

# Studying the Effect of Dark Coffee and its Extract on Protection from Hyperlipidemia in Rats

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### Abstract

Coffee contains more than 800 compounds known to have an impact on human health. The purpose of this study is to investigate how ground dark or green coffee or extracted coffee bean affects the serum lipid profile and other related parameters. Sixty eight Sprague Dawely rats were divided into 8 groups (n=8) as follows: **Group 1:** Negative control group (NC) was fed on basal diet during the experimental period. **Group 2:** Positive control group (PC), rats were fed on hypercholesterolemic diet solely. **Group 3:** Rats were fed on hypercholesterolemic diet containing 5% ground dark coffee beans. **Group 4:** Rats were fed on hypercholesterolemic diet containing 10% ground dark coffee beans. **Group 5:** Rats were fed on hypercholesterolemic diet with lower oral standard dose of dark coffee extracted beans (50 ml/ kg diet). **Group 6:** Rats were fed on hypercholesterolemic diet with the higher oral standard dose of dark coffee extracted beans (100 ml/ kg diet). **Group 7:** Rats were fed on hypercholesterolemic diet containing 5% ground green coffee beans. **Group 8:** Rats were fed on hypercholesterolemic diet containing 10% ground green coffee beans. It can be noticed that both dark or green ground coffee and extracts improved lipid profile, where decreased the total cholesterol, LDL-C, VLDL-C and triacylglycerol, however, increased HDL-C when compared to positive control group. Also they lowered the concentration of both uric acid and urea compared to positive control group. These findings indicated to the effect of consumption of coffee to improve lipid profile. To highlight our findings related to other research work we may find that the most research papers reported different doses of dark and green coffee or their extracts, different experimental animals and different sample size. Further studies are needed to include clinical trials.

**Keywords,** green coffee, dark coffee, total cholesterol, triglycerides, LDL-C, HDL-C, VLDL-C.

## دراسة تأثير القهوة الداكنة ومستخلصها على الحماية من ارتفاع دهون الدم في الفئران

## الملخص العربي

من المعروف أن القهوة تحتوي على عدد من المركبات الكيميائية التي يمكن أن يكون لها تأثير على صحة الإنسان، ويعتبر إحتساء القهوة يومياً شائع في جميع دول العالم، والهدف من هذه الدراسة هو التحقق في كيفية تأثير مسحوق القهوة الداكنة أو القهوة الخضراء أو مستخلص بذور القهوة على صورة الدهون في الدم وكلا من وظائف الكبد والكلي. في التجربة البيولوجية تم تقسيم الفئران إلى ٨ مجموعات (٨ فئران لكل مجموعة) كما يلي: المجموعة الأولى: وهي المجموعة الضابطة السالبة، تم تغذيتها على نظام غذائي أساسي خلال التجربة البيولوجية. المجموعة الثانية: (المجموعة الضابطة الموجبة) تم تغذية الفئران على الغذاء العادي ويحتوي على مسحوق الكوليسترول. المجموعة الثالثة: تم تغذية الفئران على الغذاء العادي ويحتوي على مسحوق الكوليسترول وكذلك يحتوي على ٥٪ من مسحوق بذور القهوة الداكنة. المجموعة الرابعة: تم تغذية الفئران على الغذاء العادي ويحتوي على مسحوق الكوليسترول ويحتوي على ١٠٪ من مسحوق بذور القهوة الداكنة. المجموعة الخامسة: تم تغذية الفئران على الغذاء العادي ويحتوي على مسحوق الكوليسترول ويحتوي على ٥٠ مللى من مستخلص بذور القهوة الداكنة / كجم من الغذاء. المجموعة السادسة: تم تغذية الفئران على الغذاء العادي ويحتوي على مسحوق الكوليسترول ويحتوي على ١٠٠ مللى من مستخلص بذور القهوة الداكنة / كجم من الغذاء. المجموعة السابعة: تم تغذية الفئران على الغذاء العادي ويحتوي على مسحوق الكوليسترول ويحتوي على ٥٪. المجموعة الثامنة: تم تغذية الفئران على الغذاء العادي ويحتوي على مسحوق الكوليسترول ويحتوي على ١٠٪. المجموعة التاسعة: تم تغذية الفئران على الغذاء العادي ويحتوي على مسحوق الكوليسترول ويحتوي على ١٠٠ مللى من مستخلص بذور القهوة الداكنة أظهرت أقل قيمة عند تقدير كلاً من (الكوليسترول الكلي، الدهون الثلاثية، LDL-C، VLDL-C، AST، ALT، حمض البوليك ونيتروجين اليوريا) والتي كانت قريبة من نتائج المجموعة الضابطة السلبية. كان لمسحوق القهوة الداكنة أو الخضراء ومستخلص البذور فوائد على مستويات صورة الدهون في الدم حيث انخفض كل من الكوليسترول الكلي، LDL، VLDL والدهون الثلاثية وزيادة HDL. عند مقارنتها بالمجموعة الضابطة الموجبة. كما كان لها فوائد على تركيز كل من حمض البوليك ونيتروجين اليوريا حيث انخفضت مقارنة بالمجموعة الضابطة الموجبة. وأشارت النتائج إلى توصلت لها هذه الدراسة إلى أن استهلاك القهوة يمكن أن يكون مفيداً للمرضى الذين يعانون من ارتفاع الكوليسترول في الدم. وتتوه الدراسة الحالية إلى إجراء مزيد من الأبحاث بجرعات مختلفة من مستخلص القهوة الداكنة والخضراء أو البذور.

**الكلمات المفتاحية:** القهوة الداكنة، القهوة الخضراء، الكوليسترول، الدهون الثلاثية، إنزيم اسبارتات امينوترانسفيريز، حمض البوليك.

## Introduction

Cholesterol has an essential role in human cardiovascular health. Cholesterol has qualities that are both helpful and harmful. HDL-cholesterol, is more well-known than LDL-C, however both are sources of cholesterol. People are more likely to suffer from cardiovascular problems if their blood cholesterol levels are high. When cholesterol builds up in the circulation, it causes the artery walls to thicken and harden, a condition known as plaque that defined as atherosclerosis. Reduced or halted blood flow to the heart is a result of arteries being less elastic, thicker, and more susceptible to cholesterol and plaque occlusions. When blood flow is restricted, chest pain, often known as angina, may develop.

Another kind of fat seen in the blood is triacylglycerol. Elevated levels of triacylglycerol are linked to an increased risk of cardiovascular disease. Triacylglycerol are among the most common types of human fat. An elevated risk of diabetes and cardiovascular disease is linked to elevated triacylglycerol levels. Age and gender have a role in the usual range of triacylglycerol levels. In addition to low HDL-cholesterol or high LDL-cholesterol, and increase blood triacylglycerols speed up atherosclerosis, according to Hongbao (2006). Atherosclerosis is a risk factor for cardiovascular disease and stroke.

Obesity has several negative effects on health, one of which is an increased chance of dying at a younger age. Obesity and its consequences, such as lipedema, are on the rise, and many individuals don't have the patience to adhere to diet regimens. Products claiming to burn fat have sparked an unparalleled buying frenzy throughout the globe. Expanding coffee varieties is also a part of it. Here we take a look at what research has shown about how coffee affects body weight.

Bertsch et al. (2020) states that most individuals with high lipidemia will also have lipedema, which is characterized by the accumulation of loose connective (fat) tissue on the extremities. Puberty, pregnancy, and menopause are all times when hormone levels fluctuate, which may lead to the development of lipedema. Characteristics of this condition include an overabundance of fat around the limbs and a deficiency of fat in the abdominal region. According to Pelled et al. (2016), there's no need to move the limbs because that type of obesity considered being a stubborn obesity that need special treatment strategy to success.

Drinking coffee is a brew that is created by crushing tropical plant beans into a dark brown powder that has a robust aroma and taste. (The Cambridge Dictionary, 2023)

These days, coffee is one of the most consumed beverages worldwide. It has a distinctive flavor come from a group of compounds

exceed 800 components. The two most significant coffee species, *Coffea arabica* and *Coffea canephora* var. *robusta*, account for 60–40% of global production. Robusta is primarily from Vietnam and originates from the lowlands of Central and West Africa, South Asia, and Arabica is typically from South America (primarily from Brazil) and upland and mountainous regions of East Africa (**Pancsira, 2022**).

As a functional food with antioxidant qualities, coffee lowers the risk of death and lowers the incidence of diabetes, cancer, and liver disease. It also offers protection against Parkinson's disease. In rats, green coffee bean extract lowers body weight and visceral fat while also having a hypotensive impact. These characteristics are associated with bioactive substances, including trigonelline, caffeine, theophylline and theobromine, cafestol, kahweol, tocopherols, and chlorogenic acids and their derivatives (**Sualeh et al., 2020**).

Depending on the roasting duration, green coffee beans have up to twice the amount of 5-O-caffeoylquinic acid (5-CQA) than roasted coffee. Coffee's caffeine inhibits hydrogen peroxide-induced lipid peroxidation products in human skin fibroblasts, lowers tissue lipid peroxidation and reactive oxygen species (ROS), and preserves the antioxidant system in hypoxia-induced pulmonary epithelial cells (**Sualeh et al., 2020**).

Arabica coffee is generally considered to be of higher quality than Robusta coffee, particularly among consumers and specialists. Thus, there are ways to raise the standard of Robusta coffee. The distinctively acidic flavor and taste of Arabica coffees is produced by steaming Robusta coffee. Additionally, this treatment eliminates two distinct scents from Robusta: "musty" and "earthy." Coffee beans, particularly Robusta coffee beans, can be steamed to remove compounds that are bad for the stomach, like chlorogenic acids and free diterpenes like cafestol, kahweol, dehydrokahweol, and dehydrocafestol. The steaming parameters can determine the reduction of these chemicals. (**Barrea et al., 2023**).

Coffee beans that have been specially treated with wind to produce monsooned coffee have a nice consistency, low acidity, and a pleasing scent and flavor. The technique of decaffeination, which happens before roasting, is typically connected to Arabica coffee. The most popular and affordable method of decaffeination is organic solvent extraction, which involves the use of water or vapor both before and after the extraction process. Dichloromethane or ethyl acetate are the solvents used by the coffee industry. Coffee's flavor components are lost during the decaffeination process, particularly when using water as a solvent.

Chlorogenic acids and other substances linked to them may also be lost (**Rahmawati et al., 2023**).

This study aimed to investigate the effect of feeding ground dark coffee beans powder and its extract on lipid profile (TC), (HDL-C), (LDL-C), (VLDL-C) and (TAG), liver and kidney function. in hyperlipidemic rats.

## **Materials and Methods**

### **Materials**

#### **Green Coffee**

The green coffee beans were brought and identified by Agriculture Research Center, Giza. Preparation was done on coffee according to the details in the method section.

#### **Diet of rats**

Casein, cellulose, sucrose, choline chloride, D-L methionine, vitamin and mineral components, and other rats' diets were purchased from Modern Lab Company, Dokki, Giza, Egypt. Corn starch, corn oil and sucrose were obtained from local market, Cairo, Egypt.

#### **Chemicals**

Cholesterol and chemicals used during the experiment were purchased from El-Gomhoriya Pharm. and Chem. Ind. Co., Cairo, Egypt. Other chemicals used were of the highest grade available. For biochemical analyses reagent kits were used. For biochemical analyses reagent kits (Spinreact Co., Barcelona, Spain) and Biodiagnostic reagent kit (Bio-diagnostic Worcestershire, UK) were used.

### **Methods**

#### **Chemical Analyses**

Chemical analyses were done on ground coffee after the preparation steps that included washing coffee beans with distilled water, dried in an open air then roasted in a coffee roaster machine (Cafemasy, Guangdong, China) at 220 °C for 15 minutes, finally grind into finely material with coffee machine.

#### **Preparation of Black Coffee Extract**

Dark coffee beans were roasted into ground fine powder. Aqueous extracts of the ground coffee samples were prepared using 1 g coffee per 20 or 40 ml distilled water in higher and lower dose, respectively. Each extraction process takes about 5 minutes to be done and the maximum temperature reached was 90°C according to Farah et al., (2008).

#### **Animal Model for Hyperlipidemia**

The hyperlipidemic model diet for rats was used according to the composition suggested by Rods et al. (1996), 1.5% cholesterol and 10% soybean oil added to the diets of hyperlipidemic rats.

### Experimental Animal Design

Sixty four Sprague Dawley adult male rats were purchased from The National Research Center of Egypt's Animal House, Dokki. The average weight of each rat was about  $150 \pm 10$ g. Rats were housed clean, well-ventilated cages in an air-conditioned room at  $22 \pm 2^\circ\text{C}$  with a 12 h: 12 h light-dark cycle. Rats were fed basal diet, Ain-93G diet (Reeves et al., 1993). After a week of adjusting to the standard casein diet (Reeves, et al., 1993), and tap water *ad libitum*. After adaption period, eight rats in each group were divided into eight groups as follows:

1. **Group 1:** Negative control group (NC) was fed on basal diet during the experimental period.
2. **Group 2:** Positive control group (PC), rats were fed on hypercholesterolemic diet solely.
3. **Group 3:** Rats were fed on hypercholesterolemic diet containing 5% ground dark coffee beans.
4. **Group 4:** Rats were fed on hypercholesterolemic diet containing 10% ground dark coffee beans.
5. **Group 5:** Rats were fed on hypercholesterolemic diet with lower oral standard dose of dark coffee extracted beans (50 ml/ kg diet).
6. **Group 6:** Rats were fed on hypercholesterolemic diet with the higher oral standard dose of dark coffee extracted beans (100 ml/ kg diet).
7. **Group 7:** Rats were fed on hypercholesterolemic diet containing 5% ground green coffee beans.
8. **Group 8:** Rats were fed on hypercholesterolemic diet containing 10% ground green coffee beans.

### Biological Evaluation

Biological evaluation of tested diets was carried by determination of feed intake (FI), body weight gain % (BWG%) and organs weight/ body weight % according to Chapman et al., (1959) using the following equation:

$$\text{BWG \%} = \frac{\text{Final body weight} - \text{Initial body weight}}{\text{Initial body weight}} \times 100$$

$$\text{Organ weight/ body weight \%} = \frac{\text{Organ weight (g)}}{\text{final weight (g)}} \times 100$$

### Biochemical Analyses

At the end of the experiment (6 weeks), the rats were fasted overnight, anaesthetized and sacrificed to obtain blood samples. Each blood sample was placed in a dry clean centrifuge tube, and then centrifuged for 10 min at 2500 rpm to separate the serum. Serum was carefully separated into clean dry Wassermann tubes by using a Pasteur pipette and kept frozen at  $-20^\circ\text{C}$  until analyses. Serum samples were used for determination of lipid profile; triacylglycerol (TAG), total cholesterol

(TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and very low-density lipoprotein cholesterol (VLDL-C) that was calculated using modified Friedewald formula (Friedewald et al., 1972; Francisco et al., 2008). Liver function, i.e., alanine aminotransferase (ALT), aspartate aminotransferase (AST), kidney function, i.e., serum uric and urea nitrogen were done. All biochemical analyses were carried out using reagent kits.

#### **Determination of Lipid Profile**

Triacylglycerol was done according to **Bucolo and David (1973)**, using reagent kit (Spinreact Co., Barcelona, Spain). Sample triacylglycerol incubated with lipoproteinlipase (LPL), liberate glycerol and free fatty acids. Glycerol is converted to glycerol-3-phosphate (G-3-P) and adenosine-5-diphosphate (ADP) by glycerol kinase and ATP. (G-3-P is then converted by glycerol phosphate dehydrogenase (GPO) to dihydroxyacetone phosphate (DAP) and hydrogen peroxide ( $H_2O_2$ ). In the last reaction,  $H_2O_2$  (reacts with 4-aminophenazone (4-AP) and p-chlorophenol in presence of peroxidase (POD) to give a red colored dye. The intensity of the color formed is proportional to the triacylglycerol concentration in the sample and measured spectrophotometrically (BT-260 Plus, Shanghai, China) at 505nm the results expressed as mg/dl.

#### **Determination of Serum Cholesterol**

Serum cholesterol was done according to **Allain et al., (1974)**, using reagent kit (Spinreact Co., Barcelona, Spain), and spectrophotometer (BT-260 Plus, Shanghai, China) at 578nm. The concentration of the sample was calculated and the results were expressed as mg/dl.

#### **Determination of Liver Function**

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined (**Murray, 1984a**), and (**Murray, 1984b**) respectively, using reagent kit (Spinreact Co., Barcelona, Spain), spectrophotometer (BT-260 Plus, Shanghai, China) at 340nm and the results were expressed as U/L.

#### **Determination of Kidney Function**

Serum urea and uric acid were determined according to **Kaplan et al., (1984)**, and **Fossati et al., (1980)** using reagent kit (Spinreact Co., Barcelona, Spain). For urea in the samples were hydrolyzed enzymatically into  $NH_3$  and  $CO_2$ .  $NH_3$  ions formed react with  $\alpha$ -ketoglutarate in a reaction catalyzed by glutamate dehydrogenase (GLDH) with simultaneous oxidation of NADH to  $NAD^+$ . The decrease in concentration of NADH, is proportional to urea concentration in the sample mg/dl. The development of reaction produced a color that was measured using spectrophotometer (BT-260 Plus, Shanghai, China) at 340nm. Furthermore, serum uric acid was determined and the produced



color was measured at 520nm using (BT-260 Plus, Shanghai, China). The amount of protein was calculated by the following equation:

Uric acid concentration (mg/dl) = Absorbance of sample x 60 / Absorbance of standard.

### Statistical analysis

The means, standard deviation (STD) deviation were calculated using Windows Operating System 10 Home Premium, for biochemical analyses. The statistical package for social science (SPSS) version 16 were used to compare all treated groups. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Dunncan Test. Values were expressed as mean  $\pm$  STD and  $p < 0.05$  was considered to be significant (Bailey, 1994).

### Results and Discussions

All groups indicated to significant decrease in FER and BWG (%) as compared to the negative control group especially GCP 5%, the most declining in FER was observed in positive control group as shown Table 1. The present data were in agreement with those obtained by **Lukitasari et al., (2023)** who concluded that green, roasted and decaffeinated coffee resulted in a significant differences of body weight gain and feed intake suggesting that long-term caffeine and coffee consumption may decrease body weight in humans (**Lukitasari et al., 2023**).

**Mehaya & Mohammad (2020)**, indicated that the feed intake for rats fed on Arabic coffee bean showed higher intake than the normal control and experimental rats (**Mehaya & Mohammad, 2020**). However, **Mendoza et al., (2023)** reported that there was none significant effect of any dose of coffee, caffeinated or decaffeinated, on weight gain or feed intake (**Mendoza et al., 2023**).

**Table (1):** Effect of ground dark coffee and its extract on feed intake, FER and BWG (%) in hyperlipidemic rats.

Parameters Animal groups	Mean of Feed Intake (g/day/rat)	FER Mean $\pm$ SD	BWG (%) Mean $\pm$ SD
Negative control (-)	14.1	0.96 $\pm$ 0.18 <sup>a</sup>	34.69 $\pm$ 10.52 <sup>a</sup>
Positive control (+)	4.7	0.43 $\pm$ 0.65 <sup>b</sup>	2.46 $\pm$ 1.05 <sup>bc</sup>
DCSP 5%	3.94	0.54 $\pm$ 0.32 <sup>c</sup>	4.67 $\pm$ 1.87 <sup>c</sup>
DCSP 10%	7.47	0.72 $\pm$ 0.82 <sup>c</sup>	3.47 $\pm$ 3.72 <sup>c</sup>
DCSE 50ml	8.94	0.77 $\pm$ 0.21 <sup>c</sup>	5.67 $\pm$ 1.87 <sup>c</sup>
DCSE 100ml	9.87	0.86 $\pm$ 0.05 <sup>c</sup>	18.77 $\pm$ 8.63 <sup>b</sup>
GCP 5%	4.8	0.42 $\pm$ 0.75 <sup>b</sup>	2.56. $\pm$ 1.06 <sup>bc</sup>

GCP 10%	8.94	0.56 ± 0.32 <sup>c</sup>	4.98 ± 1.87 <sup>c</sup>
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DCSP: Dark coffee seeds powder, DCSE: Dark coffee seeds extract, GCP: Green coffee powder.

\*Non-significant differences between the values had the same letter.

\*LSD: Least Significant Difference at level ( $p < 0.05$ ).

Total cholesterol (TC), triacylglycerol (TAG), low density lipoprotein (LDL-C) and very low density lipoprotein (VLDL-C) were significantly decreased ( $P \leq 0.05$ ) in all treated group when comparing with the positive control group, as shown in Table (2). From this table, it could be noticed that, rats treated with the higher dose (100 ml) dark coffee beans extract recorded the lowest mean value of lipid profile, that approach from negative control group.

On other hand high density lipoprotein (HDL-C) was significantly increased ( $p < 0.05$ ) in treated groups as compared to positive control group.

Numerous epidemiological studies have shown that one of the main underlying causes of mortality in the world is cardiovascular disease (CVD). A key factor in the development and course of CVD is dyslipidemia, which is defined by increased levels of TAG, TC, and LDL-C, and decreased levels of HDL-C. The primary cause of dyslipidemia is the interaction of genetic abnormalities with environmental factors (diet, exercise, and pharmacological effect). Dietary fatty acid composition may have a significant impact on blood lipid profiles (**Schweiker et al., 2022**).

A cholesterol test is a blood parameter that measures the amount of cholesterol and certain fats in the blood. Cholesterol is a waxy, fat-like substance that's found in blood and every cell of the body. The cells and organs need cholesterol to keep healthy pathway and function. The liver makes all the cholesterol of body needs. But cholesterol can also get from the eaten foods, especially meat, eggs, poultry, and dairy products. Foods that are high in dietary fat can also make the liver produce more cholesterol. There are two main types of cholesterol: LDL), or "bad" cholesterol, and HDL, or "good" cholesterol. Elevated levels LDL-cholesterol in blood increases the risk for CVD and other heart diseases. High LDL-C levels can cause also the buildup of a sticky substance called plaque in the arteries. Over time, plaque can narrow arteries or fully block them. When this happens, parts of body don't get enough blood (**Pebriani & Salamah, 2021**).

VLDL-C stands for very-low-density lipoprotein. The liver makes VLDL-C and releases it into bloodstream. The VLDL-C particles mainly carry triacylglycerol, another type of fat, to tissues. VLDL-C is similar to

LDL-C, but LDL-C mainly carries cholesterol to tissues instead of triacylglycerol. VLDL-C and LDL-C are sometimes called "bad" cholesterols because they can contribute to the buildup of plaque in arteries. This buildup and their symptoms are called atherosclerosis. The plaque that builds up is a sticky substance made up of fat, cholesterol, calcium, and other substances found in the blood. Over time, the plaque hardens and narrows arteries. This limits the flow of oxygen-rich blood to body. It can lead to coronary artery disease and other heart disease (Huang & Lee, 2022).

Triacylglycerols are a type of fat. They are the most common type of fat in body. They come from foods, especially butter, oils, and other fats which were eaten. Triglycerides also come from extra calories. These are the calories that eaten, but the body does not need right away. The body changes these extra calories into triglycerides and stores them in fat cells. When the body needs energy, it releases the triacylglycerols. The VLDL-C particles carry the triacylglycerol to the tissues. Having a high level of triacylglycerol can raise risk of heart diseases, such as coronary artery disease (Laufs et al., 2020).

After comparing the treatment groups with the positive control group, a significant decrease ( $p < 0.05$ ) in TC, TAG, LDL-C, and VLDL-C was seen. The HDL-C levels were much greater in the negative control group, however. The positive control group had substantially lower values for HDL-C, higher in TC, TAG, and LDL-C when compared to the normal control group, our results is agreed with literature, i.e., Al-Tamimi et al. (2024). Statistical analysis revealed that HDL-C increased significantly across all treatment groups compared to the positive control group, whereas other lipid profile decreased significantly ( $p < 0.05$ ). All the lipid profile components in this study were improved significantly in all treated groups specifically the extract and green coffee beans groups. Compared to the normal control group, HDL-C levels improved significantly in treated groups. But there was a marked improvement after taking green and roasted coffee beans as an extract.

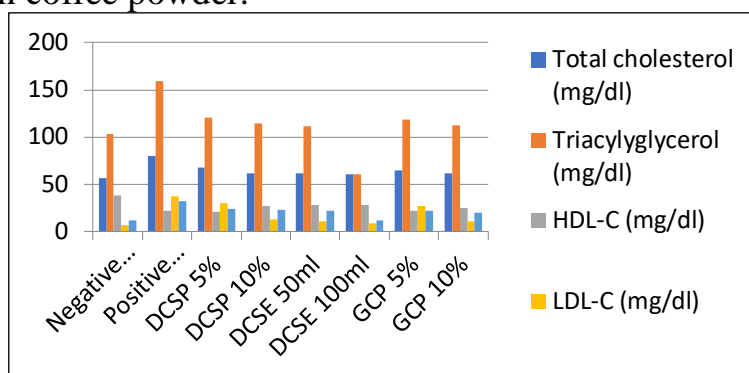
**Table (2):** Effect of dark coffee consumption and its extract on serum lipid profile (mg/dl) in hyperlipidemic rats.

i Profile Animal groups	Total cholesterol (mg/dl) Mean $\pm$ SD	Triacylglycerol (mg/dl) Mean $\pm$ SD	HDL-C (mg/dl) Mean $\pm$ SD	LDL-C (mg/dl) Mean $\pm$ SD	VLDL-C (mg/dl) Mean $\pm$ SD
Negative control (-)	57.00 $\pm$ 1.00 <sup>b</sup>	103.14 $\pm$ 0.50 <sup>ab</sup>	38.33 $\pm$ 5.00 <sup>a</sup>	6.40 $\pm$ 1.00 <sup>c</sup>	12.05 $\pm$ 0.10 <sup>c</sup>
Positive control (+)	80.00 $\pm$ 2.64 <sup>a</sup>	159.17 $\pm$ 1.36 <sup>a</sup>	21.60 $\pm$ 0.50 <sup>d</sup>	37.54 $\pm$ 0.61 <sup>a</sup>	31.73 $\pm$ 0.26 <sup>a</sup>
DCSP 5%	67.66 $\pm$ 18.58 <sup>ab</sup>	120.93 $\pm$ 1.00 <sup>ab</sup>	21.50 $\pm$ 0.50 <sup>d</sup>	29.86 $\pm$ 9.03 <sup>b</sup>	23.78 $\pm$ 1.10 <sup>ab</sup>
DCSP 10%	62.00 $\pm$ 1.00 <sup>b</sup>	114.20 $\pm$ 79.42 <sup>ab</sup>	27.00 $\pm$ 1.00 <sup>dbc</sup>	13.03 $\pm$ 4.06 <sup>c</sup>	22.97 $\pm$ 15.73 <sup>ab</sup>

DCSE 50ml	62.00 ± 0 <sup>b</sup>	111.00 ± 28.33 <sup>ab</sup>	28.00 ± 2.00 <sup>bc</sup>	10.80 ± 1.41 <sup>c</sup>	22.19 ± 5.66 <sup>ab</sup>
DCSE 100ml	61.00 ± 7.81 <sup>b</sup>	60.63 ± 4.78 <sup>cb</sup>	28.66 ± 4.72 <sup>b</sup>	8.65 ± 0.25 <sup>c</sup>	12.12 ± 0.95 <sup>cb</sup>
GCP 5%	64.66 ± 18.58 <sup>ab</sup>	118.93 ± 1.00 <sup>ab</sup>	22.50 ± 0.50 <sup>d</sup>	27.56 ± 9.03 <sup>b</sup>	21.78 ± 1.00 <sup>ab</sup>
GCP 10%	62.00 ± 1.00 <sup>b</sup>	112.20 ± 79.42 <sup>ab</sup>	25.00 ± 1.00 <sup>dbc</sup>	11.03 ± 4.06 <sup>c</sup>	19.97 ± 15.73 <sup>ab</sup>
L.S.D	14.80	61.35	5.53	7.32	12.19

\*Non-significant differences between the values had the same letter.

\*LSD: Least Significant Difference at level (p<0.05). Abbreviations: DCSP: Dark coffee seeds powder, DCSE: Dark coffee seeds extract, GCP: Green coffee powder.



**Figure (1):** Effect of dark coffee consumption and its extract on serum lipid profile (mg/dl) in hyperlipidemic rats. DCSP: Dark coffee seeds powder, DCSE: Dark coffee seeds extract, GCP: Green coffee powder.

Next to that our work using coffee and its extract enhance and improve the sharp symptoms in treated groups that may be explained by the richness of phytochemicals in coffee.

Despite occasional disagreements, several authors have attested to coffee's beneficial effects on human health. It is believed that the antioxidant and anti-inflammatory components of coffee are responsible for the good correlations among treated groups compare to positive groups specifically in liver functions. These results are agreed with Amah et al., (2020) that their research suggests that coffee may have positive effects on the liver.

In our study, when comparing to the positive control group, all treated groups showed significantly reduced levels of ALT and AST. Similar observations were found by Ikeda and co-workers (2010) as they reported that ALT, AST, and gammaglutamyl transferase (GGT) levels in the blood are inversely related to coffee intake. Similarly Amah and co-workers (2020) found after following 30 days of coffee consumption, the mean plasma activity of AST and ALT decreased significantly in female participants but was much greater in male ones. Surprisingly, the results of Amah and co-workers. (2020), found that female patients had higher increases in ALT levels compared to AST levels.

The liver is the most vital digestive organ in the body. It is responsible for metabolizing xenobiotics through different pathways, such as hydrolysis, conjugation, oxidation, and reduction and hydration.

There are various factors that are involved in the development and advancement of liver diseases. These include viral infection, inadequate nutrition, xenobiotic exposure, ethanol and drug abuse, and metabolic diseases. Being fatal, liver diseases are a major cause for concern for international public health. Most of the synthetic drugs have adverse side effects, which is why they are not presently suitable for treating liver injury. Hence, a lot of attention is being given to complementary and alternative medicines for treating hepatic disorders. There are several medicinal plants that consist of compounds having hepatoprotective activities like phenols, flavonoids, carotenoids, alkaloids, xanthines, coumarins, monoterpenes, and essential oils (**Chupradit et al., 2022**).

The antioxidant and anti-inflammatory effects of coffee are also likely to be responsible for the mechanism behind the beneficial associations between coffee consumption and varying degrees of liver disorders. Furthermore, several authorities have in time pasted documented different beneficial effects of coffee on various aspect of human health, although some of the author reported conflicting findings. Importantly, consumption of coffee has been shown in literature to possess beneficial potentials for the **liver (Amah et al., 2020)**.

All treated groups were significantly decreased in the concentration of both enzyme Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) as compared to positive control group. The obtained data were in agreement with that of Ikeda et al., (2010) who showed that coffee consumption is inversely related to serum levels of liver enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gammaglutamyl transferase (GGT) (**Ikeda et al., 2010**). Moreover, (**Amah et al., 2020**) found that in male subjects, the mean plasma activities of AST and ALT were significantly increased consumption of coffee for 30 days in female subjects significantly in plasma. In female subjects ALT increased more than AST did (**Amah et al., 2020**).

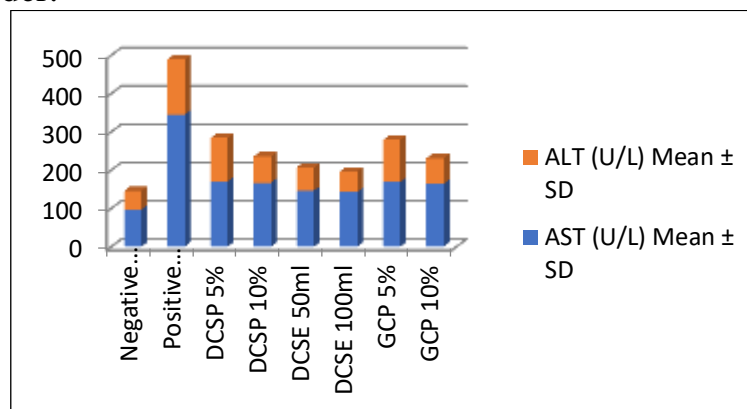
**Table (3):** Effect of dark coffee consumption and its extract on serum liver functions AST and ALT levels (U/L) in hyperlipidemic rats.

Liver function	AST (U/L) Mean ± SD	ALT (U/L) Mean ± SD
<b>Animal groups</b>		
Negative control (-)	97.50 ± 11.56 <sup>c</sup>	48.60 ± 16.19 <sup>c</sup>
Positive control (+)	345 ± 6.00 <sup>a</sup>	143.90 ± 1.05 <sup>a</sup>
DCSP 5%	170.70 ± 6.93 <sup>b</sup>	113.25 ± 1.35 <sup>b</sup>
DCSP 10%	166.95 ± 1.56 <sup>bc</sup>	68.67 ± 5.38 <sup>c</sup>
DCSE 50ml	146.70 ± 1.00 <sup>bc</sup>	60.40 ± 1.00 <sup>c</sup>
DCSE 100ml	142.70 ± 93.39 <sup>bc</sup>	54.03 ± 33.36 <sup>c</sup>
GCP 5%	168.70 ± 6.93 <sup>b</sup>	110.25 ± 1.35 <sup>b</sup>

GCP 10%	163.95 ± 1.56 <sup>bc</sup>	66.67 ± 5.38 <sup>c</sup>
L.S.D	68.688	27.259

\*Non-significant differences between the values had the same letter.

\*LSD: Least Significant Difference at level ( $p < 0.05$ ). DCSP: Dark coffee seeds powder, DCSE: Dark coffee seeds extract, GCP: Green coffee powder.



**Figure (2):** Effect of dark coffee consumption and its extract on serum liver functions AST and ALT levels (U/L) in hyperlipidemic rats. DCSP: Dark coffee seeds powder, DCSE: Dark coffee seeds extract, GCP: Green coffee powder.

Our result showed the concentration of both of uric acid and urea nitrogen were significantly decreased ( $p < 0.05$ ) as compared to positive control group (Table 4). It could be noticed that, rats treated with 100 ml dark coffee beans extract, recorded the lowest mean value (uric acid and urea nitrogen) which was near to the negative control. Chronic kidney disease is a lifestyle-related disease known to affect many Egyptians. This disease may progress and weaken the function of the kidneys, which is termed as chronic renal failure. Hemodialysis, which is one type of treatment for patients with renal failure, uses a dialysis-type artificial kidney called a dialyzer, and it is currently the most widely used form of treatment administered to patients with renal failure (Kameda, Horikoshi, Kumagai, Saito, & Yoshioka, 2020). Coffee intake reduced gout risk by decreasing urate and urea levels in plasma. These findings accentuate the benefits of coffee intake for gout management. The mediators may provide a novel insight into potential therapeutic targets for gout prevention. That is in line with our results which indicate to the decrease significantly the levels of uric acid and urea nitrogen in the treated groups. These findings suggest that coffee has potential as a gout remedy. These mediators may open the door for novel gout treatments, according to Qin et al. (2024).

Also studies that support our work, a study done by Mahmoud and co-workers (2013) which shows that individuals with hypercholesterolemia and hyperuricemia may experience coffee's positive benefits to varied degrees.

**Table (4):** Effect of dark coffee consumption and its extract on serum kidney function uric acid and urea nitrogen levels (mg/dl) in hyperlipidemic rats.

<b>Kidney function</b>	<b>Uric acid (mg/dl)</b>	<b>Urea nitrogen (mg/dl)</b>
<b>Animal groups</b>	<b>Mean ± SD</b>	<b>Mean ± SD</b>
<b>Negative control (-)</b>	<b>1.28 ± 0.01<sup>b</sup></b>	<b>32 ± 5.29<sup>b</sup></b>
<b>Positive control (+)</b>	<b>2.56 ± 1.0<sup>a</sup></b>	<b>145.00 ± 2.00<sup>a</sup></b>
<b>DCSP 5%</b>	<b>2.13 ± 1.35<sup>ab</sup></b>	<b>61.00 ± 54.56<sup>b</sup></b>
<b>DCSP 10%</b>	<b>1.80 ± 0.34<sup>ab</sup></b>	<b>37.00 ± 0<sup>b</sup></b>
<b>DCSE 50ml</b>	<b>1.85 ± 0.05<sup>ab</sup></b>	<b>38 ± 1.00<sup>b</sup></b>
<b>DCSE 100ml</b>	<b>1.36 ± 0.40<sup>ab</sup></b>	<b>35.67 ± 0.57<sup>b</sup></b>
<b>GCP 5%</b>	<b>2.23 ± 1.35<sup>ab</sup></b>	<b>65.00 ± 54.56<sup>b</sup></b>
<b>GCP 10%</b>	<b>1.90 ± 0.34<sup>ab</sup></b>	<b>39.00 ± 0<sup>b</sup></b>
<b>L.S.D</b>	<b>1.2806</b>	<b>39.848</b>

\*Non-significant differences between the values had the same letter.

\*LSD: Least Significant Difference at level ( $p < 0.05$ ). Abbreviations: DCSP: Dark coffee seeds powder, DCSE: Dark coffee seeds extract, GCP: Green coffee powder.

### Conclusion and Recommendations

Our study looked at how coffee and its derivatives affect the lipid profile, kidney and liver functions in Hyperlipidemic rats. Ground dark or green coffee and extracts had benefits on lipid profile levels where decrease both of TC, LDL-C, VLDL-C and TAG and increase in HDL-C when compared to positive control group. Also, showed benefits on the concentration of both uric acid and urea this decreased as compared to positive control group. Similar results found in liver function. These findings indicated that consumption of coffee could be beneficial for patients with hypercholesterolemia, elevated liver function enzymes or hyperuricemia. According to our results it could be recommended that the consumption of coffee could be beneficial for patients' related condition. Further clinical trials are needed.

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