

Effect of Green Coffee Bean Extract on Steroid Hormones Synthesis, Blood Lipids and Body Weight in Rats

Research Article

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Abstract

The aim of this study was to investigate the effect of Green coffee extract on body weight, blood lipids and steroid hormones synthesis on rats.

The extracts of green and roasted coffee were prepared using Methanol 80%. 35 rats have been divided to 5 equal groups, the first group was fed by normal diet without supplementing, and the other four groups were fed by high fat diet. The second groups was left without any supplementation while the third and fourth group were fed by 1 ml of green coffee extracts 0.5% and 1% respectively. The fifth group was supplemented by 1 ml of roasted coffee extract 1%. After 6 weeks the weight gain and food intake was calculated and the serum was taken to test blood lipids and ACTH, FSH, LH Hormones.

The results showed that the supplementation by 1% Green coffee extract lead to significant decrease in blood lipids and cholesterol, Also it showed significant increase in FSH hormone comparing by other groups. While there is no difference between all groups in ACTH and LH hormones. On the other hand, the high fat diet didn't cause significant change in weight gain compared to regular diet. Coffee analysis showed that the roasting process may lead to liberation of caffeine and decrease in chlorogenic acid content with no difference in phenol content.

Keywords: Green Coffee; Steroid Hormones; Blood; Rats; Human Nutrition.

Introduction

Chlorogenic Acids are one of the most important Hydroxycinnamic Acids that fall under the group of phenolic acids and these acids are found in a range of foods such as fruits, vegetables, and coffee, especially green coffee (unroasted coffee beans) which are the richest natural sources of these acids [41, 11].

Chlorogenic acids are effective in many biological activities within the body, where they have an anti-dyslipidemic, anti-hyperglycemic effects with anti-obesity properties in humans and animals. It is believed that they improve fat and glucose metabolism and thus improving insulin sensitivity through several mechanisms at the level of gene expression [41, 37].

Recent studies have indicated that consumption of green Coffee Extract (GCE) result in body weight reduction without affecting the food intake. It improved serum lipids through several proposed mechanisms. Suggested mechanisms include affecting

fat absorption, increasing beta oxidation, reducing the rate of lipid biosynthesis by inhibiting lipid and cholesterol regulating enzymes, and affecting Sterol regulatory element-binding protein (SREBP). However, the mechanisms of action of chlorogenic acid are not fully understood and need additional studies [41, 37]. Despite the encouraging results for the use of green coffee extract as a nutritional supplement supporting weight loss, there is an urgent need to know its effect on steroid hormone synthesis, as most studies have been shown that, the mechanism of chlorogenic acid effects are mostly on the levels of fat and cholesterol synthesis, and enzymes involved in its synthesis in particular [37]. It is also known that cholesterol is an essential element in the biosynthesis of steroid hormones [3], which may raise a question of the extent to which these acids affect the biosynthesis of these hormones, and what may stress this question is that some women in gulf countries using green coffee as traditional medicine to prevent pregnancy temporarily "as they claim". According to studies available to the researcher, the effect of green and roasted coffee extract on the steroid hormones synthesis and its relationship to

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the weight loss and blood lipids has never been studied.

Objectives

This study aimed to investigate the effect of fortification with green coffee extract at two concentrations of 0.5% and 1% in rats fed a high-fat diet and compared this effect with roasted coffee extract at a concentration of 1%, then to investigate its effects on the following:

1. Luteinizing Hormone (LH) and Follicle-Stimulating Hormone (FSH) levels in the serum.
2. Levels of adrenocorticotrophic hormone (ACTH) in the serum.
3. Change in body weight and level of blood lipids.

Literature Review

Cholesterol and Steroid Hormones

Cholesterol is of great importance for many vital functions within the body as it is part of cell membranes, neurotransmitters, and is an important precursor of bile acids and steroid hormones. Most cholesterol is synthesized in the liver and can be synthesized in other locations such as the intestine, adrenal cortex, and reproductive tissue [3].

The synthesis of cholesterol begins with the production of a compound [3-Hydroxy-3-methylglutaryl CoA (HMG CoA)] from Acetyl CoA molecules, after which the enzyme HMG CoA Reductase converts it into Mevalonate which enters into a subsequent reactions ending with the formation of cholesterol, this enzyme is a rate-limiting enzyme and is One of the most important key enzymes regulating cholesterol synthesis [19].

Cholesterol synthesis is regulated by several regulators that affect the rate-limiting enzyme, including: Sterol regulatory element-binding protein (SREBP), which acts as a transcription factor and increases the synthesis of HMG CoA Reductase. It was also regulated by hormonal regulation as high insulin levels will lead to increase gene expression of this enzyme which increases its synthesis. Statins used to treat hypercholesteremia act by inhibiting the same enzyme. After the cholesterol synthesis in the liver, It will be stored in the cholesterol pool, and then either transported to the gallbladder to produce bile acids that contribute to the digestion and absorption of fats, or will transported to peripheral tissues by lipoproteins in plasma [3].

Steroid hormones are one of the most important products of cholesterol metabolism, and their synthesis begins by converting cholesterol into Progenenolone which is a major precursor for the rest of steroid hormones that are synthesized in several places including the adrenal cortex, sex glands, uterus and brain [39, 3].

Steroid hormones can be classified into [56, 39, 3]:

1. **Corticosteroid hormones:** they are made in the adrenal cortex and include a group of hormones under two important classifications, glucocorticoids and mineralocorticoids.
2. **Sex hormones:** include androgens, estrogens and progestins.

Green Coffee

Green Coffee Contents

The coffee belongs to the Rubiaceae family, which includes more than 90 species [59], the most famous of which are *Coffea arabica*, which accounts for nearly three quarters of global coffee production, and *Rubista* coffee, or the so-called *Canefora* [2].

Green coffee is one of the most important and richest natural sources for chlorogenic acids, the content of which varies depending on the type of coffee, environmental and agricultural conditions, processing and storage methods [9]. *Coffea arabica* contain higher amount of fat and Trigonelline, and lower level of caffeine [2] and chlorogenic acid compared to *Rubista* coffee. chlorogenic acid estimated to be around 8.1% of Arabic coffee dry weight [15, 40], and the estimated daily intake of chlorogenic acid in coffee consumer is between 0.5-1 g/day [5].

Chlorogenic acids are compounds of cinnamic acids (Caffeic Acid, P-Coumaric Acid, and ferulic acid) that bind to ester bonds with Quinic acid. These acids are found in green coffee in several forms and are named depending on the type of cinnamic acid associated with Quinic acid such as Caffeoylquinic acids-CQA, dicaffeoylquinic acid-diCQA, feruloylquinic acids-FQA, and P-coumaroylquinic P-CoQA, each of them falls under at least 3 forms. The most important chlorogenic acid esters are Caffeoylquinic acid – CQA, which represents about 80% of the total chlorogenic acids in green coffee include 3-,4-,5 - Caffeoylquinic acid (3-,4-,5-CQA).

Impact Of Roasting Processes On Green Coffee Contents

The degrees of coffee roasting vary depending on the duration of the roasting process and the temperature used. It is noted that roasting at low temperatures for a long time results in different characteristics of coffee, and gives a different flavor as opposed to roasting at high temperatures for a short period of time which gives a higher quality coffee product [59].

When roasting coffee, Caffeine content remains relatively constant [59], and may increase due to the loss of other compounds, and it possible to decrease due to volatilization which is likely to occur but in small limits [8]. Fat, glycerol and sterols contents in coffee are thermally stable and not significantly affected by the roasting process [50]. As a result of the thermal effect, a combination of reactions occurs, such as caramelization and Millard reaction, where amino acids interact with reduced sugars to form the Melanodin compound (the final product of the Millard reaction). Melanodine is one of the compounds that contribute to the distinctive color of coffee and account for 25% of the dry weight of roasted coffee. Also, Trigonelline is converted to Nicotinic Acid, which increases as intensity of roasting increase, and is available in higher amount in heavily roasted coffee [8].

Effect of Green Coffee Consumption

Effect of green coffee on body weight

Studies on green coffee extract are still limited, and most research in this field has been focusing on studying the effect of chlorogenic acid as the effective compound in green coffee or studying the effect of coffee drinks “in general” on body weight.

Although there are no studies on the effect of roasted coffee "as an extract" on weight-according to the sources available to the researcher- the consumption of coffee in general "as a drink" had different results on body weight, some had a positive effect and others had no effect, which could be due to several factors such as coffee type, degree of roasting, active ingredients, and amount consumed [51].

One of the most important studies on green coffee extract is that of Shimoda et al., (2006) who fortified a regular diet with either green coffee extract or caffeine at a concentration of 0.5% or 1%, or chlorogenic acid at a concentration of 0.15% or 0.3% -of the food weight -, and examined the effect on body weight and fat accumulation in mice compared to the control group that consumed 0.1% of Orlistat. The study showed that adding green coffee extract to food at 0.5% and 1% may be effective in weight loss as it resulted in a significant decrease ($p \leq 0.05$) in gained weight and Visceral fat compared to the control group.

Tanaka et al. (2009) did a similar experiment, in which he examined the effect of one-month fortification of diet with green coffee extract at 1% of the food weight on rats, and they found that consumption of green coffee extract led to a significant decrease ($p \leq 0.05$) in body weight and fat tissue without affecting food intake compared to the control group fed on a normal diet only (both groups diet consisted of 5% cholesterol of food weight).

To test the effect of decaffeinated green coffee extract, [49] tried to add the extract to high-fat diet by 0.1%, 0.3%, 0.9% and chlorogenic acid by 0.015% - of food weight- and comparing its effect by 2 control groups which depend on regular diet and high fat diet without fortifications, to examine the effect of the decaffeinated green coffee extract on obesity and insulin resistance. It was concluded that the fortification with the extract resulted in a significant decrease ($p \leq 0.05$) in body weight without affecting food intake compared to the control group. This effect could be reached through down-regulating of genes responsible for the synthesis of fat and inflammatory factors in visceral fat tissue in mice.

Effect of green coffee on blood lipids

Tanaka et al [53] tried to study the effect of high-cholesterol food supplemented by 1% of green coffee extract for a group of rats for a month. The study showed that the extract significantly reduced the level of triglycerides in the serum and liver with increased oxidation of fatty acids, and reduced lipid synthesis by reducing the activity of Fatty Acid Synthase (FAS) ($p \leq 0.05$), without affecting the levels of HDL-C, TC, TC/HDL-C ratio.

In another study, supplementing a high-fat diet "20% fat" with decaffeinated green coffee extract in different proportions (0.1%, 0.3% and 0.9% of food weight) for 11 weeks resulted in a significant decrease in TAG, TC and free fatty acids levels in rats whose diet was supplemented with 0.3% and 0.9% green coffee extract compared to the control group. The study showed that green coffee extract modifying the effect of high fat diet through affecting genes responsible for fat synthesis [49].

Shimoda et al. [48] studied supplementation of 200 and 400 mg of green coffee extract, 20 and 40 mg of caffeine per kilogram of body weight which result in significant reduction ($p \leq 0.01$) on serum triglycerides in a similar effect to Orlistat, while they did not

find any effect for supplementation of 60 and 120 mg chlorogenic acid. In another experiment, it was found that supplementation of mice diet with 1% green coffee extract increased beta-oxidation activity by improving the activity of the enzyme carnitine palmitoyltransferase (CPT) ($p \leq 0.01$) in rat's liver.

On the other hand, the addition of chlorogenic or caffeic acid to a high-fat food, at a dose equal to 0.02% of the food weight, resulted in a significant decrease ($p \leq 0.05$) in plasma triglycerides and cholesterol levels compared to the high-fat group (control), and did not affect HDL-C levels. Supplementation of these acids was shown to significantly reduce fatty acid synthesis by inhibiting the activity of Fatty Acid Synthase, HMG-CoA Reductase and acyl-CoA:cholesterol acyltransferase (ACAT), and increases beta oxidation and gene expression of peroxisome proliferator activated receptor- α (PPAR- α) compared to the control group ($P \leq 0.05$) [4]. When testing the daily injection of chlorogenic acid 80 mg/kg bw into the hamster's peritoneum for 8 weeks while fed on a high-fat "15% fat" diet and comparing to a control group that relied on a high-fat diet only, chlorogenic supplemented group had a significant decrease ($p \leq 0.05$) in the levels of triglycerides, total cholesterol, free fatty acids (FFA), and increased hepatic lipase activity, decreased lipoprotein lipase (LPL) activity in muscles and increased gene expression of PPAR- α in the liver compared to the control group. The researchers suggested that one of chlorogenic mechanisms is through influencing the gene expression of PPAR- α which is considered as one of the key regulators for fat and glucose metabolism [31].

Recently, Meng et al., (2013) review many studies about the effect of chlorogenic acid on lipid and glucose metabolism, including one by Lee et al., where chlorogenic acid was found to have an inhibitory effect on the HMG-CoA Reductase (in Vitro) more than Simvastatin effect (drug used to treat high cholesterol levels).

When compare the effect of filtered coffee -roasted in two degrees "LightMedium Roast" and "medium roast"- on cardiovascular disease risk factors in a group of women who used to consume coffee on a regular basis, they were instructed to consume 3-4 cups of filtered coffee a day for a month (5 g of mild or moderately roasted coffee prepared in a 150 ml cup), and it was found that consumption of slightly and moderately roasted coffee resulted in a significant increase ($p \leq 0.05$) in LDL-C by 12% and 14%, and total cholesterol levels by 10%-12% respectively, and there was no significant difference on these effects between the two different roasting degrees. Medium-roasting coffee significantly increased the HDL-C level by 7% ($p \leq 0.05$), with no effect of coffee consumption on blood level of TAG [6].

Karabudak et al., [24] studied the effect of Turkish coffee consumption at an average of 62.3 ml/day and instant coffee at an average of 116.3 ml/day on a group of healthy people (122 individuals) and compared it to those who do not consume coffee, they found no significant difference in blood lipid levels ($P \leq 0.05$).

Effect of green coffee on steroid hormones synthesis

A number of studies have been conducted to investigate the effect of coffee drink -in general- or caffeine -as an effective compound- and its relationship to sex hormones levels or the potential impact on fertility and reproduction. Among them Kitts (1987) studied the effect of roasted coffee extract on a group of

non-adult mice for 3 consecutive days by injecting the extract into the stomach directly by 0.5g/15g of body weight, and it shows similar effect ($P \leq 0.05$) to estradiol-by 10 nanograms/15 grams of body weight- However, when injected together, the effect of estradiol is stronger because they compete for the same binding sites in the uterus, and it was concluded that coffee contains weak estrogenic compounds and the effects of coffee consumption may vary depending on the amount consumed, which requires additional studies.

In another study, the effect of caffeine and coffee consumption on ovulation-related hormones such as FSH, LH and Estradiol (E2) was examined by Lucero et al., [33] where the study was conducted on a group of ladies with an average age of 40.5 years, none pregnant or lactating and not using any type of hormonal therapy. It demonstrate a correlation between increased Body Mass Index (BMI) and low levels of Sex Hormone Binding Globulin (SHBG) ($P \leq 0.05$). Cholesterol consumption (greater than 217 mg/day) and consumption of more than one cup of coffee per day were also found to be positively correlated with E2 level ($p \leq 0.05$), and consumption of more than 500 mg/day lead to a 70% increase in E2 compared to those who consumed less than 100 mg / day. To compare the effect of consuming caffeinated or decaffeinated coffee on sex hormones (Estradiol, testosterone) and SHBG proteins, [60] conducted a small 8-weeks study on a group of healthy coffee consumers, and it showed no difference between the groups ($p \leq 0.05$). consumption of caffeinated coffee lead to increase in the total testosterone level in men and decrease it in women. Decaffeinated coffee led to a decrease in the free and total testosterone level in women, but all of these results were not significant by the end of the study. The researchers stated that these results may not reflect the true effect due to the small sample size and additional studies on larger groups were urgently needed.

When injected intravenously into a group of ovary-excised cattles at 20 mg / kg bw, caffeine was found to have no significant effect ($p \leq 0.01$) on the secretion of gonadotropin-stimulating hormones from the pituitary gland compared to the control group injected with Saline only [44].

To investigate the relationship between ovulation and caffeine intake greater than 300 mg/day, a number of pre-menopausal women of were interviewed by telephone "regarding consumption of caffeinated beverages, nature of life, demographic characteristics and functional level" with urine samples collected to examine estrogen and progesterone metabolites, but they conclude that there is no strong association between caffeine consumption and risk of fertility decline ($P \leq 0.05$) [12].

The effect of caffeine and caffeinated beverages on sex hormones has also been investigated by Schliep et al.,(2012) who conducted a study on a group of premenopausal women and the results showed that caffeine consumption greater than or equal to 200 mg per day was inversely correlated ($p \leq 0.05$) with the level of free estradiol in white women.

When Ezzat and El-Gohary, 1994, investigated the effect of male rabbit caffeine ingestion by 30-60 mg/kg for 4 weeks, it was shown to increase the level of FSH and decrease LH with no effect on ACTH ($p \leq 0.05$), and microscopic examination showed that, ingestion lead to inhibition of sperm synthesis with reduced

sperm tubule size, and fat formation around the liver, and Signs of stimulation of steroid synthesis appeared in the adrenal gland. The study concluded that long-term consumption of caffeine may suppress sperm production.

It was found that supplementing with yacon plant extract by 200 mg/kg or chlorogenic acid by 5 mg / kg for 5 weeks resulted in an increase in sperm count ($P \leq 0.05$) by 20% and 34%, respectively, and inhibiting testosterone breakdown in rat liver ($P \leq 0.05$). The two researchers once again examined the effect of yacon plant extract and ferulic acid, which is one of the metabolites of chlorogenic acid "with the same previous quantities". It was found that supplementation by yacon plant extract and ferulic acid increases the number of sperms ($P \leq 0.05$) to 43% and 37%, respectively. The study concluded that yacon extract increases sperm production, which may be due to its content of phenolic acids and its inhibition of the testosterone catabolism process in the liver.

Materials and Methods

Preparation of green and roasted coffee extracts

Green coffee beans "unroasted" of the Harrar coffee - a strain of *Coffea arabica* - were purchased from the local market in Riyadh, where past of it was roasted at a medium roasting temperature (130 ° C for 8 minutes) by a toaster connected to a scale Thermal (YÜCEL, Turkey). Then, green and roasted coffee were ground and sifted with a 1 mm² sieve to increase extraction efficiency. After that, 80g of "green or roasted" ground coffee was weighed and then submerged in 800 ml of methanol solvent at a concentration of 80% (methanol 80: 20 distilled water) and extracted using an inverter condenser on a 70° C water bath for two hours [52, 48]. Thereafter, the extracts were filtered by Whatman filter paper No. 1 (Sigma-Aldrich, St. Louis, MO, USA), and then the solvent was removed using a rotary evaporator Rotavapor R-210/215 (Büchi®, Switzerland) after setting it at a temperature of 40 ° C and a pressure of 40 and 140 revolutions per minute until the weight was constant and the extracts were condensed, then the extracts were weighed by calculating the difference between the weight of the vessel before and after evaporation [26] he result was calculated using the following equation:

$$\text{yield} = (\text{extract weight}/\text{sample weight}) \times 100$$

The extracts were then stored in opaque glass bottles at 4°C until the doses were made and the active compounds were determined.

Dosing preparation

The dose of green coffee extract in 0.5% and 1% concentration was prepared by drawing 0.5 ml and 1 ml of concentrated green coffee extract and dissolving it in 99.5 ML and 99 ml of distilled water respectively for a total volume of 100 ml, and the dose of roasted coffee extract was prepared by dissolving 1 ml of roasted coffee extract in 99 ml of distilled water. Doses are prepared weekly and kept in 4°C refrigerated in opaque glass vials until use.

Analysis of green and roasted coffee extracts active ingredients

- Determination of total phenols: Total phenols in both green

and roasted coffee extract were estimated using Folin - Ciocalteu Reagent solution following Skasonen method [25] where 2.5 ml of distilled water was added in a test tube with 0.1 ml of the extract and then 0.1 ml of undiluted Folin solution. After mixing well, it was left for 6 minutes then 0.5 ml of 20% sodium Carbonate solution was added and left for 30 minutes at a temperature of 20 °C until the reaction was completed. Then, the absorption level was then read at wavelength of 760 nm by a Spectrophotometer (Biochrom Ltd., Cambridge, England). The concentration was calculated from the standard curve for gallic acid.

- Determination of Caffeine and Chlorogenic acids: the level of chlorogenic acid and caffeine in the two extracts was estimated by Ultra-Performance Liquid Chromatography (UPLC) using two standard samples of chlorogenic acid and caffeine supplied by Sigma (Sigma-Aldrich, St. Louis, MO, USA). The samples were prepared for injection by dissolving a known amount of the sample in deionized water, and the UPLC was adjusted to the following conditions: -

Machine: Waters ACQUITY UPLC® system equipped with a quaternary pump system (Milford, MA, USA).

Column: acquity BEH C18 column (Waters, Milford) of dimension 50 mm × 2.1 mm id, and 1.7µm particle size.

Mobile phase: binary, 70% [Formic Acid +water (0.1% v/v)] + 30% Methanol.

Flow rate: 0.3 ml/min.

sample injection: 5 µL.

Experiment animals: 35 Wistar-Albino (male) rats weighing 181.7 ± 5 g were selected from the Center for experimental animals, Faculty of Pharmacy, King Saud University. The rats were placed in separate stainless steel cages with controlled temperature and humidity throughout the study period, and maintain 12-hour dark/light cycle with free access to water and food.

Experiment design: The rats were randomly distributed into 5 groups so that in each group there were 7 rats and they were acclimatized on experiment conditions for one week, after which each group underwent its own feed for 6 weeks (Table-1).

The first group was fed a normal diet in accordance with the recommendations of the American Institute of nutrition- AIN 93-Purified Rodent Diet (diets, Bethlehem, PA), while the rest four groups underwent a high Fat diet - AIN 93-Purified Rodent Diet – (32% fat, 14% beef tallow) (Dyets, Bethlehem, PA). Group I and Group II were designed to be control groups, and Group II was used to compare the impact of supplementation between groups. Third and fourth groups were given 1 ml (oral) as daily dose of green coffee extract with concentration 0.5% and 1%,

respectively, in addition to access to high-fat food. The fifth group was given 1 ml (oral) as daily dose of roasted coffee extract at 1% concentration in addition to high-fat food as previous groups.

Weight Assessment and biochemical analysis

The rats were weighed weekly to track changes in weight. After the end of the experiment period, the final weight of the rats was taken and to calculate the weight gained, the difference between the final and the initial weight was calculated. After a 12-hour fasting period the rats were anesthetized with Diethyl ether, blood samples were taken directly from the heart and collected into Serum Separator Tube (SST) containing gel without any additives. It was left for two hours at room temperature, then the serum was separated using a centrifuge (2000 rpm / 10 minutes), then the serum was kept at -20 °C until the biochemical analyzes were carried out as follows:

Blood lipids and cholesterol levels

These analyzes were performed by using pre-prepared Kits solutions provided by the United Company (United Diagnostic Industry-UDI, Dammam, KSA), using a Spectrophotometer (Biochrom Ltd., Cambridge, England). Total cholesterol levels (TC) were estimated according to [1], triglycerides (TAG) according to the method [14, 36], and high density lipoprotein cholesterol (HDL-C) according to the (Phosphotungstic Acid Method). LDL-C cholesterol was calculated mathematically according to the manufacturer's instructions.

Hormone levels affecting steroid synthesis

The levels of FSH, LH and ACTH hormones in the serum were analyzed by the Enzyme-Linked Immunosorbent Assay (ELISA) method using Kits (Cusabio Biotech Co., Wuhan, China) according to the manufacturer's instructions.

Statistical Analysis

The data were analyzed using the SPSS (Statistical Package for Social Sciences), and the results were presented as (mean ± standard error). The One Way-Analysis of Variance (One Way-ANOVA) test was used for comparison between groups at a significant level ($P \leq 0.05$). Duncan test performed in case there were differences between groups for "homogeneous data". For the heterogeneous data, we checked the differences between groups using Welch test, and if there is differences it was determined using the Games-Howell test [30].

Table 1. Distribution of animal groups according to the study design.

Group	Group(1)	Group(2)	Group(3)	Group(4)	Group(5)
Diet type	Normal diet (ND)	High-Fat Diet (HFD)	High-Fat Diet (HFD)	High-Fat Diet (HFD)	High-Fat Diet (HFD)
Green coffee extract dose	-	-	0.50%	1%	
Roasted coffee extract dose	-	-	-	-	1%
Number of rats	7	7	7	7	7

Results and Discussion

Coffee extracts and their content of the active compounds

- **Coffee extracts:** 16.6 g and 18.2 g of green and roasted coffee extracts were obtained, respectively, for each 80 gm of ground coffee (about 20.8% for green coffee weight and 22.8% for roasted coffee weight). The density was around to 1.8 g / ml for the two extracts.

- **proportion of active compounds in coffee extracts:** When estimating the content of the daily intake of total phenol, chlorogenic acid and caffeine per 1 ml of the prepared dose given to the experimental group (Table -2), it was found that the dose of green coffee extract at a concentration of 1% contains higher significant amount of chlorogenic acid and less caffeine content comparing with 1% roasted coffee extract ($P \leq 0.05$), while there were no statistically differences in the phenolic content between these two doses. The analysis show that the dose of green coffee extract 0.5% have lower ($P \leq 0.05$) content of phenolic acids, chlorogenic acid, and caffeine compared to the other two doses (1% roasted and green coffee). These results are consistent with what Jaafar et al. who reported that there are no significant differences in the total phenolic content between green coffee and light medium roast coffee [16], and are also consistent with what Farah et al. [8] reported that roasted coffee contains more caffeine than green coffee.

Effect of coffee extracts on body weight and food intake

High-fat food intake did not lead to a significant difference in weight gain between the first and second control groups (Table-3). This may be due to the short period of experimentation and the animals were not in the stage of growth.

We find that this significance did not appear by comparing the second group with both the first and fifth groups (the control group and the group fortified with 1% roasted coffee extract),

and therefore it is not possible to judge the effect of the coffee extract or roasting on the gained weight and therefore that there is an appetite suppressant effect. Rats in all groups gained weight during the experiment although the numbers varied from one group to another.

The results did not show clear significant differences between the groups through which one can conclusively judge the effect of coffee extract with its three doses on weight. While we find that, there are significant differences ($p \leq 0.05$) in weight gained between the fourth group "supplemented by 1% green coffee extract GCE" and both the second and third groups "group of high fat diet and the group supplemented by 0.5% green coffee extract" (Table-3). We find that this significance disappears by comparing the second group with both the first and fifth groups (the control group and the group fortified with 1% roasted coffee extract), and therefore it is not possible to judge the effect of the coffee extract on the weight gained, nor appetite suppressant effect. Table (3) is showing the effect of the total food intake with no significant effect between groups.

The results of this study differ with St-Onge et al. [51] stating that fortification with green coffee extract results in reduced dietary intake combined with reduced weight. This finding also differs from that reported by Song et al. (2014), which stated that fortification of food with multiple proportions of green coffee extract- up to 0.9% of food weight- did not affect food intake, and is consistent with Tanaka et al. (2009) that the supplementing food with 1% green coffee extract does not affect food intake. These differences may be due to the different type of coffee used or the method of fortification, or dosing, as this study relied on the administration of fixed daily dose while the previous two studies relied on the fortification of food only. Therefore, the amount of extract consumed per day may vary based on the food intake.

Effect of coffee extracts on blood fat and cholesterol levels

The study showed that eating a high-fat diet led to a significant increase ($P \leq 0.05$) in the level of total cholesterol TC compared

Table 2. Average (\pm standard error) amount of active compounds (mg / mL) in daily doses of green and roasted coffee extracts.

Extract	Total phenols	Caffeine	Chlorogenic acid
	(mg/ml)	(mg/ml)	(mg/ml)
Green coffee extract 0.5%	0.464 \pm 0.006 ^a	0.69 \pm 0.007 ^a	0.302 \pm 0.007 ^a
Green coffee extract 1%	0.927 \pm 0.013 ^b	1.39 \pm 0.014 ^b	0.604 \pm 0.013 ^c
Roasted coffee extract 1%	0.919 \pm 1.013 ^b	1.46 \pm 0.015 ^c	0.521 \pm 0.012 ^b

Means that do not share the same letter have significant differences ($P \leq 0.05$).

Table 3. The effect of green and roasted coffee extracts on body weight and daily intake for rats fed a high-fat diet.

Group no.	Diet type	Initial weight (g)	Final weight (g)	Weight gain (g)	Total food intake (g)	Daily food intake
1	Normal diet	302.9 \pm 2.7 ^a	338.3 \pm 7.5 ^{ab}	134.4 \pm 6.1 ^{ab}	827.9 \pm 10.9 ^{bc}	19.7 \pm 0.3 ^{bc}
2	High fat diet (HFD)	202.7 \pm 5.7 ^a	371.9 \pm 16.2 ^b	169.1 \pm 13.4 ^b	837 \pm 6.4 ^c	19.9 \pm 0.2 ^c
3	HFD + 0.5% GCE	203.4 \pm 2.9 ^a	346.3 \pm 14.6 ^{ab}	142.9 \pm 14.4 ^b	800.3 \pm 7.5 ^{ab}	19.1 \pm 0.2 ^{ab}
4	HFD + 1% GCE	203.1 \pm 3.1 ^a	305.4 \pm 13.9 ^a	102.3 \pm 12.5 ^a	773.1 \pm 12.9 ^a	18.4 \pm 0.3 ^a
5	HFD + 1% RCE	203.4 \pm 1.4 ^a	338.3 \pm 13.8 ^{ab}	134.9 \pm 12.5 ^{ab}	806.6 \pm 10.9 ^{bc}	19.2 \pm 0.3 ^{bc}

GCE: Green Coffee extract, RCE: roasted coffee extract, HFD: High fat diet

Means that do not share the same letter have significant differences ($P \leq 0.05$)

to the normal food group (Table -4) except for the high-fat group fortified with a dose of 1% roasted coffee "the fifth group", there was no significant difference. Also, it was found that the fortification with a dose of 0.5% and 1% of green coffee extract leads to a significant decrease ($P \leq 0.05$) in the level of TC compared to the second "control" group, with significant differences ($P \leq 0.05$) in the effect between the two groups supported with green coffee extract (Table -4).

The study did not show significant differences between the "first" control group and the fifth group "fortified with 1% roasted coffee extract" in LDL-C levels. Otherwise, there are significant differences ($P \leq 0.05$) between the control group and all "second, third and fourth" high-fat meals in lowering TAG levels or raising HDL-C levels.

There was no clear effect of 0.5% dose of green coffee extract on the levels of TAG, LDL-C and HDL-C, while the effect was significant ($P \leq 0.05$) when increasing the dose to 1% of green coffee extract on the levels of both TAG and LDL-C.

As a sum of the results (Table -4), it can be confirmed that there is a significant effect ($P \leq 0.05$) of fortifying high-fat meals with a dose of 1% green coffee on cholesterol blood lipids in serum. High-fat meals "whether they are not fortified or fortified with doses of 0.5% Green coffee or 1% roasted coffee" led to variable results that cannot be judged on a clear effect on blood lipid and cholesterol levels. Nevertheless, supplementation with green coffee extract or roasted in different doses in this study had a significant effect ($P \leq 0.05$) on the ratio between total cholesterol / high-density lipoprotein cholesterol (TC/HDL-C) which carry good health benefits (Table -4).

Looking at the effect of coffee in general, we find that these results was consistent with what Jaafar M. et. al. [16] who reported that consuming green or roasted coffee as "light roasting" led to a significant ($P \leq 0.05$) decrease in LDL-C levels and an increase in HDL-C levels, and that roasted coffee has less effect than green and lightly roasted coffee. On the other hand, some previous studies linked coffee consumption with high blood cholesterol [34], and this was not shown in this study.

The effects of coffee extract are generally consistent with some previous studies that reported that it improves blood lipid levels and leads to a reduction in TAG levels [4, 48, 49, 53], Total cholesterol levels [58, 49, 43, 31, 4] and lowers LDL-C levels [58, 31]. While there are other studies that do not agree with these results

and indicate that green coffee extract or chlorogenic acid leads to a decrease in the level of HDL-C [58, 31], there are even studies that have shown no effects on Triglycerides levels [21].

The results obtained in this study on the effect of roasted coffee differ with what was mentioned in some studies that coffee leads to an increase in TC and LDL-C [6, 22] and is consistent with the studies reported that coffee consumption increases the level of HDL-C [22], and these differences may be due to the different method of supplementation and preparation.

Effect of coffee extracts on hormones affecting steroid hormone synthesis

The results of the study (Table -5) indicate that there are no significant differences between the five study groups on the activity of ACTH and LH hormones, which we could conclude that there is no effects of coffee supplementation on these hormones under study conditions.

On the other hand, we find that the matter is different when looking at the effect on FSH levels (Table -5), as it was shown that green coffee extract at a dose of 1% ($P \leq 0.05$) was significant in affecting FSH compared to other groups. Other than that, there were no differences between groups, neither in the ratio of LH / FSH or FSH / LH. Despite the clear effect that appeared on the hormone by consuming a concentration of 1% of green coffee, it is not possible to conclude a recommendation regarding the effect of supplementation on human because there is no significant effect of this concentration on the hormone FSH or the ratio of LH as the two hormones work together to perform their roles in the body.

Studies that have investigated the effect of coffee on hormones that influence steroid synthesis or sex hormones remain limited. Studies in this aspect showed conflicting results, some of which showed an inverse association between coffee or caffeine and estradiol [28, 32, 45], and others showed a positive association between them [33].

The results of this study are similar to the results of Ezzat and el-Gohary [7], which showed that caffeine consumption leads to an increase in FSH while not affecting the hormone ACTH, as the researchers concluded through which caffeine consumption in long term may affect sperm production. The current study shows that the effect of green coffee on hormones was greater than roasted coffee, despite the latter was containing more caffeine.

Table 4. The effect of green and roasted coffee extracts on blood lipids in rats fed with a high-fat diet.

Group no.	Diet type	TG	LDL-C	HDL-C	TC	TC/HDL-C
		(mg/dl)	(mg/dl)	(mg/dl)	(mg/dl)	
1	Normal diet	81.6 ± 2.5 ^a	18.9 ± 3.2 ^a	71.6 ± 3.1 ^a	106.8 ± 5.5 ^a	1.5 ± 0.03 ^a
2	High fat diet (HFD)	183.1 ± 7.4 ^b	147.3 ± 7.1 ^b	23.9 ± 1.3 ^b	207.9 ± 6.9 ^b	8.8 ± 0.3 ^b
3	HFD + 0.5% GCE	162.4 ± 3.1 ^b	129.9 ± 5.9 ^b	28.1 ± 1.2 ^{bc}	190.5 ± 5.4 ^c	6.9 ± 0.5 ^c
4	HFD + 1% GCE	130.5 ± 6.3 ^c	95.2 ± 6.4 ^c	35.2 ± 2.9 ^c	156.5 ± 4.5 ^d	4.6 ± 0.4 ^d
5	HFD + 1% RCE	101.9 ± 4 ^d	53.9 ± 9.7 ^a	121.3 ± 6.9 ^c	121.3 ± 6.9 ^a	2.8 ± 0.4 ^c

HDL-C: High-density lipoprotein cholesterol, LDL-C: Low-density lipoprotein cholesterol, TC: Total cholesterol, TG: triglyceride, TC/HDL-C: ratio of total cholesterol to high-density lipoprotein cholesterol
Means that do not share the same letter have significant differences ($P \leq 0.05$)

Table 5. The effect of green and roasted coffee extracts on hormones affecting steroid hormones synthesis on rat fed a high-fat diet.

Group no.	Diet type	ACTH	LH	FSH	LH/FSH	FSH/LH
		(Pg/ml)	(mIU/ml)	{mIU/ml}		
1	Normal diet	19.4± 1.96 ^a	4.4 ±1 ^a	10.5 ±1.8 ^a	0.5 ±0.1 ^a	2.6 ±0.6 ^a
2	High fat diet (HFD)	20.2 ±2.1 ^a	3.7 ±0.7 ^a	12.6 ±1.6 ^{ab}	0.3 ±0.1 ^a	5.2 ±1.6 ^a
3	HFD + 0.5% GCE	24.6 ±2.8 ^a	3.3 ±1.1 ^a	12.3 ±1.5 ^{ab}	0.3 ±0.2 ^a	18.6 ±9.8 ^a
4	HFD + 1% GCE	19.7 ±3.04 ^a	3.02 ±0.6 ^a	28.5 ±3.1 ^c	0.1 ±0.03 ^a	14.6 ±5.3 ^a
5	HFD + 1% RCE	17.2 ±3.01 ^a	4.1 ±0.96 ^a	18.9 ±2.4 ^b	0.3 ±0.1 ^a	7.3 ±2.2 ^a

ACTH:Adrenocorticotropic Hormone, FSH:Follicle-Stimulating Hormone, LH: luteinizing Hormone
Means that do not share the same letter have significant differences ($P \leq 0.05$)

The increase in FSH level when supplementing with green coffee extract 1% may be due to its content of chlorogenic acid or because it contains other compounds that decrease with the roasting process, such as the compound Trigoniline.

The supplementation may have resulted in primary hypogonadism, which is usually associated with a low level of inhibin and elevated FSH or LH hormones, or both [35], and this may indicate a lack of sperm or Testicular failure [23]. This may occur as a result of several factors such as exposure to radiation, injury, overheating, or drugs, and the pituitary gland responds to this defect by secreting additional amounts of FSH [35]. However, these explanations cannot be determined, and extensive studies are necessitated.

Conclusion

A number of conclusions can be drawn from the results of this study, namely:

1. Under the conditions and duration of the experiment, the increase in fat intake did not lead to a significant difference in the weight gained compared to the normal diet.
2. The experiment did not show significant differences in the weight gained or the total food intake when using doses of 0.5% and 1% of green or roasted coffee extract compared to normal food.
3. There was a significant effect ($P \leq 0.05$) to fortify a high-fat diet with a dose of 1% of green coffee extract on levels of blood lipids and cholesterol. Otherwise, the high-fat food, whether un-supplemented or supplemented (with doses of 0.5% of green coffee extract or 1% of Roasted coffee extract) led to variable results that cannot be judged on a clear effect on blood lipid and cholesterol levels.
4. The results of the study did not verify any significant results or differences between the five groups on the activity of the hormones ACTH and LH, which concludes with it that in the conditions and duration of this experiment there is no effect of coffee, whether it is green or roasted, at the level of the two hormones.
5. It was evident that supplementing animal diets with green coffee extract 1% led to a significant increase ($P \leq 0.05$) in FSH level compared to lower doses of green and roasted coffee.
6. Roasting coffee may increase the release of caffeine and decrease the amount of chlorogenic acid, and it does not lead to significant effects on the phenols contents.

Recommendations

Through the results of this study, a number of recommendations can be presented that can be put on the research table:

1. It is recommended to increase the time period of the experiment to check long term effect of green coffee on blood fats, weight, LH, FSH and ACTH hormones.
2. It is recommended to study other types of coffee and to establish higher doses of extracts, to find out their effect on LH, FSH, ACTH, blood lipids and weight gain.
3. This experiment was based on the study of the effect of giving animals separate doses from the diet of coffee extract to find out the effect on the variables under study. It is suggested that to study the effect of adding coffee ground (without extraction) in different doses on the same food to compare results.
4. It is suggested to conduct direct research on human, to study the effects on the variables studied here.

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