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RESEARCH ARTICLE

Effect of *Garcinia cambogia* Extract on Fatty Liver in Rats Fed High Lipid [1]

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Summary

Garcinia cambogia is a plant which contains hydroxycitric acid and suppresses appetite and body fat accumulation. The aim of the study was to investigate the improving effect of *Garcinia cambogia*-extract on liver phospholipid, triglyceride, lipid hydroperoxide, total protein and gamma-glutamyltransferase (GGT) activity, and histopathology of liver in female rats fed high-lipid diet. One-year-old 30 female Sprague-Dawley rats were separated to three equal groups. Group 1 (control group) was fed basal diet (2% liquid vegetable oil, 0% cholesterol), while the diets of both group 2 and 3 contained vegetable oil (2% liquid and 5% hydrogenated vegetable oil) and cholesterol (3%). 4.5% (W/W) *Garcinia cambogia*-extract was added to the diet of group 3 from day 45. Rats were euthanized on day 75. Liver samples were weighed, homogenized and centrifuged to obtain post-mitochondrial fractions (PMF). PMF phospholipid levels significantly decreased in the group fed high-lipid diet compared with the control group and in the group fed *Garcinia cambogia*-extract compared with the other two groups ($P<0.05$). PMF lipid hydroperoxide levels were significantly higher and PMF triglyceride levels were significantly lower in the control group than those in the other two groups ($P<0.05$). In the result of histopathological examinations, marked fat infiltration was observed in hepatocytes of animals fed high-lipid diet. Livers of animals fed *Garcinia cambogia*-added diet showed moderate fat infiltrations of the hepatocytes. Liver fattening partly occurred in rats fed high-lipid diet. However, this did not lead to the severe cellular degeneration. *Garcinia cambogia* added to the high-lipid diet insufficiently impaired liver fattening in the present dose.

Keywords: *Garcinia cambogia, Rat, Fatty liver, Lipid hydroperoxide*

Yüksek Lipid Diyetiyle Beslenen Sıçanlarda *Garcinia cambogia* Ekstresinin Yağlı Karaciğer Üzerine Etkisi

Özet

Garcinia cambogia hidroksisitrik asit içeren bir bitki olup, iştah ve vücut yağ birikimini azaltmaktadır. Bu çalışmayla yüksek lipid diyeti ile beslenen dişi ratlarda *Garcinia cambogia* ekstresinin karaciğer fosfolipid, trigliserit, lipid hidroperoksit, total protein düzeyleri ve gamma glutamil transferaz (GGT) aktivitesine olan etkilerini ve karaciğer histopatolojisini araştırılması amaçlanmıştır. Araştırma için 30 adet 1 yaşında Sprague-Dawley sıçan kullanılmış ve üç eşit gruba ayrılmışlardır. Grup 1 (kontrol grubu) %2 sıvı nebati yağ içeren standart yemle beslenmiştir. Grup 2 ve 3'ün yemlerinin her ikisi de yükse oranlarda nebati yağ (%2 sıvı ve %5 hidrojenize) ve kolesterol (%3) içermektedir. 45. günden itibaren 3. grubun yemine %4.5 (W/W) *Garcinia cambogia* ekstresi ilave edilmiştir. Sıçanlar 75. günün sonunda ötenazi edilmiştir. Karaciğer örnekleri, post mitokondriyal fraksiyon (PMF) elde etmek üzere tartılmış, homojenize edilmiş ve santrifüjlenmiştir. Kontrol grubuna kıyasla yüksek lipid diyetiyle beslenen grupta ve diğer iki gruba kıyasla *Garcinia cambogia* ilave edilmiş yemle beslenen grupta, PMF fosfolipid düzeyleri anlamlı olarak düşmüştür ($P<0.05$). Kontrol grubunda, diğer iki gruba göre PMF lipid hidroperoksit düzeyleri anlamlı derecede yüksek ve PMF trigliserit düzeyleri de anlamlı derecede düşük bulunmuştur ($P<0.05$). Histopatolojik incelemelerin sonucunda, Yüksek lipid diyetiyle beslenen sıçanlarda hepatositlerde belirgin yağ infiltrasyonları gözlenmiştir. *Garcinia cambogia* ilave edilmiş olan sıçanların karaciğerleri ılımlı yağ infiltrasyonu göstermişlerdir. Yüksek lipid diyetiyle beslenen sıçanların karaciğerinde yağlanma kısmen meydana gelmiştir. Ancak bu durum ağır hücresel dejenerasyona yol açmamıştır. Yüksek lipid diyetine ilave edilen *Garcinia cambogia*'nın verilen dozu karaciğer yağlanmasını azaltmaya yeterli olmamıştır.

Anahtar sözcükler: *Garcinia cambogia, Sıçan, Yağlı karaciğer, Lipid hidroperoksit*

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INTRODUCTION

The increasing incidence of obesity continues to be a major health problem in developed and developing countries [1-3]. Obesity increases lifestyle-related diseases such as arthritis, elevated cholesterol, cancer and serious hormonal imbalances [2,3]. However, treatment of obesity is often unsuccessful [4]. Obesity and its related metabolic and cardiovascular complications continue to present an escalating challenge to contemporary medicine. Obesity signifies chronic imbalance between food consumption and energy expenditure [5]. Therefore, antiobesity foods and food ingredients may avoid obesity, possibly leading to prevention of lifestyle-related diseases, if they are effective in reducing body fat accumulation [6]. The main object of treatment for obese animals is weight reduction. The desired endpoint may be an ideal body weight or may be a reduction in clinical signs associated with a concurrent condition [7].

Identification of supplements that increase satiety or at least maintain satiety during energy restriction is needed. One possible way to improve satiety is to increase hepatic fatty acid oxidation [8]. Hydroxycitric acid (HCA), a herb-derived compound, found in the fruit rind of *Garcinia cambogia*, is known to reduce appetite, inhibit fat synthesis and decrease body weight without stimulating the central nervous system [9]. This compound competitively inhibits the extramitochondrial enzyme ATP-citrate-lyase, which catalyzes the cleavage of citrate to acetylcoenzyme A (CoA) and oxaloacetate, a key step, necessary for the formation of fatty acids, in *de novo* lipogenesis in the liver [10-13]. Several studies have demonstrated that oral administration of HCA dose-dependently depressed *in vivo* lipogenesis in liver tissue of rodents [8,10,14]. Pair-feeding studies also revealed a significant antilipogenic contribution of HCA treatment beyond its anorectic properties in some rat species [6].

It was shown that supplementation with flavonoids enhances the efficacy of the animal to protect themselves from hyperlipidaemia [15]. Flavonoids from *Garcinia cambogia* effectively lower lipid levels in normal and hypercholesterolemic rats. Diet intake and body weight gain are unaltered on flavonoid consumption [16].

We thought that, the lipid hydroperoxide may show cellular damage in liver, phospholipid, triglyceride and gamma-glutamyltransferase (GGT) activity may indicate degeneration degree of the liver; and the total protein levels may change in liver fattening in rats fed high-lipid diet with and without *Garcinia cambogia*-extract.

The aim of the study was to investigate the improving effect of *Garcinia cambogia*-extract on liver phospholipid, triglyceride, lipid hydroperoxide, total protein levels and GGT activity, and histopathology of liver in female rats fed high-lipid diet.

MATERIAL and METHODS

Animals and Dietary Treatment

Thirty, one-year-old female Sprague-Dawley rats were housed individually in standard cages (33x23x12 cm) under controlled conditions of temperature, lighting and humidity. Rats, weighing average 229 g, were randomly assigned to three experimental groups of ten animals each. The procedures were approved by Istanbul University Veterinary Faculty Ethic Committee (Protocol No: 2006/181). Diets and tap water were given *ad libitum*. *Garcinia cambogia* rind fruit extract was provided by General Nutrition Products, Inc., SC, USA. After 1-week adaptation to housing conditions, group 1 (control group) was fed basal diet (2% liquid vegetable oil, 0% cholesterol), while the diets of group 2 and 3 contained vegetable oil (2% liquid- and 5% hydrogenated-vegetable oil) and cholesterol (3%). Also, 4.5% (W/W) *Garcinia cambogia* extract containing 65% HCA (17, 18) was added to the diet of group 3 as from day 45. To equalize the crude protein and the metabolizable energy levels in all groups, the composition of nutrients in groups were changed. Composition and calculation of nutrients in diets are indicated in *Table 1*. The trial period was 75 days.

At the end of the trial period all animals were euthanized by diethylether overdose, sacrificed and liver samples were promptly excised and weighed. Samples were rinsed in ice-cold 1.15% KCl, dried. Samples (3 gr of each liver sample) were homogenized using a MICCRA D-1 homogenizer in four volumes of ice-cold isotonic phosphate buffer, pH 7.4, and centrifuged at 900xg for 20 min to obtain PMF samples [19].

For PMF of lipid hydroperoxid, 3 gr of liver was weighed into 50 ml centrifuge tube and homogenized with 15 ml of cold (-20°C) HPLC-grade methanol. Homogenates were centrifuged (3 min at 1400xg) and the supernatants were removed for assay [20]. All PMF samples were stored at -4°C until analyzed.

Assay Procedures

Total protein, phospholipid, triglyceride and GGT concentrations were determined using an autoanalyzer (Tokyo Boeki Medical System TMS1024) and commercial kits (Reactivos Spinreact, S.A., Girona, Spain) in PMF samples. Analyze of lipid hydroperoxide was made as described by Galobart et al.[20].

Histophatologic Examination

Liver tissue samples were fixed in 10% buffered formalin solution, routinely processed and then stained with hematoxylin-eozin (H.E.) and evaluated under light microscopy. Also, fresh samples of liver tissues were taken, sectioned with cryostate and finally stained with Sudan IV to examine the fat accumulation. The histopatological scoring was made with reference to Kleiner et al.[21] *(Table 2)*.

Table 1. *Composition of the diet (%)*

Tablo 1. *Yemin kompozisyonu (%)*

Items	Group 1	Group 2	Group 3
Ingredients (%)			
Cracked barley	23	11	5.5
Cracked wheat	42.5	42.5	52.5
Rasmol	15	15	5
Soybean meal	14.5	17.5	17.5
Fish meal	2	3	4
Liquid vegetable oil	2	2	2
Vitamin-mineral mixture	1	1	1
Cholesterol	-	3	3
Hydrogenated vegetable oil	-	5	5
Garcinia cambogia extract	-	-	4.5
Calculation of nutrients			
Metabolisable energy (MJ/kg)	12.5	13.7	13.7
Crude Protein (%)	19.5	19.5	19.5

Vitamin-mineral mix, kg: *Vitamin A 12.000 IU, Vitamin D_3 1.500 IU, Vitamin E 104 IU, Vitamin K 15 mg, Vitamin B_1 14 mg, Vitamin B_2 11 mg, Vitamin B_6 14 mg, Vitamin B_{12} 20 mg, Folic acid 2.5 mg, nicotinic acid 78 mg, Pantothenic acid 26 mg, Biotin 334 mcg, Choline chloride 1635 mg, Selenium 0.36 mg, Cobalt 0.46 mg, Iode 1.41 mg, Zinc 95 mg, Manganese 68 mg, Copper 20 mg, Iron 104 mg*

Table 2. *Histopathologic scoring of fat accumulation* [21]

Tablo 2. *Yağlanmanın histopatolojik skorlaması* [21]

Score	Fatty Change
0	< 5 %
1	5-33 %
2	33-66 %
3	> 66 %

Statistical Analysis

The statistical comparisons of PMF total protein, phospholipid, triglyceride and GGT between groups were evaluated with One way ANOVA (Duncan's multiple range test). A statistical significance was considered at $P<0.05$ [22]. SPSS statistical software package (version 10.0) was used for statistic al analysis. All results were displayed as mean ± SE.

RESULTS

Liver weights, PMF total protein level and GGT activity was not statistically different between groups *(Table 3)*.

PMF phospholipid levels significantly decreased in the group fed high-lipid diet compared with the control group and in the group fed *Garcinia cambogia*-supplement compared with the other two groups ($P<0.05$).

PMF lipid hydroperoxide levels were significantly higher and PMF triglyceride levels were significantly lower in the control group than those in the other two groups ($P<0.05$). PMF lipid hydroperoxide and triglyceride levels were not significantly different between Groups 2 and 3.

Histopathological examination of the liver was performed by H.E. staining. In the result of histopathological examinations, marked fat infiltration was observed in

Table 3. *Liver weights, PMF total protein, phospholipid, triglyceride, lipid hydroperoxide levels and GGT activity in groups (Mean ± Standard deviation, n=10)*

Tablo 3. *Gruplarda karaciğer ağırlıkları, PMF total protein, fosfolipid, trigliserit, lipid hidroperoksit düzeyleri ve GGT aktivitesi (Ortalama ± Standart deviyasyon, n=10)*

Parameters	Group 1	Group 2	Group 3
Liver weight (g)	10.55±1.25^a	11.11±1.87^a	11.53±1.32^a
Phospholipid (mg/100g)	242.63±18.69^c	186.0±51.05^b	138.90±38.67^a
Lipid hydroperoxide (µg CHP/g)	178.91±80.28^b	93.04±39.76^a	68.27±24.12^a
Total protein (g/100g)	3.38±0.13^a	3.13±0.32^a	3.2±0.38^a
Triglyceride (mg/100g)	605.13±53.10^a	695.78±52.04^b	680.70±77.02^b
GGT (IU/kg)	14.71±12.12^a	29.13±25.40^a	13.25±7.25^a

^{a,b,c} *Means within the same line with different letters differ ($P<0.05$)*

Group 1: *Control group*

Group 2: *High lipid diet fed group*

Group 3: *High lipid diet fed group and Garcinia cambogia extract supplemented (after day 45) group*

The LHP value of samples was expressed as micrograms per gram of cumene hydroperoxide (CHP) (Galobart et al.[20]

Table 4. *Scores of fat infiltration in all groups (n=10)*
Tablo 4. *Tüm gruplarda yağ infiltrasyonu skorları (n=10)*

Groups	Scores of Fat Infiltration			
	O	+1	+2	+3
Group 1 (number of animals in control group)	10	-	-	-
Group 2 (number of animals in high-fat diet group)	-	2	4	4
Group 3 (number of animals in high-fat diet+extract group)	2	4	3	1

Score 0: *Fat infiltrations were detected only in one or two hepatocytes*
Score 1: *Very few amounts of fat infiltrations were detected in the cytoplasms of hepatocytes that were grouped as two or three*
Score 2: *Most of the hepotocytes were swollen and in particular areas there were fatty changes in the cytoplasms of hepatocytes*
Score 3: *There was severe parenchyma degeneration in the hepatocytes and mild mononuclear inflammation. Fat infiltrations were detected in most of the hepatocytes*

hepatocytes of animals that were fed with high-lipid diet. Livers of animals fed with *Garcinia cambogia*-added diet showed moderate fat infiltrations of the hepatocytes.

Histopathological findings based on distribution percentages of fat infiltration in hepatocytes, were shown in *Table 4*.

Percentages of fat infiltrations in liver tissues of animals in group 1 were beneath 5% and as there were not any morphological changes detected the score were admitted as "0". Fat infiltrations were detected in all of the animals in group 2. In 8 animals fat infiltration was admitted as +2 and +3, and in 2 animals only +1. As shown in *Table 3*, the number of animals with prominent fat infiltrations was admitted as +2 and +3 was decreased in group 3.

DISCUSSION

Dietary cholesterol may stimulate biosynthesis of triglyceride through increased activity of glycerophosphate acyltransferase (EC 2.3.1.15), which catalyzes the first committed step in glycerolipid synthesis [23].

Ichi et al.[24] showed in their study that the liver weights of rats fed with cholesterol were higher than in rats fed high-fat diet. Insignificant differences in liver weights of *Garcinia cambogia* treated rats compared with control were reported [6,25]. Adaramoye et al.[19] stated that the administration of kolaviron failed to reverse the significant increase ($P<0.001$) in the relative weight of liver caused by cholesterol intake. In the present study, there were no statistically differences in liver weights between groups. These findings are parallel to those of Adaramoye et al.[19]. We suggest that high lipogenic animals appear to be insensitive to HCA treatment at usual dietary level as Saito et al.[6] reported.

Dietary cholesterol has been shown to reduce fatty acid oxidation, which, in turn, increases the levels of hepatic triglyceride. Also increase availability of exogenous fatty acids from the diet or derived from adipose tissue, stimulation of fatty acid synthesis, increase channeling of fatty acids into hepatic triglycerides, increase uptake of fatty acids by the liver, or some combination of these factors may potentially lead to increase triglyceride synthesis [23]. Feeding high-fat and cholesterol to rats caused a significant increase in PMF triglycerides compared with control [19,26]. It was reported that HCA alone has been shown to increase liver lipid content [27]. Leonhardt et al.[10] showed in their study that hepatic lipid content in rats of the HCA group was not significantly higher compared to the control group. They suggest that the high-carbohydrate diet composition have contributed to the different results. Brandt et al.[28] observed that long-term HCA treatment led to several unexpected and deleterious effects on lipid metabolism, including increased liver lipid content. They reported that long-term HCA treatment can increase liver *de novo* lipogenesis and lipid content despite decreasing body weight in rats. Koshy et al.[16] reported that in the tissues in male Sprague-Dawley rats administered with flavonoids the concentrations of triglycerides were decreased significantly when compared to the control group ($P<0.01$). In the present study PMF triglyceride levels were significantly lower in the control group than those in the other two groups ($P<0.05$). These findings are similar to the findings of Ichi et al.[24], Adaramoye et al.[19] and Davis et al.[26]. Reason of higher PMF triglyceride levels in the experimental groups was the effect of fatty feeding on the liver what may be due to blockage in the synthesis of lipoprotein, which carries triglyceride away from this organ, thus causing fat accumulation as stated by Farombi et al.[29]. PMF triglyceride levels were not significantly different between Groups 2 and 3. This data controverts to the data of Koshy et al.[16].

Fungwe et al.[23] stated that the addition of cholesterol to either the 5 or 20% fat diet did not affect total hepatic phospholipid concentration in male Sprague-Dawley rats. Dietary cholesterol failed to stimulate hepatic formation of phospholipid *in vivo* or *in vitro*. Significant reductions of phospholipid levels in liver tissues of rats receiving flavonoids were reported [16,30]. In this study PMF phospholipid levels significantly decreased in the group fed high-lipid diet compared with the control group and in the group fed *Garcinia cambogia*-supplement compared with

the other two groups (P<0.05). These findings are similar to the findings of Koshy et al.[16] and Leont'eva et al.[30].

HCA administration could also have an effect on fatty acid oxidation itself. Because extramitochondrial cleavage of citrate is the penultimate step in the conversion of glucose to malonyl CoA, suggestions have been made that administration of HCA, by reducing the acetyl-CoA concentration, could reduce cytosolic malonyl-CoA concentrations and increase fatty acid oxidation [31].

Minhajuddin et al.[32] stated that atherogenic diet resulted in a significant increase of 86% in the production of lipid hydroperoxide compared to normal fed rats. Their data indicates that the hyperlipidemic rats also showed an increase in the levels of lipid hydroperoxide and conjugated dienes. It was reported that kolaviron [19,29] and flavonoids [33] inhibit lipid hydroperoxide in plasma and *in vitro*. In the study, PMF lipid hydroperoxide levels were significantly higher in the control group than those in the other two groups (P<0.05). PMF lipid hydroperoxide levels were not significantly different between Groups 2 and 3, but the levels in Group 3 were tended to be lower than Group 2. These findings are parallel to the findings of Adaramoye et al.[19] and Farombi et al.[29].

It was reported that dietary administration of garcinol [25] and kolaviron [29] did not cause to toxicity in the liver of all rats in experimental groups. However, GGT activity in kolaviron treated rats decreased slightly compared to the control group, but showed no significance [29]. In the present study PMF GGT activity was not statistically different between groups. These findings are similar to the findings of Farombi et al.[29] and indicate that the lipid levels added to the diet were insufficient to perform serious liver damage. Moreover, *Garcinia cambogia* added diet of Group 3 was tended to lower the GGT activity in the liver tissue.

Adaramoye et al.[19] reported that, compared to the control group, kolaviron treatment in Wistar albino rats fed with cholesterol leads to a slightly and insignificant decrease in PMF protein content in the liver. In this study PMF total protein levels were not statistically different between groups. These findings are parallel with the findings of Adaramoye et al.[19]. Since the protein amounts of all diets in the present study were in basal levels, the total protein concentrations in the liver tissue remained in normal levels. Feeding high-fat diet did not change the total protein levels in the liver.

In the study of Saito et al.[6], all rats were fed with diets containing high lipid levels. Fat accumulation as judged by fat vacuolation in the liver of male Zucker obese rats was observed in all *Garcinia cambogia* treated groups, and the accumulation was conspicuous in both the highest HCA group (154 mmol/kg) and the control group (0 mmol/kg) [6]. The histopathological observations of Farombi et al.[29], including cell necrosis and fatty change, showed no visible lesions in kolaviron treated rats. They suggested that kolaviron appears to act as an *in vivo* natural antioxidant and an effective hepatoprotective agent. In the result of histopathological examinations in the present study, marked fat infiltration was observed in hepatocytes of animals fed high-lipid diet. Livers of animals fed *Garcinia cambogia*-added diet showed moderate fat infiltrations of the hepatocytes. These findings partly correspond to the data of Saito et al.[6] and Farombi et al.[32].

Liver fattening partly occurred in rats fed high-lipid diet. However, this did not lead to the severe cellular degeneration. *Garcinia cambogia* added to the high-lipid diet insufficiently impaired liver fattening in the present dose.

In conclusion, liver fattening partly occurred in rats fed high-lipid diet, but the lipid content did not lead to severe cellular liver degeneration and damage. Supplementation of *Garcinia cambogia* to the high-lipid diet was tended to inhibit the lipogenesis in the liver, but insufficiently impaired the liver fattening in the present dose. The higher doses of *Garcinia cambogia*-extract may be experienced.

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