

Ethanollic extract of *Garcinia cowa* leaves reduces adipocyte size and hepatic fat accumulation in high-fat diet-fed rats

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ABSTRACT

Garcinia cowa Roxb. ex Choisy (*G. cowa*) has traditionally been used to enhance blood circulation, aid digestion, and act as an antipyretic and expectorant. Although previous studies have demonstrated its anti-hyperlipidemic activity *in vitro*, the anti-obesity effects of *G. cowa* remain inadequately explored. This study evaluates the effects of ethanolic *G. cowa* leaf extract (GCE) in high-fat diet (HFD)-induced obese rats. Wistar rats were divided into four groups: normal diet, HFD, HFD with GCE (200 mg/kg), and HFD with GCE (400 mg/kg), treated for 8 weeks. HFD significantly increased body weight, fat mass, and feed efficiency ratio, whereas GCE supplementation attenuated these effects without affecting food intake. In addition, histological analysis revealed HFD-induced adipocyte enlargement and hepatic fat accumulation. Notably, the administration of GCE restored both adipocyte size and number while also reducing hepatic fat accumulation. However, among groups, no significant differences were observed in liver and kidney weights, serum lipid profiles, liver enzymes, or blood glucose. Hydroxycitric acid and flavonoids may contribute to the anti-obesity effects of GCE. These findings suggest that GCE treatment reduced adipocyte size and hepatic lipid accumulation in HFD-induced obese rats, demonstrating its potential anti-obesity properties.

INTRODUCTION

The global increase in obesity has become a serious public health concern for both societies and healthcare systems [1]. Globally, 2.5 billion adults are classified as overweight and 890 million as obese, representing more than a twofold increase in adult obesity since 1990 [2]. Obesity is characterized by excessive fat accumulation in adipose tissue as well as in internal organs, such as the liver, heart, pancreatic islets, and skeletal muscles [3]. It is associated with numerous diseases and conditions that are linked to increased mortality, including type 2 diabetes mellitus, cardiovascular disease, metabolic syndrome, chronic kidney disease, hyperlipidemia, hypertension, nonalcoholic fatty liver disease (NAFLD),

cancer, obstructive sleep apnea, osteoarthritis, and depression [4]. Obesity can be prevented or managed through several ways, such as diet, exercise, behavioral changes, and medication [5]. However, anti-obesity drugs are known to cause various side effects. For instance, orlistat and sibutramine have been shown to cause certain side effects, including oily stools, increased defecation, fecal urgency, increased blood pressure, dry mouth, constipation, headache, and insomnia [6,7]. Accordingly, the development of plant-based medications for obesity control has generated increased interest due to their minimal side effects. Numerous medicinal plants have been shown to exhibit anti-obesity effects in various animal models. Among these, several species of the *Garcinia* genus, such as *Garcinia cambogia*, *Garcinia pedunculata*, *Garcinia indica*, and *Garcinia atroviridis*, have been studied for their anti-obesity properties [8–12].

Garcinia cowa Roxb. ex Choisy (*G. cowa*), commonly known as Cha muang in Thai, belongs to the family Clusiaceae. In traditional medicine, the fruits and leaves of *G. cowa* have been used to enhance blood circulation, treat indigestion,

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and serve as an expectorant [13]. Meanwhile, the bark, root, and latex have been used to reduce fever [13,14]. Previous studies showed that *G. cowa* extracts have been reported to exhibit antitumor-promoting [15], antioxidant, antimutagenic [16], anti-platelet [17], antibacterial [18], cytotoxic [19], and vasorelaxant [20,21] activities.

A previous study by Pattamadilok *et al.* [22] demonstrated the anti-hyperlipidemic activity of the ethanolic extract of *G. cowa* leaves *in vitro*. The phytochemical analysis of *G. cowa* leaf extracts has identified polyphenolic glycosides, organic acids, including hydroxycitric acid and its lactone, and flavonoids [23,24]. Hydroxycitric acid is believed to aid in weight management and help combat obesity [12]. However, scientific evidence supporting the anti-obesity effects of *G. cowa* remains insufficient. Therefore, the present study aims to evaluate the anti-obesity potential of an ethanolic extract of *G. cowa* leaves in rats fed a high-fat diet (HFD).

MATERIALS AND METHODS

Plant materials and extract preparation

Garcinia cowa leaves were sourced from Chiang Rai Province, Thailand. The plant was identified by Assoc. Prof. Rawiwan Charoensup, and a voucher specimen (MFU-NPR0190) was subsequently deposited at the Natural Products Research Laboratory, School of Science, Mae Fah Luang University, Thailand.

The *G. cowa* extract used in this study is the same fraction employed in our previous research, with no additional preparation performed [20]. This extract was prepared using the same procedures described in our earlier study. Briefly, 1.70 kg of air-dried *G. cowa* leaves were ground into a powder and extracted using 95% ethanol. The resulting extract was filtered and evaporated to obtain 211.29 g of *G. cowa* ethanolic extract (GCE), corresponding to a yield of 12.42%. The extract was kept at a temperature of -20°C for future use.

LC-QTOF-MS analysis

The qualitative analysis of the chemical composition of GCE was previously analyzed in our earlier study using LC-QTOF-MS in negative ionization mode [20].

Animals

Adult male Wistar rats (6–7 weeks old) were purchased from Nomura Siam International Co., Ltd. (Thailand). They were chosen for these experiments due to their tendency to readily gain weight in response to an HFD [25]. All animals were maintained under controlled conditions at 23°C – 25°C , with a 12-hour light/dark cycle. Food and water were provided to the animals *ad libitum*. They underwent a 1-week acclimatization period before the experiments. The experimental protocol was approved by the Animal Ethical Committee of Mae Fah Luang University (Approval No. AR02/62).

Experimental design

The rats were randomly assigned to four experimental groups, with five rats per group. Sample size calculation was performed using G*Power software (version 3.1.9.7), based

on effect size estimates from a previous study [26]. According to the calculation, each group comprised five rats ($n = 5$). The experimental groups were as follows:

Group I (NC): Normal control rats fed with a standard chow diet and given a vehicle for 8 weeks

Group II (HFD): HFD control rats fed with HFD and given a vehicle for 8 weeks

Group III (HFD + GCE200): Rats fed with HFD and administered GCE 200 mg/kg for 8 weeks

Group IV (HFD + GCE400): Rats fed with HFD and administered GCE 400 mg/kg for 8 weeks

The normal control group (Group I) was fed a commercial standard rodent diet (National Laboratory Animal Center, Mahidol University, Thailand). In contrast, the HFD and experimental groups (Groups II, III, and IV) received HFD containing 45 kcal% fat (product No. D12451, Research Diet Inc., New Brunswick, NJ, USA). Both the normal control and HFD control groups (Groups I and II) received oral gavage vehicle administration, while the treatment groups (Groups III and IV) received GCE at doses of 200 and 400 mg/kg, respectively, administered once daily via oral gavage.

At the end of the experiment (day 56), the rats were anesthetized by intraperitoneal injection of thiopental sodium (40 mg/kg), following overnight fasting. Once deep anesthesia was achieved, blood samples were collected via cardiac puncture for biochemical analysis. Following euthanasia, organs such as the liver, kidneys, and visceral fat, including the greater omentum, retroperitoneal fat, and epididymal fat, were removed. The liver, kidneys, and fat tissues were washed with 0.9% saline solution, blotted dry, and weighed. A schematic representation of the experimental design is shown in Figure 1.

Body weight, food intake, and feed efficiency ratio (FER)

During the experimental period, the rats' body weight and food intake were recorded weekly. The calculation of FER was performed using the following formula [27]:

$$\text{FER (\%)} = [\text{Body weight gain (g/day)} / \text{Food intake (g/day)}] \times 100$$

Biochemical analysis

Blood samples were collected in tubes without anticoagulants for biochemical analysis, while additional samples were transferred to sodium fluoride tubes for fasting blood glucose analysis. Triglycerides, total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and fasting blood glucose were measured using an automated chemistry analyzer (COBAS INTEGRA® 400 plus analyzer, Roche, Germany) at Mengrai Lab, a medical laboratory in Chiang Rai, Thailand.

The level of very LDL-C (VLDL-C) in serum was estimated using the formula described by Friedewald *et al.* [28].

$$\text{VLDL-C} = \text{Triglyceride} / 5.$$

Histological analysis

The liver, retroperitoneal, and epididymal fat were removed, washed with 0.9% saline solution, and fixed in 10%

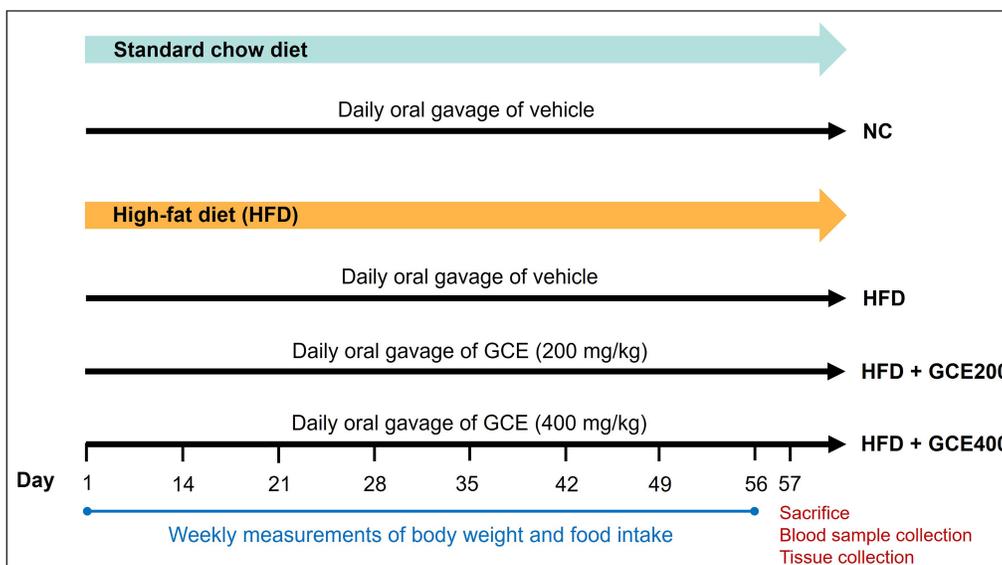


Figure 1. Schematic representation of the experimental design. NC: normal diet group; HFD: high-fat diet group; HFD + GCE200: HFD with GCE (200 mg/kg) group; HFD + GCE400: HFD with GCE (400 mg/kg) group.

formaldehyde. The tissues were embedded in paraffin, sectioned at a thickness of 5 μm , and stained with hematoxylin and eosin (H&E). Six randomly selected images of both retroperitoneal and epididymal adipose tissue were captured from each animal using a Carl Zeiss fluorescence microscope (AxioScope; Rushmore Precision Co., Ltd.) with ZEN 2.3 software. Adipose tissue images were analyzed using ImageJ (Version 1.52q) to assess adipocyte parameters, including number, diameter, perimeter, and area. For measurements of adipocyte number, diameter, perimeter, and area, 10 randomly selected cells per image per animal were analyzed. Adipocyte diameter was determined by measuring the distance between opposite cell membranes in four planes. Lipid droplet accumulation in liver tissue was evaluated using the steatosis grading component of the NAFLD activity score (NAS) [29]. Steatosis was graded based on the proportion of steatosis area in the liver parenchyma as follows: grade 0, <5%; grade 1, 5%–33%; grade 2, 33%–66%; and grade 3, >66%.

Statistical analysis

The results are presented as the mean \pm SEM. Statistical analysis was conducted using SPSS software (version 25.0). Group mean differences were analyzed by one-way ANOVA, followed by a least significant difference (LSD) *post hoc* test. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Chemical composition of GCE

The phytochemical composition of GCE was previously characterized in our earlier study using LC-QTOF-MS with negative ionization mode. This demonstrated that GCE contains hydroxycitric acid, citric acid, kaempferol, isovitexin,

apigenin, scutellarein, myricetin, eriodictyol, luteolin, quercetin, amentoflavone, isomangiferin, and garcimangosone C [20].

Effect of GCE on body weight, food intake, and FER

The HFD group exhibited significantly higher body weight and weight gain than the NC group ($p < 0.05$). Animals treated with GCE at a dose of 400 mg/kg demonstrated a marked reduction in body weight and cumulative weight gain ($p < 0.05$) (Fig. 2A, B). A significant decrease in food intake was observed in the HFD group compared to the NC group ($p < 0.001$); however, GCE treatment did not affect food intake relative to the HFD group (Fig. 2C). Furthermore, FER was markedly elevated in the HFD group ($p < 0.001$), but this increase was significantly attenuated by GCE at 400 mg/kg ($p < 0.01$) (Fig. 2D).

Effect of GCE on fat weight

As shown in Figure 3A, the total fat weight, the sum of the weight of greater omentum, epididymal fat, and retroperitoneal fat, was significantly higher in the HFD group than in the NC group ($p < 0.01$). However, administering GCE at 400 mg/kg significantly reduced total fat weight compared to the HFD group ($p < 0.05$). There were no significant differences in greater omentum weight among the groups ($p > 0.05$) (Fig. 3B). Additionally, epididymal and retroperitoneal fat weights were significantly increased in the HFD group compared to those in the NC group ($p < 0.01$). Although the epididymal fat weight exhibited a decreasing trend in the 400 mg/kg GCE-treated group compared to the HFD group, the reduction did not reach statistical significance ($p = 0.098$) (Fig. 3C). In contrast, GCE treatment at 400 mg/kg significantly reduced retroperitoneal fat weight compared to the HFD group ($p < 0.01$) (Fig. 3D).

Effect of GCE on adipocytes

Histological sections of epididymal and retroperitoneal adipose tissue from the HFD group showed a lower number

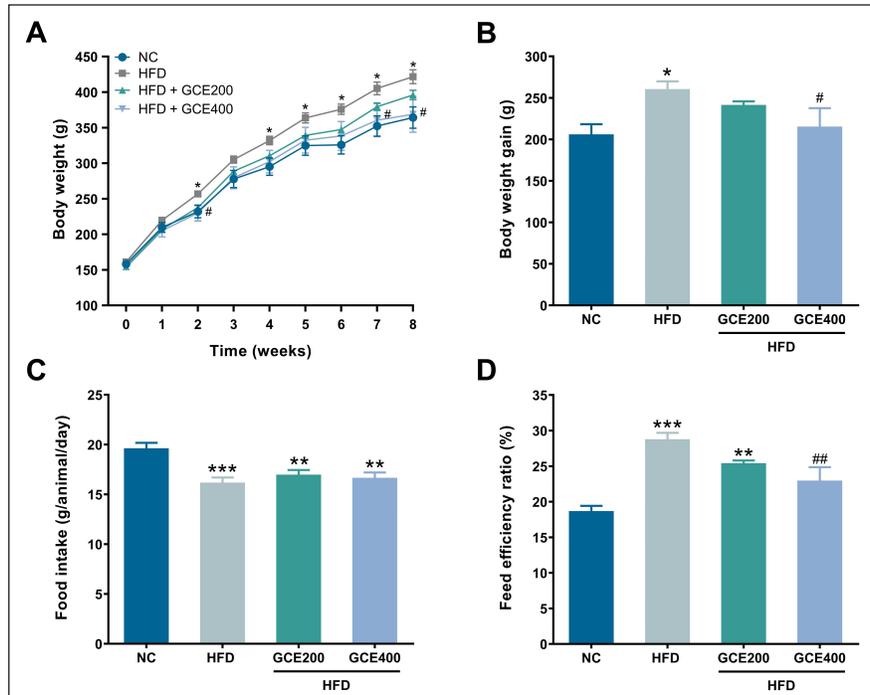


Figure 2. Effects of GCE on body weight, body weight gain, food intake, and FER. (A) Body weight changes over 8 weeks. (B) Body weight gain. (C) Food intake. (D) FER. NC: normal diet group; HFD: high-fat diet group; HFD + GCE200: HFD with GCE (200 mg/kg) group; HFD + GCE400: HFD with GCE (400 mg/kg) group. Values are expressed as mean \pm SEM ($n = 5$). * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ compared with the NC group; # $p < 0.05$ and ## $p < 0.01$ compared with the HFD group.

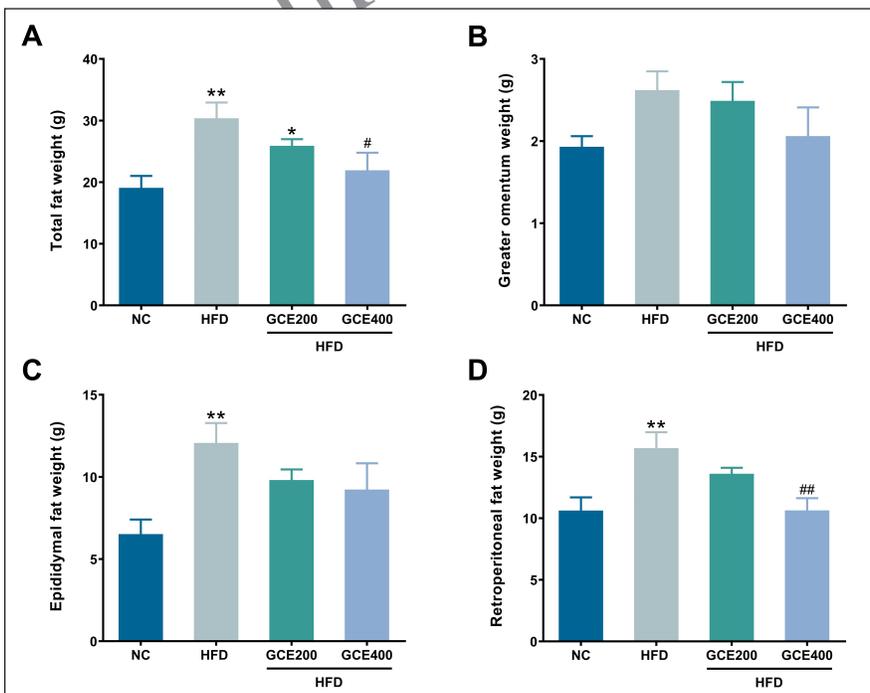


Figure 3. Effects of GCE on fat weight. (A) Total fat weight. (B) Greater omental fat weight. (C) Epididymal fat weight. (D) Retroperitoneal fat weight. NC: normal diet group; HFD: high-fat diet group; HFD + GCE200: HFD with GCE (200 mg/kg) group; HFD + GCE400: HFD with GCE (400 mg/kg) group. Values are expressed as mean \pm SEM ($n = 5$). * $p < 0.05$ and ** $p < 0.01$ compared with the NC group; # $p < 0.05$ and ## $p < 0.01$ compared with the HFD group.

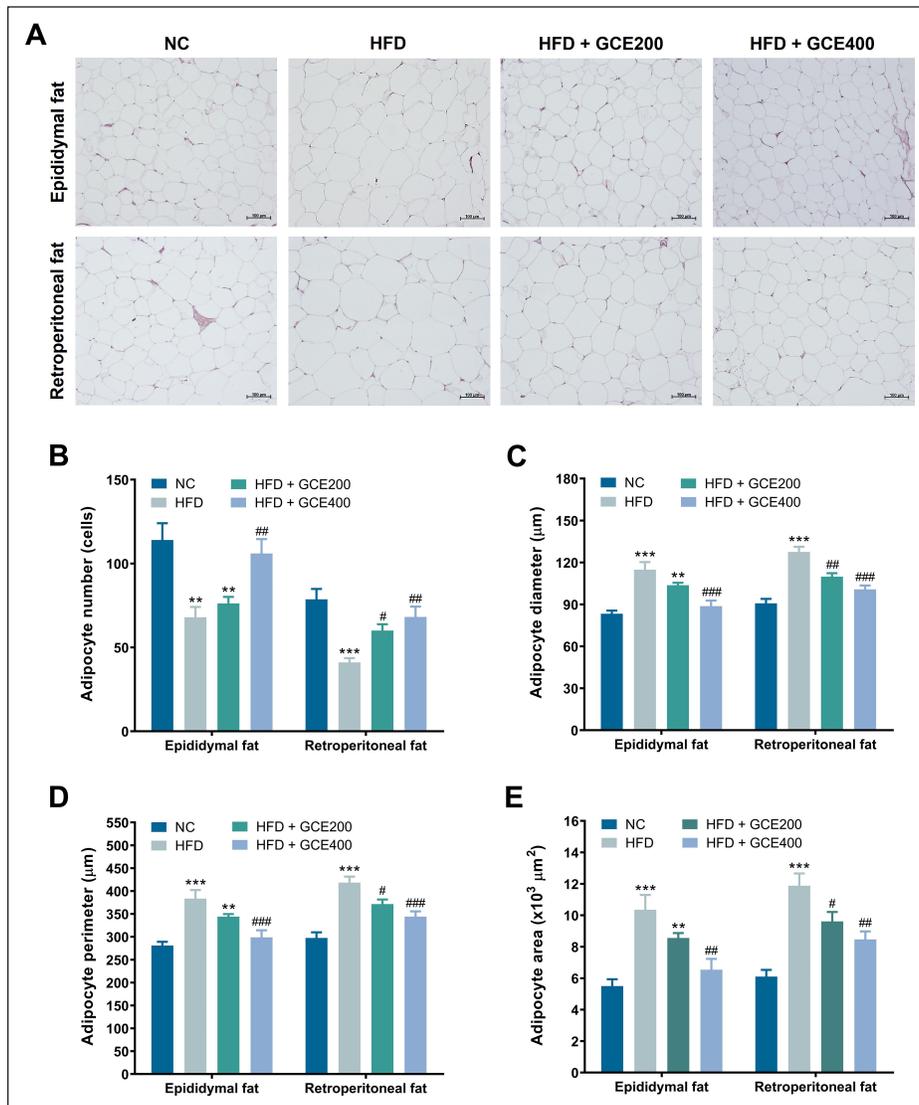


Figure 4. Effects of GCE on epididymal and retroperitoneal adipose tissue. (A) Representative images of H&E-stained adipose tissue sections. Scale bars: 100 µm. (B) Number of adipocytes. (C) Adipocyte diameter. (D) Adipocyte perimeter. (E) Adipocyte area. NC: normal diet group; HFD: high-fat diet group; HFD + GCE200: HFD with GCE (200 mg/kg) group; HFD + GCE400: HFD with GCE (400 mg/kg) group. Values are expressed as mean ± SEM (*n* = 5). ***p* < 0.01 and ****p* < 0.001 compared with the NC group; #*p* < 0.05, ##*p* < 0.01, and ###*p* < 0.001 compared with the HFD group.

per unit area of adipocytes and greater adipocyte diameter, perimeter, and area than the NC group (*p* < 0.01). Oral supplementation of GCE significantly increased the adipocyte number while reducing their diameter, perimeter, and area in both epididymal and retroperitoneal adipose tissues (*p* < 0.05) (Fig. 4).

Effect of GCE on organ weight

There were no significant changes in liver and kidney weights among the groups (*p* > 0.05) (Table 1).

Effect of GCE on histology of the liver

As presented in Figure 5A, consuming an HFD led to noticeably greater fat accumulation in liver tissue compared

Table 1. Effect of GCE on organ weight (g).

Organs	Groups			
	NC	HFD	HFD + GCE200	HFD + GCE400
Liver	9.76 ± 0.30	10.30 ± 0.31	10.11 ± 0.47	9.65 ± 0.84
Kidneys	2.34 ± 0.07	2.38 ± 0.05	2.19 ± 0.11	2.16 ± 0.15

Values are expressed as mean ± SEM (*n* = 5). No significant difference between groups (*p* > 0.05).

to the NC group. This was further supported by a significant increase in steatosis scores in the HFD group (*p* < 0.001). Notably, treatment with GCE at 400 mg/kg significantly reduced steatosis scores compared to the HFD control group (*p* < 0.01) (Fig. 5B).

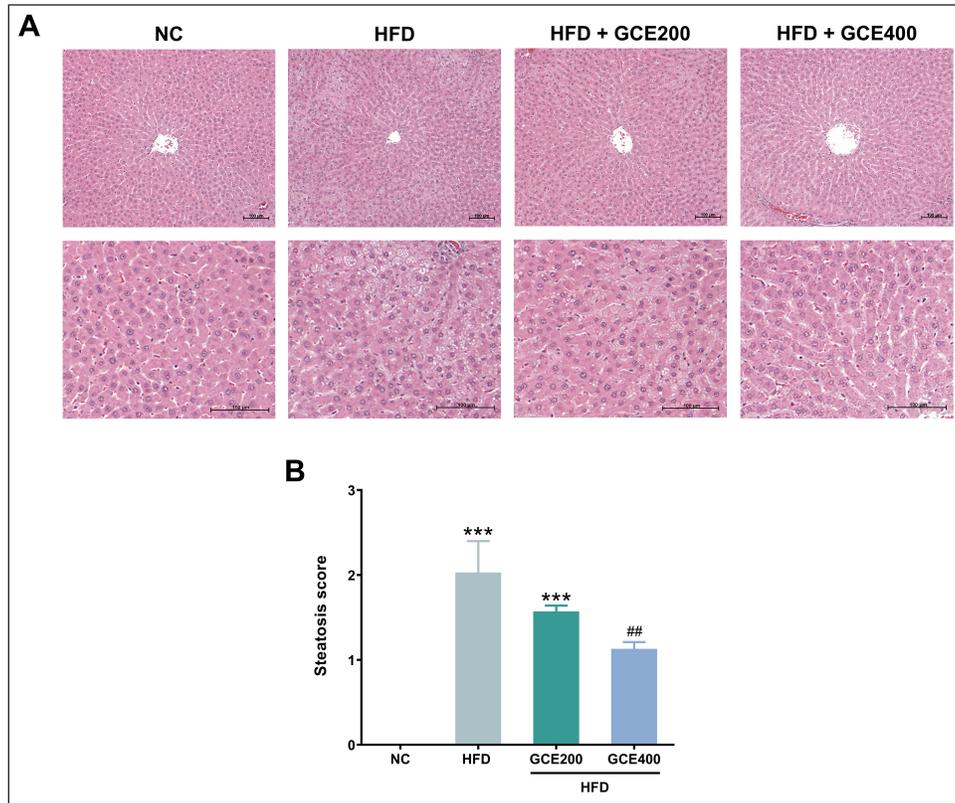


Figure 5. Effects of GCE on liver tissue. (A) Representative images of H&E-stained liver tissue sections. Scale bars: 100 μ m. (B) Steatosis score. NC: normal diet group; HFD: high-fat diet group; HFD + GCE200: HFD with GCE (200 mg/kg) group; HFD + GCE400: HFD with GCE (400 mg/kg) group. Values are expressed as mean \pm SEM ($n = 5$). *** $p < 0.001$ compared with the NC group; ## $p < 0.01$ compared with the HFD group.

Table 2. Effect of GCE on biochemical parameters.

Parameters	Groups			
	NC	HFD	HFD + GCE200	HFD + GCE400
Triglycerides (mg/dl)	69.60 \pm 9.06	61.20 \pm 1.50	61.80 \pm 5.70	67.00 \pm 12.89
Total cholesterol (mg/dl)	57.60 \pm 2.75	56.80 \pm 3.34	62.40 \pm 4.33	61.80 \pm 2.65
HDL-C (mg/dl)	42.40 \pm 3.08	35.40 \pm 4.02	39.40 \pm 1.63	36.40 \pm 1.63
LDL-C (mg/dl)	7.57 \pm 1.31	5.37 \pm 0.62	7.80 \pm 1.32	8.40 \pm 1.81
VLDL-C (mg/dl)	13.92 \pm 1.81	12.24 \pm 0.30	12.36 \pm 1.14	13.40 \pm 2.58
AST (U/l)	87.40 \pm 11.40	89.00 \pm 3.27	82.00 \pm 2.86	78.00 \pm 4.36
ALT (U/l)	30.80 \pm 3.69	24.40 \pm 1.94	26.40 \pm 0.81	24.00 \pm 2.93
Glucose (mg/dl)	149.60 \pm 9.01	148.00 \pm 5.94	152.60 \pm 7.08	149.60 \pm 11.03

Values are expressed as mean \pm SEM ($n = 5$). No significant difference between groups ($p > 0.05$).

Effect of GCE on biochemical parameters

No significant differences in triglycerides, total cholesterol, HDL-C, LDL-C, VLDL-C, AST, ALT, or blood glucose were observed between the groups ($p > 0.05$) (Table 2).

DISCUSSION

The present study investigated the anti-obesity effects of GCE in HFD-fed Wistar rats. The findings revealed that HFD consumption significantly increased both body weight and weight gain, consistent with previous research demonstrating

that HFD contributes to weight gain due to elevated energy intake [30,31]. Notably, treatment with GCE effectively reduced body weight and weight gain compared to untreated HFD-fed rats, indicating that GCE can mitigate HFD-induced weight gain. Importantly, food intake was lower in all HFD-fed rats than those fed the normal diet. The high energy content of HFD may explain the reduced food intake observed in rats, which aligns with previous studies reporting decreased food consumption in HFD-fed animals [32–34]. However, food intake did not differ significantly between the GCE-treated groups and the untreated

HFD control group, suggesting that the reduced weight gain in GCE-treated rats was not due to decreased food consumption. Additionally, the study found that the FER was higher in the HFD group, which was significantly decreased following GCE treatment. The findings imply that rats fed an HFD and treated with GCE had a reduced efficiency in converting consumed food into body mass. By lowering FER, GCE may influence energy expenditure, lipid metabolism, and nutrient absorption.

A major characteristic of obesity is the increased accumulation of fat in the body, and in rodents, intra-abdominal adipose tissue expansion occurs due to cellular hypertrophy [32]. Excessive visceral fat is a key indicator of metabolic disorders, including cardiovascular disease, atherosclerosis, dyslipidemia, and hypertension [26]. The present results reveal a significant increase in visceral fat mass in the HFD control group, consistent with previous findings showing a marked rise in fat pad weight in HFD control animals [35,36]. Additionally, HFD consumption led to adipocyte enlargement by increasing their diameter, perimeter, and area while reducing their number per unit area. Treatment with GCE significantly reduced visceral fat weight. The reduction in fat weight may result from a decrease in fat pad mass, attributed either to a decrease in new adipocyte formation from precursor cells or to a reduction in adipocyte size caused by fat storage [37]. We confirm this observation, as administration of GCE increased the number of adipocytes per unit area while significantly reducing their size, suggesting a shift toward smaller adipocytes and potentially reduced fat accumulation. These findings demonstrate the anti-obesity potential of GCE in HFD-fed rats by reducing visceral fat mass and adipocyte size.

Several studies have reported a significant increase in organ weights, such as the liver, kidney, and heart in HFD-fed animals [3,26]. With the progression of obesity, an increase in liver weight occurs as a result of abnormal glycosylation or fibrosis, which are associated with hepatocyte hypertrophy and hepatic steatosis [38]. In obesity, kidney weight also increases, resulting from swelling, inflammation, and necrotic changes in the tissue [33]. However, our findings suggest a trend of increased liver weight in the HFD group, while GCE treatment appeared to reduce it, although these changes were not statistically significant. Meanwhile, kidney weight remained consistent across all groups. These results align with previous findings by Kim *et al.* [39] who reported that the same HFD used in our study had no significant effect on liver or kidney weight.

Obesity has been found to contribute to hepatic steatosis, marked by excessive hepatic fat accumulation, and is associated with oxidative stress-induced liver cell damage [40]. Research indicates that feeding mice and rats an HFD induces liver steatosis [41,42]. In our study, the HFD control group exhibited significantly greater lipid accumulation in liver tissues compared to the normal group. Administration with GCE diminished this HFD-induced liver lipid accumulation, indicating that GCE may mitigate lipid buildup in liver tissues caused by HFD. However, serum ALT and AST levels, well-known enzymes used as indicators of liver function and important markers for predicting toxicity [43], showed no significant changes. These findings suggest that while the HFD

used in this study for 8 weeks induces hepatic steatosis, it does not cause hepatotoxicity.

Recent research has shown that consumption of HFD increases serum levels of total cholesterol, triglycerides, and LDL-C while decreasing HDL-C levels [44,45]. This results in serum lipid imbalances, which are associated with increased risk for several diseases [46]. In contrast, our findings showed no differences in triglycerides, total cholesterol, LDL-C, HDL-C, or VLDL-C levels between the experimental groups. This may be because the percentage of energy from fat in the diet used in the current study was insufficient to cause significant disruptions in lipid metabolism within the 8-week duration. The results of this study align with previous research by Bréger *et al.* [47] which reported that experimental rats fed an HFD containing 45% energy from fat, similar to the diet used in the present study, for 12 weeks exhibited no changes in plasma triacylglycerols, total cholesterol, LDL-C, or HDL-C levels relative to those on a normal diet. The *in vitro* findings demonstrated that the ethanolic extract of *G. cowa* leaves possesses anti-hyperlipidemic activity [22]. However, since no changes in blood lipid levels were observed in the experimental rats fed the HFD in this study, it was not possible to evaluate the lipid-lowering effects of the extract. Further studies should explore the anti-hyperlipidemic effects of *G. cowa* extract using a diet with a higher fat-energy percentage or an extended study duration.

In a previous study, a qualitative analysis of compounds in the ethanolic extract of *G. cowa* leaves was conducted using LC-QTOF-MS. The findings revealed that the extract contained various bioactive compounds, including hydroxycitric acid, citric acid, kaempferol, isovitexin, apigenin, scutellarein, myricetin, eriodictyol, luteolin, quercetin, amentoflavone, isomangiferin, and garcimangosone C [20]. Hydroxycitric acid is a widely recognized herbal dietary supplement known for its effectiveness in treating obesity [48]. This compound, derived from citric acid, is found in plant species native to South Asia, including *G. cambogia*, *G. indica*, and *G. atroviridis* [49]. Studies in rats have demonstrated that hydroxycitric acid reduces weight regain and suppresses food intake [50]. In humans, it promotes weight loss without stimulating the central nervous system [51] and helps reduce obesity-related visceral fat accumulation [52]. Additionally, hydroxycitric acid has been shown to decrease *de novo* lipogenesis during carbohydrate overfeeding in humans, potentially supporting weight maintenance [53]. Research also indicates that hydroxycitric acid reduces body weight regain in rats following substantial weight loss, likely due to its inhibitory effect on lipogenesis [54]. Hydroxycitric acid lowers acetyl-CoA levels, thus suppressing lipogenesis by limiting the availability of precursors required for the synthesis of fatty acids and cholesterol [55].

In addition to hydroxycitric acid, flavonoids such as kaempferol, apigenin, scutellarein, myricetin, eriodictyol, and quercetin have been shown to possess significant anti-obesity effects. A previous study by Torres-Villarreal *et al.* [56] highlighted the anti-obesity properties of kaempferol, which suppresses adipogenesis and enhances lipolysis in 3T3-L1 adipose cell lines. Furthermore, recent research indicates that kaempferol stimulates the browning of white adipose tissue

through the AMPK/SIRT1/PGC-1 α pathway, demonstrating its anti-obesity properties [57]. Apigenin has also demonstrated notable anti-obesity effects. In HFD-induced obese mice, it improved dyslipidemia, hepatic steatosis, and insulin resistance [58]. Moreover, apigenin significantly reduced visceral adipose tissue in HFD-fed mice by inhibiting adipogenesis via the signal transducer and activator of transcription 3 and cluster of differentiation 36 signaling axis [59]. Scutellarein has been shown to significantly alleviate HFD-induced obesity, hyperlipidemia, inflammation, and fatty liver [60]. Similarly, myricetin has exhibited anti-obesity effects in several animal studies. Myricetin treatment effectively reduces body weight, serum glucose, triglycerides, cholesterol, and the size of epididymal adipocytes, while also lowering fat mass in HFD-induced obese C57BL/6 mice [61]. Additionally, myricetin inhibits the differentiation of 3T3-L1 preadipocytes and enhances adipocyte lipolysis [62]. Eriodictyol supplementation in HFD-induced obese mice significantly reduced dyslipidemia and adiposity by promoting fecal lipid excretion and suppressing lipogenesis in white adipose tissue. It also alleviated hepatic steatosis by partially inhibiting hepatic lipogenesis and enhancing fatty acid oxidation in the liver [63]. In a previous study, Jung *et al.* [64] reported that quercetin reduced body weight gain, liver weight, and white adipose tissue weight, as well as lowered serum lipid levels, liver fat accumulation, and lipid droplet size in epididymal adipose tissue. Its anti-obesity effects are likely associated with the regulation of genes related to lipid metabolism. In Western diet-fed mice, chronic quercetin intake alleviated hepatic lipid accumulation, likely by reducing the expression of steatosis-linked genes [65]. Lastly, amentoflavone exhibits anti-obesity properties by inhibiting body weight gain, reducing perirenal adipose tissue weight, and lowering serum triglyceride levels in HFD-fed rats, as well as suppressing 3T3-L1 adipocyte differentiation [66]. Collectively, these phytochemicals, including hydroxycitric acid, apigenin, kaempferol, apigenin, scutellarein, myricetin, eriodictyol, quercetin, and amentoflavone, are likely responsible for the anti-obesity effects of GCE. However, additional research is required to investigate the anti-obesity mechanisms of GCE in greater detail, as well as to isolate and characterize the active constituents responsible for this activity.

The lack of significant changes in lipid profiles or glucose levels represents a limitation of this study and may be due to the study duration or dietary regimen. Future studies with a higher dietary fat content or extended treatment duration are recommended to further validate and strengthen the observed findings from this study.

CONCLUSION

This study demonstrates that GCE effectively reduces body weight, cumulative weight gain, FER, and total fat mass, particularly in retroperitoneal fat, and adipocyte size. Furthermore, GCE significantly mitigates HFD-induced hepatic fat accumulation. The anti-obesity effects of GCE in rats fed an HFD are probably due to its bioactive compounds, such as hydroxycitric acid and flavonoids. These findings indicate that GCE has the potential for development as a therapeutic or nutraceutical agent in obesity prevention and treatment.

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AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

ETHICAL APPROVALS

Ethical approvals are given in the 'Materials and Method' section

DATA AVAILABILITY

All data are available with the authors and shall be provided upon request.

PUBLISHER'S NOTE

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USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

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