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Effects of green coffee bean extract supplementation on lipid profile and oxidative stress in female rabbits

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Green coffee bean extract (GCBE), derived from raw and unroasted coffee fruits, is a major dietary source of potent polyphenols, primarily chlorogenic acids (CGAs), which provide an array of pharmacological and health benefits due to their high natural antioxidant capacity. The aim of the paper is to determine the effect of feeding an aqueous extract of green coffee bean powder on the lipid profile, which includes total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C), and triacylglycerols (TAG), and also to study its effect on the antioxidant status (GSH, SOD, and MDA) in female rabbits. The investigation was conducted on twenty healthy female rabbits, aged 4–6 months and weighing approximately 1350–1500 g, which were randomly and equally allocated into two experimental groups: a control untreated group receiving a normal diet and water, and a treated group supplemented with the extract. The feeding experiment lasted for a period of two months, from December 1, 2024, to February 1, 2025. The obtained results revealed that GCBE treatment significantly improved the enzymatic and non-enzymatic antioxidant defense systems, as evidenced by a substantial increase of 42.94 % in glutathione (GSH) levels and a 57.72 % elevation in superoxide dismutase (SOD) activity ($p \leq 0.05$) in the green coffee extract-treated group compared to the control untreated group. Concurrently, a significant 17.40 % reduction in malondialdehyde (MDA) concentration ($p \leq 0.05$) was observed in the treated group, indicating a notable decrease in lipid peroxidation and systemic oxidative damage. On the other hand, regarding the serum lipid profile, GCBE consumption induced a remarkable 89.49 % increase ($p \leq 0.05$) in high-density lipoprotein cholesterol (HDL-C) levels within the treated group compared to the control group, while the other atherogenic lipid parameters showed the exact opposite trend, with significant drops in total cholesterol by 30.48 %, triglycerides by 38.14 %, low-density lipoprotein cholesterol (LDL-C) by 43.81 %, and very low-density lipoprotein cholesterol (VLDL-C) by 53.05 %. In conclusion, the administration of green coffee bean extract successfully mitigates oxidative stress, enhances antioxidant defense status, and optimizes healthy cholesterol levels, displaying robust hypolipidemic and anti-atherogenic potential in the female rabbit model.

Keywords: Green coffee, oxidative stress, lipid profile.

Вплив застосування екстракту зелених кавових зерен на ліпідний профіль та оксидативний стрес у самок кролів

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Екстракт зелених кавових зерен (ЕЗКЗ), отриманий із сирих і необсмажених плодів кави, є основним дієтичним джерелом потужних поліфенолів, переважно хлорогенових кислот, які забезпечують низку фармакологічних ефектів та переваг для здоров'я завдяки своїй високій природній антиоксидантній здатності. Метою цієї роботи є визначення впливу згодовування водного екстракту порошку зелених кавових зерен на ліпідний профіль, який включає загальний холестерин, холестерин ліпопротеїнів високої щільності, холестерин ліпопротеїнів низької щільності, холестерин ліпопротеїнів дуже низької щільності та триацилгліцероли, а також вивчення його впливу на антиоксидантний статус у самок кролів. Дослідження проводили на двадцяти здорових самках кролів віком 4–6 місяців і масою тіла приблизно 1350–1500 г, яких випадковим чином розділили на дві рівні експериментальні групи: контрольну необроблену групу, яка отримувала звичайний раціон і воду, та дослідну групу, раціон якої доповнювали екстрактом. Експеримент із годівлею тривав протягом двох місяців, з 1 грудня 2024 року по 1 лютого 2025 року. Отримані результати показали, що застосування ЕЗКЗ значно покращило ферментативну та неферментативну системи антиоксидантного захисту, про що свідчить істотне підвищення рівня глутатіону на 42,94 % та активності супероксиддисмутази на 57,72 % ($p \leq 0,05$) у групі, що отримувала екстракт зеленої кави, порівняно з контрольною групою. Одночасно у дослідній групі спостерігалося значне зниження концентрації малондіальдегіду на 17,40 % ($p \leq 0,05$) у порівнянні з контрольною групою, що вказує на помітне зменшення перекисного окиснення ліпідів та системного оксидативного пошкодження. З іншого боку, щодо ліпідного профілю сироватки крові, споживання ЕЗКЗ викликало значне підвищення рівня холестерину ліпопротеїнів високої щільності на 89,49 % ($p \leq 0.05$) у дослідній групі порівняно з контрольною групою, тоді як інші атерогенні ліпідні параметри демонстрували абсолютно протилежну тенденцію зі значним зниженням загального холестерину на 30,48 %, тригліцеридів на 38,14 %, холестерину ліпопротеїнів низької щільності на 43,81 % та холестерину ліпопротеїнів дуже низької щільності на 53,05 %. На завершення, введення екстракту зелених кавових зерен успішно знижує оксидативний стрес, підвищує статус антиоксидантного захисту та оптимізує рівень здорового холестерину, виявляючи потужний гіполіпідемічний та антиатерогенний потенціал на моделі самок кролів.

Ключові слова: зелена кава, оксидативний стрес, ліпідний профіль.

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Introduction

Coffee is a major agricultural export product as well as one of the most important beverage crops in the world; it is the second-most commonly traded commodity in the world, following oil [1]. Green coffee refers to unroasted, raw and unprocessed coffee fruit [2]. Green coffee has previously been hypothesized to have good benefits on health [3].

The chemical structure of green coffee from different geographical origins is characterised by the presence of phenolic acids (caffeic acid and chlorogenic acids) and alkaloids (caffeine) [4]. Chlorogenic acids are esters of trans-cinnamic acids that are dramatically reduced in green coffees throughout the roasting process. Green coffee has the highest antioxidant activity (AA) and has been claimed to be the primary and natural source of antioxidants [5]. Its total antioxidant value ranges from 0.15–0.30 mg/g. Antioxidants are required for a variety of bioactivities in the human body. Many researchers indicated that coffee extracts displayed a variety of essential biological actions, including antiviral, antibacterial, antioxidant, anti-inflammatory, and free radical scavenging [6].

Green coffee beans have more chlorogenic acid than conventional roasted coffee beans, which offers several health benefits; decaffeinated coffee enriched with chlorogenic acid elevates a person's conduct and attention. CGA and related chemicals help to increase fat metabolism in the liver. It also alters lipid metabolism and glucose in both healthy individuals and those with genetically metabolic-related diseases [7–9].

Genetics, lifestyle, and diet all have an impact on one's lipid profile [10]. Despite being controllable, dyslipidemia is a leading risk factor for cardiovascular diseases like coronary heart disease and metabolic syndrome [11, 12]. Today, the initial and preventive treatment of dyslipidemia involves modifications to one's diet and lifestyle, as well as medication [13].

Green coffee bean extract is a supplement that is derived from fresh coffee beans that have not undergone fermentation or roasting. It is reported to provide several health benefits [14–16]. GCBE contains caffeine and chlorogenic acid, which may explain some of its therapeutic benefits [17]. The influence of GCBE supplementation on the lipid profile is still unclear. A recent clinical study found that GCBE supplementation reduced low-density lipoprotein cholesterol (LDL-C), serum total cholesterol (TC), and plasma free fatty acids in obese women [18]. In comparison, other studies have failed to show an improvement in lipid parameters measured in the blood after treatment with GCBE [19, 20].

Coffee is an excellent dietary source of antioxidants due to its high concentration of polyphenols known as chlorogenic acids, and its consumption has been linked to increased plasma antioxidant capacity [21]. Polyphenols are the most prevalent antioxidants in our diet [22]. Chlorogenic acids are found in many plants; chlorogenic acids are esters of cinnamic acids and quinic acid [23]. Green coffee beans are the principal sources of chlorogenic acids (CGAs), because a significant amount of CGAs are lost during the roasting process of coffee [24]. The 5-caffeoylquinic acid is the most abundant CGA ester in the green coffee bean [25].

It has been reported that CGAs possess anti-inflammatory, anti-oxidative, anti-diabetes, anti-obesity, anti-hypertensive, and anti-lipidemic properties [26]. Many studies have shown that CGAs can reduce oxidative stress, as demonstrated by reducing malondialdehyde [27, 28] or concentrations of oxidized low-density lipoprotein [27, 29] and improving the overall antioxidant capability [30] of plasma [31].

The aim of the study

The aim of the paper is to determine the effect of feeding an aqueous extract of green coffee bean powder on the lipid profile, which includes total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, very low-density lipoprotein cholesterol, and triacylglycerols, and also to study its effect on the antioxidant status in female rabbits.

Materials and methods

Animal of the Study

The investigation was conducted over a two-month period, beginning on December 1, 2024, and ending on February 1, 2025. Twenty female rabbits (weighing approximately 1350–1500 g) were housed in the animal house at the Faculty of Veterinary Medicine, University of Kerbala. The environmental conditions were maintained at 22–24 °C with a 12-hour light/dark cycle. The rabbits were acclimatized in the animal house for two weeks before the beginning of the research. The animals were fed a diet of freshly prepared food pellets and vegetables obtained from local markets, with water provided ad libitum.

Materials and Green Coffee Extract Preparation

The green coffee utilized in the experiment originated from Brazil. To prepare the aqueous solutions, the beans were ground into a fine powder using an electrical mixer. The obtained powder was extracted using water at 70 °C for 20 minutes, in accordance with the traditional Arabian method of preparing coffee. After that, the extract was filtered and lyophilized to remove any remaining traces of water. The ultimate physical form of the extract was a green powder. The extract was designated as green coffee water extract (GCWE) and was dissolved in distilled water at a concentration of 100 mg/ml [32].

Experimental Design

Twenty female rabbits were randomly allocated into two equal groups (10 rabbits each). Group 1 served as the control group, receiving only a normal diet and water, whereas Group 2 received the normal diet and water supplemented with the green coffee extract. The experiment lasted for two months. At the end of the experimental period, blood samples were gathered without sacrificing the animals via cardiac puncture.

Blood Collection

The animals were anesthetized with intramuscular injections of ketamine (90 mg/kg) and xylazine (40 mg/kg). Blood samples were collected after two months of the experiment using a 5 ml disposable syringe. The samples were placed in serum tubes for 30 minutes before undergoing centrifugation at 3000 rpm for 10 minutes. The separated serum was then transferred to Eppendorf tubes and stored frozen at -20 °C until biochemical examinations were performed. The tubes were stored at 4 °C until they were subjected to biochemical analysis.

Statistical Analysis

The data were statistically evaluated using an independent two-sample t-test with a computer-based statistical program (SPSS, version 22 for Windows). P-values < 0.05 were considered to indicate statistically significant differences.

Results and discussion

Lipid profile results

The assessment of the serum lipid profile parameters in female rabbits after a two-month experimental period revealed notable changes following the administration of green coffee bean extract, as summarized in ***Table 1***.

Table 1

Effect of green coffee bean extract (GCBE) on the serum lipid profile of female rabbits (Mean ± S.D., n = 10)

Parameter, mg/dl	Control Group	GCBE-Treated Group
Total Cholesterol	79.83±6.94	55.50±11.05*
Serum Triglycerides	98.33±6.80	60.83±8.18*
HDL Cholesterol	8.47±1.69	16.05±2.55*
LDL Cholesterol	40.33±8.01	22.66±3.66*
Very Low Density Lipoprotein	18.38±6.00	8.63±2.36*

Note: Asterisks (*) indicate statistically significant differences compared with the control group (p≤0.05).

In this investigation, we found that GCBE treatment decreased total serum cholesterol, triglycerides, LDL cholesterol, and VLDL-cholesterol levels significantly in the GCBE-treated group when compared with the control untreated group. Specifically, the administration of green coffee bean extract led to a substantial reduction in total cholesterol by 30.48 % and serum triglycerides by 38.14 %. The most pronounced hypolipidemic effects were observed in the atherogenic lipoprotein fractions, where LDL cholesterol and VLDL-cholesterol levels dropped by 43.81 % and 53.05 %, respectively. Conversely, the level of anti-atherogenic HDL-cholesterol increased significantly in the GCBE group, showing a remarkable elevation of 89.49 % when compared with the control untreated group. These percentage alterations demonstrate that GCBE supplementation exerts a robust modulating effect on lipid metabolism, shifting the lipid profile toward a significantly less atherogenic state.

Oxidative stress results

The evaluation of oxidative stress biomarkers and antioxidant enzyme activities in female rabbits after a two-month experimental period demonstrated significant alterations following the administration of green coffee bean extract, as summarized in ***Table 2***.

Table 2

Effect of green coffee bean extract (GCBE) on the serum oxidative stress parameters of female rabbits (Mean ± S.D., n = 10)

Parameter, Units	Control Group	GCBE-Treated Group
GSH, µg/l	5.10±1.46	7.29±1.13*
MDA, nmol/ml	26.21±3.35	21.65±2.89*
SOD, µg/l	4.47±1.15	7.05±1.18*

Note: Asterisks (*) indicate statistically significant differences compared with the control group (p≤0.05).

In this investigation, the results of oxidative stress showed a significant increase in GSH and SOD levels in the GCBE-treated group when compared with the control group, while the MDA results showed the opposite trend between the same groups. Specifically, GCBE administration enhanced the enzymatic antioxidant defense system, resulting in a 57.72 % elevation in SOD activity and a 42.94 % increase in non-enzymatic GSH levels. Concurrently, the treatment successfully mitigated lipid peroxidation, as evidenced by a significant 17.40 % reduction in serum MDA concentrations compared to the untreated control group. These percentage variations strongly indicate that green coffee bean extract possesses potent antioxidant capabilities, effectively scavenging free radicals and reducing systemic oxidative stress in experimental animals.

Antioxidant-rich meals have been shown to reduce blood cholesterol, triglyceride, and low-density lipoprotein levels [33]. In this research, we found that administering the green coffee bean extract to female rabbits reduced total serum cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL-C), and very low-density lipoprotein cholesterol (VLDL-C) levels considerably when compared to the control untreated group. Green coffee bean extract (GCBE) contains chlorogenic acid (CGA), which is a strong antioxidant. Green coffee contains CGA as an active chemical that has been shown to increase metabolic rate [34], increase the oxidation of fatty acids [34, 35], and reduce hepatic triglycerides [36] and levels of total cholesterol [37]. Aside from CGA, coffee contains polyphenols that possess the ability to decrease visceral fat accumulation [38].

In this investigation, we also found that GCBE has a favorable influence on serum HDL levels. Serum HDL levels increased in the GCBE-treated groups compared to the control group. This outcome is consistent with a prior study by Salamat et al. (2018), which showed that GCBE supplementation caused a significant increase in HDL-C in adult men with dyslipidemia who took 800 mg/day of green coffee extract for 8 weeks [20]. However, this finding is in disagreement with Wan et al. (2013), where 28 days of green coffee extract intake at a dose of 40 mg/kg/day lowered high-density lipoprotein levels in male rats, an effect that may be explained by the regulation of PPAR-α expression in the liver [39].

The discrepancy between trials may be attributable to the preparation method of the administered coffee, which should be noted due to the differences in caffeine and phenolic content among coffee brews. The latter is attributed to the coffee-to-water ratio, brewing temperature, cup capacity, and degree of roasting [40]. These findings could also be clarified by the notion that CGA exclusively works through the pro-atherogenic pathway of cholesterol metabolism. Furthermore, a clinical study conducted on obese women between the ages of 20 and 45 showed that GCBE combined with a calorie-restricted diet had an effect on lipid metabolism by causing notable changes in blood levels of low-density lipoprotein, free fatty acids, and total cholesterol [18].

Additionally, Tanaka et al. (2009) found that green coffee bean extract contains a significant amount of chlorogenic acid, which reduces LDL-C, triglyceride, and total cholesterol levels in the liver and serum [41]. The effect of chlorogenic acid in lowering blood lipids is linked to preventing the liver's synthesis, transport, and intestinal absorption of lipids and cholesterol [42]. On the other hand, one of the metabolic effects of chlorogenic acid, present in green coffee, is the suppression of lipogenesis and the amplification of lipolysis [41].

Oxidative stress is usually quantified by comparing oxidative stress markers, such as glutathione (GSH), malondialdehyde (MDA), and superoxide dismutase (SOD), against the formation of free radicals; by monitoring these parameters, the level of oxidative stress in the body can be calculated. In the present study, levels of GSH (5.10 µg/l in control vs. 7.29 µg/l in treated) and SOD (4.47 µg/l in control vs. 7.05 µg/l in treated) were significantly ($p \leq 0.05$) increased in the GCBE-treated group compared to the control group, suggesting that the animals' antioxidant defense status had improved. Conversely, MDA levels were greater in the control group (26.21 nmol/ml) compared to the GCBE group (21.65 nmol/ml), indicating a decrease in lipid peroxidation and a reduction in systemic oxidative stress.

Scientists have become increasingly interested in polyphenol-rich foods because of their antioxidant qualities [43]. Polyphenols (natural compounds), which are present in fruits, vegetables, nuts, and plants, possess potent antioxidant properties and shield the body from oxidative damage. Thus, polyphenols act as therapeutic compounds against oxidative stress [44]. Chlorogenic acid (CGA) is a polyphenol often found in human foods, particularly green coffee beans. CGA promotes endogenous antioxidant mechanisms, which guard against and scavenge free radicals. By encouraging Nrf2 to translocate from the cytosol to the nucleus, it activates sets of antioxidant genes that protect cells from harm. Numerous studies have shown that in sophisticated animal models of a range of chronic and metabolic diseases, CGA supplementation improves the treatment of oxidative damage and free radical generation. Molecular simulations have provided evidence for chlorogenic acid's significant antioxidant action and hydrogen-donating characteristics [45].

Conclusions

Our findings revealed that green coffee bean extract (GCBE) significantly improves the serum lipid profile and reduces systemic oxidative stress levels in female rabbits ($p \leq 0.05$). Specifically, a two-month administration of GCBE led to a substantial reduction in atherogenic lipid fractions (total cholesterol, triglycerides, LDL-C, and VLDL-C) by 30.48–53.05 %, while remarkably elevating anti-atherogenic HDL-C levels by 89.49 %. Furthermore, the extract effectively enhanced the antioxidant defense system, increasing SOD and GSH levels by 57.72 % and 42.94 % respectively, and mitigated lipid peroxidation by decreasing MDA concentrations by 17.40 %. These results add robust scientific evidence supporting the therapeutic potential of GCBE to enhance metabolic health, successfully manage hyperlipidemia, and alleviate oxidative damage.

DECLARATIONS

Ethical Statement

All animal handling, maintenance, and experimental procedures involving female rabbits in this study were conducted in strict accordance with international ethical standards and veterinary-sanitary guidelines for the care and use of laboratory animals in scientific research.

This research was conducted under the official reference number UOK.VET.PH.2025.122 at the anatomical laboratory of the College of Veterinary Medicine, University of Kerbala, Iraq. All efforts were made to minimize animal suffering, and blood samples were collected via cardiac puncture under deep anesthesia without sacrificing the animals.

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Conflict of interest

The authors state that there is no conflict of interest.

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Declaration of AI and AI-assisted technologies

The author declare that no artificial intelligence or AI-assisted technologies were used in the preparation of this manuscript.

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