

EFFECT OF THE ANTI-OBESITY AGENT *GARCINIA CAMBOGIA* EXTRACT ON SERUM LIPOPROTEIN (a), APOLIPOPROTEINS A1 AND B, AND TOTAL CHOLESTEROL LEVELS IN FEMALE RATS FED ATHEROGENIC DIET

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ABSTRACT

The aim of the study was to investigate the effect of the anti-obesity agent *Garcinia cambogia* extract, on serum lipoprotein (a), apolipoproteins A1 (apo A1) and B (apo B), and total cholesterol levels in atherogenic diet fed rats. Thirty female, one year old Sprague-Dawley rats were separated to three equal groups. Group 1 (control group) was fed basal diet (2% liquid vegetable oil, 0% cholesterol). The diets of Groups 2 and 3 contained vegetable oil (2% liquid and 5% hydrogenated) and cholesterol (3%) in high levels. 4.5% (w/w) *Garcinia cambogia* extract was added to the diet of Group 3 after Day 45 up until the end of trial period. Blood samples were withdrawn on Days 0, 45 and 75. Serum lipoprotein (a), apo B, apo A1 and total cholesterol levels were determined by colorimetric methods. Serum lipoprotein (a) and apolipoprotein B levels were not significantly different between groups throughout the study. Serum apo A1 levels increased ($P < 0.05$) towards the end of the study in Groups 2 and 3. Serum total cholesterol levels were significantly higher in Groups 2 and 3 than in the control group on Days 45 and 75. *Garcinia cambogia* extract did not have any significant effect on the analysed indices. The rising in serum apo A1 levels in Groups 2 and 3 was surprising, since apo A1 is a primary protein of high density lipoprotein and is protector from atherosclerosis. In conclusion, a 65% HCA containing diet was insufficient to lower atherosclerotic lipoprotein levels. Therefore, a higher dose of *Garcinia cambogia* extract should be experienced in future studies.

Key words: *Garcinia cambogia*, Apolipoprotein, Lipoprotein (a), Cholesterol, Rat.

INTRODUCTION

Obesity is one of the serious risk factors for so-called lifestyle-related diseases such as diabetes, cardiovascular diseases (CVD) and hypertension (Jebb, 1999). Anti-obesity foods and food ingredients may avert obesity, hence leading to the prevention of such lifestyle-related diseases, if they are effective in reducing fat accumulation (Saito *et al.*, 2005).

Garcinia cambogia, also known as Malabar tamarind, is a plant native to Southeast Asia. The dried rind has been extensively used for centuries throughout Southeast Asia as food preservative, flavoring agent and carminative, and is now popularly used as ingredient of dietary supplements for weight loss in developed countries (Saito *et al.*, 2005). (-)-Hydroxycitric acid (HCA) is a principal acid in the fruit rinds of *Garcinia cambogia* (Loon *et al.*, 2000; Lewis and Neelakantan, 1965) which is a competitive inhibitor of ATP-citrate-lyase, a cytosolic enzyme that catalyses the extramitochondrial cleavage of citrate to oxaloacetate and acetyl-CoA (Sullivan *et al.*, 1977). The action of HCA should reduce the acetyl-CoA pool, thus limiting the availability of two-carbon units required for fatty acid and cholesterol biosynthesis (Hayamizu *et al.*, 2003b). This has led to suggestions that administration of HCA

could inhibit lipogenesis. HCA might induce weight loss through increased satiety by increasing fatty acid oxidation, by inhibition of acetyl-CoA and subsequently of malonyl-CoA formation that would stimulate carnitine transferase activity, or by increasing the rate of glycogen synthesis in the liver (Hellerstein and Xie, 1993).

The presence of increased cholesterol levels in the diet has been demonstrated to elevate serum and aortic tissue cholesterol and, as such, increase aortic atherosclerosis (Clarkson *et al.*, 1962). A linear correlation between dietary cholesterol intake and mortality from coronary heart disease has been established (Verschuren *et al.*, 1995). The hardening and narrowing of the arterial wall owing to cholesterol deposition increases the resistance to blood flow, thereby increasing blood pressure (Guyton and Hawthorns, 1994).

Lipoprotein (a) is a high atherogenic lipoprotein which is considered as an independent risk factor for myocardial infarction (Akman *et al.*, 2008). It has been suggested that low density lipoproteins (LDL) cause cholesterol deposition in the arterial wall whereas high density lipoproteins (HDL) promote effect of cholesterol from this site (Sreenivasan *et al.*, 2011). Apolipoproteins are the protein constituent of lipids. Apolipoprotein A1 (apo A1) is the major component of HDL, which both have cardioprotective properties, whereas apolipoprotein

B (apo B) and LDL are atherogenic (Dange *et al.*, 2001). HDL plays an important role in estimating risk for CVD. The major protein component of HDL is apo A1 and circulating levels of apo A1 negatively correlate to CVD mortality (Smith *et al.*, 2006).

The antiatherogenic action of antioxidants is commonly linked to the inhibition of lipoprotein oxidation (Heinecke, 1998). Several dietary flavonoids have been shown to lower LDL levels and inhibit the oxidative modification of LDL *in vitro* (Catapano, 1997) and have the potential to reduce LDL oxidation and atherogenesis *in vivo*. Flavonoid compounds from *Garcinia cambogia* offer promising therapeutic value in preventing advancement of atherosclerosis and related cardiovascular anomalies, by inhibiting cholesterol synthesis and alleviating hyperlipidemia (Koshy *et al.*, 2001).

The LD₅₀ of HCA was greater than 5 g/kg body weight when administered once orally via gastric intubation to fasted male and female albino rats. There was also no evidence of acute systemic toxicity among rabbits that were dermally administered HCA at 2 g/kg body weight (Ohia *et al.*, 2002). Therefore, HCA-containing *Garcinia* products have been deemed to be safe (Saito *et al.*, 2005). The study was designed to investigate the effect of *Garcinia cambogia* extract on serum lipoprotein (a), apolipoproteins A1 and B, and total cholesterol levels in rats fed atherogenic diet.

MATERIALS AND METHODS

Animals and Dietary Treatment: Thirty female, one year old Sprague-Dawley rats (pathogen-free), weighing average 229 g were used in this study. They were housed individually in standard cages (33x23x12 cm) under controlled conditions of temperature, lighting and humidity. Rats were randomly assigned to three experimental groups of ten animals each. Appropriate diets of all groups (Table 1) and tap water were given *ad libitum* from the beginning (day 0) up until the end of trial period (day 75).

Garcinia cambogia fruit rind 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 Groups 2 and 3 contained vegetable oil (2% liquid and 5% hydrogenated vegetable oil) and cholesterol (3%) in high levels. 4.5% (w/w) *Garcinia cambogia* extract containing %65 HCA (Leonhardt *et al.*, 2001; Leonhardt *et al.* 2004) was added to the diet of Group 3 after day 45 up until day 75. The composition of diets is indicated in Table 1.

Blood Sampling and Analysis: Blood samples were withdrawn from animals on days 0, 45 and 75 of the trial period through coccygeal venipuncture after overnight

fasting and anesthetizing with diethyl ether. Sera were separated by centrifugation at 2700 g for 15 min and stored at -80°C until analysis. Serum lipoprotein (a), apo A1 and B levels were determined using commercially available spectrophotometric kits (BEN SRL, Italy) through standard colorimetric enzymatic methods. Serum total cholesterol levels were determined by a commercial kit (Spinreact, S.A. Ctra. Santa Coloma, 7-E-17176 Sant Esteve de Bas, Spain) adapted for the autoanalyzer (Tokyo Boeki Medical System TMS1024, Tokyo, Japan).

Statistical Analysis: Results are presented as mean ± SD. Data were compared by using analysis of variance (ANOVA coupled with Duncan's multiple range test) between groups within each blood sampling week for all blood indices at significance level of P<0.05 (Ergün and Aktas, 2009). All statistical analyses were performed using software package program (SPSS for windows, Standard version 10.0, 1999, SPSS Inc., Headquarters, Chicago, IL, USA).

RESULTS AND DISCUSSION

The results of serum lipoprotein (a), apo B, apo A1 and total cholesterol levels in female rats fed atherogenic diet and *Garcinia cambogia* extract were shown in Table 2. In the present study, there was an insignificant decrease in the mean serum lipoprotein (a) level of the *Garcinia*-supplemented group (Group 3) on Day 75, although the levels were not statistically different between groups throughout the trial. Lipoprotein (a) is a sinking pre-beta lipoprotein band that represented an antigenically distinct component of low-density lipoproteins (LDL) fraction. Lipoprotein (a) is an apolipoprotein B-containing lipoprotein in which apolipoprotein B is linked via disulfide bridge to another protein component, apolipoprotein (a) (Fless *et al.*, 1986). The accumulations of lipoprotein (a) in the arterial wall, or its potential to inhibit fibrinolysis have been proposed as the possible underlying reasons for the increased risk of cardiovascular and atherosclerotic diseases (Wang *et al.*, 2005). Genetic factors are considered to be major determinants of lipoprotein (a) levels (Boerwinkle, 1992). About 70% to 95% of the variation in plasma lipoprotein (a) levels has been attributed to genetic variation at the apolipoprotein (a) locus with the remainder being due to variations in race, age, environmental factors, drugs, pregnancy and diabetes. The elevated levels of lipoprotein (a) in childhood may predict a later risk of cardiovascular and atherosclerotic diseases. Lipoprotein (a) levels are reported to be unaffected by gender, age, pubertal stage, diet, glycemia, environmental factors or anthropometric measures (Wang *et al.*, 2005). Berg *et al.* (2003) reported that neither caloric restriction and weight changes nor the nutrient quality showed any influence on the lipoprotein (a) levels in the patients investigated.

Koshy *et al.* (2001) noted that flavonoids from *Garcinia cambogia* effectively lowered lipid levels in normal and hypercholesterolemic rats. In the present study, the reason of this insignificant decrease may be partly the results of Koshy *et al.* (2001).

Serum apo B levels were not significantly different between groups throughout the study. The levels lowered in all groups after Day 45. This study shows that, dietary cholesterol and/or *Garcinia cambogia*-extract did not affect the serum apo B concentrations. The protein part of LDL, apo B, undergoes fragmentation and crosslinking by radical oxidation (Ichi *et al.*, 2007). It can be assumed that the decreases in total and LDL-cholesterols are caused by a reduction in apo B-containing lipoprotein particles (Krauss, 1991). Berg *et al.* (2003) reported that the changes in LDL-cholesterol were significantly correlated with reductions in apo B in all groups. Fungwe *et al.* (1993) found that dietary cholesterol stimulated secretion of apo B in a dose-dependent manner.

Serum apo A1 levels were significantly higher in the *Garcinia*-supplemented group (Group 3) than in the control group (Group 1) on Days 45 and 75. The levels were significantly higher in Group 3 than in Group 2 on Day 45, whereas there was not a significant difference between these two groups on Day 75. The levels increased with advancing days in all groups. Apo A1 and A2 are the main apolipoproteins of high-density lipoproteins (HDL). Berg *et al.* (2003) reported that apo A1 showed a small reduction in patients with increased coronary heart disease risk fed an oat bran enriched diet. They also noted that the reduction of apo A1 as well as HDL-cholesterol was significantly enhanced by fat restriction. Although the increase in the *Garcinia cambogia*-supplemented group was an expected situation, the increase in the fatty-feeding group was surprising. Latter may be random. Also surprising was the significant variation in groups on day 0. All rats were divided in groups randomly at the beginning of the study and we suggest that this variation could be caused by individual differences.

As expected, serum total cholesterol levels were significantly higher in groups fed atherogenic diet (Groups 2 and 3) than in the control group on Days 45 and 75. *Garcinia cambogia* extract containing %65 HCA (Leonhardt *et al.*, 2001; Leonhardt *et al.* 2004) was added to the diet of Group 3 after day 45 up until day 75. *Garcinia*-supplemented feeding insignificantly increased the serum total cholesterol levels on Day 75. Some studies reported that, feeding cholesterol to rats significantly raises plasma total cholesterol levels (Minhajuddin *et al.*, 2005; Adaramoye *et al.*, 2005). Ichi *et al.* (2007) reported that dietary cholesterol raises LDL cholesterol levels and very high intake of cholesterol causes atherosclerosis. However, some investigators (Fillias *et al.*, 1956, Minhajuddin *et al.*, 2005) reported

that rats have a strong capability to maintain their plasma cholesterol and are particularly resistant to the development of hypercholesterolemia and atherosclerosis. Total cholesterol is a well established cardiovascular disease risk factor (Wang *et al.*, 2005). Epidemiological and metabolic studies have shown that serum cholesterol levels are strongly influenced by the amount and type of dietary fat, as well as by daily cholesterol intake (Schaefer *et al.*, 1995). Fibre-rich foods may have a protective effect against atherosclerosis by lowering serum cholesterol, brought about by reducing the intestinal absorption of cholesterol and enhanced excretion of the same (Zollner *et al.*, 1997).

Leonhardt *et al.* (2004) determined that plasma levels of total cholesterol did not differ among dietary HCA (3%) group and control group. Brandt *et al.* (2006) noted that long-term HCA treatment (1.8% of diet, 4-wk period) led to several unexpected and deleterious effects on lipid metabolism, including the increased liver lipid content and postprandial cholesterolemia. They could not determined group differences in plasma cholesterol levels. Similarly, Barth *et al.* (1972) stated that long-term HCA treatment increased plasma cholesterol, which would not be predicted from the acute effect of HCA to reduce acetyl-CoA, the substrate for cholesterol synthesis. Hayamizu *et al.* (2003a) treated the subjects for 12 weeks with *Garcinia cambogia* (containing 1g of HCA per day) or placebo. They found that the mean values of total cholesterol did not change in the *Garcinia cambogia* group. Hayamizu *et al.* (2003b) reported that serum total cholesterol levels of dietary *Garcinia cambogia*-treated (3.3%) mice tended to be lower than those of control mice ($P < 0.1$). Whereas, Koshy *et al.* (2001) gave *Garcinia cambogia* at a dose of 1 mg/100 g body weight/day by oro-gastric tube to the rats, and determined that the serum concentration of cholesterol decreased significantly in the experimental animals when compared to the control group. Preuss *et al.* (2004) found that serum total cholesterol level significantly decreased in HCA group (155 mg/dl, individually 4.7 g HCA/day) compared to placebo group (160 mg/dl) in moderately obese subjects in Week 8.

In the current study, the lack of effect on serum total cholesterol is consistent with some (Leonhardt *et al.*, 2004, Brandt *et al.*, 2006, Barth *et al.*, 1972, Hayamizu *et al.*, 2003a, b) but not all (Koshy *et al.*, 2001, Preuss *et al.*, 2004) previous reports. Possibilities are that differences in the doses and efficacy of HCA preparations used in these studies or in the treatment periods may have contributed to the contrary results.

The conversion of citrate into acetyl-CoA by ATP-citrate-lyase only occurs when energy intake exceeds the energy requirements of the body. Hence, the energy requirements of the body are not met; carbohydrate will be used in the citric acid cycle to produce ATP for energy rather than to form citrate, the

substrate for *de novo* fatty acid synthesis. It is therefore likely that the ATP-citrate-lyase is relatively inactive when the subjects are in a negative energy balance. Consequently, HCA would be ineffective in inhibiting fat synthesis (Schaller, 1999). The ineffectiveness of HCA, when the subjects are in a negative energy balance has

also been observed in other studies (Heymsfield *et al.*, 1998). Similarly, Kovacs *et al.* (2001) reported that HCA is not effective in inhibiting fat synthesis or stimulating hepatic glycogen formation in condition of moderate negative energy balance.

Table 1: Composition of experimental diets fed.

	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
<i>Garcinia cambogia</i> 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 D₃ 1.500 IU, Vitamin E 104 IU, Vitamin K 15 mg, Vitamin B₁ 14 mg, Vitamin B₂ 11 mg, Vitamin B₆ 14 mg, Vitamin B₁₂ 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.

Table 2: Serum lipoprotein (a), apolipoprotein B, apolipoprotein A1 and total cholesterol levels in female rats fed atherogenic diet and *Garcinia cambogia* extract.

	Day 0	Day 45	Day 75
Lipoprotein (a) (mg/dl)			
Group 1	0.278 ± 0.149	0.080 ± 0.041	0.058 ± 0.040
Group 2	0.323 ± 0.129	0.072 ± 0.026	0.093 ± 0.062
Group 3	0.240 ± 0.222	0.059 ± 0.043	0.064 ± 0.020
Apolipoprotein B (mg/dl)			
Group 1	0.116 ± 0.059	0.122 ± 0.025	0.086 ± 0.056
Group 2	0.120 ± 0.044	0.122 ± 0.007	0.081 ± 0.047
Group 3	0.105 ± 0.051	0.138 ± 0.062	0.079 ± 0.051
Apolipoprotein A1 (mg/dl)			
Group 1	0.096 ± 0.035 ^{ab}	0.106 ± 0.057 ^b	0.143 ± 0.044 ^b
Group 2	0.114 ± 0.028 ^a	0.127 ± 0.040 ^b	0.315 ± 0.092 ^a
Group 3	0.076 ± 0.016 ^b	0.190 ± 0.071 ^a	0.345 ± 0.095 ^a
Total cholesterol (mg/dl)			
Group 1	63.8 ± 10.2	102 ± 36.6 ^b	91.6 ± 16.2 ^b
Group 2	66.7 ± 12.3	175 ± 63.1 ^a	230 ± 70.4 ^a
Group 3	60.6 ± 6.76	147 ± 73.2 ^a	258 ± 111 ^a

^{a,b}: Values within same column followed by different superscript differ significantly (P<0.05)

Mean ± standard deviation, n = 10.

Group 1: Control group

Group 2: Group fed high-lipid diet

Group 3: Group fed high-lipid diet and *Garcinia cambogia* extract.

Conclusion: *Garcinia cambogia* extract did not have any significant effect on the indices analysed in the study. The effect of atherogenic diet feeding raised the total cholesterol level as expected but in our opinion it wasn't sufficient to cause an atherogenic effect, because no difference could be detected in atherogenic lipoprotein

levels. The rise in serum apo A1 levels in groups fed fatty diet may be a response to fatty feeding of metabolism, since apo A1 is a primary protein of HDL and is protector from atherosclerosis. Although it is suggested that flavonoid compounds of *Garcinia cambogia* could inhibit lipogenesis, such an effect couldn't be found with a diet

containing %65 HCA in the study. Therefore, a higher dose of *Garcinia cambogia* extract should be experienced in future studies.

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