid ratios (TC/HDL-C, TAG/HDL-C, AIP, Non HDL-C/HDL-C), CRP, glucose, insulin, HOMA-IR and increased adiponectin levels in fructose fed rats. Treatment with GE reduced liver TAG content, hepatic expression of SREBP-1c (86%), ACC (79%), FAS (81%), COX-2 (86%), oxidative stress and PKC-θ phosphorylation (67%) in skeletal muscle of fructose fed rats. GE supplementation decreased fructose induced hepatic fat accumulation and improved insulin sensitivity.

Keywords
Fructose, Hepatic steatosis, Inflammation, Oxidative stress, Insulin resistance, Garcinia cambogia

Introduction

Nutrients can either be beneficial or detrimental for healthy well-being depending on the nature and quantity of the consumed dietary ingredients. The incidence of consumption of high energy foods such as western style diets rich in refined sugars has been increasing alarmingly (Alwahsh *et al*. 2014). Among the usual dietary sugars, fructose has been extensively used in the production of beverages and processed ready-to-eat foods owing to its sweet taste, easy availability and reasonable cost (Dills 1993). Fructose when ingested is completely absorbed, rapidly metabolized in the liver and undergoes a strikingly dissimilar metabolic fate from that of glucose (Tappy and Lê 2010).

Evidence suggests that fructose consumption enhances hepatic triacylglycerol (TAG) synthesis in rats and humans (Fried and Rao 2003). Increased TAG production causes deposition of fat in organs such as liver, skeletal muscle and thereby enhances visceral fat mass (Stanhope and Havel 2008). Moreover, the burden of excess fructose metabolism generates reactive oxygen species (ROS), initiates various proinflammatory pathways, reduces antioxidant status and also causes dysregulation of adipokines (Carvalho *et al*. 2010). Factors such as lipotoxicity, dysregulated adipokine secretion, oxidative stress and chronic low-grade inflammation decrease insulin sensitivity and thereby contribute for the development of insulin resistance (Qatanani and Lazar 2007).

Chronic intake of excess fructose has been reported to be a critical contributor for the incidence of Metabolic syndrome (MetS) and its associated co-morbidities (Miller and Adeli 2008). MetS encompasses a cluster of complications such as abdominal obesity, dyslipidemia, hypertension, hyperglycemia, hyperinsulinemia and insulin resistance. These metabolic alterations enhance the risk of the progression of several diseases such as diabetes mellitus, cardiovascular disorders, hepatic steatosis and certain kinds of cancer (Ford and Giles 2003). The pharmacological management of MetS is challenging as the therapeutic strategies have to focus on tackling individual metabolic complications. Hence there is an intensive search for effective compounds with fewer adverse effects which may alleviate the health threatening effects of fructose and prevent the occurrence of MetS.

Several plant extracts, phytochemicals, herbs and dietary supplements have been investigated and employed in the management of MetS and its related complications. One such plant is *Garcinia cambogia* (Gaertn.) Desr. (Clusiaceae), also known as *Garcinia gummi-gutta*, Malabar tamarind and Brindleberry. It is a tropical plant grown in Southeast Asia, South India, and Africa. The fruit of the plant is edible, has a characteristic sweet and sour taste, and hence widely used as food preservative, carminative and flavouring agent in various culinary preparations (Saito *et al*. 2005). In India, *Garcinia cambogia* extract (GE) has been used in traditional medicine for the treatment of diarrhea, dysentery, ulcers, haemorrhoids and certain types of cancers (Duke *et al*. 2002). The fruit rind has been stated to contain organic acids such as (-) hydroxycitric acid (HCA), citric acid, malic acid, flavonoids, xanthones, benzophenones such as garcinia/cambogia and isoxanthohumol (Chuah *et al*. 2013).

Studies have documented the beneficial effects of certain individual constituents of the *Garcinia cambogia* fruit such as (-)-HCA, garcinol and flavonoids. (-)-HCA, a competitive inhibitor of ATP citrate lyase (ACLY), has been shown to reduce triacylglycerol levels when administered to

both high fat diet induced and genetically modified obese animal models (Sullivan *et al*. 1974, Asghar *et al*. 2007). *In vitro* experiments have revealed the potent antioxidant and anti-inflammatory properties of the polyisoprenylated benzophenone, garcinol (Kolodziejczyk *et al*. 2009). It has been reported that flavonoids extracted from *Garcinia cambogia* fruit reduced lipid levels in hypercholesterolemic rats (Asha *et al*. 2001).

However, few reports state the hypolipidemic properties of the individual components of *Garcinia cambogia* fruit; the underlying molecular mechanisms remain unclear. To the best of our knowledge, the synergistic effects of the various vital phytochemical ingredients of the *Garcinia cambogia* fruit extract on the molecular basis of hepatic steatosis and insulin sensitivity in high fructose diet induced animal models have not been addressed till now. Since the whole fruit rind of *Garcinia cambogia* is widely used in several food and medicinal preparations, we intended to investigate the effectiveness of the *Garcinia cambogia* fruit in the manner that it is being commonly used. Hence, the present study was designed to investigate the effects of *Garcinia cambogia* fruit extract on hepatic lipid accumulation and insulin sensitivity in high fructose induced insulin resistant rats.

Materials and methods

Chemicals

All the chemicals used were of molecular or analytical grade, and were procured from Sigma Aldrich (USA), Merck (India) and Sisco Research Laboratories (SRL) (India). The proteolytic inhibitors used for tissue homogenisation [Phenylmethylsulfonylfluoride (PMSF), Aprotinin, Dithiothreitol, Pepstatin A and Benzamidine HCL] were purchased from Sigma-Aldrich, USA. Okadaic acid was obtained from Acros Organics (Thermo Fisher Scientific Inc., Belgium). The primary antibodies for Sterol regulatory element binding protein -1c (SREBP-1c), Fatty acid synthase (FAS), Peroxisome proliferator-activated receptor - α (PPAR-α) and Protein kinase C- θ (PKC-θ) were procured from Abcam (Cambridge, UK). The primary antibodies for acetyl CoA carboxylase (ACC) and cyclooxygenase-2 (COX-2) were obtained from Cell Signaling Technology Inc. (Danvers, MA). The primary antibodies for Insulin receptor-β (IR-β) was purchased from Pierce (Thermo Fisher Scientific Inc. USA) and β-actin from Biolegend (SanDiego, CA). The primary antibody for glyceraldehyde -3- phosphate dehydrogenase (GAPDH), horse radish peroxidase (HRP) coupled secondary antibodies; anti-rabbit IgG and anti-mouse IgG were procured from Santa Cruz (Santa Cruz Biotechnology, Santa Cruz, CA, USA). The nitrocellulose membranes (0.45µm) were obtained from Sigma-Aldrich, USA. The CL X-posure films and enhanced chemiluminescence (ECL) substrate West Pico Super Signal were procured from Pierce, (Thermo Fisher Scientific Inc., Marietta, USA).

Plant material

Fresh fruits of *Garcinia cambogia* were procured from Kerala, India. The fruit was authenticated (Accession No.25821 dated 10/01/2012) by Dr. B. R. Ramesh, Director of Research, French Institute of Pondicherry, Puducherry, India.

Preparation of ethanolic extract of *Garcinia cambogia*

The fresh fruits of *Garcinia cambogia* were washed, the seeds were removed and the fruit rinds were sun dried. The dried rinds were then ground finely and crude extraction with 70% v/v ethanol was performed by constant shaking for 24 h at room temperature. The filtrate was evaporated under reduced pressure to attain a semi-solid residual material which was stored at 4°C until use (Oluyemi *et al*. 2007).

Animals and Interventions

After obtaining approval from the Institutional Animal Ethics Committee, the study was conducted in the Department of Biochemistry, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry. Five month-old male Wistar rats (n = 40) were obtained from the Institute Central Animal House and were housed in plastic polycarbonate cages. The animals were provided food and water available *ad libitum* and were maintained at 22±2°C with a 12/12 h light/dark cycle. The animals were acclimatized for one week and were then divided into different groups based on their body weight. The animals were divided into four groups with ten animals in each.

Group 1: Control [C] – rats were fed with a standard rodent diet.
Group 2: Control+GE [C+GE] - rats were fed with standard rodent diet + GE
Group 3: Fructose [F] - rats were fed with 60% high fructose diet
Group 4: Fructose +GE [F+GE] - rats were fed with 60% high fructose diet + GE

The standard rodent diet was procured from Pranav Agro Industries Ltd., Maharashtra, India. The high fructose diet was prepared by mixing 60% wt/wt fructose with standard rodent diet (Hwang *et al*. 1987). The GE was dissolved in drinking water and a dose of 400mg/kg body wt/day (Oluyemi *et al*. 2007) was administered to each rat in groups 2 and 4 by oral gavage. All rats in the four groups received their respective diets and GE for an experimental duration of ten weeks.

Sample collection

Blood samples were collected at the end of ten weeks. After 15 h of fasting, blood samples were drawn from all the rats in Ethylenediaminetetraacetic acid (EDTA) vials. The blood samples were centrifuged at 3500 rpm for five min, the plasma was separated and stored at - 80°C for subsequent investigations. At the end of the experiment, all the animals were sacrificed, organs such as liver, adipose tissue, skeletal muscle and epididymal fat were collected and weighed. The liver and skeletal muscle tissues were frozen immediately in liquid N_2 and preserved at -80°C for subsequent proteomics analyses. A part of the liver tissue was fixed in 10% neutral buffered formalin for histopathological analyses.

Estimation of parameters

Analyses of biochemical parameters

The plasma levels of glucose, total cholesterol (TC), triacylglycerol (TAG), high density lipoprotein cholesterol (HDL-C), aspartate transaminase (AST), alanine transaminase (ALT), total protein and albumin were analysed using standard reagents kits adapted to fully automated random access discrete clinical chemistry analyser (AU-400, OLYMPUS, Japan). The lipid ratios were calculated as follows; Non HDL-C = TC- (HDL-C), TC/HDL-C ratio, TAG/HDL-C ratio, Atherogenic index of plasma (AIP) = log10 of TAG/HDL-C and Non HDL-C/HDL-C.

Estimation of Insulin, C-reactive protein (CRP) and Adiponectin

The plasma levels of Insulin, CRP and Adiponectin were analyzed using appropriate rat Enzyme-linked immunosorbent assay (ELISA) kits. The ELISA kit for Insulin was procured from Crystal chem Inc., USA, CRP from Immunology Consultants Laboratory, Inc. Portland, USA and Adiponectin from Ray Biotech^R, Inc. Norcross, USA.

Assessment of insulin resistance

Homeostasis Model Assessment Index for Insulin Resistance (HOMA-IR) was used to assess insulin resistance. It was calculated by the formula (Matthews *et al*. 1985)

HOMA-IR = [fasting glucose (mmol/L) x fasting insulin (µIU/mL)]/22.5

Estimation of TAG in liver and muscle

The liver and muscle tissues were homogenized in chloroform and methanol (2:1, v/v) mixture (1 g tissue in 20 ml of solvent mixture). 0.2 ml (4 ml for 20 ml) of water or 0.9% NaCl solution was added to the tissue homogenate, mixed and centrifuged at 2000 rpm to separate into two phases. The lower chloroform phase containing lipids was collected and evaporated. The TAG content was estimated using commercial enzyme kits (Genuine Biosystems, Chennai, India) and the TAG concentrations were expressed as mg of TAG per g of tissue (Folch *et al*. 1957).

Estimation of oxidative stress parameters in skeletal muscle

The skeletal muscle tissues were homogenized with 0.1M Tris-HCl buffer (pH 7.4, 10% W/V) (Sellamuthu *et al*. 2013). The samples were then centrifuged at 14,000 g for 15 min at 4°C. The supernatants were separated and used for the analyses of total oxidant status (TOS) and total antioxidant status (TAS). The oxidative stress index (OSI) was calculated using the formula (TOS/TAS) x 100. The protein concentrations in the muscle homogenates were determined.

Estimation of total oxidant status

To 225 µL of reagent 1(150 µM xylenol orange, 140 mM NaCl and 1.35 M glycerol), 35 µL of the sample was added and an initial absorbance was measured at 560nm. To this, 11 µL of reagent 2 (5 mM ferrous ammonium sulphate and 10 mM o-dianisidine dihydrochloride) was added, mixed and after 4 min, the absorbance was measured at 560 nm when the reaction trace draws a plateau line. A standard graph was plotted using different concentrations of hydrogen peroxide (H_2O_2) (0-200). The results were extrapolated from the standard graph and expressed as µmol H_2O_2 Equiv. /L (Erel 2005).

Estimation of total antioxidant status

To 300 µl of the Ferric reducing antioxidant power (FRAP) reagent (acetate buffer, TPTZ and $FeCl_3$), 10 µl of the sample was added, mixed and kept at room temperature for 4 min. The solution was measured at 593 nm and the results were obtained from a standard graph of $FeSO_4$ (0-1000 µMol/L) (Benzie and Strain 1996).

Estimation of protein by Lowry method

100 µl of the muscle homogenate was mixed with 900 µl of distilled water. From this 20µl was taken, mixed with 2.5 ml of alkaline copper reagent and incubated for 10 min. 250 µl of Folin-Ciocalteu reagent was added to this mixture and incubated for 30 min in dark. The solution was measured at 660 nm and the protein concentrations were calculated from the standard graph plotted using varying concentrations of bovine serum albumin. The values were expressed as µg/mL (Lowry *et al*. 1951).

Histological examination of liver tissues by haematoxylin and eosin staining

The liver tissue samples fixed in formalin were dehydrated and embedded in paraffin wax. Thin sections (5 µm) were cut and stained with haematoxylin and eosin for examination of morphological changes. The images were captured using Olympus BX43 phase contrast microscope

(Olympus, Japan) under 20X resolution.

Oil Red O staining of liver

The deposition of lipid droplets (steatosis) was analysed by staining the liver sections with Oil Red O stain. The portions of the liver samples were frozen in tissue freezing medium, then sectioned to 5 µm thickness and were stained with Oil Red O followed by Ehrlich's hematoxylin as counterstain. The images were captured using Olympus BX43 phase contrast microscope (Olympus, Japan) under 20X resolution.

Western blotting

The liver and skeletal muscle tissues were homogenized in modified Radioimmunoprecipitation assay (RIPA) buffer (pH 8.0 containing 150mM NaCl, 1% nonidet P-40, 0.5 % sodium deoxycholate, 0.1 % sodium dodecyl sulphate (SDS), 50mM Tris, 5mM Ethylenediaminetetraacetic acid (EDTA), 1mM Ethylene glycol tetraacetic acid (EGTA), 10mM sodium fluoride, 20mM Dithiothreitol (DTT), 1mM Benzamidine HCL, 1mM Phenylmethylsulfonyl Fluoride (PMSF), 1µM Aprotinin, 1 µg/ml pepstatin A and 0.5 µg/ml okadaic acid). The tissue homogenates were centrifuged at 10,000 rpm for 30 min at 4°C. The supernatant was separated and the protein concentration was analysed by Lowry's method (Lowry *et al*. 1951). The different proteins were resolved by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE, Mini Protean II System, Bio-Rad, China) (Laemmli 1970) and the percentages of the gels used vary depending on the molecular weight of the proteins (12%- PPAR-α, 10% - SREBP-1c, COX-2, 8% -IR-β, total PKC-θ and 6%-ACC, FAS). After separation, the proteins were transferred onto the nitrocellulose membranes (Sigma, USA) using Trans SD semi-dry transfer (15 V, 35 min) (Bio-Rad, China) and the membranes were blocked with 5% (w/v) bovine serum albumin (BSA) or non – fat milk powder at room temperature for 1 hour. The membranes were then probed with specific primary antibodies (SREBP-1c, ACC, FAS, PPAR-α, COX-2, IR-β, total PKC-θ) in 5% (w/v) BSA or non – fat milk powder in Tris Buffered Saline - 0.1% Tween 20 (TBS-T) as per the manufacturer's recommended dilution and kept for incubation overnight at 4°C with shaking. After incubation the membranes were washed with TBS-T thrice and followed by incubation with species specific, horseradish peroxidase-conjugated secondary antibodies and incubated at room temperature for 1 hour. After washing thrice with TBS-T, the blots were visualized by enhanced chemiluminescence method using ECL reagent kit (Pierce, Thermoscientific Inc, USA). The density of the bands were captured by Image Densitometer GS – 800 (Bio-Rad, China) and analysed using Quantity One software. The same blots were then stripped in stripping buffer (62.5mM Tris-HCl, pH 6.8, 2% SDS, and 100mM β-mercaptoethanol) at 50 - 60°C for 20-30 min with shaking and then reprobed with different antibodies such as β-actin, GAPDH and phospho PKC-θ. The same procedure was repeated as mentioned above.

Statistical analysis

The data were expressed as mean ± SD. Statistical analyses were carried out using Statistical Package of Social Science (SPSS, Version 19.0). One way analysis of variance (ANOVA) with Tukey's *post hoc* test was used to identify differences between the groups. A p value < 0.05 was considered as statistically significant.

Results

Table 1 Effects of GE on relative organ weights, plasma lipid ratios and hepatic TAG content in control and fructose fed rats

Parameters	Control	C+GE	Fructose	F+GE
Liver wt (g/100g BW)	2.51 ± 0.30	2.48 ± 0.31	3.30 ± 0.20[a]	2.81 ± 0.21[b]
Adipose wt (g/100g BW)	1.31 ± 0.21	1.27 ± 0.16	2.11 ± 0.27[a]	1.68 ± 0.31[b]
Epididymis wt (g/100g BW)	0.71 ± 0.12	0.67 ± 0.13	1.18 ± 0.20[a]	0.90 ± 0.19[b]
TC/HDL-C	1.66 ± 0.21	1.49 ± 0.18	3.05 ± 0.83[a]	2.02 ± 0.29[b]
TAG/HDL-C	2.63 ± 0.46	2.46 ± 0.44	6.66 ± 1.13[a]	3.92 ± 0.55[b]
AIP	0.41 ± 0.07	0.38 ± 0.08	0.82 ± 0.07[a]	0.59 ± 0.06[b]
Non HDL-C/HDL-C	0.66 ± 0.21	0.49 ± 0.18	2.05 ± 0.83[a]	1.02 ± 0.29[b]
Liver TAG (mg/g of tissue)	4.42 ± 0.65	4.28 ± 0.41	10.11 ± 0.89[a]	6.73 ± 0.79[b]

Data were represented as Mean ± SD. (n=10), [a] Significant as compared with control group ($p<0.05$)[b], Significant as compared with fructose group ($p<0.05$). C-Control, F-Fructose, GE- Garcinia cambogia extract, BW- Body weight, TC-Total cholesterol, TAG-Triacylglycerol, HDL-C–High density lipoprotein cholesterol, non HDL-C–Non high density lipoprotein cholesterol, AIP- Atherogenic index of plasma

Table 1 shows the relative organ weights; liver, adipose tissue, epididymal fat mass, plasma lipid ratios; TC/HDL-C, TAG/HDL-C, AIP, Non HDL-C/HDL-C and hepatic TAG content were significantly increased in rats fed with high fructose diet when compared with control rats. Administration of GE significantly reduced organ weights, lipid ratios and hepatic TAG content in fructose fed rats. GE supplementation along with control diet did not show any significant change in relative organ weights, plasma lipid ratios and hepatic TAG content.

Table 2 Effects of GE on plasma AST, ALT, total protein, albumin, CRP, glucose, insulin, HOMA-IR and adiponectin levels in control and fructose fed rats

Parameters	Control	C+GE	Fructose	F+GE
AST (IU/L)	94.30 ± 8.58	92.70 ± 6.82	110.10 ± 6.21[a]	98.80 ± 5.29[b]
ALT (IU/L)	50.10 ± 5.72	47.60 ± 6.48	67.30 ± 6.70[a]	55.30 ± 5.98[b]
Total protein (g/dl)	7.15 ± 0.22	7.31 ± 0.23	6.92 ± 0.13	7.08 ± 0.30
Albumin (g/dl)	3.97 ± 0.52	4.13 ± 0.46	3.72 ± 0.42	3.88 ± 0.26
CRP (µg/mL)	551.45 ± 81.62	545.32 ± 68.21	892.05 ± 91.51[a]	672.58 ± 79.27[b]
Glucose (mg/dl)	78.30 ± 10.01	76.50 ± 9.66	98.70 ± 9.88[a]	84.50 ± 8.54[b]
Insulin (ng/mL)	0.48 ± 0.10	0.45 ± 0.08	1.04 ± 0.17[a]	0.67 ± 0.14[b]
HOMA-IR	2.34 ± 0.49	2.17 ± 0.55	6.41 ± 1.36[a]	3.60 ± 1.09[b]
Adiponectin (ng/mL)	450.49 ± 90.25	455.02 ± 80.25	185.45 ± 42.33[a]	367.57 ± 81.36[b]

Data were represented as Mean ± SD. (n=10), [a] Significant as compared with control group ($p<0.05$)[b], Significant as compared with fructose group ($p<0.05$). C-Control, F-Fructose, GE-Garcinia cambogia extract, AST- Aspartate transaminase, ALT- Alanine transaminase, CRP- C reactive protein, HOMA- IR - Homeostasis model of insulin resistance.

Table 2 shows high fructose feeding significantly elevated plasma AST, ALT, CRP, glucose, insulin, HOMA-IR and reduced adiponectin levels when compared with control rats. Treatment with GE along with a fructose diet reduced AST, ALT, CRP, glucose, insulin, HOMA-IR and increased adiponectin levels. There was no significant change in the total protein and albumin levels in fructose fed rats and with fructose+GE treated rats. Supplementation of GE with a control diet did not show any significant change in plasma AST, ALT, total protein, albumin, CRP, glucose, insulin, HOMA-IR and adiponectin levels.

Table 3 Effects of GE on TAG content and oxidative stress parameters in skeletal muscle of control and fructose fed rats

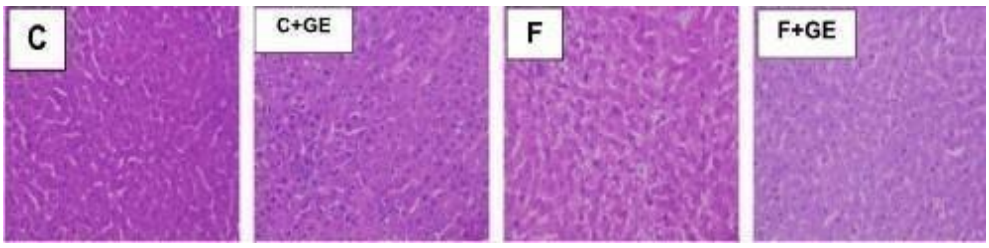
Parameters	Control	C+GE	Fructose	F+GE
TAG (mg/g of tissue)	2.51 ± 0.57	2.48 ± 0.60	8.82 ± 1.34[a]	5.56 ± 1.11[b]
TOS (µmol H_2O_2 Equiv./mg of protein)	1.34 ± 0.13	1.31 ± 0.12	2.97 ± 0.26[a]	1.86 ± 0.20[b]
TAS (µmol/mg of protein)	64.33 ± 7.30	66.76 ± 8.22	42.08 ± 6.38[a]	58.07 ± 7.12[b]
OSI	2.10 ± 0.28	2.00 ± 0.37	7.20 ± 1.21[a]	3.22 ± 0.36[b]

Data were represented as Mean ± SD. (n=10), [a] Significant as compared with control group ($p<0.05$)[b], Significant as compared with fructose group ($p<0.05$). C-Control, F-Fructose, GE-Garcinia cambogia extract, TAG-Triacylglycerol, TOS–Total oxidant status, TAS–Total antioxidant status, OSI – Oxidative stress index.

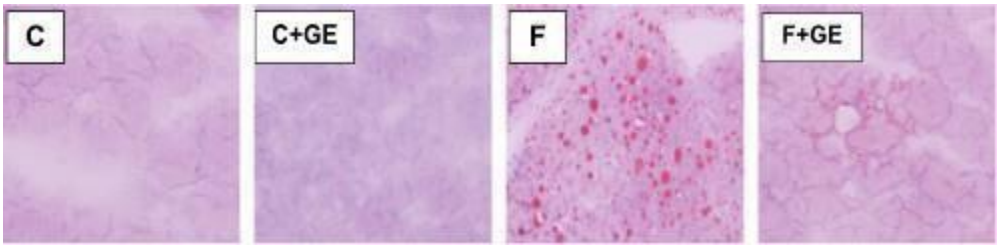
Table 3 shows significant elevation in the TAG content, TOS, OSI and reduction in the TAS in the skeletal muscle of fructose fed rats. Administration of GE decreased the muscle TAG content, TOS, OSI and increased TAS in fructose fed rats. No significant change was observed in TAG content, TOS, TAS and OSI in control rats supplemented with GE.

Figure 1: Representative images showing the effects of GE on hepatic steatosis in control and fructose fed rats by A) Haematoxylin and eosin staining and B) Oil Red O staining.

A)



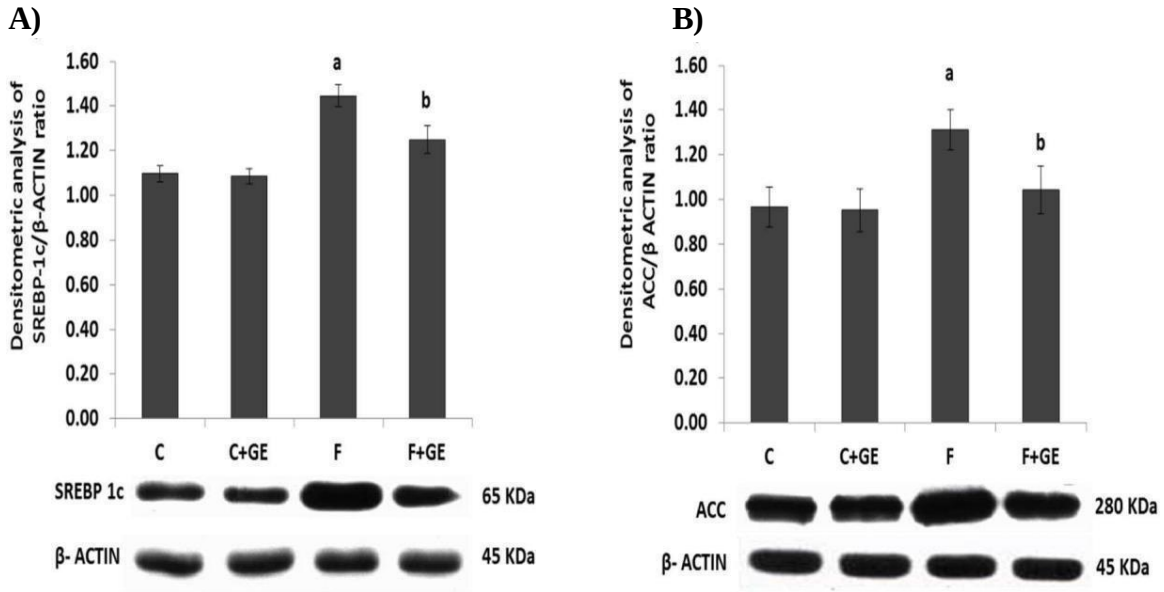
B)



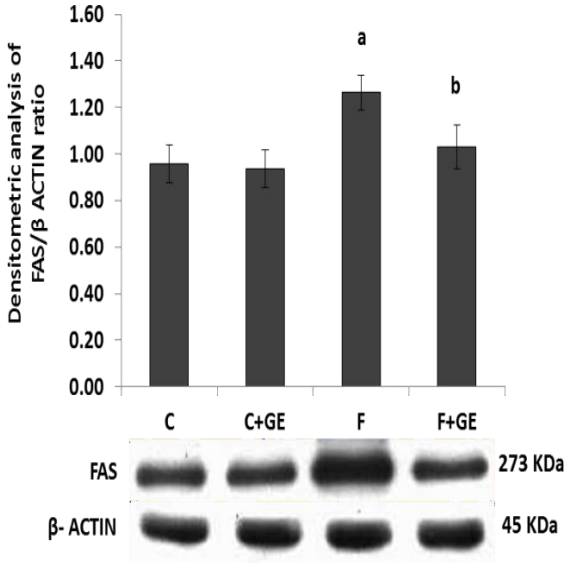
The images were captured using phase contrast microscope Olympus BX43, Japan under 20X resolution. C and C+GE groups - show normal liver morphology and no fat accumulation. F group - shows mild microvesicular centrizonal steatosis, 15-20%/hepatic lobule and shows marked fat accumulation. F+GE group - show normal liver morphology and reduced fat accumulation. C-Control, F-Fructose, GE- *Garcinia cambogia* extract

Figure 1 shows the effects of GE on hepatic fat accumulation in control and fructose fed rats by A) Haematoxylin; Eosin and B) Oil Red O staining. Fructose fed rats showed mild microvesicular centrizonal steatosis with 15-20%/hepatic lobule and also revealed marked hepatic fat accumulation. Treatment with GE along with fructose diet significantly reduced the liver fat deposition and maintained normal liver morphology. The control rats supplemented with GE showed normal liver morphology and no fat accumulation.

Figure 2: Immunoblot and densitometric analysis of the effects of GE on the hepatic expression of A) SREBP-1c, B) ACC C) FAS D) PPAR-α and E) COX-2 in control and fructose fed rats.



C)



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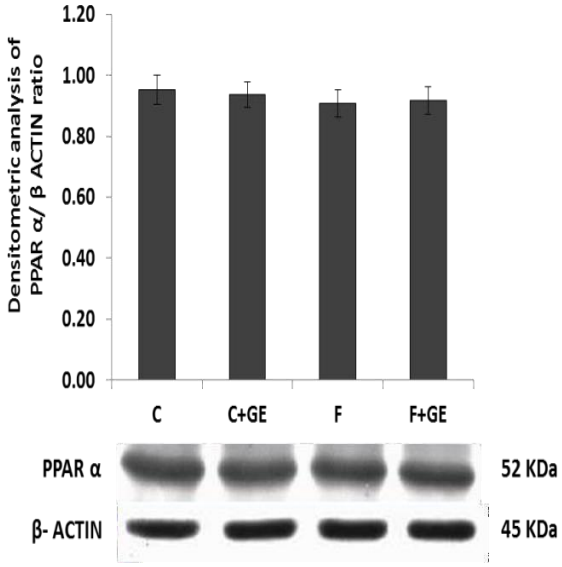

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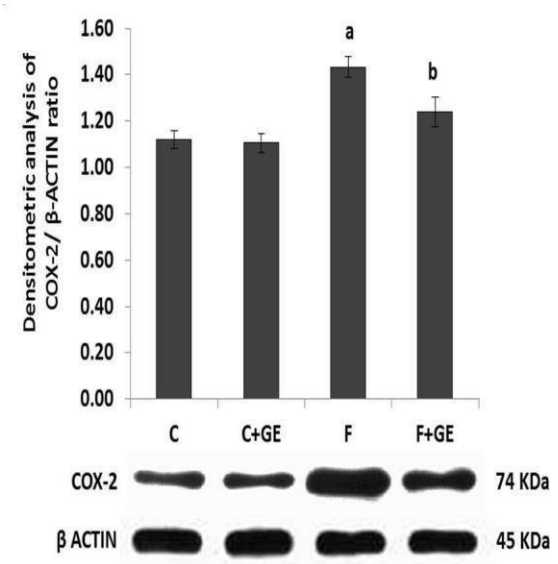
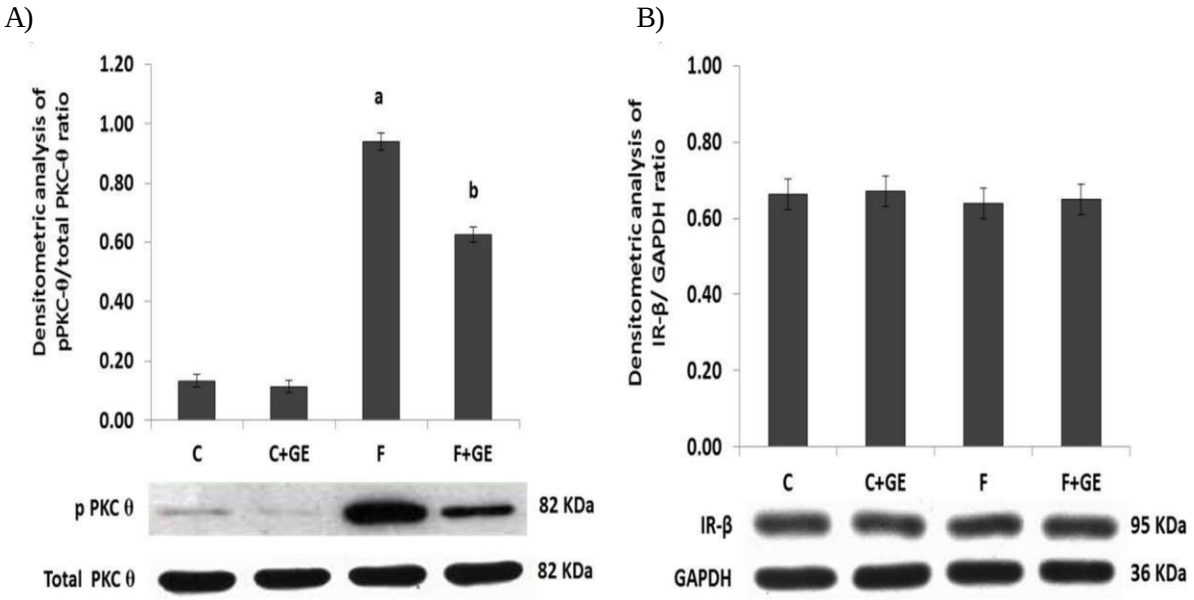


Data were expressed as mean ± SD, (n=3). Differences between groups were analysed using one way analysis of variance with Tukey's *post hoc* test using SPSS version 19.0. [a] Significant as compared with the control group ($p<0.05$), [b] Significant as compared with fructose group ($p<0.05$). C-Control, F- Fructose, GE- Garcinia cambogia extract.

Figure 2 shows high fructose feeding upregulated the hepatic expression of key lipogenic proteins; SREBP-1c, ACC, FAS and inflammatory protein COX-2. Treatment with GE significantly downregulated the expression of SREBP-1c (86%), ACC (79%), FAS (81%) and COX-2 (86%) in fructose fed rats. There was no significant difference in the hepatic expression of PPAR-α in fructose

fed rats and with fructose+GE treated rats.

Figure 3: Immunoblot and densitometric analysis of the effects of GE on the expression of A) IR-β and B) PKC-θ in skeletal muscle of control and fructose fed rats.



Data were expressed as mean ± SD, (n=3). Differences between groups were analysed using one way analysis of variance with Tukey's post hoc test using SPSS version 19.0. a Significant as compared with the control group ($p<0.05$), b Significant as compared with fructose group ($p<0.05$). C-Control, F- Fructose, GE- Garcinia cambogia extract.

Figure 3 shows enhanced phosphorylation of PKC-θ in the skeletal muscle of fructose fed rats and supplementation of GE along with fructose diet significantly reduced the phosphorylation of PKC- θ (67%). No significant changes were observed in the skeletal muscle expression of IR-β in fructose fed rats and with fructose+GE treated rats. There was no significant change in the expression of SREBP-1c, ACC, FAS, PPAR-α, COX-2, IR-β and PKC-θ in control rats supplemented with GE.

Discussion

In our study, rats fed with a fructose rich diet displayed elevated plasma lipid ratios in association with increased liver, adipose tissue, and epididymal fat mass. Raised plasma levels of AST, ALT and elevated liver TAG content were observed in these rats. Histological examination of the liver sections revealed marked hepatic fat accumulation upon high fructose feeding. Administration of GE significantly reduced fructose induced increment in the relative organ mass, plasma lipid ratios, plasma activities of enzymes; AST, ALT and hepatic TAG content. Noticeable reduction in the liver fat deposition was observed upon treatment with GE. Taken together, it is evident that administration of GE significantly reduced fructose mediated hyperlipidemic changes.

To elucidate the molecular mechanisms of the hypolipidemic effects of GE, we investigated the expression of certain key proteins involved in the lipid homeostasis. Chronic fructose

consumption induces a key transcriptional factor, SREBP-1c which further activates a cascade of enzymes involved in fatty acid biosynthesis such as ACLY, ACC, FAS and thereby leads to enhanced lipogenesis (Horton et al. 2002, Miyazaki et al. 2004). Evidence suggests that oxidative stress enhances hepatic fat accumulation through up regulation of SREBP-1c expression (Sekiya et al. 2008). In the present study, we found rats fed with a high fructose diet showed increased hepatic expression of SREBP-1c, ACC and FAS. Activation of these lipogenic enzymes could be attributed for the observed fructose mediated hyperlipidemic changes. Administration of GE markedly decreased the hepatic expression of SREBP-1c, ACC and FAS. The reduction in the SREBP-1c expression might have down-regulated the enzymes ACC, FAS and thereby decreased fatty acid synthesis. This could partly account for the observed hypolipidemic effect in GE treated rats.

The fruit rind of Garcinia cambogia has been reported to contain several components such as (-) HCA, citric acid, malic acid, flavonoids, xanthones, benzophenones like garcinia/cambogia and isoxanthohumol (Chuah et al. 2013). Among the organic acids, HCA is a known competitive inhibitor of the citrate cleavage enzyme, ACLY (Sullivan et al. 1974). It has been reported that the inhibitory effect of HCA on ACLY decreased the availability of acetyl-CoA and thereby led to the suppression of lipogenesis (Sullivan et al. 1972). Further, our previous study has shown that supplementation of HCA markedly decreased oxidative stress and activation of stress proteins via its hypolipidemic properties in rats fed with fructose rich diet (Sripradha et al. 2016a). In another study, we have also reported the total polyphenol content (82.82 ± 7.64 mg of gallic acid equivalents (GAE)/g of GE), ferric reducing antioxidant power (260.49 ± 10.18 µM FRAP/g of GE) and the phytochemical constituents; flavonoids, saponins, tannins, of GE thereby indicated its potent antioxidant and anti- inflammatory properties (Sripradha et al. 2016b). The synergistic effects of these phytoconstituents in GE might have accounted for the suppression of SREBP-1c and its target lipogenic enzymes; ACC, FAS and thereby led to reduced synthesis of lipids. Our findings were in agreement with Asha et al (2001), who have reported that administration of flavonoids isolated from Garcinia cambogia fruit reduced serum and tissue lipids in rats fed with cholesterol rich diet by decreasing the activities of hepatic enzymes and enhancing degradation of lipids. In our study, both high fructose feeding and treatment with GE did not cause any significant effect in the hepatic expression of PPAR–α. Thus, administration of GE attenuated fructose induced hyperlipidemia and hepatic fat accumulation via inhibition of lipogenic enzymes.

Enhanced lipogenesis is positively associated with fat deposition in various organs like liver, adipose tissue and skeletal muscle (Stanhope and Havel 2008). Accumulation of lipids induces the release of several inflammatory mediators and causes low grade inflammation (Gregor and Hotamisligil 2011). In the present study, rats fed with a high fructose diet showed increased plasma CRP levels in association with elevated liver, adipose tissue and epididymal fat mass which are the characteristic hallmarks of chronic inflammation. Furthermore, the increased liver mass in fructose fed rats was positively associated with marked hepatic fat accumulation. It has been reported that hepatic steatosis causes activation of various signaling pathways involved in inflammation and oxidative stress (Zeng et al. 2014). Consistent with this, high fructose feeding caused increased hepatic expression of the inflammatory protein COX-2 in the present study. Administration of GE reduced plasma CRP levels, hepatic activation of COX-2 and thereby ameliorated fructose induced inflammation. The observed decrease in the liver, adipose and epididymal fat mass upon GE treatment further associates the decline in the inflammatory response.

In our study, fructose fed rats displayed systemic insulin resistance which was evidenced by increased plasma glucose, insulin, HOMA-IR and reduced adiponectin levels. Insulin resistance is a

state in which there is reduced response of the target cells to normal levels of circulating insulin. Administration of GE reduced plasma levels of glucose, insulin, HOMA-IR, increased adiponectin levels and thereby improved insulin sensitivity. Furthermore, to elucidate the mechanisms underlying fructose induced insulin resistance and the effects of treatment we analysed TAG content, oxidative stress parameters and the expression of IR-β and PKC-θ in the skeletal muscle of fructose fed rats. We observed increased TAG, TOS, OSI and reduced TAS in the skeletal muscle of fructose fed rats. It has been documented that elevated free fatty acid levels and hyperglycemia are the chief contributors for the production of free radicals (Evans et al. 2002). In the present study, the observed hypertriglyceridemia and activation of lipogenic enzymes are indicative of elevated free fatty acid levels. Administration of GE reduced TAG content, TOS, OSI and enhanced TAS in the skeletal muscle of fructose fed rats. The presence of various antioxidants and phytochemicals in the GE are likely to be responsible for the observed decline in the fructose induced skeletal muscle fat accumulation and oxidative stress. In line with our findings, several in-vitro reports have also documented the antioxidant properties of the GE (Subhashini et al. 2011, Shivapriya et al. 2013).

Inflammation and oxidative stress are the major contributors for the genesis of insulin resistance (Shoelson et al. 2007). The major molecular defects in insulin signaling pathway leading to insulin resistance could be due to defective binding of insulin to insulin receptor (IR), posttranslational modifications/mutations of the IR and enhanced insulin receptor substrate-1 (IRS-1) serine/tyrosine phosphorylation instead of normal IRS-1 tyrosine/serine phosphorylation (Kolterman et al. 1980, Taylor and Arioglu 1998). Elevated free fatty acid levels induces PKC-θ, a novel Ca2+ independent enzyme and the most abundant PKC isoform in the skeletal muscle. PKC-θ is involved in the serine/threonine phosphorylation of proteins in several signal transduction pathways (Griffin et al. 1999). In our study, high fructose feeding caused enhanced phosphorylation of PKC-θ without any change in the IR total content. Increased skeletal muscle TAG content observed in the fructose fed rats could have led to the activation of PKC-θ. Evidence suggests that activation of PKC-θ enhances IRS- 1/2 serine phosphorylation, reduces IRS-1/2 tyrosine phosphorylation and thereby causes insulin resistance (Qatanani and Lazar 2007). GE treatment decreased activation of PKC-θ and did not cause any change in the total IR content. Thus the antioxidant and lipid lowering properties of GE markedly improved peripheral insulin sensitivity and thereby decreased insulin resistance. Recently, Dong et al (2023) have reported that Garcinia cambogia attenuated hepatic lipid accumulation and insulin resistance and thereby exerted weight loss in mice fed with a high fat diet. Our findings are in line with the results of Dong et al suggesting the beneficial effects of Garcinia cambogia on hepatic steatosis and insulin sensitivity.

Excess fructose intake was positively associated with lipid derangements, inflammation, redox imbalance and insulin resistance. Administration of GE down-regulated key proteins involved in lipogenesis, markedly decreased oxidative stress, inflammation and thereby improved insulin sensitivity. The cumulative effects of HCA, various antioxidants, anti-inflammatory constituents and other major phytochemicals present in GE are most likely to be responsible for the observed beneficial effects. Further, as a significant number of people partake of food that includes Garcinia cambogia fruit, its beneficial effects could be surmised and our study thus established a scientific explanation for the healthful effects of Garcinia cambogia.

Conclusion

Chronic ingestion of a fructose rich diet causes lipid derangements which lead to hepatic steatosis and insulin resistance. Supplementation of GE markedly alleviated the fructose mediated hyperlipidemic changes and insulin resistance, thereby attenuated hepatic fat accumulation and

improved insulin sensitivity. While restriction of high refined sugar containing foods is to be encouraged, consumption of Garcinia cambogia fruit helps in decreasing the metabolic derangements caused by such foods.

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