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COMPARATIVE STUDY ON THE USES OF GREEN COFFEE BEANS, ROASTED, ARABIC COFFEE FORMULA AND THEIR EXTRACTS TO REDUCE OBESITY IN MALE RATS

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ABSTRACT: *This study was conducted to investigate the effect of use of extract and powder of green coffee, roasted coffee, Arabic coffee and mix of green and roasted coffee to improve the level of serum lipid profile, high liver enzyme and renal function. Fifty four mature male albino rats weighting 150±10g were divided into two main groups; control negative group (6 rats) was fed on basal diet and control positive group (48 rats) which was fed on hyperlipidemic diet for four weeks to induce hyperlipidemia. Rats in the second main group supplements were divided into eight groups (each group consisted of 6 rats and fed on basal diet) as a following: The first group was left as a control positive , the second group fed diet containing 5 % Arabic coffee / kg in diet. The third group fed diet containing 5 % green coffee / kg in diet .The fourth group fed diet containing 5 % roasted coffee / kg in diet. The fifth group was administrated orally with 5 % Arabic coffee extract. The sixth group was administrated orally with 5 % green coffee extract, the seventh group was administrated orally with 5 % roasted coffee extract. The eighth group was administrated orally with 5 % mix of green and roasted coffee extract. Feeding experiment lasted for 28 days. Serum total cholesterol, triglycerides, lipoprotein fractions (HDLc, LDLc and VLDLc), atherogenic index (AI), liver enzymes(ALT, AST, ALP),total protein, albumin, globulin, albumin/globulin (A/G) ratio, uric acid, urea, creatinine, leptin hormone and testosterone hormone were assessed . Histopathological changes of liver, heart and testis were examined. The obtained results concluded that feeding with arabic coffee (5%), green coffee (5%) and roasted coffee (5%) improved liver , kidney functions, lipid profile, level of leptin and testosterone hormones, liver , heart and testis tissues changes. According to the results, arabic ,green and roasted coffee could be used for to improve the health situation of obese rats along with weight loss.*

Key words: *Obesity, green coffee, roasted coffee, Arabic coffee, lipid profile, liver enzymes, leptin hormone, testosterone hormone and histopathological changes.*

INTRODUCTION

Obesity is a complex disease of multifaceted etiology. It meets the medical definition of disease in that it is a physiological dysfunction of the human organism with environmental, genetic and endocrinological etiologies (Conway and Rene, 2004). Obesity is a medical condition in which excess body fat has accumulated to the extent that it may have a negative effect on health, leading to reduced life expectancy and/or increased health problems. People are considered obese when their body mass index (BMI), a measurement obtained by dividing a person's weight by the square of the person's height, exceeds 30 kg/m2, with the range 25-30 kg/m2 defined as overweight (Haslam and James, 2005).

Obesity increases the likelihood of various diseases, particularly heart disease, type 2 diabetes, obstructive sleep apnea, certain types of cancer, and osteoarthritis (Kushner and Robert, 2007).

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).

Obesity has reached epidemic proportions throughout the globe, and this

has also impacted people of the Arabic-speaking countries, especially those in higher-income, oil-producing countries. The prevalence of obesity in children and adolescents ranges from 5% to 14% in males and from 3% to 18% in females (Badran and Laher, 2011) .

Several recent human studies have shown that coffee antioxidants, especially chlorogenic acids, promote weight loss in overweight people. One study found that taking a daily dose of green coffee extract helped overweight men and women to lose weight without having to change their diet (Vinson *et al.,* 2012).

Fresh coffee beans are a light green color. During roasting, the beans darken to their more familiar dark brown color. Chlorogenic acids are a group of key coffee antioxidants with several potential health benefits, one of which is helping to promote weight loss. During roasting, the high temperatures cause chemical reactions that alter the amount of these antioxidants in coffee beans. One study found that lightly roasting coffee can actually increase the amount of chlorogenic acids in coffee beans, but medium and dark roasted coffee beans may lose over 50% of their original amounts of these compounds(Farah *et al.,* 2005).

Another interesting fact is that the roasting process increases the amount of some other kinds of antioxidants in coffee, such as N-methylpyridinium(NMP), as well as melanoidins (antioxidants formed during roasting that help give roasted seeds (Onakpoy *et al*., 2011). Green & roasted coffee study found that the mixture was effective in reducing body fat and also improving the levels of certain biomarkers used to estimate how well the body is able to defend against oxidative stress (Bakuradze *et al.,* 2011).

Thereupon, comparative study on the uses of green coffee beans, roasted, arabic coffee formula and their extracts to reduce obesity in male rats seems to be indispensable.

MATERIALS AND METHODS

1-Food material:

Green and roasted coffee beans were obtained from Cairo but Arabic coffee beans was obtained from Saudi Arabia. Standard diets were supplemented with Arabic coffee, green and roasted coffee beans at concentration 5 % for 28 days. There often from 5g of different coffee beans, extracts were prepared by boiling in 100 ml water for 5 min and filtration, then administered orally at dose 2 ml / rat for 28 successive days. Chemical analysis of coffee kinds were conducted in Food Technology Research Institute in Giza, as shown (Table 1 and 2).

2-Hyperlipidemic diet

Hyperlipidemic diet was prepared from fine ingredients per 100g according to Rashwan (1994), the diet had the following composition fat20%(sunflower oil 10% + sheep tallow19%),sugar 10%, salt mixture 4%, vitamin mixture1%, casein (protein content 14%), mithionine 0.3%, and corn starch up to 100.

3-Chemicals:

Casein, Vitamins mixture and salt mixture and all other basal ingredients diet were purchased from El-Gomhoria Company, Cairo, Egypt.

4- Experiments animals:

Fifty four male albino rats, weight 150±10 g, of Sprague Dawley Strain were used. They were obtained from the animal house laboratory of Ophthalmic Research Institute, Giza.

Methods:

Biological experiments:

Diet: The basal diet (casein – basal diet) was composed of 12.3g casein (10% protein), 10g corn oil (10% fat) , 4g cellulose (4% fiber), mineral mixture (4%). Vitamin in mixture(1%) and corn starch up to 100g according to(NRC, 1995) . The salt mixture used in the experiment was composed according to Hegsted (1941). vitamin mixture used in the experiment was that of Campbell (1963) .

Table (1): Chemical analysis for three samples of coffee

sample	Test results (wet weight)%					
	Moistue	Protein	Fat	Crude Fibers	ZN (ppm)	Ash %
Green coffee	7.09	13.04	1.52	44.91	7.64	3.37
Roasted coffee	3.01	12.49	16.23	41.49	7.57	3.83
Arabic coffee	6.18	12.51	9.61	34.11	7.20	3.53

Table (2): Phenolic compounds for three samples of coffee

Phenolic compounds	***Test Methods**	**Test results (µg/100g)**		
		Green Coffee	**Roasted coffee**	**Arabic coffee**
Syringic	Journal. Sci. Food Agric.,79: 1625-1634 (1999)	5012.40	8815.79	5318.71
Gallic		125.96	784.75	936.96
Pyrogallol		1777.76	27874.05	8276.03
4- Aminobenzoic		184.60	824.96	347.80
Protocatechuic		3815.31	6999.94	4385.07
Catechein		34913.90	8092.75	10040.97
Chlorogenic		163343.06	171334.71	677992.67
Catechol		58986.83	196026.41	398821.15
Epicatechein		69112.21	--	--
Caffeine		78633.60	442069.07	379230.95
P.OH.benzoic		3307.11	5933.89	--
Caffeic		1542.56	30801.19	2591.49
Vanillic		1814.96	9410.63	16559.65
Ferulic		687.09	1461.92	1318.50
Iso-ferulic		--	3715.97	1303.50
E- Vanillic		93489.45	221210.09	514309.47
Ellagic		829.56	10727.69	1899.19
α-Coumaric		53.63	948.81	507.54
Benzoic		3685.54	12256.89	23680.23
Salicylic		3921.48	7786.85	14624.32
3,4,5-methoxy-cinnamic		--	3150.32	---
Coumarin		542.11	3468.38	1250.41
p-Coumaric		1690.55	5469.04	2781.80
Cinnamic		22.98	504.87	142.28

Experimental design and animal groups:

After this week , the rats were divided into two main groups as follows:-

- **The first main group (6 rats)** was fed on basal diet and kept as the control negative .
- **The second main group (48 rats)** was fed on hyperlipidemic diet for four weeks to induce hyperlipidemia according to Rashwan (1994). Rats in the second main group (48 rats) were divided into eight groups (each group consisted of 6 rats) as the following:
- **The first group** was left as a control positive and fed on hyperlipidemic diet. The second group was fed on basal diet and supplemented with mix of Arabic coffee 5%.The third group was fed on basal diet and supplemented with green coffee bean 5%.The fourth group was fed on basal diet and supplemented standard with roasted coffee bean 5%.The fifth group was fed on basal diet and administrated orally with 2ml Arabic coffee extract 5%.The sixth group was fed on basal diet and administrated orally with 2ml green coffee bean extract 5%.The seventh group was fed on basal diet, and administrated orally with 2ml roasted coffee bean extract 5%.The eighth group was fed on basal diet and administrated orally with 2ml mixture of three types of coffee bean extract, 5%.

Biological evaluation: During the experiment period (28 days) , the quantities of diet consumed and / or wasted were recorded every day . In addition , rats weight was recorded weekly .

At the end of the experiment period , the rats were 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).

Liver, kidneys, spleen, heart and lungs 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: After rats at the end of the experiment, blood samples were collected from aorta . Each sample sacrificed, was placed in a dry clean centrifuge tube , then centrifuged for 10 minutes at 3000 round per minute "r.p.m" to separate the serum . Serum was carefully separated into dry clean Wasserman tubes by using a Pasteur pipette and kept frozen till analysis.

Serum urea, creatinine, serum uric acid, total protein, albumin (A), globulin (G), A/g ratio, AST, ALT, total cholesterol, triglyceride, HDL-c, LDL-c, VLDL-c, testosterone and leptin hormones were determined. according to Patton and Crouch (1977), Faulkner and King (1976), Barham and Trinder (1972) and Fossati *et al*., (1980), Sonnenwirth and Jaret (1980), Drupt (1974), Catherine *et al*., (2003), Reitman and Frankel (1957), Allain *et al*., (1974), Trinder and Ann (1969), Lopes - Virella *et al.,* (1977), Friedwald *et al*., (1972) , Kikuch-Hayakawa *et al.*, (1998), Rosner *et al*., (2007)and Heymsfield *et al*., (1999), respectively.

Statistical analysis: Data were expressed as (Mean ± SD). Differences between control and treated groups were tested for significance using a one way analysis of variance (ANOVA test) according to Armitage and Berry(1987) followed by Duncan's multiple range test. Differences were considered of significance at a level of

P ≤ 0.05 using SPSS (version 20.0) computerized program.

Histopathological examination of hearts and livers:

The livers of sacrificed rats were taken and immersed in 10% neutral formalin solution. The fixed specimens were then trimmed, washed and dehydrated in ascending grades of alcohol. They were then cleared in xylol, embedded in paraffin, sectioned at 4-6 microns thickness and stained with Heamtoxylin and Eosin for examining heart and liver structure according to Carleton(1979).

RESULTS AND DISCUSSION

Nutrition evaluations:

5.1Biological changes:

5.1-1Boody weight gain, feed intake and feed efficiency ratio:

Feed intake values showed significant decrease (P≤0.05) in control (C+) group as compared to normal rats group (2.28 ± 0.12& 2.39 ± 0.09 g/day, respectively). All treated groups indicated significant decrease as compared to positive control group except Arabic , roasted and mix extracts as well as roasted powder which showed non-significant changes (P≤0.05), as shown in table (3) .

In relation to body weight gain (BWG%), it could be observed that the mean value of the positive control group was non-significantly higher than negative control group (1.304 ± 0.09 & 1.28 ± 0.08 %, respectively). All groups indicated significant differences as compared to the positive control group except green powder and roasted powder, showed significant increases (P≤0.05), as shown in table (3) .

Calculation of feed efficiency ratio, results of (FER) illustrated significant increases (0.09 ± 0.05 and 0.08 ± 0.04, respectively) of control (+) compared to (-) group. All treated groups recorded significant increases (P<0.05) values except green extract group compared to control (-) rats, as shown in table (3).

The present data were in agreement with those obtained by Sadeek *et al.,* (2010) who concluded that green, roasted and decaffeinated coffee resulted in a significant differences (p ≤ 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. However, Bonita *et al.*, (2007) established that there was non-significant effect of any dose of coffee, caffeinated or decaffeinated, on weight gain or feed consumption.

5.1-2 Relative organs weight:

Relative liver weight value showed a significant increase in the control (C+) group as compared to the normal rats group, it was 3.55 ± 0.39 and 2.93 ± 0.88 g, respectively. Rates fed on supplemented diet with (Arabic powder , Arabic extract, roasted extract and mix of green & roasted extract) showed significant decreases (P≤0.05) as compared to positive control group, as shown in table (4).

Lecoultre *et al*.,(2014) found that coffee consumption attenuates hepatic insulin resistance but not the increase of IHCLs induced by fructose overfeeding. This effect does not appear to be mediated by differences in the caffeine or chlorogenic acid content. Also, Walton *et al.,(*2013) reported that coffee drinking is associated with a reduced prevalence of cirrhosis in patients with chronic liver disease. However, there was no significant difference in the amount of coffee drunk by liver patients and the control groups.

Relative heart weight value showed non-significant increase in control (C+) group as compared to normal rats group was (0.37 ± 0.07 & 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 in table (4) for heart , liver and testis 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) they 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 (T2D).

Relative testis weight value showed non-significant decrease in control (C+) group as compared to normal rats group was (0.89 ± 0.05&1.02 ± 0.51, respectively). All treated groups indicated non-significant differences as compared to positive control group, as shown in table (4) .

Table (3): Effect of different shapes of coffee on feed intake, body weight gain (BWG %), and feed efficiency ratio (FER) of obesitic and treated rats (n= 6 rats)

Groups	FI g/day	BWG%	FER
C (-)	2.39 ± 0.09 ^{a}	1.28 ± 0.08 ^{bc}	0.08 ± 0.04^{d}
C (+)	2.28 ± 0.12^{bc}	1.304 ± 0.09^{bc}	0.09 ± 0.05 ^{bc}
Arabic powder	2.28 ± 0.03 ^{d}	1.34 ± 0.12^{ab}	0.09 ± 0.06bc ^{bc}
Green powder	2.26 ± 0.03^{d}	1.43 ± 1.02^{a}	0.10 ± 0.01^{a}
Roasted powder	2.27 ± 0.09^{cd}	1.43 ± 0.11^{a}	0.09 ± 0.06ab ^{ab}
Arabic extract	2.27 ± 0.05^{cd}	1.34 ± 0.03^{ab}	0.10 ± 0.02^{ab}
Green extract	2.29 ± 0.09^{ab}	1.21 ± 0.04^{c}	0.09 ± 0.04^{cd}
Roasted extract	2.28 ± 0.13^{bc}	1.27 ± 0.07^{bc}	0.09 ± 0.06^{bc}
Mix extract	2.28 ± 0.07^{bc}	1.32 ± 0.06^{b}	0.09 ± 0.03^{bc}

Values denote arithmetic means ± Standard deviation of the mean.Means with different letters (a, b, c, d, etc.) in the same column differ significantly at p≤0.05 using Duncan Range Multiple test, while those with similar letters are non-significantly different

Table (4): Effect of different shapes of coffee on relative organs weight of obesitic rats (n= 6 rats)

Groups	Heart(g)	Liver(g)	Testis(g)
C (-)	0.33 ± 0.04 ^{b}	2.93 ± 0.88 ^{bc}	1.02 ± 0.51 ^{a}
C (+)	0.37 ± 0.07 ^{ab}	3.55 ± 0.39^{a}	0.89 ± 0.05^{a}
Arabic powder	0.31 ± 0.04 ^{b}	2.43 ± 0.02^{cd}	1.17 ± 0.16^{a}
Green powder	0.39 ± 0.06 ^{a}	2.39 ± 0.07^{d}	1.1 ± 0.39^{a}
Roasted powder	0.34 ± 0.04 ^{ab}	3.19 ± 0.49^{ab}	0.87 ± 0.04^{a}
Arabic extract	0.31 ± 0.07 ^{b}	2.42 ± 0.04^{cd}	0.95 ± 0.03^{a}
Green extract	0.31 ± 0.06 ^{b}	3.31 ± 0.49^{ab}	1.16 ± 0.305^{a}
Roasted extract	0.36 ± 0.04^{ab}	2.62 ± 0.02^{cd}	0.93 ± 0.05^{a}
Mix extract	0.36 ± 0.02^{ab}	2.46 ± 0.01^{cd}	0.96 ± 0.02^{a}

Values denote arithmetic means ± Standard deviation of the mean.Means with different letters (a, b, c, d, etc.) in the same column differ significantly at p≤0.05 using Duncan Range Multiple test, while those with similar letters are non-significantly different.

6- Biochemical evaluations :

6-1 Serum lipid profile:

Data of table (5) show the mean values of triglycerides (TG). TG showed significant increase in positive control group as compared to negative control group; it was 172.5 ± 26.94 & 90±9.4 mg/dl, respectively. All treated group showed significant decreases (P≤0.05) when comparing with the positive control group, as shown in table (5) .

The obtained results (Tables 5&6) are in agreement with that obtained by Abd El-Fattah, (2008); he found that low and high doses of coffee caused significant elevation in serum total lipids (TL), TC, TG and LDL-C but a significant decrease of HDL-C. In accordance to the present study Shimod *et al*.,(2006) 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 beeffective on hepatic TG, and not adipose TG.

Data of table (6) revealed that the levels of HDL which was significantly higher in (C-) rats declined in case of (C+) group (47±3.35 & 33.5 ± 1.76 mg/dl, respectively). All treated groups indicated a significant differences as compared to the positive control group except that of green powder group, as shown in table (6).

The mean value of LDL in (C-) group was extremely and significant lower than the (C+) group (59.67±10.51 & 142±17.61 mg/dl, respectively). All supplemented diets showed significant decreases, (P≤0.05) as compared to positive control rats. Green extract revealed the best result for decreasing LDL of obesitic rats showing similer level when compared to the negative control group, as shown in table (6) .

The mean value of VLDL in (C-) group was extremely significant lower than the (C+) group being (18±1.88 & 34.5±5.39 mg/dl, respectively). All treated groups showed significant decreases, (P≤0.05) than for positive control group, as shown in table (6).

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 was significant decreases (P≤0.05) in atherogenic index (AI) as compared to (C+), as shown in table (6) .

The obtained results were in agreement with those obtained by Sadeek *et al*., (2010),they found that green, roasted and decaffeinated coffee resulted in a significant decrease (p≤0.05) in triacylglycerol (TAG); LDL-C; VLDL-C and in LDL\ HDL ratio as well as TC \ HDL ratio. On the other hand a significant increase (p≤0.05) in serum HDL-C is observed in green, roasted and decaffeinated coffee groups compared to diabetic rats compared to normal control with the highest value for green coffee ,with non- significant effect on serum total-cholesterol(TC) . 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(+) group. The best reduction in the lipids profile was recorded for the Arabic green coffee supplement; including the levels of total lipids, total cholesterol, triglycerides and LDL. A significant increase in the HDL level was observed for the Arabic green and light coffee bean supplement; and no significant difference in the HDL-C level was observed for normal control. The results showed that Arabic dark coffee supplemented diets, however were of lower effect against diabetic than green and light coffee.

6.2 Liver function:

6.2.1 Liver enzymes(GOT& GPT)

The mean value of GPT (ALT) showed a significant increase in the positive control group as compared to the negative control group, it was 37 ± 2.37 and23.5 ± 2.34U/L, respectively . All treated groups indicated a significant decreases as compared to positive control group, as shown in table (7), with exception of roasted powder group.

Table (5): Effect of different shapes of coffee on lipid profiles of obesitic rats (n= 6 rats)

Groups	Total Cholesterol mg/dl	Triglyceride mg/dl
C (-)	124.67 ± 13.2^{d}	90 ± 9.4^{cd}
C (+)	210 ± 13.9^{a}	172.5 ± 26.94^{a}
Arabic powder	152.3 ± 29.8^{b}	80 ± 14.41^{d}
Green powder	132 ± 11.8c^{d}	98.67 ± 3.61^{bc}
Roasted powder	136.6 ± 6.47^{bcd}	111 ± 5.44^{b}
Arabic extract	143.33 ± 6.47^{bcd}	105.67 ± 4.5^{bc}
Green extract	127.33 ± 7.61^{d}	102 ± 3.58^{bc}
Roasted extract	133± 5.48c^{d}	112.59±113.5^{b}
Mix extract	149 ± 17.3^{bc}	89.17 ± 19.11^{cd}

Values denote arithmetic means ± Standard deviation of the mean.Means with different letters (a, b, c, d, etc.) in the same column differ significantly at p≤0.05 using Duncan Range Multiple test, while those with similar letters are non-significantly different.

Table (6): Effect of different shapes of coffee on lipoproteins profile and atherogenic index (AI) of obesitic rats (n= 6 rats)

Groups	HDL mg/dl	LDL mg/dl	VLD L mg/dl	AI
C (-)	47±3.35^{ab}	59.67 ±10.51^{c}	18 ±1.88 ^{cd}	1.65 ± 0.19^{de}
C (+)	33.5 ±1.76^{d}	142 ±17.61^{a}	34.5 ±5.39 ^{a}	5.29 ± 0.66^{a}
Arabic powder	42.5 ±3.45^{bc}	93.83 ±32.15 ^{b}	16 ±2.89 ^{d}	2.56 ± 0.503^{cde}
Green powder	37.67 ±3.75^{d}	74.6 ±10.84 ^{bc}	19.73 ± 0.72 ^{bc}	2.59 ± 0.76^{e}
Roasted powder	37 ±3.09^{d}	77.47 ±4.001^{bc}	22.2 ±1.09 ^{b}	2.55 ± 0.45^{bcd}
Arabic extract	45.33 ±4.59^{abc}	76.87 ±5.56 ^{bc}	21.13 ± 0.9 ^{bc}	2.70 ± 0.13^{b}
Green extract	48 ±2.68^{a}	58.93 ±6.22^{c}	20.4 ±0.715 ^{bc}	2.18 ± 0.22^{bc}
Roasted extract	42.5 ±3.83^{bc}	67.8 ±0.88 ^{c}	22.7 ±2.52 ^{b}	1.65 ± 0.15^{bcd}
Mix extract	42 ±3.03^{c}	89.17 ±20.24 ^{b}	17.83 ±3.83 ^{cd}	2.14 ±0.15^{b}

Values denote arithmetic means ± Standard deviation of the mean.Means with different letters (a, b, c, d, etc.) in the same column differ significantly at p≤0.05 using Duncan Range Multiple test, while those with similar letters are non-significantly different.

Table (7): Effect of different shapes of coffee on liver enzymes of obesitic rats (n= 6 rats)

Groups	GPT U/L	GOT U/L
C (-)	23.5 ± 2.34 ^{f}	29.67 ± 2.5^{c}
C (+)	37 ± 2.37^{a}	42.00 ± 4.73^{a}
Arabic powder	31 ± 5.62 ^{bcd}	32.5 ± 4.89^{bc}
Green powder	31.33 ± 2.87^{bcd}	37.83 ± 9.3^{ab}
Roasted powder	32.83 ± 2^{abc}	33 ± 5.16^{a}
Arabic extract	29.17 ± 7.17^{cde}	36.33 ± 4.5^{abc}

Green extract	25.33 ± 1.37 ^{ef}	36.5 ±0.55^{abc}
Roasted extract	34 ± 3.29^{ab}	38.33 ±6.77^{ab}
Mix extract	28 ± 2.37^{def}	32.17 ± 4.79^{bc}

Values denote arithmetic means ± Standard deviation of the mean.Means with different letters (a, b, c, d, etc.) in the same column differ significantly at p≤0.05 using Duncan Range Multiple test, while those with similar letters are non-significantly different.

In relation to GOT(AST), it could be observed that the mean value of the positive control group was significantly higher than the negative control group (42.00 ± 4.73 & 29.67 ± 2.5 U/l respectively). All treated groups indicated significant as numerical decreases compared to positive control group, as shown in table (7).

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 gamma-glutamyl transferase (GGT). Moreover, Anaelechi *et al*., (2011) 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 at ($p \leq 0.05$) and ($p \leq 0.001$) respectively . In female subjects ALT increased more than AST did.

Protein fractions:

Total Protein, Albumin, Globulin and Albumin/Globulin (A/G) ratio*:*

The mean value of total protein, as shown in table (8) showed a significant decrease in the positive control group as compared to the negative control group, it was 5.37± 0.21and 5.9 ±0.31 g/dl, respectively. All treated groups indicated significant increases (P≤0.05) as compared to the positive control group.

In the same table results presented the mean values of albumin showed significant decrease in the positive control group as compared to the negative control group, it was 3.55±0.18 and 4.03±0.24 g/dl, respectively. Mostly all supplemented diet significant differences as compared to positive control group, as shown in table (8)

In relation to globulin it could be observed that the mean value of the positive control group was non-significantly lower than the negative control group (1.82±0.16 & 1.87±0.8 mg/dl, respectively). All treated groups indicated numerical differences as compared to positive control group, as shown in table (8).

Also in the same table the obtained results showed that there was non-significant decreases in albumin/globulin(A/G) ratio in (C+) as compared to normal rats(1.97±0.2 & 2.16 ± 0.08 mg/dl, respectively). In rats fed on all treatment diets , there was non-significant differences in A/G as compared to positive control group, as shown in table (8).

Anaelechi *et al*., (2011) found that in male subjects, the mean plasma values of total protein and albumin were not significantly altered ($p \geq 0.05$). Consumption of coffee for 30 days in female subjects did not significantly raise the mean plasma total protein and albumin ($p \geq 0.05$). In addition, Alicja *et al*.,(2009) ,however showed a serum albumin concentration in coffee drinkers significantly higher than in nondrinkers of coffee groups.

7. Kidney functions :

7.1 Uric acid, urea and creatinine

The mean value of urea values showed significant increase in control (C+) group as compared to normal rats group (60.83 ±0.17& 43.83 ± 8.33 mg/ dl, respectively). All treated groups indicated significant decreases as compared to positive control group, as shown in table (9).

In relation to creatinine, it could be observed that the mean value of positive control group was significantly higher than negative control group (1.33 ± 0.41 &0.62 ±0.07 mg/ dl respectively). All treated groups indicated significant decreases (P≤0.05) as compared to positive control group, as shown in table (9).

Calculation of uric acid results illustrated non-significant increase in control (+) groups compared to control (-), (1.83±0.39 and 1.73±0.22 mg/ dl, respectively). Mostly all treated groups recorded non-significant compared to control (+),as shown in table (9) .

Our result were in agreement with those obtained by Abrahão *et al.,* (2013) they found that daily doses of coffee drink caused significant increases in creatinine , urea and non- significant increases in serum uric acid. Also, Mahmoud *et al*,.(2013) found that the consumption of Arabic coffee, at different levels, could be beneficial for patients with hypercholesterolemia or hyperuricemia.

8.Testosterone and leptin hormones

The mean value of testosterone hormone showed significant decrease in control (C+) group as compared to normal rats group (1.57 ±0. 14 & 2.27 ±0.22 mg/ ml, respectively). All treated groups indicated significant differences as compared to positive control group except that of roasted extract rats , as shown in table (10).

Table (8): Effect of different shapes of coffee serum proteins of obesitic rats (n= 6 rats)

Groups	Total protein g/dl	Albumin g/dl	Globulin g/dl	A/G ratio
C (-)	5.9 ± 0.31^{bc}	4.03 ±0.24 ^{a}	1.87 ± 0.8^{c}	2.16 ± 0.08^{a}
C (+)	5.37 ± 0.21^{d}	3.55 ±0.18 ^{b}	1.82 ± 0.16^{c}	1.97 ± 0.2^{abcd}
Arabic powder	6.03 ± 0.27^{ab}	4.03 ±0.17 ^{a}	2 ± 0.11^{bc}	2.02 ± 0.06^{abc}
Green powder	5.83 ± 0.27 ^{bc}	3.73 ±0.44 ^{ab}	2.1 ± 0.27^{ab}	1.82 ± 0.44^{bcd}
Roasted powder	6.23 ± 0.14 ^{a}	3.97 ±0.19 ^{a}	2.27 ± 0.05^{a}	1.75 ± 0.12^{cd}
Arabic extract	5.8 ± 0.09b ^{c}	3.93 ±0.27 ^{a}	1.87 ± 0.22^{c}	2.15 ± 0.41^{a}
Green extract	5.73 ± 0.14 ^{c}	3.63 ±0.1 ^{b}	2.1 ± 0.24^{ab}	1.75 ± 0.23^{cd}
Roasted extract	6.05 ± 0.16^{ab}	3.8 ±0.11 ^{ab}	2.25 ± 0.05 ^{a}	1.69±0.01^{d}
Mix extract	5.83 ± 0.26 ^{bc}	3.95 ±0.18 ^{a}	1.88 ± 0.09^{c}	2.01 ± 0.07^{ab}

Values denote arithmetic means ± Standard deviation of the mean.Means with different letters (a, b, c, d, etc.) in the same column differ significantly at p≤0.05 using Duncan Range Multiple test, while those with similar letters are non-significantly different

Table (9): Effect of different shapes of coffee on kidney functions of obesitic rats (n= 6 rats)

Groups	Urea mg/dl	Creatinine mg/dl	Uric acid mg/dl
C (-)	43.83 ± 8.33^{cd}	0.62 ±0.07^{c}	1.73±0.22 ^{b}
C (+)	60.83 ±0.17^{a}	1.33 ±0.41^{a}	1.83±0.39 ^{b}
Arabic powder	40.33 ± 4.8^{d}	0.62 ±0.2^{c}	1.8 ±0.29 ^{b}
Green powder	45 ±2.68^{cd}	0.87 ±0.14^{b}	2.03 ±0.26 ^{ab}
Roasted powder	50.67 ±1.86^{bc}	0.83 ±0.05^{bc}	2.33 ±0.36 ^{a}
Arabic extract	55 ± 3.98^{ab}	0.73 ±0.14^{bc}	1.9 ±0.18 ^{b}

Green extract	47.33 ±1.37^{bcd}	0.93 ±0.55^{b}	2.13 ±0.27 ^{ab}
Roasted extract	44±3.29^{cd}	0.9±0.22 ^{b}	2.35±0.16^{a}
Mix extract	44.5 ±4.04^{cd}	0.77 ±0.1^{bc}	1.87 ±0.55 ^{b}

Values denote arithmetic means ± Standard deviation of the mean.Means with different letters (a, b, c, d, etc.) in the same column differ significantly at p≤0.05 using Duncan Range Multiple test, while those with similar letters are non-significantly different

Table (10): Effect of different shapes of coffee on testosterone and leptin hormones of obesitic rats (n= 6 rats)

Groups	Testosterone mg/ml	Leptin mg/ml
C (-)	2.77 ± 0.22 ^{b}	0.9 ± 0.089 ^{de}
C (+)	1.47 ± 0.14 ^{c}	2.5 ± 0.237 ^{a}
Arabic powder	4.3 ± 1.71^{a}	1.23 ± 0.441^{bc}
Green powder	4.47 ± 2.27 ^{a}	0.8 ± 0.155^{de}
Roasted powder	2.83 ± 0.46^{b}	1.03 ± 0.103^{cd}
Arabic extract	2.84 ± 0.29 ^{b}	1 ± 0.237 ^{cde}
Green extract	2.87 ± 0.04 ^{b}	0.93 ± 0.137 ^{cde}
Roasted extract	1.23 ± 0.34^{c}	1.37 ± 0.45 ^{b}
Mix extract	2.6 ± 0.47 ^{b}	0.7 ± 0.08 ^{e}

Values denote arithmetic means ± Standard deviation of the mean.Means with different letters (a, b, c, d, etc.) in the same column differ significantly at p≤0.05 using Duncan Range Multiple test, while those with similar letters are non-significantly different.

The obtained results were in agreement with those obtained by Wedick *et al*., (2012), they found that differences in hormone concentrations between the treatment groups. Among men, consumption of caffeinated coffee increased total testosterone and decreased total and free estradiol. Among women, decaffeinated coffee decreased total and free testosterone and caffeinated coffee decreased total testosterone. Also, Ramlau *et al.,* (2008) found that sons of mothers drinking 4-7 cups/day had lower testosterone levels than sons of mothers drinking 0-3 cups/day (P = 0.04). Current male caffeine intake was associated with increasing testosterone levels (P = 0.007). Men with a high caffeine intake had approximately 14% higher concentration of testosterone than those with a low caffeine intake (P = 0.008). In addition, Wedick *et al*.,(2012) they found non- significant differences between treatment groups for any of the studied outcomes at week 8. At 4 weeks, decaffeinated coffee was associated with a borderline significant increase in SHBG in women, but not in men. At days 28, several differences observed in hormone concentrations between the treatment groups. Among men, consumption of caffeinated coffee increased total testosterone and decreased total and free estradiol. Among women, decaffeinated coffee decreased total and free testosterone, and caffeinated coffee decreased total testosterone.

In relation to leptin hormone 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 (10) .

The obtained results were in agreement with those obtained by Yamashita *et al*., (2012), 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) 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) 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+caffeine diet.

9.Histopathological changes of livers.

Photo (1 to 9) indicated that due to obesity inducation control (+) rats certain histopathological changes in particular portal infiltration with inflammatory cells (photo 2).No histopathological changes occurred when rats fed with different kinds of coffee (photos 3,5,6,7,8,9), with exception of Arabic coffee powder group (photo 4) where local congestion of central vein & hepatic sinusoids may be occurred .

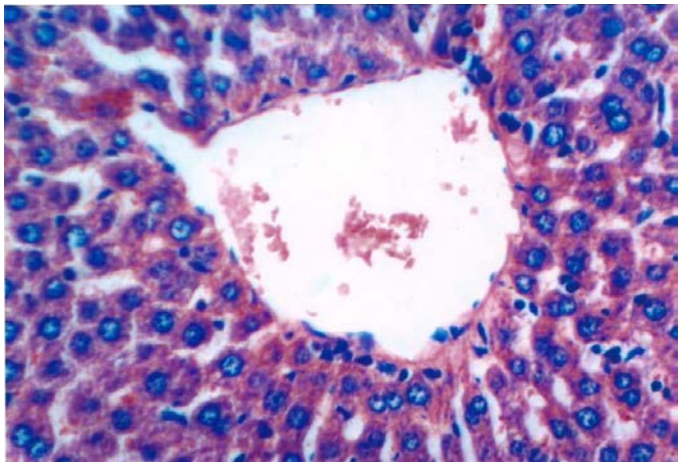

Photo (1): Liver of rat from group 1 (control "-")showing the normal histological structure of hepatic lobule.(H and E, X400).

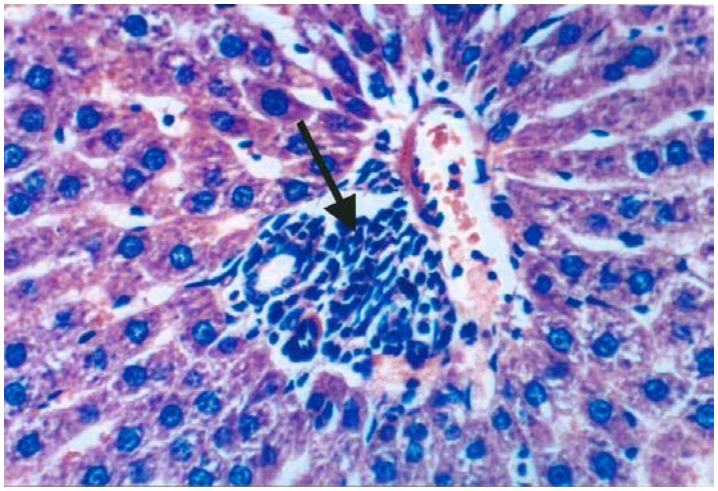

Photo (2): Liver of rat from group 2(control "+") showing portal infiltration with inflammatory cells.(H and E, X400)

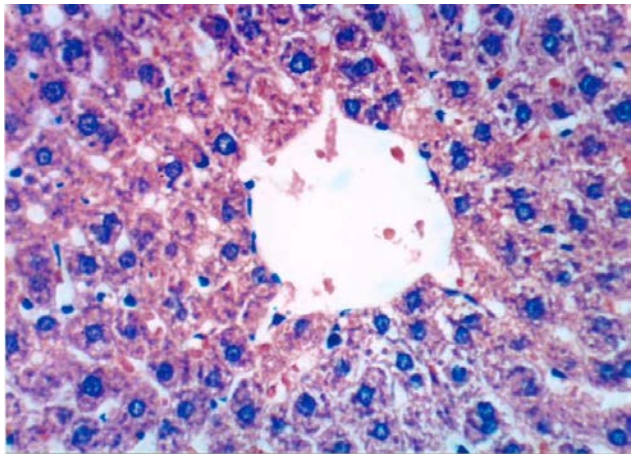

Photo (3): Liver of rat from group 4(mix of green and roasted coffee) showing no histopathological changes (H&EX400).

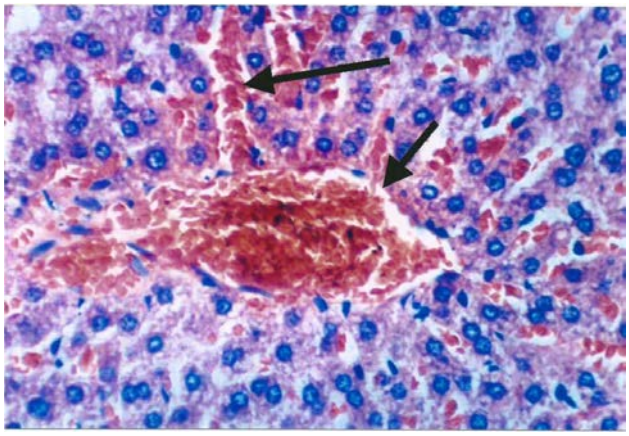

Photo (4): Liver of rat from group 8(Arabic coffee powder) showing local congestion of central vein and hepatic sinusoids (H & E X 400).

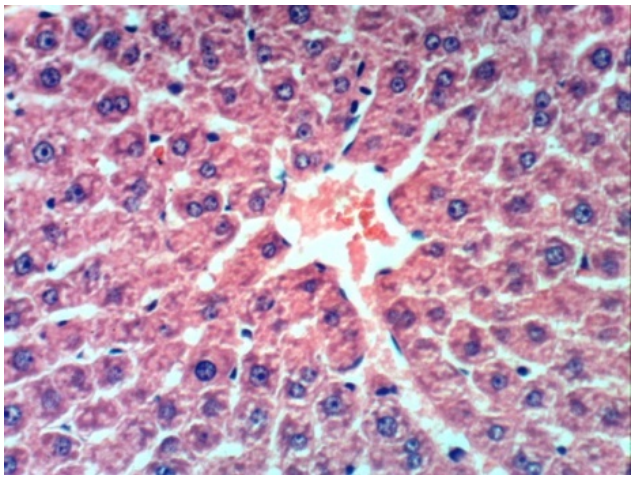

Photo (5): Liver of rat from group 3 (green coffee extract) showing no histopathological changes (H & E X 400).

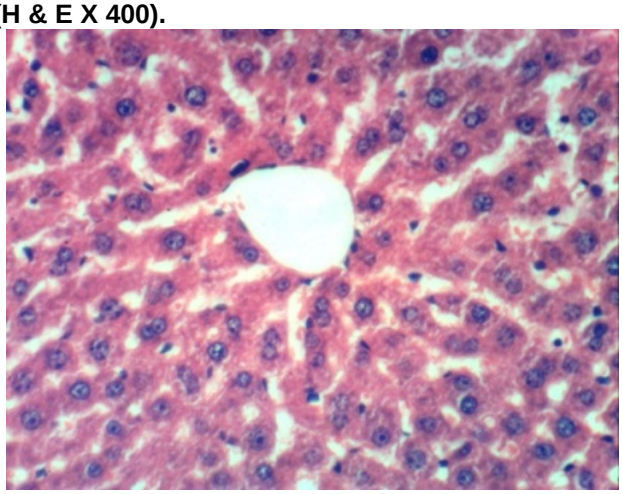

Photo (6): Liver of rat from group 5 (roasted coffee extract) showing no histopathological changes (H & E X 400).

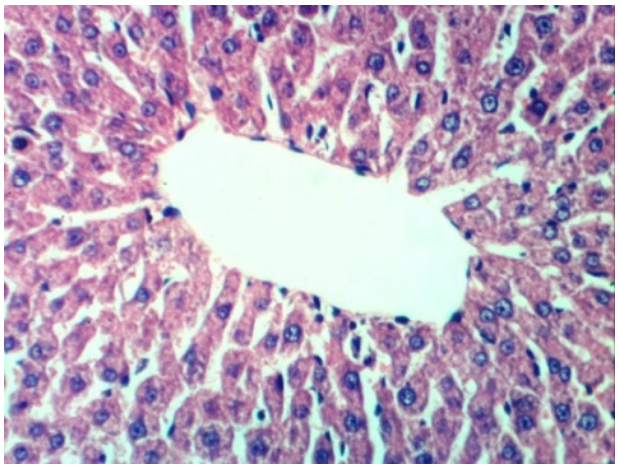

Photo (7): Liver of rat from group 6 (Arabic coffee extract) showing no histopathological changes (H & E X 400).

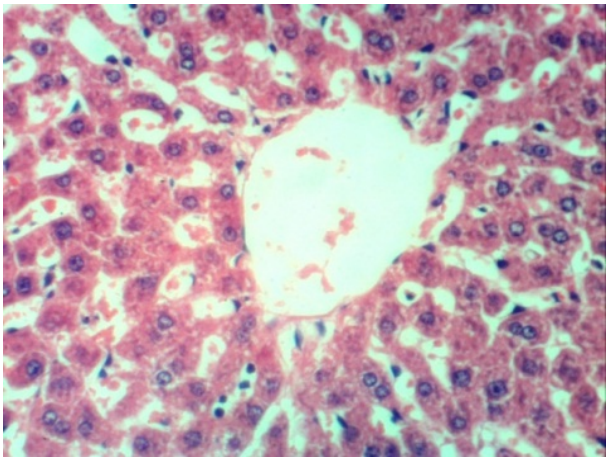

Photo (8): Liver of rat from group 7(green coffee powder) showing apparent normal hepatic lobule (H & E X 400).

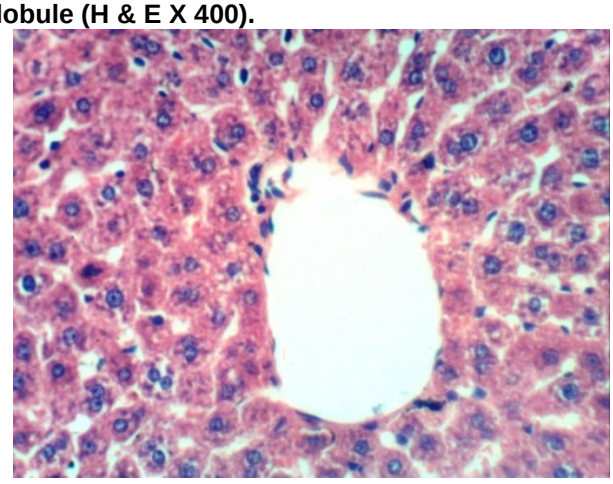

Photo (9): Liver of rat from group 9 (roasted coffee powder) showing no histopathological changes (H & E X 400).

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دراسة مقارنة عن استخدام حبوب القهوة الخضراء والمحمصة وخلطة القهوة العربية ومستخلصاتهم للحد من السمنة عند ذكور الفئران

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الملخص العربي

أجريت الدراسة الحالية لمعرفة تأثير مستخلص ومسحوق كلا من القهوة الخضراء والمحمصة والعربي والخليط بين كلا من القهوة الخضراء والمحمصة في تحسين مستوي دهون الدم وإنزيمات الكبد المرتفعة ووظائف الكلي. تم استخدام ٥٤ فأر ابيض ذكر بالغ يتراوح وزن كل منها ١٠±١٥٠ جرام. تم تقسيمهم الى مجموعتين اساسيتين مجموعه ضابطه سالبه نتكون من ٦ فئران تغذت على الغذاء الاساسي ومجموعه ضابطه موجبة من ٤٨ فأراً تغذوا علي وجبه عاليه الدهن الحيواني لمده أربعة اسابيع لرفع دهون الدم. الفئران تم تقسيمهم الى مجموعتين اساسيتين مجموعه ضابطه سالبه نتكون من ٦ فئران تغذت على الغذاء الاساسي) والثانية قسمت الى ثماني مجموعات فرعية كالتالي: المجموعة الأولى الفرعية تركت كمجموعة ضابطة موجبة. المجموعة الثانية الفرعية تغذت على الغذاء المحتوى على ٥% من القهوة العربي لكل كجم من الغذاء. المجموعة الثالثة الفرعية تغذت على الغذاء المحتوى على ٥% من القهوة الخضراء لكل كجم من الغذاء. المجموعة الرابعة الفرعية تغذت على الغذاء المحتوى على ٥% من القهوة المحمصة لكل كجم من الغذاء. المجموعة الخامسة الفرعية أعطيت فمويا مستخلص القهوة العربي ٥%، المجموعة السادسة الفرعية أعطيت فمويا مستخلص القهوة الخضراء ٥%. المجموعة السابعة الفرعية أعطيت فمويا مستخلص القهوة المحمصة ٥%، المجموعة الثامنة الفرعية أعطيت فمويا مستخلص خليط بين كلا من القهوة الخضراء والمحمصة ٥%. وتم قياس الكولسترول الكلى، الجلوسريدات الثلاثية، الليبوبروتينات، (HDL–C, VLDL–C, LDL–C)، معامل تصلب الشرايين، إنزيمات الكبد(ALT,AST,ALP) البروتين الكلى، الألبيومين، الجلوبيولين، النسبة بين الألبيومين الى الجلوبيولين، اليوريا، حمض اليوريك، الكرياتنين وهرموني اللبتن والتستيرون. وكذلك إجراء الفحص الهستوباثولوجى للكبد والقلب والخصيتين. وقد أظهرت النتائج أن تناول القهوة الخضراء والمحمصة والعربي (٥%) قد نتج عنه تحسن في دهون الدم ووظائف الكبد والكلي ومستوى هرمونات اللبتن والتستيرون وتحسن في تغيرات انسجه الكبد والقلب والخصيتين. وطبقا لهذه النتائج فإنه يمكن استخدام القهوة الخضراء والمحمصة والعربي لتحسين الحالة الصحية للفئران السمينة وانقاص الوزن لديهم.

الكلمات المفتاحية: السمنة, القهوة الخضراء، القهوة المحمصة ،القهوة العربي, دهون الدم، إنزيمات الكبد، هرمون اللبتن, هرمون التستيرون, التغيرات الهستوباثولوجية.