

The Potential Role of Green Coffee Bean Extract Consumption on Some Biomarkers in Female Rabbits

Saba Ibrahim Salih¹, Nada Abdulhussein Alkafaji², Sajaa R. Al-Saedi¹,
Rana Fadhil Mousa¹

¹ Physiology, Biochemistry and Pharmacology Department, Veterinary Medicine College, University of Kerbala, Karbala, Iraq.

² College of agriculture, university of kerbala , Karbala, Iraq.

Corresponding author: saba.ibrahim@uokerbala.edu.iq

Received:9/5/2026

Accepted:9/6/2026

Published:6/9/2026

Abstract— Green coffee is coffee prepared from raw, unroasted coffee and has many benefits because it has antioxidants effects on the body. This study aim to investigate the effects of green coffee extracts on body weight, blood parameters and some liver enzyme in female rabbits. The current study was conducted on 20 female rabbits aged ranged around 3months and the experiment last for two months. The rabbits were divided into two groups of 10 each. The control group received regular feed and water, while the treated group received green coffee bean extract at a dose of 100 mg/kg/day. Blood samples were collected and samples prepare to estimate the levels of related biomarkers. The results show a significant decrease ($P \leq 0.05$) in body weight after 2 months of treatment with GCBE, also, the results indicated that there was a significant increase ($P \leq 0.05$) in RBC, PCV and Hb concentration in green coffee treated group when compare with control untreated group, while there is a significant decrease ($P \leq 0.05$) in WBC in green coffee treated group when compare with control untreated group. On the other hand, there is a significant decrease in liver enzyme in green coffee treated group when compare with control. As conclusion, it is best to take green coffee bean extract because its improve health state by improves blood parameters and liver enzymes, in addition to helping with weight loss.

Key words: GCBE, Chlorogenic acid, liver enzyme

INTRODUCTION

For centuries, plants and their extracts have been used as natural remedies to treat various ailments. Research has identified bioactive compounds in plants that exhibit therapeutic properties, making them potential treatments for chronic diseases (1). The second most traded commodity in the world after oil is coffee, a popular beverage valued for its flavor (2). Green coffee, which has many health benefits because of its special therapeutic and medicinal qualities, has gained popularity as a result of increased worries about side

effects, even if standard coffee's benefits are linked to caffeine (3).

Unroasted coffee beans are the source of Green Coffee Bean Extract (GCBE), which has demonstrated promise in aiding weight loss, improving liver function to increase fat metabolism, and lowering body fat buildup without limiting caloric intake.

Additionally, GCBE's high antioxidant content—specifically, chlorogenic acid, caffeine, n-coumaric acid, and ferulic acid—is largely responsible for its health advantages and may have therapeutic effects (4).

The main phenolic molecule in green coffee, chlorogenic acid (CGA), is rich in polyphenols and has a number of possible health benefits. It is known to: Prevent the development of fat cells (adipocytes) (5). Coffee beans can be extracted using techniques such hot water extraction (6,7) to extract CGA, which has been found to have health advantages (Figure 1). CGA levels vary depending on factors such as: coffee bean type coffee origin, preparation method and roasting degree (8), these variables impact the overall nutritional value of green coffee.

Roasting coffee beans alters several physical properties, including: tapped density, bulk density, sensory characteristics, flow ability and chemical composition (10)

Notably, Chlorogenic Acid (CGA) levels are significantly affected by temperature, decreasing substantially at higher roasting temperatures (11). Research highlights CGA's potential health benefits as a novel antioxidant, particularly in: hypertension management cardiovascular health, diabetes prevention, metabolic syndrome treatment and neuroprotection (12).

This study aimed to investigate the effect of an extract of green coffee bean powder on the weight, hematological profile and liver enzymes function in female rabbits.

MATERIALS AND METHODS

The study took place over two months. Twenty healthy

female rabbits weighing 1.35 to 1.5 kg each were carefully chosen, and they were given a cozy home in the animal house of the University of Kerbala's Faculty of Veterinary Medicine. The rabbits lived in a comfortable setting with a balanced 12-hour light and 12-hour dark cycle and a temperature between 22 and 24°C. The rabbits were given two weeks to acclimate to their new surroundings prior to the start of the investigation. They were given a healthy diet of freshly made pellets, fresh produce from nearby markets, and unrestricted access to clean water during the study. This arrangement allowed us to guarantee the bunnies' general health and wellbeing.

Experimental Design

Twenty female rabbits were randomly allocated into two groups (10 each). Group 1 served as the control group, receiving only normal diets and water, whereas Group 2 received normal diets and water as well as green coffee extract in dose of 100 mg/kg/ day according to Al Amri et al,2020 (13). The experiment lasted for two months. At the end of the experiment, blood samples were gathered without slaughtering animals via heart puncture.

Methods

Green coffee Extract preparation

Green coffee sources in Iraq are limited, as it is not a major domestic agricultural product. Green coffee beans are often imported from other countries such as Brazil, Ethiopia, Colombia, and others. The green coffee used in the experiment was imported from Brazil. For preparation of water solutions, an electrical mixer was used to ground the beans into a fine powder. In keeping with the traditional Arabian method of making coffee, the resulting powder was extracted using water at 70 °C for 20 minutes. The extract was then filtered and lyophilized to eliminate any remaining water. The extract's final physical form was green powder. The extract was identified as a green coffee bean extract (GCBE) that had been dissolved at a concentration of 100 mg/ml in distilled water (13).]

Ethical approve

This research was conducted under the official reference number UOK.VET.PH.2025.170 at the state-of-the-art anatomical laboratory of the College of Veterinary Medicine, University of Kerbala, Iraq, a leading institution for veterinary research and education.

STATISTICAL ANALYSIS

Data analysis was performed using an independent two-sample T-test via SPSS software (version 22 for Windows, USA), with results considered statistically significant at a P-value of 0.05 or less..

RESULT AND DISCUSSION

Body weight

The Effects of green coffee bean extract on the body weight at the beginning and 2 months after its using is show in table 1. Results show no significant differences($p \geq 0.05$) between the green coffee bean extract and control group at the beginning of experiment (column1), two months after using GCBE extract the results showed a significant decrease ($p \leq 0.05$) in the body weight in green coffee bean extract when compare with control group(column2) (table 1).

Table 1. Body weight of rabbits before and after using of GCBE extract

Periods Groups	Weight (gm) before using the extract Mean $\pm$ SD	Weight (gm) after using the extract Mean $\pm$ SD
Control	1421.83 $\pm$ 52.59A	1430.83 $\pm$ 49.71A
GCBE	1428.66 $\pm$ 55.94A	1370.00 $\pm$ 31.87B

Values are explained as mean $\pm$ SD, n=10/group, and significant differences ($p \leq 0.05$).

The findings align with Hausenblas and Huynh (14), who discovered that chlorogenic acid in Green Coffee Bean Extract (GCBE) leads to significant weight loss in obese individuals.

Additionally, research by Park et al. (15) supports this result, showing that chlorogenic acid cause decreases release of adipokines (leptin and adiponectin), restricts growth of lipid droplets and accumulation of intracellular triglycerides and ultimately reduces fat accumulation and body weight. These studies confirm the potential of GCBE in aiding weight loss.

According to additional studies, Chlorogenic Acid (CGA) may decrease the protein-level expression of genes related to fat formation, such as Fatty Acid Synthase (FAS), Sterol Regulatory Element Binding Protein-1c (SREBP-1c), and Peroxisome Proliferator-Activated Receptor Gamma (PPAR γ) (16). Our results are similarly consistent with those of Gorji et al. , who found that supplementing with Green Coffee Extract (GCE) improves obesity indicators, especially in those whose initial BMI was 25 kg/m² or higher (17). Its potential as a weight management tool is further supported by the fact that the weight loss linked to GCBE may be explained by decreased fat absorption brought on by pancreatic lipase inhibition (18).

Hematological parameters

The results presented in Table 2 demonstrate significant increase ($P \leq 0.05$) in RBC, Hb and PCV in GCBE treated group when compare with control group, while there is significant decrease ($P \leq 0.05$) in WBC count in GCBE treated group when compare with control group.

Table 2. Blood parameters of rabbits 2 months after using of GCBE extract

Group Parameter	Control group Mean $\pm$ S.D	GCBE experimental group Mean $\pm$ S.D	P value
RBC. Count (millions/ mm ³)	4.45 $\pm$ 0.68	5.26 $\pm$ 0.55	$P \leq 0.05$
WBC. Count (cell x 10 ³ /mm ³)	8.29 $\pm$ 1.90	4.48 $\pm$ 0.71	$P \leq 0.05$
Hb concn. (g/dl)	9.28 $\pm$ 1.44	11.97 $\pm$ 1.54	$P \leq 0.05$
PCV (Hematocrit) (%)	28.84 $\pm$ 4.14	34.95 $\pm$ 2.61	$P \leq 0.05$

Values are explained as mean $\pm$ SD, n=10/group, and significant differences ($p \leq 0.05$).

The RBC count showed a significant increase in the experimental group (5.26 ± 0.55 million/mm³) compared to the control group (4.45 ± 0.68 million/mm³). This implies that the antioxidant addition, phenolics' antioxidant activity prevents liver qualities of green coffee extract, which shield red blood cells from tissue damage (24). These findings suggest GCBE may support oxidative stress and lengthen their lifespan, may promote liver health and function.

erythropoiesis. Previous investigations have revealed similar results, A study by Koriem et al. who found that even low levels of showing that polyphenols in plant extracts enhance oxygen transport. Chlorogenic Acid pretreatment significantly protected the capacity and induce erythropoiesis (19).

The experimental group WBC count was significantly lower level of AST, ALT and ALP, compared to the ($4.48 \pm 0.71 \times 10^3/\text{mm}^3$) than that of the control group ($8.29 \pm 1.90 \times 10^3/\text{mm}^3$). This decline would suggest that green coffee extract has contributed to Chlorogenic Acid's potent antioxidant properties, immune-modulatory properties. One of the main bioactive which neutralize harmful free radicals and prevent liver cell components of green coffee, chlorogenic acid, has been damage (25).

demonstrated to influence immunological responses by lowering the In conclusion, the results of this study demonstrate that production of pro-inflammatory cytokines (20). This may be the administering GCBE to adult female rabbits has a positive reason for a reduced WBC count.

Hemoglobin concentration increased significantly in the weight loss. These positive effects may be attributed to the experimental group, with a mean value of 11.97 ± 1.54 g/dl effects of Chlorogenic acid by enhancing metabolic health compared to 9.28 ± 1.44 g/dl in the control group. This implies through aiding weight loss and its anti-inflammatory and increase in oxygen-carrying capacity, which could be brought about antioxidant properties in addition to protecting the liver. by green coffee extract's potent antioxidants, which help shield hemoglobin molecules from oxidative damage (21).

Hematocrit, another name for packed cell volume (PCV), was significantly higher in the experimental group ($34.95 \pm 2.61\%$) than in the control group ($28.84 \pm 4.14\%$). This lends more credence to the idea that green coffee extract promotes erythropoiesis and may also increase oxygen delivery and blood viscosity. Studies on plant-derived polyphenols improving hematological profiles have shown a similar impact (22).

Liver enzyme

The effect of green coffee bean extract consumption on liver function can be seen based on blood AST, ALT and ALP levels as shown in Table 3. The results show significant decrease ($P \leq 0.05$) in AST and ALT level in GCBE experimental group when compare with control group, also there is a decrease in ALP in GCBE experimental group when compare with control group but didn't reach to significant level ($P \geq 0.05$).

Table 3. ALT, AST, ALP activity in rabbit blood serum 2 months after using of GCBE extract

Group	Control group	GCBE	
Parameter	Mean $\pm$ S.D	experimental group Mean $\pm$ S.D	P value
AST	66.9283 $\pm$ 9.901	54.2517 $\pm$ 7.397	P $\leq$ 0.05
ALT	72.4433 $\pm$ 11.211	58.7917 $\pm$ 9.099	P $\leq$ 0.05
ALP	48.1083 $\pm$ 13.296	41.9117 $\pm$ 6.762	P $\geq$ 0.05

Values are explained as mean $\pm$ SD, n=10/group, and significant differences ($p \leq 0.05$).

Our results in Table 3 confirm that Green Coffee Bean Extract (GCBE) has no adverse effects on liver function. The Chlorogenic Acid (CGA) in green coffee acts as antioxidants to neutralize free radicals, anti-inflammatory agents to reduce inflammation, cell protectors to prevent apoptosis (23). Additionally, phenolic compounds in green coffee exhibit hepatoprotective activity to safeguard liver health; caffeine

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