



RESEARCH ARTICLE

# Green coffee - supplemented yoghurt modulates lipid profile and liver enzymes in obese rats

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## Abstract

Therapeutic effects of yogurt supplemented with different concentrations of green coffee bean extract (GCBE) in comparison with the commercial fat burner C4 RIPPED in rats fed a high-fat diet. Twenty-five male albino rats, weighing  $200 \pm 10$  g, were used in this study. After an acclimatisation period, the rats were randomly divided into 2 main groups. The first group ( $n = 5$ ) was fed a standard basal diet and served as the negative control group (A). The second group ( $n = 20$ ) was fed a high-fat diet for 4 weeks to induce obesity. Following obesity induction, the obese rats were further divided into 4 subgroups ( $n = 5$  each) and fed experimental diets for an additional 4 weeks as follows: group (B1) served as the positive control and continued on the high-fat diet; group (B2) received the high-fat diet supplemented with C4 RIPPED at a dose of 1.2 mg/g body weight; group (B3) received yogurt supplemented with 1 % green coffee bean extract; and group (B4) received yogurt supplemented with 2 % green coffee bean extract. The selected concentrations of green coffee bean extract (1 % and 2 %) were based on previously published studies reporting their safety and biological efficacy in improving lipid metabolism and metabolic health. All experimental procedures involving animals were conducted in accordance with the ethical guidelines for the care and use of laboratory animals and were approved by the Institutional Animal Ethical Committee (IAEC) of the relevant institution.

**Keywords:** C4 RIPPED; green coffee; lipid profile; yogurt

## Introduction

Obesity is described as a natural state in which surplus fat builds up in the body to the extent that it causes negative health effects, leading to a reduction in the individual's average lifespan or the occurrence of increasing health problems (1). Recent studies indicate a significant increase in obesity rates worldwide and no one factor has been pinpointed as the sole cause of obesity; rather, there are multiple contributing factors. It was previously believed that high caloric intake combined with a sedentary lifestyle that does not burn off these calories were the primary causes of obesity, the increased consumption of sugars and fats in large amounts, which leads to excessive caloric intake, coupled with a lack of physical activity and poor eating habits, are among the main causes of obesity (2). In addition to being a condition by itself, obesity is also associated with numerous other diseases, particularly heart diseases, hypertension, diabetes and elevated cholesterol levels, high triglycerides, oxidative stress, liver diseases, various types of cancer and sleep apnea, as well as an undesirable body appearance. All of these factors can result in a reduction in average lifespan or contribute to worsening health issues (3). Several experts resort to pharmaceutical medications to decrease body weight and blood lipids, such as the commonly used C4 RIPPED fat burner, which contains a blend of biologically active ingredients that work through different mechanisms to reduce fat accumulation and decrease body fat percentage (4).

Since these medications can lead to certain side effects and health hazards, a majority of individuals have opted for alternative treatment approaches. One example of this approach is the use of functional foods (5), including medicinal plants, which work through various mechanisms, including regulating the body's energy balance, either by affecting the nervous system and increasing satiety, or by reducing energy consumption, increasing energy expenditure, decreasing fat absorption, increasing fat breakdown, or reducing the differentiation and proliferation of fat cells, among other mechanisms (6). In addition, these plants are recognised for their plentiful nature, affordability and reduced side effects in comparison to chemical medications, which frequently lead to adverse reactions and weaken the body's immune system (7). One illustration of this type of plant is green coffee, is advantageous because it postpones the uptake of triglycerides and sugars in the small intestine, owing to its constituents of phenolic acid, caffeine and antioxidants (8). Another type of therapeutic food is probiotics. Studies have indicated that the consumption of probiotic-containing products significantly reduced blood fat levels. Due to the insufficient research and data regarding the complications associated with weight loss medications, many of which are taken without prescriptions, this research aimed to examine the influence of these medications on decreasing blood fat levels and to compare them with herbal remedies and probiotics, in addition to evaluating their effects on rats consuming a high-fat diet (9, 10).

## Materials and Methods

### Chemicals and experimental drug

#### C4 Ripped

C4 Ripped was purchased from a local pharmacy. The product is manufactured in the USA by Cellucor. The recommended human therapeutic dose is 435 mg/kg body weight. Based on the manufacturer's guidelines, the equivalent rat dose was calculated and adjusted to 1.2 mg/g body weight (9).

#### Plant material

Green coffee beans (*Coffea robusta*) were obtained from a local market in Tikrit, Iraq. The beans were air-dried in a hot-air oven at 43 °C until complete dehydration.

#### Preparation of green coffee extract (GCE)

Green coffee beans were extracted with 80 % ethanol at a ratio of 1:10 (w/v) in sealed containers. The mixture was stirred at room temperature ( $25 \pm 2$  °C) for 4 hr and filtered. The residues were re-extracted under identical conditions. The combined filtrates were concentrated using a rotary evaporator at temperatures below  $40 \pm 2$  °C. The obtained extract was stored at  $20 \pm 2$  °C until use.

#### Identification of bioactive compounds by high-performance liquid chromatography (HPLC)

Total phenolic and flavonoid contents of the green coffee extract (GCE) were identified and quantified using high-performance liquid chromatography (HPLC) according to the standard method (10).

#### Preparation of probiotic yoghurt fortified with green coffee extract (GCE)

Probiotic stirred yoghurt fortified with GCE was prepared following the standard method (11). Cow's milk fat content was standardized to 1 % by cream separation. The milk was divided into 3 equal portions (2 L each): a control and 2 treatments supplemented with 1% (T1) and 2% (T2) GCE respectively.

Milk samples were heated at 90 °C for 10 min, cooled to 43 °C and inoculated with 3 % starter culture containing *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. Incubation was carried out at 37 °C until pH reached 4.6. After coagulation, the yoghurt was stirred at low speed (< 30 rpm) for 3 min, dispensed into 150 mL plastic cups, sealed and stored at  $4 \pm 2$  °C.

#### Experimental animals

Twenty-five healthy adult male albino rats (Western strain), weighing 175–185 g, were obtained from the College of Veterinary Medicine, Tikrit University, Salah Al-Din, Iraq. Animals were housed in wire cages (5 rats per cage) under hygienic, temperature-controlled laboratory conditions with free access to food and water.

#### Experimental diets

Standard diets were obtained from the Central Animal House, College of Veterinary Medicine. The basal diet consisted of 3.5 % fat, 22 % protein, 60 % carbohydrates, 12 % fibre and 2.4 % ash. A high-fat diet was prepared by supplementing the basal diet with cholesterol (1 %), bile salts (0.25 %) and fat (15 %) (12).

#### Experimental design

Rats were randomly allocated into the following groups:

- **Group A (Negative control, n = 5):** Fed the basal diet and tap water for 8 weeks.

- **Group B (Obese rats, n = 20):** Fed a high-fat diet for 4 weeks to induce obesity, then subdivided into 4 groups (n = 5 each) and treated for 28 days as follows:

- **B1:** Positive control, high-fat diet only
- **B2:** High-fat diet + C4 Ripped
- **B3:** High-fat diet + 2 g/day yoghurt supplemented with 1 % GCE
- **B4:** High-fat diet + 2 g/day yoghurt supplemented with 2 % GCE

#### Body weight measurement

Body weight was recorded before and after the experimental period using a digital electronic balance (accuracy 0.01 g). The average daily body weight gain was calculated (13):

$$\text{Average daily weight gain (g/day)} =$$

$$\frac{\text{Final body weight} - \text{Initial body weight}}{\text{Number of experimental days}}$$

$$\frac{\text{Final body weight} - \text{Initial body weight}}{\text{Number of experimental days}}$$

#### Biochemical analysis

Blood samples were collected by cardiac puncture using a 5 mL syringe after overnight fasting and centrifuged at 3000 rpm for 10 min to obtain serum. Serum total cholesterol, triglycerides and HDL-C were determined (14). LDL-C and VLDL-C were calculated using the Friedewald equations (15):

- $\text{VLDL-C (mg/dL)} = \text{Triglycerides} / 5$
- $\text{LDL-C (mg/dL)} = \text{Total cholesterol} - \text{HDL-C} - \text{VLDL-C}$
- $\text{LDL-C (mg/dL)} = \text{Total cholesterol} - \text{HDL-C} - (\text{Triglycerides} / 5)$

Liver enzymes, including alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), were measured using commercial diagnostic kits (16).

#### Statistical analysis

The experiment was conducted using a completely randomised design (CRD), with individual animals considered as experimental units. Data were analysed using the statistical analysis system (SAS) software version 9.2 (17). Differences among means were assessed using Duncan's multiple range test and statistical significance was set at  $p \leq 0.05$  (18).

## Results and Discussion

### Bioactive compounds of green coffee

Table 1 presents the results of the statistical analysis of the alcoholic extract of green coffee, showing a total phenolic content of 46.17 mg/100 g and a total flavonoid content of 6.53 mg/100 g. These findings are consistent with those reported by early works, who confirmed that the polyphenolic fraction of green coffee is predominantly composed of flavonoids (19). Polyphenols, particularly chlorogenic acid, represent the major bioactive constituents of green coffee and are known to exert antioxidant, anti-inflammatory and metabolic regulatory effects through modulation of glucose and lipid metabolism. These results are also in agreement with previous works, who reported that GCE is rich in polyphenols exhibiting various biological activities, including immune-stimulating, anti-allergic, anti-cancer, anti-inflammatory and antiviral properties (20). Many researchers have also

demonstrated the presence of several pharmacologically active compounds in green coffee, such as phenolic compounds and soluble dietary fibre, which contribute to its functional and

**Table 1.** Bioactive compounds in green coffee

Compound	Content (mg/100 g)
<b>Total phenolic content (TPC)</b>	46.17 ± 1.24
<b>Total flavonoid content (TFC)</b>	6.53 ± 0.68

metabolic benefits (21).

#### Effect of green coffee on body weight of obese rats

Table 2 shows the effect of feeding rats a balanced diet (A), a high-calorie diet (B1), a high-calorie diet supplemented with C4 Ripped (B2) and a high-calorie diet supplemented with yogurt enriched with green coffee at concentrations of 1 % and 2 % (B3 and B4) on body weight gain and average daily weight increase after 4 weeks. The results indicated that rats fed the standard diet showed a daily weight gain of 0.253 g/day and a final weight gain of 7.10 g, whereas rats fed a high-calorie diet exhibited the highest daily and final weight gains (1.089 g/day and 30.76 g respectively).

Conversely, rats treated with C4 Ripped showed no daily weight gain and a final weight loss of -21.91 g. Similarly, rats fed a high-calorie diet supplemented with yogurt and green coffee (B3 and B4) showed no daily weight gain, with final weight losses of -25.59 g and -33.95 g respectively.

The increase in body weight observed in rats fed a high-calorie diet may be attributed to the high energy density and enhanced palatability of the diet, which promote excess fat deposition. High-fat diets are known to reduce satiety signaling and encourage short-term overeating, leading to increased caloric intake and body mass index (22). In contrast, weight loss observed in rats treated with C4 Ripped may be explained by the drug's ability to inhibit pancreatic and gastric lipase enzymes, thereby reducing fat absorption and increasing fecal fat excretion (23). These findings are consistent with those of early works, who reported a significant weight-loss effect associated with C4 administration (9).

The reduction in body weight observed in rats receiving

yogurt fortified with green coffee may be attributed to the high content of bioactive compounds, particularly polyphenols and flavonoids, which enhance lipid oxidation and suppress lipogenesis. In addition, the caffeine content of green coffee may contribute to appetite suppression and increased energy expenditure, thereby promoting weight loss (19). These results are in agreement with previous reports, who demonstrated that green coffee consumption significantly reduced body weight by stimulating metabolic activity and accelerating fat utilisation (24).

#### Effect of green coffee on lipid profile

The biochemical analysis results, shown in Table 3, revealed significant variations ( $p \leq 0.05$ ) in the biochemical tests of the experimental groups. The cholesterol level increased in the B1 group to 209.90 (mg/dL) compared to 103.61 (mg/dL) in the A group. Meanwhile, treatments B2, B3 and B4 led to a significant decrease in cholesterol levels, with values of 158.03, 140.26 and 136.11 (mg/dL), respectively. Furthermore, the table shows a significant increase in triglyceride levels in the B1 group, which reached 147.35 (mg/dL) compared to 80.12 (mg/dL) in the A group. However, a significant decrease in triglyceride levels was observed in the blood serum of animals in the B2, B3 and B4 groups, with values of 134.21, 126.56 and 124.86 (mg/dL) respectively. Additionally, the results showed a significant decrease ( $p \leq 0.05$ ) in the high-density lipoprotein (HDL) levels in the B1 group, which reached 28.34 (mg/dL) compared to 44.03 (mg/dL) in the A group. In contrast, HDL-C levels in the treated animal groups (B2, B3 and B4) significantly increased, with values of 33.50, 38.92 and 39.02 (mg/dL), respectively. The table also indicates a significant increase in the low-density lipoprotein (LDL) levels in the B1 group, which reached 152.09 (mg/dL) compared to 43.56 (mg/dL) in the A group. In contrast, a significant decrease in LDL-C levels was observed in the blood serum of the animals in the B2, B3 and B4 groups, with values of 97.69, 76.03 and 72.12 (mg/dL) respectively.

The findings supported the early findings, which showed that eating fatty foods has a substantial negative influence on health by promoting the formation of cholesterol, elevating bad cholesterol at the expense of good cholesterol, increasing blood serum triglyceride levels and causing plaque to form in arterial walls (25). This, in turn, leads to obstructed blood flow within blood vessels, resulting in numerous diseases. As for the treated group with C4

**Table 2.** Effect of various treatments on body weight parameters of obese rats

Treatment	Initial body weight (g)	Final body weight (g)	Body weight gain (g)	Average daily weight gain (g/day)
A	203.38 ± 2.57 <sup>b</sup>	210.48 ± 1.67 <sup>b</sup>	7.10	0.253
B1	234.55 ± 3.54 <sup>a</sup>	265.31 ± 3.08 <sup>a</sup>	30.76	1.098
B2	231.18 ± 3.89 <sup>a</sup>	209.27 ± 2.75 <sup>b</sup>	-21.91	-0.78
B3	232.82 ± 2.18 <sup>a</sup>	207.23 ± 2.55 <sup>b</sup>	-25.59	-0.91
B4	234.06 ± 3.75 <sup>a</sup>	200.11 ± 2.55 <sup>b</sup>	-33.95	-1.21

Values are expressed as mean ± standard error (SE).

Different superscript letters (<sup>a</sup>, <sup>b</sup>) within the same column indicate significant differences among treatments at  $p < 0.05$ .

**Table 3.** Effect of feeding yogurt enriched with green coffee extracts on lipid profile (mg/dL) in obese rats

Treatment	TC (mg/dL)	TG (mg/dL)	HDL (mg/dL)	VLDL (mg/dL)	LDL (mg/dL)
A	103.61 ± 1.78 <sup>e</sup>	80.12 ± 1.33 <sup>d</sup>	44.03 ± 1.55 <sup>a</sup>	16.02 ± 0.07 <sup>c</sup>	43.56 ± 1.35 <sup>d</sup>
B1	209.90 ± 2.20 <sup>a</sup>	147.35 ± 2.40 <sup>a</sup>	28.34 ± 1.28 <sup>d</sup>	29.47 ± 0.68 <sup>a</sup>	152.09 ± 2.71 <sup>a</sup>
B2	158.03 ± 2.72 <sup>b</sup>	134.21 ± 2.35 <sup>b</sup>	33.50 ± 1.08 <sup>c</sup>	26.84 ± 0.87 <sup>b</sup>	97.69 ± 2.13 <sup>b</sup>
B3	140.26 ± 2.13 <sup>c</sup>	126.56 ± 1.84 <sup>c</sup>	38.92 ± 1.63 <sup>b</sup>	25.31 ± 0.84 <sup>b</sup>	76.03 ± 1.64 <sup>c</sup>
B4	136.11 ± 3.25 <sup>d</sup>	124.86 ± 2.17 <sup>c</sup>	39.02 ± 1.57 <sup>b</sup>	24.97 ± 1.34 <sup>b</sup>	72.12 ± 2.46 <sup>c</sup>

Values are expressed as mean ± standard error (SE).

Different superscript letters (<sup>a</sup>–<sup>e</sup>) within the same column indicate significant differences among treatments at  $p < 0.05$ .

Ripped medication, the improvement in blood lipid profiles may be attributed to the high arginine content in this supplement, which is considered an active substance in reducing fat accumulation and lowering fat levels. This occurs by reducing adipocytes and may also involve increased oxidation of fatty acids released from adipose tissues in the liver and skeletal muscles, as well as triglycerides (9). The mice in the last group were fed yogurt that had been supplemented with green coffee. The findings are in line with those of previous works, who highlighted the potential of probiotics and yogurt to increase HDL-C and decrease blood cholesterol levels (26, 27). This effect was attributed to the bacteria's ability to break down fats by producing enzymes that do so and use the fat as a source of carbon. The results were in line with early works, who found that consuming yogurt enriched with green coffee significantly reduced lipid markers (28). This decline was explained by the binding of cholesterol and bile acids to the fibres, which increased excretion through faeces and decreased the creation of cholesterol production through the generation of short-chain fatty acids. These impacts might be connected to the thickness of these fibres, since the existence of these fibers postpones the uptake of triglycerides and sugars in the small intestine, thereby reducing blood fat levels.

### Effect of green coffee on liver enzymes

Table. 4 illustrates that rats fed a high-calorie diet exhibited significantly elevated serum ALT, AST and ALP activities compared with the control group, indicating hepatic stress or dysfunction. These findings are consistent with those reported by previous

**Table 4.** Effect of yogurt enriched with green coffee extracts on liver enzyme activities in obese rats

Treatment	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
A	29.85 ± 1.24 <sup>d</sup>	36.32 ± 1.73 <sup>d</sup>	73.64 ± 2.17 <sup>c</sup>
B1	48.17 ± 1.38 <sup>b</sup>	61.56 ± 2.03 <sup>a</sup>	88.69 ± 3.54 <sup>a</sup>
B2	60.95 ± 1.14 <sup>a</sup>	64.08 ± 1.85 <sup>a</sup>	90.04 ± 2.11 <sup>a</sup>
B3	35.24 ± 1.75 <sup>c</sup>	43.82 ± 1.11 <sup>b</sup>	81.22 ± 2.81 <sup>b</sup>
B4	34.15 ± 1.26 <sup>c</sup>	40.39 ± 2.47 <sup>c</sup>	80.01 ± 2.97 <sup>b</sup>

Values are expressed as mean ± standard error (SE).

Different superscript letters (<sup>a</sup>, <sup>b</sup>, <sup>c</sup>, <sup>d</sup>) within the same column indicate significant differences among treatments at  $p < 0.05$ .

Abbreviations: ALT = Alanine aminotransferase; AST = Aspartate aminotransferase; ALP = Alkaline phosphatase; IU/L = International units per liter.

workers, who suggested that excessive intake of saturated fatty acids leads to hepatic lipid accumulation and increased liver enzyme activity (29).

Rats treated with C4 Ripped showed further increases in liver enzyme levels, which may be related to hepatic impairment caused by certain drug components, resulting in elevated liver biomarkers such as ALT, AST and ALP (30). In contrast, rats receiving yogurt fortified with green coffee, particularly at the 2 % concentration, showed a marked reduction in liver enzyme activities. This hepatoprotective effect may be attributed to the antioxidant capacity of green coffee polyphenols, which reduce oxidative stress and protect hepatocytes from cellular damage.

Moreover, the presence of lactic acid bacteria in yogurt may enhance hepatic metabolic efficiency and improve liver function by

modulating metabolic indicators. These findings are consistent with early works, who demonstrated the beneficial role of yogurt fortified with green coffee in reducing liver enzyme levels (8, 31).

### Study limitations

Despite the significant findings of the present study, some limitations should be acknowledged. Inflammatory biomarkers such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF- $\alpha$ ) were not measured, which limits the ability to fully elucidate the anti-inflammatory mechanisms associated with green coffee supplementation. Additionally, changes in gut microbiota composition were not directly assessed; therefore, the proposed role of probiotics and green coffee in modulating the gut-liver axis remains inferential. Future studies incorporating inflammatory markers and microbiome analysis are recommended to provide a more comprehensive understanding of the underlying mechanisms.

### Conclusion

The present study demonstrates that a high-calorie diet significantly elevates liver enzyme activities (ALT, AST and ALP) in rats, indicating potential liver stress or impairment. Administration of C4 Ripped medication further increased these enzyme levels, suggesting a possible hepatotoxic effect of the drug. In contrast, supplementation with yogurt enriched with green coffee at 1 % and 2 % effectively reduced liver enzyme concentrations, highlighting its hepatoprotective potential. These findings suggest that green coffee-fortified yogurt may serve as a natural intervention to mitigate liver dysfunction associated with high-calorie diets and drug-induced hepatic stress.

### Authors' contributions

All authors contributed equally in the preparation of the manuscript. All authors read and approved the final manuscript.

### Compliance with ethical standards

**Conflict of interest:** Authors do not have any conflict of interests to declare.

**Ethical issues:** None

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