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Effect of Arabic and Green Coffee Beans on Lowering Lipid Profile Parameters in Male Rats

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ABSTRACT

This research was carried out to evaluate the effects of using powder and extract of Arabic and green coffee to improve body weight, the serum total cholesterol; triglycerides, lipoprotein fractions (HDLc, LDLc and VLDLc), atherogenic index (AI) and leptin hormone in different groups hypercholesterolemia male rats. Caffeine, chlorogenic acid and total phenolic content were determined in Arabic and green coffee. The results showed that the green coffee had contained the highest amounts from caffeine, chlorogenic acid and total phenolic content (2.10%, 9.74% and 366.0 mg/100g) whereas, Arabic coffee was 1.5%, 7.80% and 206.0 mg/100g, respectively. At the end of experimental biological (4 weeks) the resultant observed that the rats group fed orally on green coffee powder were significantly decreased in body weight and feed efficiency ratio than rats group fed orally on Arabic coffee powder. The serum total cholesterol; triglycerides, lipoprotein fractions (HDLc, LDLc and VLDLc) and atherogenic index (AI) were improved in hypercholesterolemia male rats fed orally on green and Arabic coffee may be the green and Arabic coffee had contained the highest amounts in antioxidant and total phenolic acid. The rats fed orally on green and Arabic coffee showed that the lowering leptin hormone may be caused significant positive associations with adiponectin and total and low-density lipoprotein cholesterol, and inverse associations with leptin. The results concluded that feeding with Arabic coffee and green coffee had contained the highest amounts in antioxidant and total phenolic acid and it was improved lipid profile parameters and level of leptin hormone. From the obviously results, it could be recommended that the Arabic and green coffee used for to improve weight serum lipids and leptin hormone.

INTRODUCTION

Coffee is among the most widely consumed pharmacologically active beverages in the world. Caffeine is the most widely consumed psychoactive substance. Coffee is rich in phenolic compounds with a strong antioxidant activity (Parliament, 2000). Phenolic compounds are secondary metabolites and generally involved in plant adaptation to environmental conditions (Vaast *et al*., 2006). They are well recognized as potentially protective factors against human chronic degenerative diseases, such as cancer and cardiovascular disease (Nkondjock, 2009). Regular drinking of coffee can reduce the oxidation of human Low-Density Lipoprotein (LDL) and the oxidation of LDL, decreasing the risk of atherosclerosis (Delgado-Andrade and Morales, 2005).

Roasting is an essential step in coffee production for generating aroma, flavor and color of the coffee beans. The mode of heat transfer and the applied temperature profile are the most critical processing parameters that affect the physical and chemical properties of roasted coffee beans (Schenker *et al*., 2002). The chemical reaction changes include Maillard reaction or non-enzymatic reaction, browning reaction and Strecker degradation of proteins, sugar, polysaccharides and other components. The degrees of roasting are controlled by roasting time and temperature and are necessary for the required chemical reactions without burning the beans

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and compromising the flavor of the beverage (Mendes, 2001). The degrees of roasting were qualitatively assessed from color and classified as a light, medium or dark roast (Clarke, 1985). However, over-roasted coffee could reduce antioxidant activity (Del Castillo *et al*., 2002 and Summa *et al*., 2007). Also Parliament (2000), found the major compositional changes occurring are the decrease of phenolic compounds and the formation of brown, water-soluble polymers called melanoidins, although decrease in protein, amino acids and other compounds is also described.

Coffee is one of the most commonly consumed beverages in the world that its beneficial effects on human health have been a subject of many studies (Tanaka *et al.* 2009). One of the common traditional forms of coffee is green coffee extract (GCE) that prepared from green or raw (unroasted) coffee been. It is also present in roasted coffee, but much of the GCE is destroyed during the roasting process (Higdon and Frei, 2006).GCE has been introduced as the richest sources of cholorogenic acid and most of weight losing effects of GCE has proposed to be related to its cholorogenic acid content (Song *et al.* 2014). It is also found in prunes (Stacewicz-Sapuntzakis *et al.* 2001), but the richest source of cholorogenic acid is green coffee bean extract (Higdon and Frei, 2006). Therefore, the objective of this paper was to review the results of the studies assessing the efficacy of GCE as a weight loss supplement.

Weight management is a long-standing goal of achieving a healthy lifestyle. Therefore, it is important to find a safe and effective way to reduce the body weight of overweight or obese people. In this regard, studies focusing on the components of the diet are very important. The components of the diet may work synergistically to prevent or promote weight management. Recently, green coffee introduced as the richest sources of cholorogenic acid that can play a role in weight loss (Mehnoosh *et al.* 2015).In 2005, the World Health Organization stated that 1.6 billion people were overweight and 400 million were obese. It estimates that by the year 2015, 2.3 billion people will be overweight and 700 million will be obese (WHO, 2015).

Greer *et al*. (2001) revealed that caffeine ingestion promotes glucose consumption with an increase in blood epinephrine, while pre-exercise consumption promotes ventilation and enhances lipolysis (Ryu *et al*., 2001). Chlorogenic acid, another main constituent of coffee beans, has recently been reported to selectively inhibit hepatic glucose-6-phosphatase Arion *et al.* (1997) which is a rate-limiting enzyme involved in gluconeogenesis. However, roasting of coffee beans has been shown to reduce the content of chlorogenic acid in coffee (Del Castillo *et al*., 2002). Green coffee beans are rich in chlorogenic acid and its related compounds that show hypotensive effect (Suzuki *et al*., 2002).

Chlorogenic acid is found in high concentration in coffee beans, has recently been identified as a selective inhibitor for the production of glucose in liver Naldini *et al.* (2002).It was found that raw coffee beans consist of higher concentration of chlorogenic acid as compared to roasted coffee beans Olthof *et al.* (2001).Meanwhile, caffeine, the main component in coffee enhances physical endurance & capabilities, hence promotes energy utilization and lipolysis.

Thus, the purpose of the present study was to assess in vivo some nutritional properties derived from regular consumption of green and Arabic coffee bean and their extracts especially its potential effect on lipid profile and on antioxidant status in male rats

MATERIALSAND METHODS

Materials:

Arabic coffee beans and green coffee beans were obtained from local market in Saudi Arabia.

Caffeine and 3-O-caffeoylquinic acid (chlorogenic acid, 3-CQA) were purchased from Sigma-Aldrich Chemical.

Kits of lipid parameters and leptin hormone were obtained from Bicon Diagnosemittel GmbH and Co. KG Hecke 8 made in Germany.

Methods:

Roasting green coffee beans:

The green coffee beans were roasted within 24h of evaluation in order to ensure a fresh brew using a laboratory roasted (Probat BRZ 4, Rhein, Germany). The beans were roasted to medium level roast and allowed to rest for at least eight hours. The roasted samples were ground using a sample grinder (Probat vtv-633T, Rhein, Germany) not more than 15 min.

Determination of total phenolic contents for Arabic and green coffee beans:

Total phenolics acids were determined with Folin Ciocalteu reagent according to the method of Singleton et al. (1999) using gallic acid as a standard phenolic compound and the absorption was measured at 750 nm using a spectrophotometer. The concentration of total phenolic compounds of all fractions of Arabic and green coffee beans were determined as milligrams of gallic acid equivalent/100g sample (GAE/100g sample).

Analysis of chlorogenic acid and caffeine by HPLC:

For the determination of caffeine and chlorogenic acid levels in the coffee brews, a hot water extraction method was employed (Vitorino *et al*., 2001); the extraction was followed by dilution with 100 mL/0.5 g of distilled water and, finally, HPLC analysis with a Shimadzu Brand chromatograph (model M10AVP, Japan) equipped with a C-18 reverse-phase column (Shimadzu 100 mm long x 0.3 mm ID, 4, 6 µm particle size, Japan). The HPLC was coupled to a UV/visible spectrophotometric detector (Shimadzu SPD-10A model) connected by an interface (CBM-101) to a microcomputer for data processing. The conditions of analysis used were as follows: flow (1 mL/ min); mobile phase (methanol, water and acetic acid in a ratio of 20:80:1); injection volume was 20 µL and wavelength detection at 272 nm. The concentrations of the compounds were determined with standard concentration curves.

Biological experimental:

Preparation of brews:

A filter coffee brews was prepared according to the methodology described by Lima et al. (2010), 100 g of each Arabic and green coffee powder were added in commercial filter paper, and then, 1000 mL of deionized water at 90 °C were poured into the coffee contained in the filter. The coffee brews extracts were prepared at the time and taken orally to different rat groups.

Experimental design and animal groups:

Male albino rats Sprague Dawley strain (36 animals) weighing 170-180g. Animals were housed in individual cages with screen bottoms and fed on basal diet for one week. It consisted of casein 120g, corn oil 80g, cellulose 10g, salt mixture 50g, vitamin mixture 10g, colin 0.4g and corn starch 729.6gaccording to Sheyla *et al.* (2005).

The first main group (6rats) was fed on basal diet and considerable as control negative. The second main group (30 rats) was fed on hypercholesterolemia diet for eight weeks to induce hyperlipidemia after that the second main group was divided into five groups (each group consisted of 6 rats).The main characteristics of the experimental diets can be summarized as reported in the Table (1) for four weeks according to Sheyla *et al.* (2005).

Table 1: Composition of the diets (g/1000g of diet)

Ingredients	Control negative	Control positive	Group 1	Group 2	Group 3	Group 4
Casein	120	120	120	120	120	120
Corn starch	729.6	719.6	619.6	619.6	719.6	719.6
Soybean oil	80	80	80	80	80	80
Cholesterol	00	10	10	10	10	10
Coline	0.4	0.4	0.4	0.4	0.4	0.4
Salt mix.	50	50	50	50	50	50
Vitamin mix.	10	10	10	10	10	10
Cellulose	10	10	10	10	10	10
Coffee	------	-----	100	100	------	------
Coffee extract	------	-----	-----	------	2 ml/day	2 ml/day

Group 1 fed on basal diet substituted with 10% Arabic coffee
Group 2 fed on basal diet substituted with 10% green coffee
Group 3 fed on basal diet and administered orally with 2ml/day Arabic coffee
Group 4 fed on basal diet and administered orally with 2ml/day green coffee

Biological evaluation:

During the experiment period (28 days), the quantities of diet consumed and / or wasted were recorded every day. In addition, rat's weight was recorded weekly. At the end of the experiment period, the rats was fasted overnight before sacrificed, and the blood samples were collected from each rat and centrifuged to obtain the serum. Serum was carefully separated and transferred into dry clean Ebendorf tubes and kept frozen at-20ºc till analysis as described by Schermer (1967).

Hearts were removed from each rat by careful dissection, cleaned from the adhesive matter, washed by a saline solution, dried by filter paper, weighed and kept in formalin solution (10%), according to the method described by Drury and Wallington(1980).

Biological Parameters:

Food intake (FI), body weight gain (BWG), feed efficiency ratio (FER) and organ relative weights as a percent of total body weight were calculated according to Chapman *et al.* (1959).

Biochemical analysis:

Serum total cholesterol, triglyceride, HDL-c, LDL-c, VLDL-c and leptin hormones were determined according to Allain *et al.* (1974), Trinder and Ann (1969), Lopes – Virella *et al.* (1977), Friedwald *et al.* (1972), Catherine *et al.*(2003) and Heymsfield *et al.* (1999), respectively.

Statistically analysis:

The data obtained in the present study was analyzed by ANOVA. For all analyses, when a significant difference ($p \leq 0.05$) was detected in some variable, the data means test was applied to evaluate the difference between the samples. The results were analyzed with the aid of the software SAS System for Windows SAS (2008).

RESULTS AND DISCUSSION

Antioxidant and total phenolic acid in Arabic and green coffee:

Antioxidant as caffeine and chlorogenic acid (CGA) and total phenolic acid were determined in Arabic and green coffee and the results are reported in Table (2). From the resultant it could be noticed that the green coffee was higher in caffeine, CGA and total phenolic acid (2.10%, 9.74% and 366.0 mg/100g) than Arabic coffee was 1.5%, 7.80% and 206.0mg/100g, respectively. The results showed that the green coffee beans, significantly decreases in caffeine, chlorogenic acid and total phenolic acid after roasting may be caused the thermal effect on the antioxidant during the drying of green coffee.

Antioxidant activity of coffee is related to chlorogenic, ferulic, caffeic, and *n*-coumaric acids contained in it (Nicoli *et al*., 1997). In roasted coffee, melanoidins (brown pigments) are synthesized these are strong antioxidants (Steinhart et al., 2001). In some publications, caffeine and trigonelline are considered to be antioxidants also (Farah and Donangelo, 2006). Phenyl alanines which are formed during the roasting process show high antioxidant activity also (Farah and Donangelo, 2006), as do heterocyclic compounds (Fuster *et al*., 2000).

Interestingly, the compound, such as chlorogenic acid and polyphenols, which contributed to the antioxidant activity in coffee, is geographically related (Mullen *et al.*, 2013). The coffee fruit was found to have more chlorogenic acids (CGA) in Arabica coffee fruit planted in Mexico and India compared to the coffee fruit grew in China. In addition, evidence indicates that extraction procedures could affect the antioxidants contents in coffee fruit as well as the caffeine content (Mullen *et al*., 2011). It has been shown that the antioxidant activity was high in coffee fruit extract with low caffeine concentration in comparison with coffee fruit powder.

Table 2: Antioxidant and total phenolic acid in Arabic and green coffee:

Raw materials	Caffeine %	Chlorogenic acids %	Total phenolic acids mg/100g
Arabic coffee	1.50±0.12	7.80±0.76	206.0±5.43
Green coffee	2.10±0.25	9.74±0.82	366.0±7.36

Total phenolic calculated and expressed as mg gallic acid equivalent per 100 g dry weight (mg GAE/100g dry weight).

Biological evaluations:

Body weight gain, feed intake and feed efficiency ratio:

At the end experimental biological the feed intake values showed significant decrease ($P \leq 0.05$) in control positive (C ve+) group as compared to normal rats group (12.28 ± 0.12 and12.39 ± 0.09 g/28day, respectively). All treated groups indicated significant decrease as compared to positive control group as shown in Table (3).

From the same Table (3) it could be observed that the mean value of the positive control group in body weight gain (BWG %) was non-significantly higher than negative control group (11.30 ± 0.09 and11.28 ± 0.08%, respectively). All groups indicated significant differences as compared to the control group positive except coffee green extract was significant decreased. The decreased in body weight and feed efficiency ratio in the rats group fed on green coffee powder and orally may be caused the green coffee rich amounts from caffeine and chlorogenic acid as natural antioxidant and total phenolic acid.

Calculation of feed efficiency ratio, results of (FER) illustrated significant increased of control positive compared to control negative group (3.29 ± 0.05 and 3.25 ± 0.04, respectively). Whereas all groups were recorded that significant increasing ($P<0.05$) values except green coffee extract group was than control negative and positive rats. The present data are in agreement with those obtained by Sadeek *et al.* (2010) who concluded that green, roasted and decaffeinated coffee resulted in a significant differences ($p \leq 0.05$) of body weight gain and feed intake suggesting that long-term caffeine and coffee consumption may decrease body weight in humans. Gafaar *et al*. (2013) indicated that the feed intake of the diabetic control rats was higher than the normal control and experimental rats fed on Arabic coffee bean.

Table 3: Effect of Arabic and green coffee on feed intake/day (FI), body weight gain (BWG %), and feed efficiency ratio (FER) of hypercholesterolemia rats (M±SD)

Groups	FI/day	BWG	FER
Control negative	12.39 ± 0.09 [a]	11.28 ± 0.08[bc]	3.25 ± 0.04[d]
Control positive	12.28 ± 0.12[bc]	11.30 ± 0.09[bc]	3.29 ± 0.05[bc]
Group 1	12.28 ± 0.03 [d]	11.34 ± 0.12[ab]	3.30 ±0.06[bc]
Group 2	12.26 ± 0.03[d]	11.43 ± 1.02[a]	3.33 ± 0.01[a]
Group 3	12.27 ± 0.05[cd]	11.34 ± 0.03[ab]	3.30 ± 0.02[ab]
Group 4	12.29 ± 0.09[ab]	11.21 ± 0.04[c]	3.26 ± 0.04[cd]

Different letters (a, b, c, d, etc.) differ significantly at p≤0.05, while those with similar letters are non-significantly different.

Relative heart weight:

Relative heart weight value showed no significant increase in control positive group as compared to normal rats group (0.37 ± 0.07 and 0.33 ± 0.04, respectively). All treated groups indicated non-significant differences as compared to positive control group, as shown in Table (4).

These results for heart were in line with that of Lopez-Garcia *et al.* (2006) who reported that there is no evidence that coffee consumption increases the risk of CHD. In accordance to the present study, Bonita *et al.* (2007) found that only heavy consumption (> 6 cups/day) of boiled unfiltered coffee is harmful to the heart. Also, Floegel *et al.* (2012) found that coffee consumption does not increase the risk of chronic disease, but it may be linked to a lower risk of type 2 diabetes.

Table 4: Effect of Arabic and green coffee forms on relative heart weight of hypercholesterolemia rats (M±SD)

Groups	Heart (g)
Control negative	0.33 ± 0.04 [b]
Control positive	0.37 ± 0.07[ab]
Group 1	0.31 ± 0.04 [b]
Group 2	0.39 ± 0.06 [a]
Group 3	0.31 ± 0.07 [b]
Group 4	0.31 ± 0.06 [b]

Different letters (a, b, c, d, etc.) differ significantly at p≤ 0.05, while those with similar letters are non-significantly different.

Biochemical evaluation:

Serum lipid profile:

Data of Table (5) showed that the mean values of total cholesterol levels was recorded significant increase in positive control group as compared to negative control group (210 ± 13.9 and 124.67 ± 13.2mg/dl), respectively. Green extract group (127.33mg/dl) near to normal rats followed by Arabic extract and green powder were 132.0 and 143.33mg/dl, respectively.

In regarded to triglycerides (TG)levels showed significant increase in positive control group as compared to negative control group (172.5 ± 26.94 and 90±9.4 mg/dl), respectively. All treated group showed significant decreases (P≤0.05) when comparing with the positive control group especially Arabic powder group which lower than normal values.

The obtained results are agreement with Shimod *et al*. (2006) who reported that serum and hepatic TG levels were lowered with intravenous administration of chlorogenic acid. However, the triglycerides (TG) level in the adipose tissue was not lowered. Therefore, chlorogenic acid is suspected to be effective on hepatic TG, and not adipose TG.

Table 5: Effect of Arabic and green coffee forms on lipid profiles of hypercholesterolemia rats (M±SD)

Groups	Total Cholesterol mg/dl	Triglyceride mg/dl
Control negative	124.67 ± 13.2[d]	90 ± 9.4[cd]
Control positive	210 ± 13.9[a]	172.5 ± 26.94[a]
Group 1	152.3 ± 29.8[b]	80 ± 14.41[d]
Group 2	132.0 ± 11.8c[d]	98.67 ± 3.61[bc]
Group 3	143.33 ± 6.47[bcd]	105.67 ± 4.5[bc]
Group 4	127.33 ± 7.61[d]	102 ± 3.58[bc]

The levels of HDL which was significantly higher in control negative rats declined in case of control positive group (47±3.35 and 33.5 ± 1.76 mg/dl, respectively). All treated groups indicated significant differences as compared to the positive control group except for green powder group, as shown in Table (6).

The mean value of LDL in normal group control negative was extremely significant lower than the control positive group (59.67±10.51 and 142±17.61mg/dl, respectively). All supplemented diets showed significant decreases, (P≤0.05) as compared to positive control rats. Green extract recoded the best result for decreasing LDL of hypercholesterolemia rats showing similar level when compared to the negative control group.

In the same table the resultant showed that the mean value of VLDL of control positive group was extremely significant higher than the control negative group (18±1.88 and 34.5±5.39 mg/dl, respectively). All treated groups showed significant decreases, (P≤0.05) than for positive control group.

In the same table the obtained results showed that there was significant and pronounced increase of atherogenic index (AI) in positive control group as compared to normal rats. In rats fed on all treatment diets, there were significant decreased (P≤0.05) in atherogenic index (AI) compared with positive control

The obtained results were in agreement with those obtained by Sadeek *et al.* (2010)who found that green, roasted and decaffeinated coffee resulted in a significant decrease (P≤0.05) in triacylglycerol (TG); LDL-C; VLDL-C and in LDL/HDL ratio as well as TC/HDL ratio. In addition, Gafaar *et al.* (2013) revealed that diabetic control group showed a significant increase in the values of TC, TL, TG and a significant increase (p<0.05) in LDL when compared with normal control group. All treated groups showed a significant decrease in TC, TL and TG and a significant increase of HDL compared with control positive group. The best reduction in the lipids profile was recorded for the Arabic green coffee supplement. The results showed that Arabic dark coffee supplemented diet show were of lower effect against diabetic than green and light coffee.

Table 6: Effect of Arabic and green coffee forms on lipoproteins profile and atherogenic index (AI) of hypercholesterolemia rats (M±SD)

Groups	HDL mg/dl	LDL mg/dl	VLD L mg/dl	AI
Control negative	47 ± 3.35^{ab}	59.67 ± 10.51^{c}	18 ± 1.88^{cd}	1.65 ± 0.19^{de}
Control positive	33.5 ± 1.76^{d}	142 ± 17.61^{a}	34.5 ± 5.39^{a}	5.29 ± 0.66^{a}
Group 1	42.5 ± 3.45^{bc}	93.83 ± 32.15^{b}	16 ± 2.89^{d}	2.56 ± 0.503^{cde}
Group 2	37.67 ± 3.75^{d}	74.6 ± 10.84^{bc}	19.73 ± 0.72^{bc}	2.59 ± 0.76^{e}
Group 3	45.33 ± 4.59^{abc}	76.87 ± 5.56^{bc}	21.13 ± 0.9^{bc}	2.70 ± 0.13^{b}
Group 4	48 ± 2.68^{a}	58.93 ± 6.22^{c}	20.4 ± 0.715^{bc}	2.18 ± 0.22^{bc}

Different letters (a, b, c, d, etc.) differ significantly at p≤ 0.05, while those with similar letters are non-significantly different.

leptin hormone:

In relation toleptinhormone it could be observed that the mean value of positive control group was significantly higher than negative control group (2.5 ±0.237 &0.9 ±0.089 mg/ ml, respectively). All treated groups indicated a significant decrease (P≤0.05) as compared to positive control group, as shown in Table (7).

The obtained results were in agreement with those obtained by Yamashita *et al.* (2012) who found that coffee consumption showed significant positive associations with adiponectin and total and low-density lipoprotein cholesterol, and inverse associations with leptin. In addition to, Ann *et al.* (2013) who reported that groups treated with caffeine/ephedrine (CE) and leptin- caffeine/ephedrine (LCE) lost significant amounts of weight and whole body fat mass compared to leptin only group. Only treatment with LCE significantly reduced visceral fat mass. There were no differences in lean mass between treatment groups. Moreover, Zheng *et al.* (2014) who found a decrease in the body weight of mice fed the coffee components including chlorogenic acid (CGA) and caffeine diet. There was a significant decrease in the serum and hepatic concentrations of total cholesterol, TAG and leptin of mice fed the CGA plus caffeine diet.

Table 7: Effect of Arabic and green coffee forms on leptin hormone of hypercholesterolemia rats (M±SD)

Groups	Leptin (mg/ml)
Control negative	0.9 ± 0.089^{de}
Control positive	2.5 ± 0.237^{a}
Group 1	1.23 ± 0.441^{bc}
Group 2	0.8 ± 0.155^{de}
Group 3	1 ± 0.237^{cde}
Group 4	0.93 ± 0.137^{cde}

Different letters (a, b, c, d, etc.) differ significantly at p≤0.05, while those with similar letters are non-significantly different.

Consequently, from the obviously results it was concluded that the best diet proposed in the present work was group 4 followed by group2which fed separately on basal diet and administrated orally with 2ml/day green and Arabic coffee since it promoted an increase in LDL-cholesterol, a decrease in the HDL fraction and affected less the hepatic function of the animals.

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