

EFFECT OF *Garcinia cambogia* EXTRACT ON SERUM LEPTIN, GHRELIN, ADIPONECTIN AND INSULIN LEVELS AND BODY WEIGHTS IN RATS FED WITH HIGH LIPID DIET

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Abstract: *Garcinia cambogia* (malabar tamarind, bitter kola) is seen abundantly in the evergreen forests of Konkan in South India. Hydroxycitric acid, mainly obtained from *Garcinia cambogia*, was identified by the end of the 1960's as a strong competitive inhibitor of the extramitochondrial enzyme adenosine triphosphate citrate lyase. The objective of this study was to present the effect of *Garcinia cambogia* extract known as weakener on the supportive or preventive hormones (leptin, ghrelin, adiponectin, insulin) of obesity in the rats fed the diet containing hydrogenated-vegetable oil and cholesterol. For this purpose, thirty female 5-6 months-old Sprague-Dawley rats were randomly assigned to three groups. Each group consisted of 10 rats. Group 1 (as control group) was fed with a basal diet while the diets of Groups 2 and 3 contained the hydrogenated-vegetable oil (20%) and cholesterol (1%) beside of other nutrients. *Garcinia cambogia* extract containing 6% hydroxycitric acid was added to the diet w/w of Group 3 after Day 30 up until Day 60. Blood samples were taken from animals on Days 0, 30 and 60 of the trial period. Body weights were weighed in the blood sampling days before sampling. Serum leptin, ghrelin, adiponectin, insulin levels and body weights were not significantly different between groups in the each sampling day. Serum leptin levels were significantly higher on Day 60 than on Days 0 and 30 in Groups 2 and 3. Serum ghrelin levels in Group 3 significantly decreased on Day 30 and significantly increased on Day 60. Serum adiponectin levels in Group 1 were significantly lower on Day 0 than on other days. The levels in Group 3 were significantly higher on Day 60 than on Day 0. The serum insulin levels and body weights were not significantly different between the days in each group. In conclusion, the high lipid diet in doses used in the study (20% hydrogenated-vegetable oil and 1% cholesterol) did not create fat accumulation or obesity in Sprague Dawley rats. Addition of *Garcinia cambogia* to diet (6%, w/w) of rats fed with the high lipid diet indicated no effect on weight gain. The evidences obtained from this research do not support the role of *Garcinia cambogia* plant spreadly used as a weight loss facilitator.

Key words: adiponectin; *Garcinia cambogia*; ghrelin; insulin; leptin

Introduction

Obesity is an inevitable consequence of chronic positive energy balance (1). Although genetic factors most certainly play an important role in having a tendency towards obesity, the real culprit is the environment leading to a sedentary lifestyle and excessive food consumption. Hypertension is a health problem included in or contributing

to serious diseases such as type-2 diabetes, coronary artery disease and paralysis and is on the rise worldwide (2). Weight loss can usually be achieved with dieting, behavioral modification, and exercise as well as with pharmacotherapy or surgery. Unfortunately, body weight is usually rapidly regained after the termination of such interventions (3). Therefore, pharmacological and dietary treatments that might counteract overeating are of increasing interest (4).

Garcinia cambogia (malabar tamarind, bitter kola) is seen abundantly in the evergreen forests

of Konkan in South India. Many traditional recipes in Kerala use it for its different flavor (5). Historically, *Garcinia cambogia* has been used by the Yoruba tribe in West Africa in the treatment of the respiratory tract infections such as throat ache and coughing. Preliminary studies carried out on *Garcinia cambogia* seeds have demonstrated their antifungal, anticancer, antihistamine, antiulcerogenic, antimicrobial and antiviral effects (6). Various *Garcinia* types are still being used in traditional medicine for the treatment of hepatitis, laryngitis and oral infections (7). Liquid extracts of the *Garcinia huillensis* tree bark are used against sexual diseases, wounds, bronchitis, pneumonia, angina, measles and dermatitis in traditional Zaire medicine (8).

Farombi et al. (9) reported that *Garcinia kola*, a powerful and natural antioxidant, may be used in the chemotherapeutic prevention of cancer and other diseases. Hydroxycitric acid, mainly obtained from *Garcinia cambogia* and *Garcinia indica* extracts, was identified by Watson and Lowenstein (10) towards the end of the 1960's as a strong competitive inhibitor of the extramitochondrial enzyme adenosine triphosphate citrate lyase. It is an enzyme catalyzing the division of citrate into acetyl-CoA and oxaloacetate, which is an important step in the adenosine triphosphate citrate lyase lipogenesis (11). Hydroxycitric acid that is the main acid in fruit and rind suppresses *de novo* fatty acid synthesis by inhibiting this enzyme, and increases the rates of hepatic glycogen synthesis (12). Hydroxycitric acid also decreases the hyperglycemic and hyperinsulinaemic responses to oral or intragastric glucose loads (13).

Leonhardt et al. (14) reported that substances that block the fatty acid synthesis might be useful to prevent the body weight gain because weight gain in adults, and in particular weight regain after the body weight loss, usually comprises a buildup of fat. Researchers emphasized that hydroxycitric acid may be most effective in decreasing the food intake and preventing the body weight gain when the energy intake exceeds energy expenditure and the fatty acid synthesis is increased.

Adipose tissue plays an active role in regulation of the energy balance and nutrient metabolism (15). This tissue synthesizes and secretes a number of cytokine hormones that are involved in the regulation of energy homeostasis, insulin action, and lipid metabolism. Leptin and adiponectin are the adipose tissue-specific factors. The variation

in these hormones may indicate the recovery in metabolic and cardiovascular risk profile after weight loss. Adiponectin, leptin and ghrelin are all involved in the regulation of energy homeostasis, the obesity and the moderate weight loss (1). Leptin has direct effect on some cell types including liver, bone and platelets, *in vitro* (16). While the amount of fat in body is responsible for 50-60% of plasma leptin, the factors such as age, gender, diurnal variation, hormones (primarily insulin) and cytokines (mainly TNF- α) are also effective on plasma leptin levels. Hyperleptinemia is known to be a component of metabolic syndrome (17).

Loss of fat mass causes increase of the circulating ghrelin levels, since ghrelin is a strong orexigenic signal (18). Plasma ghrelin levels in obese individuals have been found to be lower than those of thin individuals in the control group (19). Adiponectin in humans has been reported as a good marker of metabolic health (20). Adiponectin has sensitive to insulin, anti-atherosclerotic and anti-inflammatory properties (21). Weight loss induced by especially low-carbohydrate diet has been reported to increase the adiponectin concentrations in humans (22). Blood adiponectin concentrations in humans are negatively correlated with the abdominal fat mass and adipose tissue size (23).

Objective of this study was to determine the effect of *Garcinia cambogia* extract known as weakener on the supportive or preventive hormones (leptin, ghrelin, adiponectin, insulin) of obesity in rats fed the diet containing hydrogenated-vegetable oil and cholesterol.

Materials and methods

The study was approved by the Ethical Committee of Istanbul University (No:62/29042010), and was carried out according to the legal requirements of the relevant national authority. Thirty female 5-6 months-old Sprague-Dawley rats (pathogen-free) were used in the study. They were housed individually to standard cages (33x23x12 cm) under the controlled conditions of temperature, lighting and humidity. Rats were randomly assigned to the three experimental groups of 10 animals each. The tap water and the appropriate diets of all groups (Table 1) were given as *ad libitum* from the beginning up until the end of trial period (Day 60). *Garcinia cambogia* fruit rind

extract was provided by General Nutrition Products (General Nutrition Products, Inc., SC, USA). Group 1 (as control group) was fed with a basal diet while the diets of Groups 2 and 3 contained the hydrogenated-vegetable oil (20%) and cholesterol (1%) beside of other nutrients. *Garcinia cambogia* extract containing 6% hydroxycitric acid was added to diet (*w/w*) of Group 3 after Day 30 up until Day 60. Body weights were weighed in the blood sampling days before sampling.

Blood samples were taken from all animals through coccygeal venipuncture on Days 0, 30 and 60 of the trial period after the fasting overnight and after the anesthetized with Ketamine-HCL (100 mg/kg *i.p.*). Serums were separated by centrifugation at 3000 rpm for 20 minutes, and stored at -86°C until analysis. Serum leptin, ghrelin, adiponectin and insulin analyses were done by using commercially available kits with ELISA equipment (μ Quant, Bio-Tek). Serum leptin levels were determined by RayBio Rat Leptin ELISA kit (ELR-Leptin-001, RayBiotech Inc., Norcross GA 30092, USA). Serum ghrelin levels were determined by Rat Ghrelin ELISA kit (E90991Ra, USCN Life Science Inc., Houston, TX 77082, USA).

Serum adiponectin levels were determined by AssayMax Rat Adiponectin ELISA kit (ERA2500-1, AssayPro, MO 63304, USA). Serum insulin levels were determined by Rat Insulin ELISA kit (CSB-E05070r, Cusabio Biotech, Wuhan, Hubei Province 430223, P.R.China).

Results were presented as mean \pm standard error. Data were compared at the significance level of $p \leq 0.05$ by using the one way analysis of variance (Tukey's multiple range test) between the groups on each blood sampling day, and between the days within each group. All statistical analyses were performed by using the software package program (SPSS for windows, Standard version 10.0, 1999, SPSS Inc., Headquarters, Chicago, IL, USA).

Results

The comparisons between the groups and between the days were indicated in Figures. Serum leptin, ghrelin, adiponectin, insulin levels and body weights were not significantly different between groups in the each sampling day. Serum

Table 1: Composition of experimental diets

Composition of nutrients (%)	Group 1	Group 2	Group 3
Cracked barley	23	10	4.5
Fish meal	2	3	4
Cracked wheat	42.5	30.5	40.5
<i>Garcinia cambogia</i> extract	-	-	6
Hydrogenated vegetable oil	-	20	20
Cholesterol	-	1	1
Rasmol	15	15	3.5
Liquid vegetable oil	2	2	2
Soybean meal	14.5	17.5	17.5
Vitamin-mineral mixture	1	1	1
Calculation of nutrients			
Crude Protein (%)	19.5	19.5	19.5
Metabolisable energy (MJ/kg)	12.5	13.7	13.7

Vitamin-mineral mixture, kg: Vitamin A 12.000 U, Vitamin D₃ 1.500 U, Vitamin E 104 U, 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 μ g, 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

Group 1: Control group

Group 2: Group fed with the high lipid diet

Group 3: Group fed with the high lipid diet and *Garcinia cambogia* extract

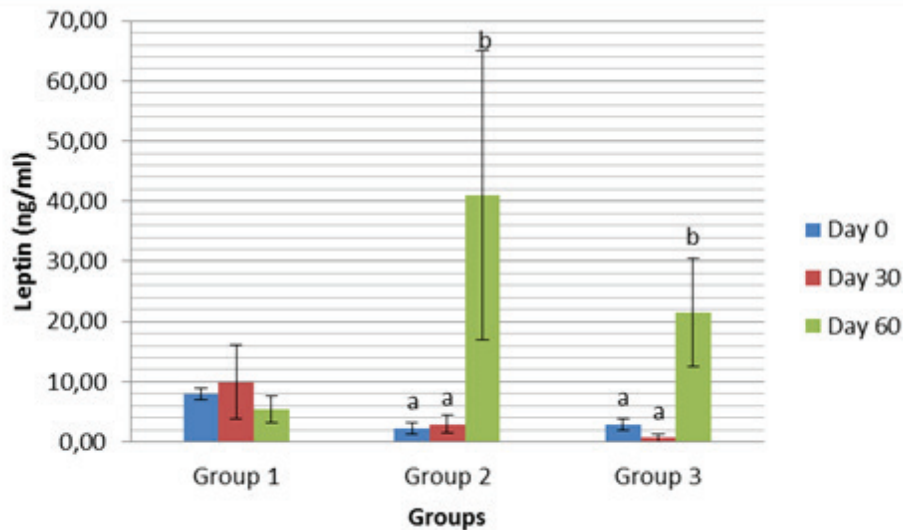


Figure 1: Serum leptin levels in rats fed with the high lipid diet

a,b: Different superscripts indicate significant differences between days ($p \leq 0.05$)
 Mean \pm standard error
 Group 1: Control group
 Group 2: Group fed with the high lipid diet
 Group 3: Group fed with the high lipid diet and *Garcinia cambogia* extract

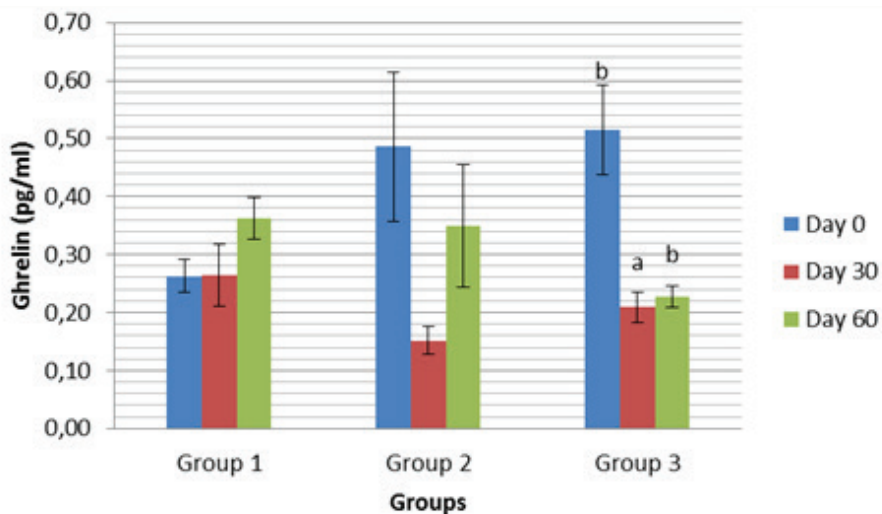


Figure 2: Serum ghrelin levels in rats fed with the high lipid diet

a,b: Different superscripts indicate significant differences between days ($p \leq 0.05$)
 Mean \pm standard error
 Group 1: Control group
 Group 2: Group fed with the high lipid diet
 Group 3: Group fed with the high lipid diet and *Garcinia cambogia* extract

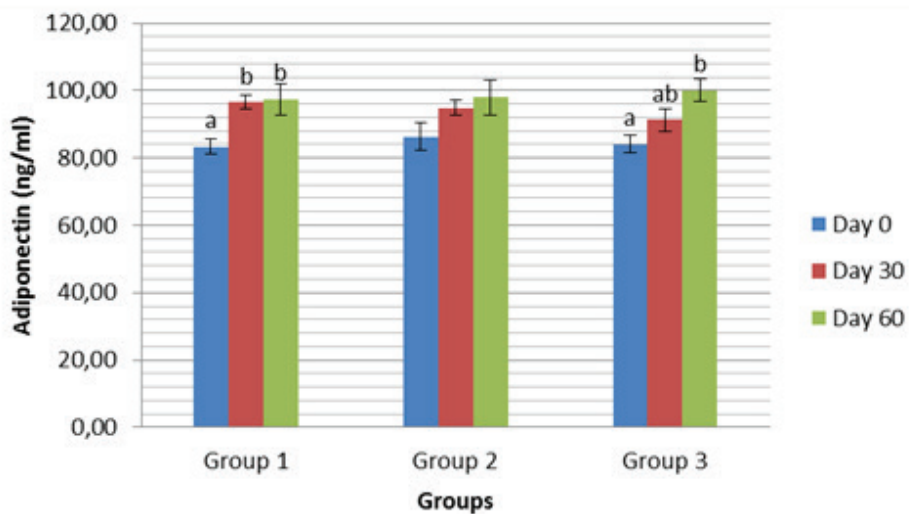


Figure 3: Serum adiponectin levels in rats fed with the high lipid diet

a,b: Different superscripts indicate significant differences between days ($p \leq 0.05$)
 Mean \pm standard error
 Group 1: Control group
 Group 2: Group fed with the high lipid diet
 Group 3: Group fed with the high lipid diet and *Garcinia cambogia* extract

Figure 4: Serum insulin levels in rats fed with the high lipid diet

Mean \pm standard error

Group 1: Control group

Group 2: Group fed with the high lipid diet

Group 3: Group fed with the high lipid diet and *Garcinia cambogia* extract

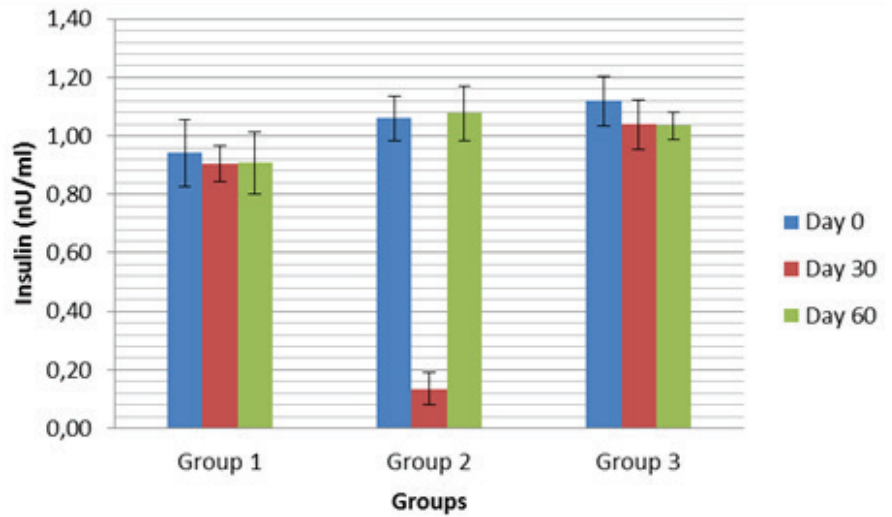


Figure 5: Body weights in rats fed with the high lipid diet

Mean \pm standard error

Group 1: Control group

Group 2: Group fed with the high lipid diet

Group 3: Group fed with the high lipid diet and *Garcinia cambogia* extract

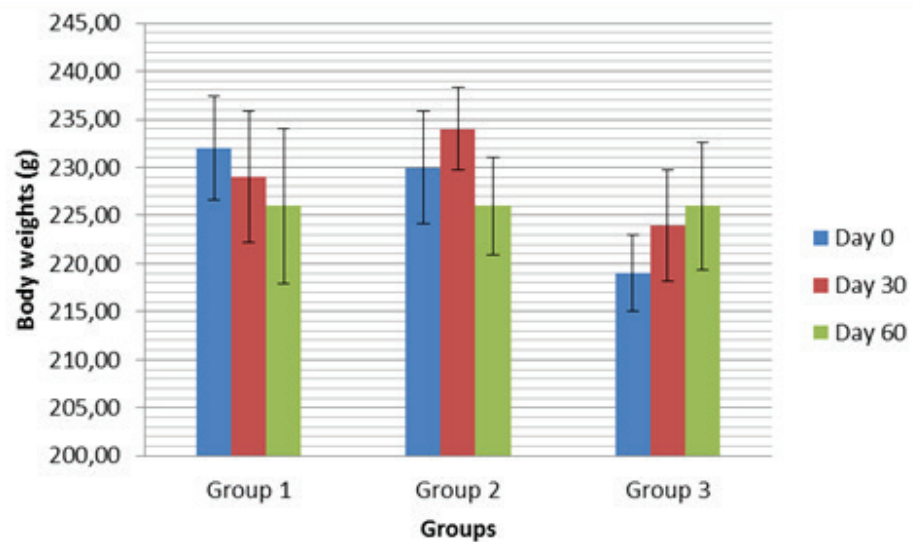


Table 2: The correlations between serum indices

Indices		Adiponectin	Leptin	Ghrelin
	r	-0.083		
Leptin	p	0.518	-	-
	n	63		
	r	-0.101	+0.022	
Ghrelin	p	0.118	0.868	-
	n	58	58	
	r	+0.012	-0.173	-0.011
Insulin	p	0.922	0.174	0.936
	n	74	63	58

r: Correlation coefficient

p: Significance level

n: Total sample number

leptin levels were significantly higher on Day 60 than on Days 0 and 30 in Groups 2 and 3. Serum ghrelin levels in Group 3 significantly decreased on Day 30 and significantly increased on Day 60. However, they were not significantly different between Days 0 and 60. Serum adiponectin levels in Group 1 were significantly lower on Day 0 than on other days. They increased with the advancing days in Group 3, and they were significantly higher on Day 60 than on Day 0. The serum insulin levels and body weights were not significantly different between the days in each group. The correlations between serum indices were indicated in Table 2. No positive or negative significant correlations were determined between the total serum leptin, ghrelin, adiponectin and insulin levels.

Discussion

Preuss et al. (24) gave 4677 mg/day hydroxycitric acid to subjects for 8 weeks, and determined that serum leptin levels gradually significantly decreased towards the end of 8 weeks. Gatta et al. (25) gave 2 g hydroxycitric acid with lunch to the male subjects and took the regular blood samples until the evening meal. They found the mean plasma leptin concentration to be 3.24 ng/ml in controls and 3.72 ng/ml in the hydroxycitric acid group. The researchers did not find a significant difference between two groups. Saito et al. (26) determined the leptin levels in Zucker obese rats, as 63.2 ng/ml in the control group and as 68.9 ng/ml in the experimental group that is given 154 mmol/kg hydroxycitric acid. The researchers reported that leptin did not mediate the unappetizing effect of hydroxycitric acid, and there was no significant difference regarding the serum leptin concentrations between the experimental groups.

Hayamizu et al. (27) reported that no statistically significant difference was found between experimental and control groups in the study where mice were given 3.3% *Garcinia cambogia* with water containing 10% saccharose for 4 weeks. The researchers determined leptin levels as 11.7 ng/ml in the control group and 5.8 ng/ml in the experimental group. They reported that *Garcinia cambogia* administration decreased the serum leptin levels and the leptin/white adipose tissue ratio without producing any changes in the appetite although leptin is

a hormone with appetite controlling activity. In the present study, serum leptin levels were not significantly different between all groups. They were significantly higher on Day 60 than on Days 0 and 30 in Groups 2 and 3. This significant rising of serum leptin concentrations in Groups 2 and 3 on Day 60 were due to the fatty feeding because fatty feeding increases leptin synthesis (28). The fact that there is no significant difference between control and experimental groups is similar to the findings of Gatta et al. (25), Saito et al. (26) and Hayamizu et al. (27).

Faraj et al. (1) determined fairly low plasma ghrelin levels in subjects with morbid obesity. Cummings et al. (29) reported that plasma ghrelin levels increase after weight loss induced by diet. Gatta et al. (25) determined the mean plasma ghrelin level to be 1.11 ng/ml both in the control group and in the male subjects taking 2 g hydroxycitric acid with lunch. In the present research, no significant difference in serum ghrelin levels was found between groups. The feeding with the high lipid diet did not lead to significant changes in serum ghrelin levels in the study. At the same time, the addition of *Garcinia cambogia* to food did not cause any significant change in serum ghrelin levels and this is similar to the findings of Gatta et al. (25).

Gatta et al. (25) determined plasma insulin concentration as 18.3 μ U/ml in the control group and as 18.64 μ U/ml in the subjects taking 2 g hydroxycitric acid with lunch, and they could not find a significant difference between 2 groups. In the present study serum insulin concentrations were not significantly different between the groups in different days or between the days within each group. The finding that hydroxycitric acid has no effect on serum insulin levels was similar to the findings of Hayamizu et al. (30), Leonhardt et al. (31) and Gatta et al. (25).

Faraj et al. (1) reported that adiponectin is not a parameter associated with weight. Engström et al. (32) reported that adiponectin is indirectly proportional to insulin. In the study, serum adiponectin levels were not significantly different between 3 groups, and the levels significantly increased with the advancing days in Group 3. The levels were significantly higher on Day 30 than on Day 0 in the control group. It was not encountered any literature similar to the subject of this study as a result of the comprehensive literature searches.

Koshy et al. (5), added 1 mg/100 g/day

of *Garcinia cambogia* flavonoids to the diets containing cholesterol, and did not observe significant changes in weight gain in the experimental animals compared to the control animals during 90 day duration of the study. Farombi et al. (9) added 200 mg/kg body weight/day of kolaviron being a seed extract of *Garcinia kola* to the diet of rats for a week, and did not determine a significant change in the body weights compared to control group. The feeding with the high lipid diet or the high lipid diet and *Garcinia cambogia* extract did not cause the significant differences in the body weights of rats in the present study. Also, the significant changes did not occur in the body weights of rats in all groups with the advancing days. These findings are in consistent with the findings of Koshy et al. (5) and Farombi et al. (9).

In conclusion, the high lipid diet in doses used in the study (20% hydrogenated-vegetable oil and 1% cholesterol) did not create fat accumulation or obesity in Sprague Dawley rats. Addition of *Garcinia cambogia* to diet (6%, *w/w*) of rats fed with the high lipid diet indicated no effect on weight gain. The differences in the dose and effectiveness of *Garcinia cambogia* extract used in this study could be reason for the opposite findings obtained in the present study compared to the findings obtained in other some studies. For example, hydroxycitric acid that is an active ingredient of *Garcinia cambogia* can be found sometimes in the free acid form and sometimes in the lactone form, and it is thought to be the biologically active inhibitor of adenosine triphosphate citrate lyase of only free acid form (33). As a result, the evidences obtained from this research do not support the role of *Garcinia cambogia* plant spreadly used as a weight loss facilitator.

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Authors declare that there are no known conflicts of interest associated with this publication and that there has been no significant financial support for this work that could have influenced its outcome.

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VPLIV IZVLEČKA RASTLINE *Garcinia cambogia* NA RAVEN LEPTINA, GRELINA, ADIPONEKTINA IN INZULINA TER NA TELESNO TEŽO PRI PODGANAH, HRANJENIH S HRANO Z VISOKO VSEBNOSTJO MAŠČOB

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Povzetek: *Garcinia cambogia* je pogosta rastlina v tropskih gozdovih južne Indije. Hidroksicitronska kislina, ki je v velikih količinah v rastlini *Garcinia cambogia*, je bila v šestdesetih letih prejšnjega stoletja prepoznana kot močan kompetitivni zaviralec zunajmitohondrijskega encima ATP-citratna liaza. Namen raziskave je bil ugotoviti, ali izvleček rastline *Garcinia cambogia* lahko vpliva na izločanje hormonov, ki sodelujejo pri urejanju telesne teže (leptin, grelin, adiponektin, inzulin) pri podganah, hranjenih s hrano z visoko vsebnostjo maščob (rastlinska olja in holesterol). V raziskavo je bilo vključenih 30 podganjih samic seva Sprague-Dawley, starih 5 do 6 mesecev, ki so bile naključno razporejene v eno od treh poskusnih skupin. Podgane iz prve skupine so prejemale navadno hrano, podgane iz druge skupine pa hrano z dodanimi 20 % rastlinskih olj ter 1 % holesterola. Izvleček *Garcinie cambogije* je bil dodajan v hrano podganam iz tretje skupine, tako da so prejemale v hrani 6 % hidroksicitronske kisline. Podgane so bile v poskusu 60 dni, s tem da so živali iz tretje skupine prejemale v hrani izvleček *Garcinie cambogije* od 30. do 60. dneva poskusa. Podganam smo odvzeli kri 1., 30. in 60. dan poskusa, ko so bile tudi stehtane. Raven leptina je bila v skupinah 2 in 3 statistično značilno višja na 60. dan v primerjavi s 1. in 30. dnevom. Raven grelina je bila v skupini 3 30. dan poskusa statistično značilno nižja, 60. dan pa statistično značilno višja. Raven adiponektina je bila v skupini 1 statistično značilno višja 30. in 60. dan poskusa. Raven inzulina ter telesna teža pa se nista razlikovali med skupinami v nobeni časovni točki. Ugotovimo lahko, da hrana z visoko vsebnostjo maščob ni povzročila v časovnem okviru raziskave debelosti, prav tako pa izvleček rastline *Garcinia cambogia* ni vplival na telesno težo ali na raven hormonov in torej ni primeren kandidat za naravno zdravilo za pomoč pri hujšanju.

Ključne besede: adiponectin; *Garcinia cambogia*; ghrelin; inzulin; leptin.