Green Coffee Extract Modifies Body Weight, Serum Lipids and TNF- α in High-Fat Diet-Induced Obese Rats

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Research note

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Abstract

Objective: Currently there are many efforts to find functional nutrients for obesity management and green coffee extract is a potential candidate. This study aimed to examine the effect of green coffee extract on body weight, serum lipids and TNF- α level in obese rats.

Results: Administration of green coffee extract to high-fat diet-induced male Wistar rats (*Rattus norvegicus*) reduced body weight, serum total cholesterol, and triglyceride at the dose of 2, 4, and 8 mg/kgBW/day; lowered LDL-cholesterol and TNF- α at the dose of 4 mg/kgBW/day ($p < 0.05$), in a dose-dependent manner. The effective dose to decrease serum TNF- α level was 4 mg/kgBW/day, while the effective dose to improve the lipid profile was 2 mg/kgBW/day. These results supported the potential use of green coffee extract as a functional nutrient in the management of obesity.

Introduction

Obesity is a universal public health problem characterized by increased adiposity, particularly in abdominal region, which associated with increased cholesterol level. Obesity characteristically presents with dyslipidemia where there is high level of triglycerides (TGs) and low density lipoprotein (LDL)-cholesterol along with low level of high density lipoprotein (HDL)-cholesterol [1, 2]. Obesity is also a systemic inflammatory condition with elevated TNF- α level [3]. Obese rats have 22% higher TNF- α level compare to normoweight rats [4]. Obese subjects are known to produce excess TNF- α in adipose and muscle tissues [5] where it plays a role in insulin resistance [6].

Epidemiological study showed that drinking coffee can have a weight-loss effect due to its chlorogenic acid content [7]. Recently, there is an increased in study examining the effect of green coffee extract (GCE) on obesity. GCE contains chlorogenic acid, a phenolic compound with antioxidant property. Chlorogenic acid increases lipid metabolism, decreases triglyceride and cholesterol levels, and increases plasma adiponectin level [8]. Moreover, GCE has been shown to significantly decrease visceral fat accumulation [9]. In in vivo studies in mice and rats, chlorogenic acid has been shown to regulate glucose and lipid metabolism, increased insulin sensitivity and improved obesity [10]. As far as our concern, there are limited studies examining the effect of GCE on serum lipid profile and TNF- α in the context of obesity. The objectives of the current study were to observe the effect of GCE on body weight, serum lipid profile and serum TNF- α of obese rats.

Methods

Animals and diets

This study was approved by the Ethics Committee of the Faculty of Medicine Andalas University (No.381/KEP/FK/2017) and was conducted according to the institutional guidelines for animal research. Eight-week old male Wistar rats (*Rattus norvegicus*) weighed around 200 g were obtained from an experimental animal breeding company (Tiput Abadi Jaya Peternakan Hewan Uji, Yogyakarta, Indonesia)

and were acclimatized while fed with standard chow *adlibitum*. The rats were housed in a 25°C room with 12 h light:dark cycle. After acclimatization, a group of male rats (n=5) were randomly picked and assigned as the negative control group which was fed standard chow *adlibitum* during the experiment. Other groups of rats were fed with standard chow supplemented with cheese (high-fat diet) to induce obesity for eight weeks. Obesity induction was considered successful when there is at least 20 g of weight gain from initial weight. Positive control group (n=5) was fed high fat diet only throughout the experiment. Treatment groups (each n=5) were given high fat diet and GCE at 2, 4, and 8 mg/kg body weight (BW)/day, respectively, for 13 days. The outcomes of the treatment were body weight, serum lipids levels and serum TNF- α level.

GCE

GCE used in this study was the commercial product of *Hendel Exitox Green Coffee Bean*[®] (Jakarta, Indonesia). Each capsul of this product contains 500 mg of GCE with 20.5 – 56.5 mg of chlorogenic acid.

Serum lipids and TNF- α analysis

After 13 days of treatment with GCE, at day 14, rats were anesthetized and blood were drawn from the orbital sinus for measurement of serum lipids (total cholesterol, triglycerides, LDL-cholesterol, HDL-cholesterol) and TNF- α . Blood samples were transferred into a tube and were centrifuged to separate the sera. Sera were analyzed at the Laboratory of Biochemistry Faculty of Medicine Andalas University according to standard methods. TNF- α in serum samples were analyzed at the Laboratory of Biomedicine Faculty of Medicine Andalas University by using ELISA kit (Rat TNF- α ELISA Kit; Elabscience). Rats were sacrificed by cervical dislocation.

Statistical analysis

Data were checked for normal distribution by Saphiro-Wilk test. Differences in groups' means were analyzed by *One Way ANOVA* followed by Bonferroni *post-hoc* test. Data were considered statistically significant when p-value <0.05.

Results

All animals (n=25) were healthy throughout the experiment. First, we assessed the success of high-fat diet-induced obesity by comparing rats fed standard chow (n=5; negative control) with those fed standard

chow plus cheese (n=20; induced obesity group). Afterwards, the induced obesity group was randomly separated into four groups (each n=5) and subjected to GCE treatments and further analysis.

The effect of GCE on body weight of high fat diet induced obese rats

Animals fed high-fat diet for eight weeks all showed significant increased in body weight (Figure 1A). Treatment with GCE for various doses (2, 4, and 8 mg/kgBW/day) for 13 days resulted in a statistically significant weight loss compare to control group (Bonferroni test; $p < 0.001$) in a dose-dependent manner (Figure 1A).

The effect of GCE on serum TNF- α of high fat diet induced obese rats

GCE treatment for 13 days on high fat diet induced obese male rats lowered TNF- α at the dose of 8 mg/kgBW/day, as shown in Figure 1B (Bonferroni test; $p < 0.05$).

The effect of GCE on serum lipids of high fat diet induced obese rats

Obese male rats treated with GCE at 2, 4, and 8 mg/kgBW/day for 13 days showed statistically significant lower serum total cholesterol and triglycerides levels compared to control group (Figure 2A and Figure 2B, respectively). Treatment with GCE at dose of 4 and 8 mg/kgBW/day also resulted in statistically significant lower serum LDL-cholesterol levels (Figure 3A). GCE treatment showed no effect on serum HDL-cholesterol levels except at the dose of 8 mg/kgBW/day, where HDL-cholesterol level decrease slightly (Bonferroni test; $p < 0.05$) (Figure 3B).

Discussion

The effect of GCE on body weight

This study shows that high fat diet induces obesity in male rats and administration of GCE resulted in weight loss in these obese rats. Chlorogenic acid content in GCE has been shown to have anti-obesity effect [7, 8, 11]. Previously, a study in rats showed that GCE has anti-obesity effect by supressing lipogenesis and stimulating lipolysis [12].

Our results are in line with previous findings in rats that showed chlorogenic acid affects obesity by lowering body fat accumulation through adipogenesis regulation [13]. Chlorogenic acid increases lipid metabolism in HFD-induced obese rats [8, 11]. GCE significantly reduced visceral fat accumulation, improved insulin resistance [9] and when combined with energy-restricted diet may lead to significant reduction in body mass index, fat mass and waist hip ratio [14].

The effect of GCE on lipid profile

Antioxidant rich-food are known to lower serum cholesterol, LDL and triglyceride levels. In this study, we found that GCE administration lowers serum total cholesterol, triglycerides and LDL-cholesterol levels. GCE contains chlorogenic acid, a strong antioxidant compound. It has been shown that chlorogenic acid increases lipid metabolism, decreases triglyceride and total cholesterol levels, possibly by its effect on fatty acid oxidation [15]. Chlorogenic acid in green coffee is an active compound capable of increasing metabolism rate [16], increasing fatty acid oxidation [8, 15] and decreasing hepatic triglyceride [7]. Apart from chlorogenic acid, the polyphenols in coffee also has a property in lowering visceral fat accumulation [17].

In the current study, we found that the GCE has a negative effect on serum HDL level. Serum HDL were lower in groups receiving 8 mg/kgBW/day dose, while those receiving lower dose (2 and 4 mg/kgBW/day) showed higher HDL levels despite not being statistically significant. This result is in line with a previous study where 28 days of chlorogenic acid intake lowered HDL level in male rats through regulation of hepatic PPAR α expression [15, 18]. This results might be explained by the possibility that chlorogenic acid works specifically through pro-atherogenic pathway of cholesterol metabolism. A clinical study in obese women aged 20-45 years showed that green coffee extract combined with calorie-restricted diet affected lipid metabolism through significant change in serum total cholesterol, LDL and free fatty acid [14].

The effect of GCE on TNF- α

Our result showed that GCE decreased serum TNF- α statistically significantly in group receiving 8 mg/kgBW/day dose. We showed that the effect on TNF- α is dose-dependent, however, normal level of TNF- α (10-100 pg/ml) could not be attained with the given doses. Higher dose or longer intake of GCE may result in further decrease of TNF- α . Chlorogenic acid may downregulate the activation of NF- κ B which lead to lower level of ROS that inhibit the production of pro-inflammatory cytokine like TNF- α [8]. Chlorogenic acid is a powerful antioxidant that may downregulate pro-inflammatory cytokine through inhibition of prostaglandin E2, nitric oxidation and production of TNF- α [19, 20].

Conclusion

Our in vivo study confirmed that GCE has beneficial effect on bodyweight and lowers serum total cholesterol, triglyceride, LDL, and TNF- α levels in high-fat diet-induced obese rats. Our findings strengthen the scientific evidence on the property of GCE in the management of obesity and hyperlipidemia.

LIMITATION

This study is a replication study, aiming to confirm the findings of previous independent studies on the effect of green coffee extract on serum cholesterol and TNF- α levels in the context of obesity.

Abbreviations

ANOVA

analysis of variance

BW

body weight

ELISA

enzyme-linked immunosorbent assay

GCE

green coffee extract

HDL

high density lipoprotein

HFD

high fat diet

LDL

low density lipoprotein

NF- κ B

nuclear factor-kappa B

TG

triglyceride

TNF- α

tumor necrosis factor-alpha

Declarations

Ethics approval and consent to participate

All procedures involving laboratory animals were approved by the Ethics Committee of Faculty of Medicine, Andalas University (No. 381/KEP/FK/2017).

Consent for publication

Not applicable.

Availability of data and materials

Data from this study is available from CI on a reasonable request.

Competing interests

None of the authors had any personal or financial conflict of interests.

Funding

None.

Authors' contributions

FF carried out the data collection, data analysis and wrote the manuscript. ZDR provided suggestions for the study design, data analysis and manuscript writing. MR provided insightful feedback on data analysis and manuscript writing. CI was responsible for designing the study, providing feedback on data analysis and on manuscript writing and the overall management. All authors read and approved the final manuscript.

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Figures

Figure 1

1A. Weight of male rats after high fat diet (HFD) induction followed by 13 d of green coffee extract (GCE) treatment. Control group was fed HFD and placebo; treatment groups were fed HFD + GCE at 2, 4, and 8 mg/kgBW/day, respectively. *** $p < 0.001$ (Bonferroni test compared to control group). 1B. Serum TNF- α (pg/dl) in male rats after high fat diet (HFD) induction followed by 13 d of green coffee extract (GCE) treatment. Control group was fed HFD and placebo; treatment groups were fed HFD + GCE at 2, 4, and 8 mg/kgBW/day, respectively. * $p < 0.05$ (Bonferroni test compared to control group).

Figure 2

2A. Serum total cholesterol (mg/dl) in male rats after high fat diet (HFD) induction followed by 13 d of green coffee extract (GCE) treatment. Control group was fed HFD and placebo; treatment groups were fed HFD + GCE at 2, 4, and 8 mg/kgBW/day, respectively. ** $p < 0.01$; *** $p < 0.001$ (Bonferroni test compared to control group). 2B. Serum triglycerides (mg/dl) in male rats after high fat diet (HFD) induction followed by 13 d of green coffee extract (GCE) treatment. Control group was fed HFD and placebo; treatment groups were fed HFD + GCE at 2, 4, and 8 mg/kgBW/day, respectively. *** $p < 0.001$ (Bonferroni test compared to control group).

Figure 3

3A. Serum LDL-cholesterol (mg/dl) in male rats after high fat diet (HFD) induction followed by 13 d of green coffee extract (GCE) treatment. Control group was fed HFD and placebo; treatment groups were fed HFD + GCE at 2, 4, and 8 mg/kgBW/day, respectively. * $p < 0.05$; ** $p < 0.01$ (Bonferroni test compared to control group). 3B. Serum HDL-cholesterol (mg/dl) in male rats after high fat diet (HFD) induction followed by 13 d of green coffee extract (GCE) treatment. Control group was fed HFD and placebo;

treatment groups were fed HFD + GCE at 2, 4, and 8 mg/kgBW/day, respectively. * $p < 0.05$ (Bonferroni test compared to control group).