Antihyperlipidemic And Antioxidant Activities of The Methanol Seed Extract of Garcinia Cambogiaon Fat Diet-Fed Rats

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Abstract- *In this project work, the study studied the antihyperlipidemic and antioxidant activities of the methanolseed extract of Garcinia cambogia on fat diet-fed rats. The phytochemical constituents, total polyphenolcontent and ferric reducing antioxidant power (FRAP)were estimated in the G. cambogia seed extract. Male Wistarrats were fed with either standard rodent diet or 30% fat diet and administered with seed extract at a dose of 400 mg/kg body weight/day for 10 weeks. At the end, lipid profile and oxidative stress parameters were estimated. The analyses revealed the presence of carbohydrates, proteins, sterols, tannins, flavonoids and saponins in extract. The total polyphenol content and FRAP of GE were82.82 ± 7.64 mg of gallic acid equivalents and 260.49 ± 10.18μM FRAP per gram of the extract. Fat feeding elevated plasma total cholesterol (TC), triacylglycerol (TAG), non high-density lipoprotein-cholesterol (non-HDL-C), malondialdehyde(MDA), reduced HDL-C and blood antioxidants, glutathione (GSH), glutathione peroxidase (GPx), catalase. Increase in total oxidant status (TOS), oxidative stress index(OSI) and decrease in the total antioxidant status (TAS) were observed in plasma, liver, and kidney of fat-fed rats. Administration of extract decreased food intake, plasma TC,TAG, non-HDL-C, MDA, increased HDL-C and blood antioxidants GSH, GPx, catalase. Seed extract also reduced TOS, OSI an delevated TAS in plasma and liver of fat-fed rats. Renal OSI was significantly reduced upon extract treatment.*

I. INTRODUCTION

Studies have shown that many of these antioxidant compounds possess anti-inflammatory, antiatherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial and antiviral activities. The ingestion of natural antioxidants will reduce the risk of cardiovascular disease, diabetes and other diseases of cancer associated with ageing. The natural plant-based antioxidants play an important role in the maintenance of human health, Synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have been used as antioxidants since the beginning of this century. But restrictions on these antioxidants have been imposed because of the corcinogene city of these compounds. Thus, the interest in natural antioxidants has increased considerably. It has been reported that diet-induced obesity triggers deposition of excess fat in adipose as well as non-adipose target tissues such as liver and kidney causing excessive generation of highly reactive molecular species (ROS) leading to oxidative damage [1-9]. Inadequate neutralization of the oxidants by the antioxidants such as superoxide dismutase (SOD), catalase, glutathione S-transferase (GST) and glutathione peroxidase (GPx) further aggravates the free radical-mediated damage. Persistent imbalance between the production of highly reactive free radicals and antioxidant defenses results in oxidative stress [10]. Oxidants generated due to oxidative stress trigger the activation of several phosphorylation cascades and leads to the stimulation of mitogen-activated protein kinases (MAPKs) and nuclear factor κB (NF-κB) [11]. In the absence of an appropriate compensating antioxidant network, the system becomes overwhelmed, leading to the activation of stress sensitive signaling pathways which causes generation of several gene products, resulting in damage to cellular components like nucleic acids, lipids, and proteins [12]. Hence it is imperative to prevent the detrimental physiological alterations induced by oxidative stress.

Garacinia Cambogia

Kingdom : Plantae
Class : Angiosperms
Order : Malpighiales
Family : Clusiaceae

Genus : Garcinia
Species : Cambogia
Binomial name : *Garcinia cambogia*

Consumption of diets enriched with antioxidants or plant products having vital phytochemicals may provide beneficial effects in alleviating these stress-mediated complications with minimal adverse effects than currently available pharmacotherapeutic strategies [13]. *Garcinia cambogia* also called Malabar tamarind belongs to the family Clusiaceaeand is grown in Southeast Asia. The fruit has a characteristic sweet and sour taste, commonly used as food preservative, carminative, and flavoring agent [14]. The extract of *G. cambogia* is used in Indian medicine for the treatment of ulcers, hemorrhoids, diarrhea, dysentery, and certain types of cancer [15]. Animal experiments have reported anti-inflammatory and antiulcerogenic properties of *G. cambogia*extract [17, 18]. To the best of our knowledge there are no previous reports stating the beneficial effects of *G. cambogia*seed extract on oxidative stress associated with diet-induced obesity. Hence the present study was designed to investigate the beneficial effects of *G. cambogia*seed extract against dyslipidemia and oxidative stress in fat-fed rats.

II. METHODOLOGY

2.1 Animals

Five-month-old male albino Wistar rats (n = 40) were obtained from the Animal house. The animals were housed in polycarbonate cages (2 rats/cage) and maintained at 25 ± 1 °C with a 12 h light/12 h dark cycle. The animals had free access to food and water provided ad libitum. The study was approved by the Institute Scientific Advisory Committee and conducted in the Department of Biochemistry. Animal handling and experimental procedures were approved by the Animal Ethics Committee.

2.2 Collection of the seeds

The seeds of *G. cambogia* were purchased from local market and were grinded to fine powder form using mixie. The powder was used for further solvent extraction purpose.

2.3 Solvent extraction

G. cambogia seeds powder was extracted with methanol using Soxhlet apparatus. Extract was concentrated in rotor evaporator. The extracts were dried and stored at -20 ºC until further use.

2.4 Experimental study

Animals were randomly divided into four groups with ten rats in each.

Group 1: Control, rats received standard rodent diet.
Group 2: Rats received standard rodent diet and *G. cambogia*.
Group 3: Rats received 30% fat diet.
Group 4: Rats received 30% fat diet and *G. cambogia*.

The control rats were fed with standard rodent diet and the total energy of the standard rodent diet was 3.2 kcal/g. The purified fat diet mixture was prepared according to Hsu et al. [20], with 30.35% of total calories derived from carbohydrate, 21.8% from protein and 53.78% from fat. The total energy of the fat diet mixture was found to be 5.02 kcal/g. The composition of fat diet and control diet is given in Table 1. Pilot studies were conducted with various concentrations of *G.cambogia* and 400 mg/kg body weight were chosen as the effective dose. The *G. cambogia* was dissolved in drinking water and a dose of 400 mg/kg body weight [19] of the *G.cambogia*seed extract was administered to each rat in groups 2 and 4 through oral gavage every day throughout the experimental period. The rats in the different groups received their respective diets and seed extract for an experimental duration of ten weeks. At the end of the experiment, fasting blood samples were collected from all rats via tail vein. The blood samples were then centrifuged at 3,500 rpm for 10 min, the plasma was separated and stored at -80°C till analysis. The animals were sacrificed, tissues like liver and kidney were frozen immediately in liquid nitrogen and kept at -80 °C for further analysis. The chemicals used for all assays were of analytical grade, obtained from Merck (India).

2.5Estimation of parameters

2.5.1Analysis of phytochemical constituents, total polyphenol, and ferric reducing antioxidant power (FRAP) of the *G.cambogia*

G.cambogia seed extract was dissolved at a concentration of 1 mg/mL in dimethyl sulfoxide (DMSO) [21] and used for all the in vitro analysis performed. The phytochemical constituents such as carbohydrates, proteins, sterols, tannins, flavonoids and saponins were analysed qualitatively using standard procedures [22].

2.5.2 Determination of total polyphenol content

The amount of total polyphenols in the seed extract of *G.cambogia* was estimated using Folin-Ciocalteu reagent by the method of Singleton and Rossi and Velioglu et al. [23, 24]; 0.1mLof the seed extract solution (1mg/mL) was mixed

with 0.75mL of Folin-Ciocalteu reagent (1: 10 diluted with distilled water). The mixture could stand for 5min at 22 °C. To this 0.75mL of 6%sodium carbonate is added, mixed, and incubated for 90min at 22°C in the dark. The absorbance of the samples was read at 725 nm using UV/visible spectrophotometer. Results were obtained from a standard graph of gallic acid (0–0.1mg/mL) and expressed as mg of gallic acid equivalents (GAE)/g of the seed extract. The assay was repeated thrice under the same conditions for three consecutive days and the mean value was obtained.

2.5.3Determination of reducing ability by FRAP assay

FRAP assay of *G.cambogia* seed extract was assessed by the method of Benzie et al. [25]: 300 µl of the FRAP reagent (acetate buffer, 2,4,6-tripyridyl-s-triazine (TPTZ) and FeCl3) was mixed with 10 µL of the seed extract solution (1 mg/mL) and incubated at room temperature for 4min. The absorbance was read at 593 nm using UV/visible spectrophotometer. Results were obtained from a standard graph of FeSO4 (0–1000 µmol/L) and expressed as µM FRAP/g of the seed extract. The assay was repeated thrice under the same conditions for three consecutive days and the mean value was obtained.

2.6 Estimation of lipid profile and oxidative stress parameters

Specific reagent kits were used for the estimation of plasma TC (Genuine Biosystems, Chennai, India), TAG (Agappe Diagnostics, Kerala, India), HDL-C (Lab-care Diagnostics, India) and these kitswere adapted to automated clinical chemistry analyzer (AU-400,Olympus, UK) for further analyses. The non-HDL-C was calculated bysubtracting TC and HDL-C. The whole blood reduced glutathione (GSH)content was measured by the method of Beutler et al. [26]. The activities of the erythrocyte antioxidant enzymes GPx and catalase were estimated by Wendel et al. [27] and Aebi et al. [28], respectively. Plasmamalondialdehyde (MDA) level was measured by high-performance liquid chromatography by the method of Rajiv Agarwal et al. [29].Liver and kidney tissues were homogenized with 0.1 M ice cold Tris-HCl buffer (pH 7.4, 10%w/v) [30]. The homogenates were centrifuged at14,000 × g for 15 min at 4°C and the supernatants were used for the estimation of total oxidant status (TOS) and total antioxidant status(TAS). The protein concentration in the liver and kidney homogenates was determined by the method of Lowry et al. [31]. TOS in plasma and tissue samples was estimated by the method of OzcanErel [32]. TAS in plasma and tissue samples was measured by the ferric reducing ability of plasma based on the method of Benzie et al. [25]. Oxidative stress index (OSI) was calculated by the formula (TOS/TAS) × 100.

2.7Statistical analysis

The results were represented as mean± SD. Differences between the groups were analysed by one-way analysis of variance (ANOVA) with Tukey as post hoc test using Statistical Package of Social Service (SPSS version 19). A p-Value < 0.05 was considered as statistically significant.

III. RESULTS

3.1 Phytochemical screening, total polyphenol content and FRAP of *G.cambogia* seed extract (Tables 2&3)

Phytochemical screening of the *G.cambogia* seed extract showed the presence of carbohydrates, proteins, sterols, tannins, flavonoids and saponins. The total polyphenol content was found to be 82.82 ± 7.64 mg GAE/g of the seed extract. FRAP of extract was found to be 260.49 ± 10.18 µmol/L FRAP/g of the extract.

Table 1. Composition of high-fat diet and control diet.

Protein concentration (µg) Fat diet ingredients	(g/100 g)
Casein	26.0
Corn starch	16.0
Sucrose	16.0
Cellulose	6.1
Safflower oil	1.0
Butter	29.0
AIN-76vitamin mixture	1.2
AIN-76mineral mixture	4.2
Choline	0.2
DL-methionine	0.3

Table 2. Phytochemical analyses of the seed extract

Sl. No	Phytochemical compounds	Results
1	Carbohydrates	+
2	Proteins	+
3	Sterols	+
4	Tannins	+
5	Flavonoids	+
6	Saponins	+

+ Present

Table 3. Total polyphenol content and ferric reducing antioxidant power (FRAP) of the seed extract

Concentration of the Extract	Total polyphenol content*	Ferric reducing antioxidant power**
1mg/mL	80.76± 5.86	239.32± 7.50

Data were represented as mean ± SD (n = 3). *Expressed as mg gallic acid

equivalents/g of the GE and **expressed as µM FRAP/g of the extract.

3.2 Effect of *G.cambogia* seed extract on food intake, plasma lipid profile in control and fat-fed rats

The average food intake per day for each group (10 rats) throughout the entire experimental duration of ten weeks is represented in Table 4. There was no significant change in the food intake between control and high-fat-fed rats. Supplementation of seed extract decreased food intake in rats fed with fat diet. High-fat feeding significantly elevated plasma TC, TAG, non-HDL-C and reduced HDL-C levels when compared to control rats. Administration of extract along with fat diet significantly reduced plasma TC, TAG, non-HDL-C and increased HDL-C levels.

Table 4. Effect of seed extract on food intake, plasma lipid profile in control and fat-fed rats.

Sl. No	Parameters	Control	Control+ extract	HFD	HFD + extract
1	Food intake (g/day)	15.13± 1.22	15.34± 1.20	15.90± 1.10	15.00± 1.22
2	Total cholesterol (mg/dL)	48.24± 2.30	46.13± 4.22	68.15± 6.41*	56.59± 7.30*
3	Triacylglycerol (mg/dL)	75.42± 5.10	74.38± 8.54	127.35± 3.22*	114.10± 7.72*
4	HDL-C (mg/dL)	29.00± 2.13	31.65± 2.80	20.40± 2.00*	24.12± 2.85*
5	Non-HDL-C (mg/dL)	18.56± 3.55	15.33± 3.00	49.80± 3.40*	33.70± 3.40*

Data were represented as mean ± SD (n = 10). *Significant as compared with control rats ($p < 0.05$).

3.2 Effect of *G.cambogia* seed extract on oxidative stress parameters in control and fat-fed rats (Tables 5-8)

Rats fed with fat-rich diet showed significant increase in the plasma MDA, decrease in the GSH content and activities of the antioxidant enzymes GPx and catalase when compared to control rats. There was elevation in the levels of TOS, OSI and reduction in the TAS of plasma, liver, and kidney of fat-fed rats. Treatment with seed extract significantly increased the blood antioxidants-GSH content and activities of GPx and catalase. There was also decrease in the plasma MDA levels upon seed extract supplementation. extract also reduced TOS, OSI and elevated TAS in plasma and liver of rats fed with fat-rich diet. Reduction in the renal OSI was observed with administration of extract along with fat diet.

Table 5. Effect of extract on blood antioxidant enzymes, plasma oxidative stress, hepatic oxidative stress and renal oxidative stress parameters in control and fat-fed rats

Sl. No	Parameters	Control	Control+ extract	HFD	HFD + extract
	Blood antioxidant enzymes				
1	GSH (mg/gHb)	4.16± 0.40	4.24± 1.20	2.60± 1.02*	3.10± 0.45*
2	GPx (U/gHb)	80.24± 5.30	83.13± 4.80	49.15± 5.41*	56.59± 7.30*
3	Catalase (K/mL)	75.42± 5.10	79.38± 5.52	44.35± 5.10*	49.46± 6.72*
	Plasma oxidative stress				
4	MDA (µmol/L)	1.90± 0.55	1.82± 0.40	4.90± 0.62*	3.80± 0.55*
5	TOS (µmol H_2O_2equiv/L)	14.56± 2.14	12.56± 2.56	24.34 ± 5.42*	21.56± 2.50*
6	TAS (µmol/L)	538.13±1.30	551.10±5.03	365.00±6.44*	432.12±6.80*
7	OSI	2.75± 0.50	2.56± 0.65	8.75± 1.43*	5.70± 0.82*
	Hepatic oxidative stress				
8	TOS (µmol H_2O_2equiv/mg of protein)	2.86± 0.48	2.82± 0.24	3.78± 0.60*	3.00± 0.40*
9	TAS (µmol/mg of protein)	72.25± 3.15	71.33± 2.10	49.88 ± 5.00*	60.06± 2.62*
10	OSI	3.65±1.22	3.59± 1.12	8.00±2.72*	5.52±2.30*
	Renal oxidative stress				
11	TOS (µmol H_2O_2equiv/mg of protein)	2.26± 0.21	2.12± 0.48	2.90± 0.22*	2.62± 0.35*
12	TAS (µmol/mg of protein)	60.86± 3.10	62.56± 2.90	50.34 ± 3.24*	58.02± 2.50
13	OSI	3.63±1.00	3.51± 2.04	6.10± 3.26*	4.80± 1.66*

Data were represented as mean ± SD (n = 10). *Significant as compared with control rats ($p < 0.05$).

IV. DISCUSSION AND CONCLUSION

In our study we investigated the beneficial effects of *G.cambogia* seed extract against fat-induced dyslipidemia and redox imbalance. In our study, no significant change was observed in the food intake between control and fat-fed rats. Treatment with seed extract reduced the food intake in fat-fedrats. The observed reduction in the food intake due to seed extract administration could be due to the vital phytochemicals present in it. We also found rats fed with fat diet showed increased body weight compared to control rats and administration of extract significantly reduced the body weight in fat-fed rats [34]. Fat feedingresulted in dyslipidemic changes as illustrated by elevated plasma levels of TC, TAG, non HDL-C and reduced HDL-C when compared to control rats. Our findings agreed with Jang et al. 2013 who have reported similar effects on high-fat intake [35]. The non-HDL-C fraction comprises of apolipoprotein B containing lipoproteins such as very low-density lipoprotein-cholesterol (VLDL-C), intermediate density lipoprotein-cholesterol (IDL-C), LDL-C, chylomicronremnants and lipoprotein a. Non-HDL-C is anaccepted index of apoB in clinical practice and is considered as a marker of cardiovascular disease [36]. There is evidence that dyslipidemia is positively associated with various metabolic and vascular complications [6, 7]. We found that administration of seed extract along with fat diet significantly reduced plasma levels of TC, TAG, non-HDL-C and elevated HDL-C. The cumulative effect of various organic acids like(–) HCA, citric acid, malic acid, secondary metabolites such as xanthones, flavonoids, benzophenones like garcinol present in the seed extract is responsible for the observed hypolipidemic effects [16]. Among the organic acids, HCA is a competitive inhibitor of the citrate cleavage enzyme, ATP-citratelyase and this inhibitory effect leads to reduced rate of lipogenesis resulting in hypolipidemic effects [37].

Flavonoids, xanthones and benzophenones are metabolites having antioxidant properties [38]. It has been shown that administration of flavonoids isolated from G. cambogia seed reduced lipid levels in rats fed with cholesterol-rich diet by decreasing lipogenesis and enhancing degradation of lipids[39]. Oluyemi et al. have reported that the seeds of G. cambogia also show erythropoietic and anti-obesity effects in fat-fed rats [19]. In line with these findings, we suggest that the vital phytochemicals in seed extract contribute for the antihyperlipidemic property and supplementation of extract may help in the amelioration of obesity-related metabolic and vascular complications. We

found rats fed with fat diet showed elevated plasma MDA levels in association with reduced activities of blood antioxidant enzymes – GSH, GPx, and catalase. There were also increased levels of TOS, OSI and reduced TAS in plasma, liver, and kidney of fat-fed rats. In obesity, altered lipid homeostasis leads to excess fat accumulation in adipose and non-adipose tissues like liver, kidney and causes cellular damage. This in turn elevates the production of cytokines which creates local inflammation and generates ROS [40]. Imbalance between the production of ROS and the antioxidant defense leads to oxidative stress and associated damage [10]. The body has an effective defense mechanism consisting of endogenous antioxidants such as GST, SOD, glutathione reductase(GRD), catalase, GPx and GSH to offer protection against these free radical mediated damages. Overproduction of ROS causes damage to biomolecules like proteins, lipids and DNA and leads to cellular damage [41]. Lipid peroxidation by ROS leads to the generation of highly toxic by product MDA [42]. This causes damage by diffusing to more distant cellular targets from their origin since they have longer half-life than ROS [43].

Administration of seed extract along with fat diet decreased plasma and hepatic TOS and OSI. There was significant decrease in the renal OSI and plasma MDA levels upon treatment with extract. Supplementation also showed increase in the blood antioxidants – GSH, GPx, catalase, plasma, and hepatic TAS. The phytochemical analyses of extract showed the presence of carbohydrates, proteins, sterols, saponins, tannins and flavonoids. Saponins and tannins have been reported to have anti-inflammatory effect [44, 45]. Flavonoids are a group of polyphenols, having antioxidant and anti-inflammatory activities [46]. Furthermore, the total polyphenol content of the extract was found to be 82.82 ± 7.64 mg GAE/g of the extract. Phenolic compounds are a large group of naturally occurring secondary plant metabolites, possessing antiapoptotic, antiinflammatory, antioxidant, antiaging and anticancer properties[47]. The Ferric Reducing Antioxidant Power of the extract was found to be 260.49 ± 10.18 μM FRAP/g of the extract. Thus, itis evident that the antioxidants present in the extract contribute for the improvement of the redox imbalance. From our study we found hyperlipidemia and oxidative stress play critical role in the pathogenesis of various complications in diet-induced obesity. The antioxidants present in the extract contribute to the prevention of these metabolic derangements(Figure 1) and this suggests the use of *G.cambogia* seed extract as a therapeutic strategy for relieving the stress-mediated complications in obesity. The present study confirmed that excess intake of fat-rich diet leads to the development of obesity-associated complications like dyslipidemia and oxidative stress. We found that *G.cambogia* seed extract is effective in improving fat diet induceddyslipidemia and redox imbalance. Thus, we suggest *G.cambogia* seed could be included as a dietary supplement which will mitigate these diet-induced complications.

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