Effect of dietary *Garcinia cambogia* extract on serum lipid profile and serum enzymes in rats fed high-lipid diet

Ateş, A.[1*]; Esen Gürsel, F.[1]; Bilal, T.[2] and Altıner, A.[1]

[1]Department of Biochemistry, Faculty of Veterinary Medicine, Istanbul University, Avcilar, 34310 Istanbul, Turkey; [2]Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine, Istanbul University, Istanbul, Turkey

[*]**Correspondence:** A. Ateş, Department of Biochemistry, Faculty of Veterinary Medicine, Istanbul University, Avcilar, 34310 Istanbul, Turkey. E-mail: atiates@istanbul.edu.tr

(Received 6 Jul 2010; revised version 25 Jun 2011; accepted 10 Jul 2011)

Summary

The aim of the study was to investigate the preventive effects of dietary *Garcinia cambogia* extract on lipid metabolism and serum activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyle transferase (GGT) in rats fed high-lipid diet. Thirty female, one-year-old Sprague-Dawley rats were used and separated into 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. Blood samples were withdrawn from all rats on days 0, 45 and 75. Serum levels of total protein, LDL-cholesterol and phospholipid were lower in the control group than in the other two groups, and there were no significant differences between these two experimental groups at the end of the study ($P<0.05$). Serum triglyceride concentrations rose significantly in the *Garcinia cambogia*-supplemented group (group 3). HDL-cholesterol levels were significantly different between the three groups ($P<0.05$). The highest levels were in the control group. Serum ALT activities were not significantly different between the groups at the end of the study. Serum AST and GGT activities were significantly lower in the groups 2 and 3 than those in the controls, respectively. Fat feeding caused rising lipid indices in serum, while *Garcinia cambogia* supplementation to the fatty diet failed to decrease the rise in serum lipid indices in the present dose. The higher doses of *Garcinia cambogia* extract should be investigated.

Key words: *Garcinia cambogia*, Rat, Serum lipids, High-lipid diet

Introduction

Obesity, which is defined as an increase of adipose mass resulting from a chronic imbalance between caloric intake and energy expenditure, is a multifactorial condition (Weiser *et al*., 1997). The intake of fat and energy is included in the problem of excessive energy intake (Hayamizu *et al*., 2003). Obesity is a serious risk factor that complicates and contributes to serious diseases such as diabetes, cardiovascular disease and hypertension (Brandt *et al*., 2006). Low caloric diet can lead to temporary weight loss; however, exercise and diet alone is not always a solution (Preuss *et al*., 2004). Therefore, body fat accumulation reducing foods and food ingredients may prevent obesity (Saito *et al*., 2005).

Many plants have been used as raw materials in drug development (Oluyemi *et al*., 2007). One of them is a tropical plant native to southeast Asia, named *Garcinia cambogia* (Abraham *et al*., 2006). Its dried fruit rinds have been used for many years for culinary purposes (Lewis and Neelakantan, 1965) and are now used popularly as an ingredient in dietary supplements of weight loss in developed countries (Ishihara *et al*., 2000). Harmful effects were not reported with the consumption of *Garcinia cambogia*

(Triscari and Sullivan, 1984). Lewis and Neelakantan (1965) isolated the principle acid in the fruit rinds of *Garcinia cambogia* and identified it as (-)-hydroxycitric acid (HCA).

Commercial samples of *Garcinia cambogia* extracts contain calcium salt of HCA for its stability (Lewis and Neelakantan, 1965). HCA inhibits the cytosolic enzyme, adenosine triphosphate-citrate lyase. This enzyme is widely distributed in animal tissues, catalyses the extramitochondrial cleavage of citrate to oxaloacetate and acetyl coenzyme A and it is the precursor for *de novo* fatty acid synthesis (Ishihara *et al*., 2000; Leonhardt and Langhans, 2002). Activity of citrate cleavage enzyme varies in accordance with the nutritional status of the animal. During starvation or when fed a high-fat diet, the adenosine triphosphate-citrate lyase levels fell drastically, and during feeding of high-carbohydrate diet the elevated levels of enzyme were determined (Jena *et al*., 2002).

HCA reduces acetyl coenzyme A pool, thus limiting the availability of two carbon units required for fatty acid and cholesterol biosynthesis (Sugden *et al*., 1982). It has been reported that oral administration of HCA depressed *in vivo* lipogenesis in liver, adipose tissue, and intestine of rodents (Sullivan *et al*., 1974). HCA also reduces food intake and body weight regain after substantial body weight loss in adult rats, and anorectic effect of HCA depends on diet composition (Leonhardt and Langhans, 2002).

Zucker obese rats and other animal species with higher lipogenic properties are insensitive to HCA treatment at the usual dietary levels (Saito *et al*., 2005). Epidemiologic and metabolic studies have indicated that serum cholesterol levels are fairly influenced by the amount and type of dietary fat, as well as by the daily cholesterol intake (Schaefer *et al*., 1995). Fibre-rich foods may lower serum cholesterol, brought about by reducing the intestinal absorption of cholesterol and enhanced excretion of cholesterol (Zollner *et al*., 1997). The aim of this study was to investigate the improving effect of *Garcinia cambogia* extract containing 50% HCA and flavonoids on serum lipid profile and enzymes in rats fed high-lipid diet.

Materials and Methods

Thirty, one-year-old female Sprague-Dawley rats were housed individually in standard cages (33 × 23 × 12 cm) under controlled conditions of temperature, lighting and humidity. Rats, weighing an average of 229 g, were randomly assigned to three experimental groups of ten animals each. 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 (Leonhardt *et al*., 2001, 2004) was added to the diet of group 3 starting from day 45. To equalize the crude protein and the metabolizable energy levels in all groups, the composition of nutrients in the groups were changed. Composition and calculation of nutrients in diets are indicated in Table 1. The trial period was 75 days.

3 ml blood samples were collected from the tails of all rats on days 0, 45 and 75. Sera were prepared by centrifugation for 10 min at 3000 g, and transferred into 1.0 ml microcentrifuge tubes. Serum samples were stored at -20°C until analysed. The clear supernatants were used for the estimation of serum indices.

Serum total protein, triglyceride, low-density lipoprotein-cholesterol (LDL-cholesterol), high-density lipoprotein-cholesterol (HDL-cholesterol), phospholipid concentrations and alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyle transferase (GGT) activities were determined using an auto analyser (Tokyo Boeki Medical System TMS1024, Tokyo, Japan) and commercial kits (Spinreact, S.A. Ctra. Santa Coloma, 7-E-17176 Sant Esteve de Bas, Spain).

Total protein was measured by Biuret colorimetric method. Triglyceride, LDL-cholesterol, HDL-cholesterol and Phospholipids were measured by enzymatic-

Table 1: Composition and calculation of nutrients in diets

	Group 1	Group 2	Group 3
Composition of nutrients (%):			
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 mixture, 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, Iodine 1.41 mg, Zinc 95 mg, Manganese 68 mg, Copper 20 mg, Iron 104 mg

colorimetric method. ALT and AST activities were measured by Reitman-Frankel colorimetric method. GGT activitiy was measured by carboxy substrate kinetic method. All methods are based on the clinical chemistry principles of Kaplan and Pesce (1984).

Results are expressed as the mean±SD (n=10). One-way analysis of variance (ANOVA) was used for data analysis. Significant differences between groups were detected in the ANOVA using Duncan's multiple range test at $P<0.05$ (Snedecor and Cochran, 1980).

Results

The mean body weight of rats in group 3 was 234.03 ± 18.22 g. Their feed intake was 12.47 ± 0.32 g. Consumption of *Garcinia cambogia* of the rats in group 3 was 2.39 g/kg bodyweight/day of.

Serum total protein, triglyceride, LDL-cholesterol, HDL-cholesterol, phospholipids, ALT, AST and GGT activities in rats fed high-lipid diet and *Garcinia cambogia* extract are indicated in Table 2.

Serum total protein level was significantly lower in the control group than in the groups fed fatty diet on days 45 and 75 ($P<0.05$). There were no significant differences between groups 2 and 3 on days 45 and 75. Serum triglyceride concentrations were not significantly different between the three groups on day 45. However, the levels tended to increase in groups fed fatty diet compared with control group. Serum triglyceride level was significantly higher in the *Garcinia cambogia*-supplemented group than in the other groups ($P<0.05$) on day 75. Serum phospholipid levels were not significantly different between the three groups, but tended to increase in the groups fed fatty diet compared with control group on day 45. They were significantly higher in groups fed fatty diet than in control group ($P<0.05$) on day 75.

Serum LDL-cholesterol levels were significantly higher in the groups fed fatty diet than in control group on days 45 and 75. However, there were no significant differences between the groups fed fatty diet on days 45 and 75. Serum HDL-cholesterol was not different between the three groups on day 45. However, it was the lowest in group 2 and the highest in group 1 on day 75 ($P<0.05$).

Serum ALT activity was higher in group 2 than in group 3 ($P<0.05$) on day 45. The levels in the control group were not significantly different from those in the other two groups on day 45. There were no significant differences between the three groups on day 75. Serum AST activity was significantly higher in the control group than in the *Garcinia cambogia*-supplemented

Table 2: Serum lipid profile and enzymes in rats fed high-lipid diet and *Garcinia cambogia* extract

Parameters	Group 1	Group 2	Group 3
Total protein (g/dl)			
Day 0	7.53 ± 0.33	7.54 ± 0.39	7.46 ± 0.27
Day 45	7.78 ± 0.49^{b}	8.56 ± 0.66^{a}	8.27 ± 0.91^{ab}
Day 75	7.88 ± 0.51^{b}	8.66 ± 0.43^{a}	8.75 ± 0.87^{a}
Triglyceride (mg/dl)			
Day 0	103.9 ± 38.2	126.4 ± 32.9	94.7 ± 28.5
Day 45	162.6 ± 52.4	200.2 ± 116.3	248.7 ± 165.3
Day 75	111.7 ± 74.8^{b}	176.6 ± 73.5^{b}	357.8 ± 106.0^{a}
LDL-cholesterol (mg/dl)			
Day 0	5.50 ± 2.37	4.60 ± 1.71	4.38 ± 1.85
Day 45	12.0 ± 8.35^{b}	31.5 ± 17.4^{a}	26.6 ± 21.8^{ab}
Day 75	5.43 ± 1.62^{b}	31.9 ± 15.7^{a}	39.7 ± 24.0^{a}
HDL-cholesterol (mg/dl)			
Day 0	36.1 ± 5.35	34.1 ± 4.88	36.4 ± 6.37
Day 45	33.1 ± 15.1	24.3 ± 16.2	17.3 ± 8.95
Day 75	28.5 ± 6.78^{a}	7.02 ± 4.99^{c}	15.6 ± 6.22^{b}
Phospholipid (mg/dl)			
Day 0	142.0 ± 27.9	145.3 ± 22.1	120.3 ± 30.6
Day 45	214.5 ± 11.0	257.8 ± 39.5	233.3 ± 69.2
Day 75	170.5 ± 12.8^{b}	272.0 ± 29.3^{a}	306.4 ± 58.6^{a}
ALT (IU/L)			
Day 0	45.7 ± 8.10	46.5 ± 13.1	35.0 ± 10.1
Day 45	74.0 ± 25.6^{ab}	80.6 ± 14.6^{a}	59.7 ± 8.92^{b}
Day 75	65.4 ± 18.6	69.3 ± 16.5	78.9 ± 18.8
AST (IU/L)			
Day 0	105.0 ± 31.9	108.6 ± 32.5	99.4 ± 14.1
Day 45	145.8 ± 44.9^{a}	141.8 ± 47.6^{ab}	107.5 ± 19.6^{b}
Day 75	151.1 ± 54.6^{a}	100.4 ± 39.7^{b}	124.9 ± 44.2^{ab}
GGT (IU/L)			
Day 0	1.50 ± 0.71	1.70 ± 0.68	1.22 ± 0.44
Day 45	2.00 ± 0.47^{a}	1.33 ± 0.50^{b}	1.40 ± 0.70^{b}
Day 75	2.14 ± 0.90^{a}	1.56 ± 0.53^{ab}	1.10 ± 0.32^{b}

Means in a row that are not followed by a common letter are different ($P<0.05$). Mean±SD, n=10. Group 1: Control group, Group 2: Group fed high-lipid diet, Group 3: Group fed high-lipid diet and *Garcinia cambogia* extract

group on day 45. Similarly, it was higher in the control group than in group 2 on day 75 ($P<0.05$). Serum GGT activities were the highest in the control group on days 45 and 75 ($P<0.05$). There were no significant differences between groups fed fatty diet on days 45 and 75.

Discussion

Development in treatment and prevention of obese animals has been accomplished in recent years, but its effectiveness is still controversial (Roudebush *et al.*, 2008). If anti-obesity foods and food ingredients are effective in reducing body fat accumulation, they may avert obesity, possibly leading to prevention of life style-related diseases such as diabetes, cardiovascular disease and hypertension (Saito *et al*., 2005; Brandt *et al*., 2006). Besides some studies (Baghbanzadeh and Babapour, 2007; Moghaddam *et al*., 2010) that described neurochemical control of food intake, it is known that HCA, derived from the fruit rind of *Garcinia cambogia* reduces appetite, inhibits fat synthesis and decreases body weight without stimulating the central nervous system (Preuss *et al*., 2004). Oral

intake of HCA has been shown to be effective in reducing food intake and inhibiting *de novo* fatty acid synthesis in liver and adipose tissue and body fat accumulation in the experimental animals (Jena *et al*., 2002). Brandt *et al*. (2006) observed that long-term HCA treatment led to several unexpected and deleterious effects on lipid metabolism, such as increase in liver *de novo* lipogenesis and lipid content of liver despite the decreasing body weight in rats. However, Zambell *et al*. (2003) reported that HCA acts as an inhibitor of *de novo* lipogenesis only if cytoplasmic acetyl CoA is produced by the citrate cleavage enzyme reaction, and not if an alternative source of cytoplasmic acetyl CoA, e.g., acetate, is available. Preuss *et al*. (2004) demonstrated that HCA could effectively cause fat degradation and beneficially regulate lipid profiles.

Minhajuddin *et al*. (2005) stated that there was a significant increase in the plasma triglyceride levels of the atherogenic fed rats compared to the normal fed rats. Contrarily, Fungwe *et al*. (1993) showed that the plasma triglyceride concentrations were not affected by high amounts of dietary cholesterol in the group fed 20% corn oil. In the present study, group 2, fed with the fatty diet, had higher serum triglyceride levels than group 1 (control group) on days 45 and 75, but the differences between these 2 groups were not significant. These findings are similar to the findings of Fungwe *et al*. (1993). The reason for the higher triglyceride levels in group 2 was the stimulation of triglyceride synthesis by 3% dietary cholesterol as reported by Fungwe *et al*. (1993).

In the study of Hayamizu *et al*. (2003), serum triglyceride levels were significantly lower in the *Garcinia cambogia* group than those of the control mice. However, Brandt *et al*. (2006) observed that long-term HCA treatment led to several unexpected and deleterious effects on lipid metabolism and no improvement in postprandial plasma triglyceride levels. They found that plasma triglyceride levels after lipogenic meals were not reduced in HCA-treated rats after 5, 15, or 23 days. Adaramoye *et al*. (2005) stated that hypertriglyceridaemia induced by cholesterol administration was unchanged following administrations of kolaviron, a *Garcinia kola* seed extract, and that there were no significant differences in plasma levels of triglyceride in rats fed kolaviron only, compared with controls. In the present study, group 3 had significantly higher serum triglyceride concentrations than those in control group and group 2 on day 75. The reason for this may be unexpected and deleterious of long-term *Garcinia cambogia* treatment effects on lipid metabolism as reported by Brandt *et al*. (2006).

Fungwe *et al*. (1993) demonstrated that feeding of high amounts of cholesterol to rats for 3 weeks (with 20% corn oil) decreased plasma concentrations of phospholipid. In contrast, in the present study, serum phospholipid concentrations increased in the group fed the fatty diet (group 2) compared to the group fed the standard diet (group 1) on day 45 and, increased significantly on day 75. These two studies may show different phospholipid concentrations because of different timed trial terms. Koshy *et al*. (2001) showed that flavonoid fed male Sprague-Dawley rats indicated a decrease in serum phospholipid levels compared to control group. However, in the present study, there were no significant differences between the fatty diet fed group (group 2) and the fatty diet and the *Garcinia cambogia* extract fed group (group 3) on days 45 and 75. For this, it was thought that the dose of *Garcinia cambogia* was insufficient to lower serum phospholipid levels in group 3.

In the study of Minhajuddin *et al*. (2005), there was an increase in LDL-cholesterol levels. Fungwe *et al*. (1993) reported that feeding of high amounts of cholesterol to rats for 3 weeks (with 5% corn oil), increased the concentrations of plasma LDL-cholesterol. Similarly, in the present study, LDL-cholesterol levels were significantly higher in the group fed fatty diet than in the control group on days 45 and 75. Preuss *et al*. (2004) and Ramos *et al*. (1995) observed that intake of HCA made a reduction in LDL-cholesterol levels. Whereas, in the present study, LDL-cholesterol levels were not significantly different between groups 2 and 3. It was assumed that the added amount of *Garcinia cambogia* extract was insufficient to reduce

LDL-cholesterol levels in group 3.

Minhajuddin *et al*. (2005) stated that there was a small increase in plasma HDL-cholesterol levels in atherogenic fed rats compared to the normal diet fed rats. Fungwe *et al*. (1993) reported that plasma HDL-cholesterol concentrations decreased by dietary cholesterol in the group fed 5% corn oil. Similar to the findings of Fungwe *et al*. (1993), in the present study, HDL-cholesterol levels were significantly lower in group 2 than in the control group on day 75 ($P<0.05$). Adaramoye *et al*. (2005) stated that there were no significant differences in plasma levels of HDL-cholesterol in kolaviron-treated animals compared with untreated hypercholesterolaemic animals. However, Preuss *et al*. (2004) and Ramos *et al*. (1995) observed that HCA supplementation increased HDL-cholesterol levels. Similarly, in the present study, HCA supplementation to the fatty diet significantly increased the serum concentrations of HDL-cholesterol ($P<0.05$). This is parallel to the findings of Preuss *et al*. (2004) and Ramos *et al*. (1995).

Farombi *et al*. (2000) described in their study, with male Wistar albino rats, that there were no significant changes in the activities of ALT, AST and GGT enzymes in the kolaviron-treated animals compared to the controls. Similarly, in the present study, obvious changes associated with tissue cell injury were not determined in any group, and the values of all enzymes were in their physiological ranges. Similar results have also been obtained in the study of Saito *et al*. (2005). Some significant differences between groups in enzyme levels may be due to individual differences in the study.

The fatty feeding caused an increase in serum lipid indices, while *Garcinia cambogia* supplementation to the fatty diet failed to decrease the rises in serum lipid indices in the present dose. The higher doses of *Garcinia cambogia* extract should be investigated.

Acknowledgement

This study was supported by the Research Fund of Istanbul University. Project number: 1481/15102007.

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