MERIT RESEARCH JOURNALS
www.meritresearchjournals.org

Merit Research Journal of Medicine and Medical Sciences (ISSN: 2354-323X) Vol. 3(7) pp. xxx-xxx, July, 2015
Available online http://www.meritresearchjournals.org/mms/index.htm

Original Research Article

Impacts of different preparations of coffee on body weight, serum uric acid and liver enzymes in experimental rats

Ahdab Abdo Elmaadawy[1], Hesham Hamdy Saad[2], Nora Mohamed Al-Qahiz[3] and Mohamed Saleh Ismail[2*]

[1]Home Econ. Dept. Faculty of Specific Education, Zagazig University, Egypt

[2]Nutrition and Food Sci. Dept. Faculty of Home Economics, Menoufia University, Egypt

[3]Nutrition and Food Sciences Dept. Princes Nora Bint Abdul Rahman University, Riyadh

***Corresponding Author's E-mail: drmohsaleh@yahoo.com**

Abstract

Coffee beverage is a globally consumed and is prepared in a wide variety of formats. This study aimed at clarifies the effect of different preparations of coffee on body weight, serum uric acid and liver enzymes in experimental rats. Forty male albino rats (130±2.3 g) were divided into five equal groups (n=8), control, Turkish coffee medium roasting (TMC), Turkish coffee dark roasting (TDC), Nescafe (NesC), and Arabic coffee. Each group received 2 ml oral solution containing the dose of coffee (0, 4.3, 4.3, 14.3, 8.6 mg/100 g BW respectively). The experiment continued for thirty days, and at the end rats were anesthetized and killed for collection of blood samples that used for determination of uric acid, urea, ALT, and AST. Samples of liver were collected for histopathology. Results showed that rats fed different preparations of coffee had significantly smaller weight gain. Meanwhile, group fed Nescafe lost considerable amount of body weight. Different preparations of coffee especially Turkish coffee dark roasting, Nescafe and Arabic coffee reduced serum uric acid. TMC group had significantly (P<0.05) the lowest values of AST and ALT, then NesC group. In conclusion, moderate amounts of Nescafe have the most favorable effects on body weight, serum uric acid, and liver enzymes.

Key words: Acid, ALT, AST, Body Weight, Coffee, Liver, Nescafe, Uric Rats

INTRODUCTION

Coffee beverage is a globally consumed and is prepared in a wide variety of formats. Population groups consuming coffee in a different formats (e.g. 37 °C–88 °C; 0%–80% milk; 0 g–16 g of sugar; 25 mL–880 mL in volume; foamed milk; cream; ice; flavorings; brew adjuncts or co-adjuncts) (Chen et al. 2012).

Turkish coffee (*Türk kahvesi*) is a method of preparing coffee, in which coffee bean roasted and then finely ground, the grounded coffee are boiled in a pot, usually with sugar, and served in a cup where the grounds are allowed to settle (wikipedia.org).

Nescafé is a brand of instant coffee made by Nestlé. It comes in many different product forms. The name is a portmanteau of the words "Nestlé" and "café". Nestlé first introduced their flagship powdered coffee brand in Switzerland on April 1, 1938 (http://www.nescafe.com).

Arabic coffee (*Coffee Arabica*) is a name that refers mainly to the Saudi coffee, or ''Al-Qahwa'' which made from coffee beans roasted very lightly or heavily from165 C (329F)to210 C(410 F) and cardamom (Mahmoud et al., 2013). Sometimes prepared with other spices like saffron (to give it a golden color),cloves, and cinnamon (Habeeb and James, 2010).

Several studies found that coffee might increase the risk of chronic diseases. For example, Jee et al. (1999) found that coffee consumption for more than one time daily led to a slight increase in blood pressure. Similarly, Chown et al. (2001) and Keijzers et al. (2002) found that the consumption of high amounts of coffee resulted in impaired glucose tolerance. Moreover, coffee

consumption may increase the risk of acute myocardial infarction (Baylin et al., 2006) and stroke (Mostofsky et al., 2010). These deleterious effects might be caused by cafestol plus kahweol esters that occur naturally in coffee beans and found to elevate the serum activity of alanine aminotransferase (ALT) in serum (Urgert et al., 1995 and Urgert and Katan, 1997).

On the other hand, epidemiologic studies indicated that drinking large amounts of coffee drastically reduced the incidence of type-2 diabetes (van Dam and Feskens, 2002 and Salazar-Martinez et al. 2003). Similarly, coffee consumption has been found to be associated with a reduced risk of chronic liver disease (Saab et al. 2014).

Roasted coffee contains naturally antioxidants and other compounds that are formed during the roasting process (Kempfet al. 2010). Caffeine and chlorogenic acids have been extensively studied because they may reduce the risk of insulin resistance (Ranheim and Halvorsen 2005; and Van Dieren et al., 2009), and development and progression of atherosclerosis (Butt and Sultan, 2011) and they might decrease blood pressure (BP) (Yamaguchi et al., 2008; and Medina-Rem et al., 2010).

We concluded from several studies that the diterpenoid alcohols cafestol and kahweol might cause deleterious effects of coffee (de Rooset al., 2001; Tofovic et al., 2002; Ranheim and Halvorsen, 2005; and Bonita et al., 2007), with the coffee polyphenols producing benefits (Bonita et al., 2007) and caffeine showing contrasting results, including increases in cholesterol (Tofovic et al., 2002). Finally, the results obtained by Sunil et al. (2012) suggest that all these components were active and the effects observed were cumulative.

Importantly, the concentration of these compounds depends on how coffee is prepared. Boiled coffee has higher concentrations because diterpenes are extracted from the coffee beans by prolonged contact with hot water. By comparison, brewed/filtered coffee, because of the much shorter contact with hot water and retention of diterpenes by filter paper, has a much lower concentration of cafestol and kahweol (Bak and Grobbee, 1989).

Data related to the effect of different preparations of coffee on body weight, serum uric acid or liver enzymes are lack; therefore, we carried out this study to clarify the effects of different preparations of coffee (Turkish coffee - both of mediumor dark roasting, Nescafe, and Arabic coffee) on body weight, serum uric acid or liver enzymes in experimental animals.

MATERIALS AND METHODS

Preparation of coffee

All coffee used in this study were obtained freshly from local markets in Cairo, Egypt, except for Arabic coffee which obtained from local markets at Riyadh, Saudi Arabia. The coffee were prepared by traditional methods, but without adding sugars, sweeteners, creamer, or milk.

1. Turkish coffee (medium and dark roasting)

In this study, we used two type of Turkish coffee; medium roasting (in which fresh coffee beans roasted to brown color at temperature ranged from 210 to 220 C) and dark roasting (in which fresh coffee beans roasted to brown color at temperature ranged from 240 to 250 C). After roasting, the beans are ground to the finest possible powder; finer than for any other way of preparation. The brew was prepared by immersing the coffee powder (5 g coffee / 100 ml water) in hot water, forjust as the coffee comes to the boil, the pot is removed from the heat, then allowed to cool and the solution separated and used in the experiments (The coffee foam were removed).

2. Nescafe (Instant coffee)

In this study, we used Nescafe brand produced by Nestle Egypt Company(6 October City, Giza, Egypt). It is instant coffee and ready to use. The brew was prepared by dissolving 5 gram of instant coffee in 100 ml of hot water.

3. Arabic coffee (Coffee Arabica)

The coffee seeds were roasted for 10 min then milled and turned into powder. The brew was prepared by boiling 30 g of coffee powder in one liter of water for 20 min (Mahmoud et al., 2013).

Coffee doses

The rat's dose from studied coffees was calculated according to the corresponding amounts consumed by the adult person. Researchers suggested that adult person who weighs 70 kg consume on average:

1- Two small cups (beaker) of Turkish coffee daily (about 60 ml/day), this amount contain about 3 gram of Turkish coffee powder. Therefore, the normal dose of coffee for human would be 3000 mg/ 70 kg of body weight and for rat would be 4.3 mg/ 100 gram of body weight per day.

2- One medium cup of Nescafe daily (about 200 ml/day), this amount contain about 10 gram of Nescafe. Therefore, the normal dose of Nescafe for human would be 10000 mg/ 70 kg of body weight and for rat would be 14.3 mg/ 100 gram of body weight per day.

3- Five small cups of Arabic coffee daily (about 150 ml/day), this amount contain about 6 gram of Arabic coffee powder. Therefore, the normal dose of Arabic coffee for human would be 6000 mg/ 70 kg of body weight and for rat would be 8.6 mg/ 100 gram of body weight per day.

Animals

Forty male Albino rats weighing 115 – 135 grams (130±2.3 g) were purchased from Animal Unit at Helwan, Ministry of Health, Egypt. The rats were housed

Table 1. Body weights and food intakes of experimental rats

	Control (n=8) Mean±SD	TMC (n=8) Mean±SD	TDC (n=8) Mean±SD	NesC (n=8) Mean±SD	AC (n=8) Mean±SD	ANOVA F	P value
Initial body weight (g)	132.2±3.7a	123.0±2.3b	121.0±2.9b	117.2±2.0b	126.6±1.1b	109.8	0.001
Final body weight (g)	155.6±24.8 a	132.4±18.8b	127.7±11.6b	112.3±13.7c	133.0±14.0b	6.0	P<0.05
Change in body weight (%)	+ 17.7%	+7.6%	+5.5%	-4.2%	+ 5.1%		
Food intake (g/day)	12.8±2.8 a	11.3±2.2 ab	10.8±2.2 ab	9.0±2.0 b	10.8±1.7 ab	1.6	0.209

TCM= Turkish coffee (medium roasting); TCD= Turkish coffee (dark roasting); NesC= Nescafe; AC= Arabic coffee. ANOVA= Analysis of variance. SD= Standard deviation. Mean values subscribed with different letters show significant differences between these values as calculated by one way ANOVA and LSD at P<0.05.

individually in cages, and were kept at 22 C, 56% humidity (40 to 70%) and in a 12-h: 12-h light: dark cycle and were allowed free access to food and tap water.

After 7 days of acclimatization, rats were randomly allocated to five groups (8 rats for each). The study was conducted in the animal lab at Faculty of Home Economics, Minufiya University, Shibin El-Kom, Egypt.

Each group was fed a defined basal diet plus water ad libitum. The standard diet is composed of protein (20%), sucrose (5%), fats (10%), vitamin mixture (1%), salt mixture (4%), fiber (4%), and starch up to 100%.

Experimental feeding groups

The control group was kept on the basal diet only. The first group (TMC) received single oral dose of Turkish coffee medium roasting (4.3 mg/ 100 g/day). The second group (TDC) received single oral dose of Turkish coffee dark roasting (4.3 mg/ 100 g/day). The third group (NesC) received single oral dose of Nescafe (14.3 mg/ 100 g/day). The fourth group (AC) received single oral dose of Arabic coffee (8.6 mg/ 100 g/day). All coffee solutions were fed to animals orally on daily basis.

The experiment continued for 30 days. The weights of the rats were measured at the beginning and at the end of the experimental period. By the end of the period, the rats were fasted for 8 hours, then anesthetized with diethyl ether and killed by exsanguinations. Blood samples were collected in heparinized tubes. All blood samples were immediately centrifuged (3,000 rpm, 20 min, and 4℃) for the separation of serum. The serum was stored at -20℃ until analysis. Liver samples were taken for histological examination.

Biochemical analysis

The following parameters were determined in the serum: urea (Tietz,1970); uric acid (Buchanan et al. 1965); and ALT and AST (Rej, 1984).

Histopathological examination

Samples of liver was taken and fixed in 10% neutral buffered formalin for 24 hours. Paraffin sections 6 µm thick were prepared and stained with hematoxylin and eosin (H and E) for the examination by light microscopy. The histopathology was carried out in histology lab at Faculty of Veterinary Medicine, Cairo University, Egypt.

Statistical analysis

All values were expressed as means±SD. Data were initially analyzed using the analysis of variance for each group (One Way ANOVA). When a significant F-value ($p < 0.05$) was obtained, LSD multiple test was performed for post hoc analysis.

RESULTS

As shown from table 1, the body weight gain of control group was significantly (P<0.05) the highest (+17.7%), while the NesC group lost 4.2% of their weight (P<0.05). In parallel, the food intake of NesC group was significantly lower than control group (P<0.05).

As shown in table 2, the mean values for AC group were the lowest (1.7±0.6 mg/dl) while the mean values for control and TMC groups was the highest (2.3±1.2 and 2.3±0.8 mg/dl respectively). The same table showed that mean value of urea for control group were significantly the lowest while the mean value for NesC group was significantly the highest. Regarding AST there was no significant differences between groups. As for ALT, the mean value for TMC group was significantly the lowest (41.6±8.4 U/L) while the value of TDC was the highest.

Histopathological changes associated with Turkish coffee (medium roasting), Turkish coffee (dark roasting), Nescafe, and Arabic coffee in liver tissues of rats are presented in figures 1 to 5.

Table 2. Concentration of serum uric acid, urea, AST, and ALT of experimental rats

	Control (n=8) Mean±SD	TMC (n=8) Mean±SD	TDC (n=8) Mean±SD	NesC (n=8) Mean±SD	AC (n=8) Mean±SD	ANOVA F	P value
Uric acid (mg/dl)	2.3±1.2 b	2.3±0.8 b	1.8±0.9 a	1.8±0.9 a	1.7±0.6 a	0.6	0.676
Urea (mg/dl)	57.0±3.5 a	70.4±15.7 ab	71.6±33.9 ab	90.8±24.3 b	82.4±15.9 ab	1.7	0.191
AST (U/l)	251.6±54.9 a	230.2±57.8 b	265.0±85.0 a	247.5±67.5 a	267.6±29.3 a	0.3	0.874
ALT (U/l)	54.4±17.2 a	41.6±8.4 b	60.8±32.1 a	46.8±16.9 a	52.4±11.8 a	0.7	0.589

TCM= Turkish coffee (medium roasting); TCD= Turkish coffee (dark roasting); NesC= Nescafe; AC= Arabic coffee. ANOVA= Analysis of variance. SD= Standard deviation. Mean values subscribed with different letters show significant differences between these values as calculated by one way ANOVA and LSD at $P<0.05$.

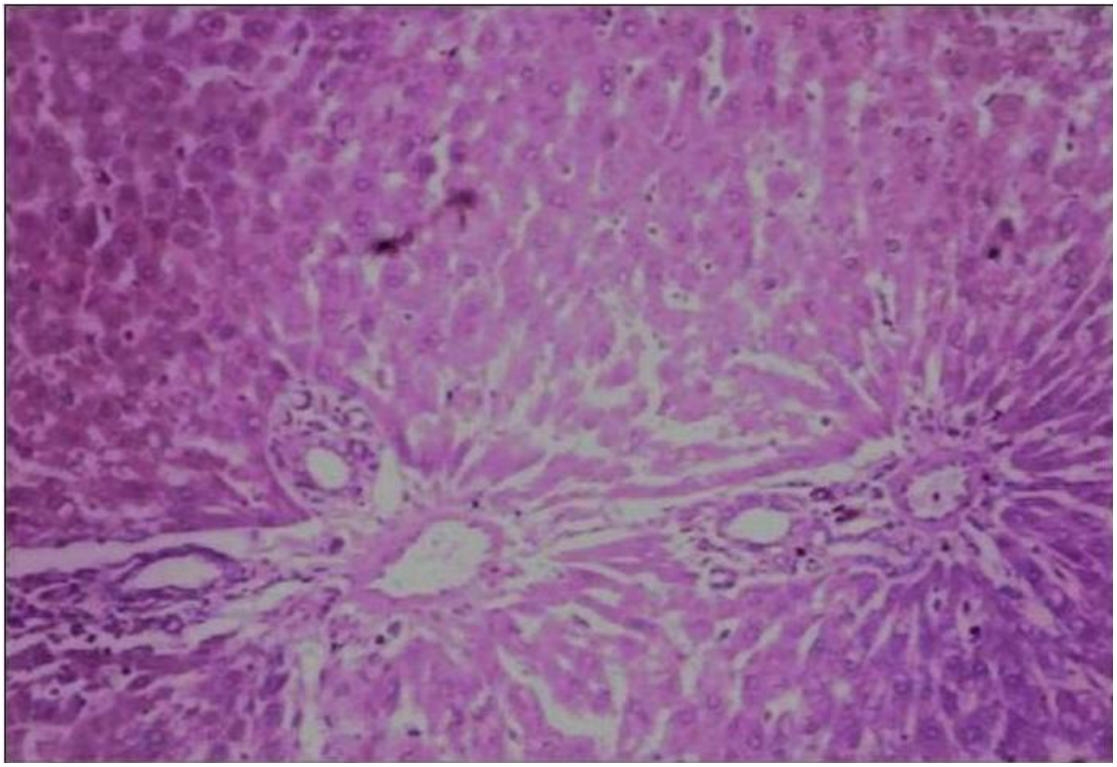
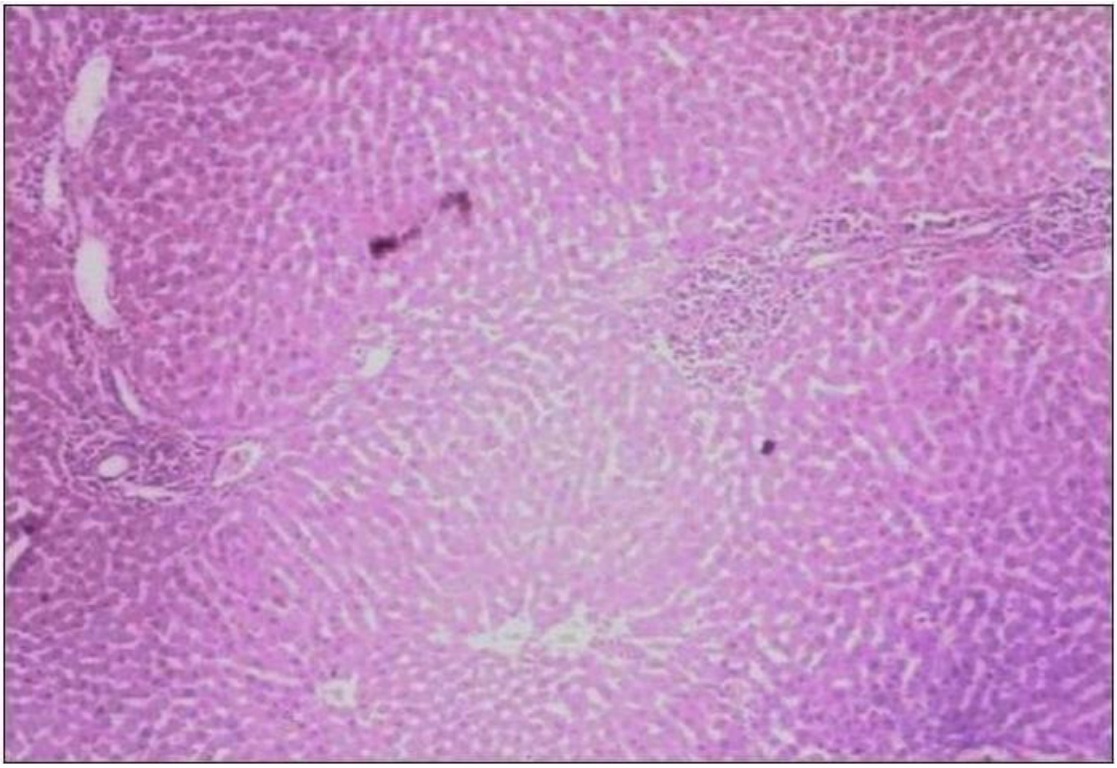


Figure 1. Liver of control group. As shown, there are normal hepatocytes, central viena and portal tracts.



Figuer 2. Liver of Turkish coffee (medium roasting) group. As shown, there are minimal degenerative changes with infiltration of portal tracts with mono-nuclear cells.

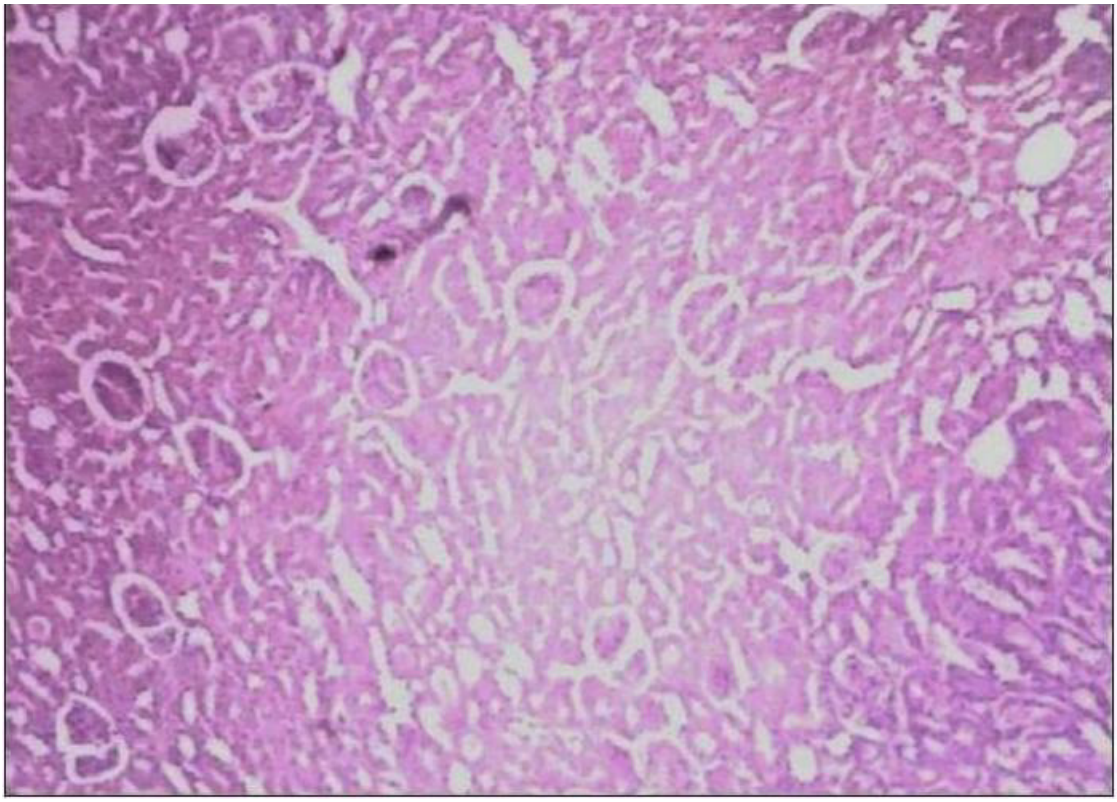


Figure 3. Liver of Turkish coffee (dark roasting) group. As shown there are mild degenerative changes of the tubular epithelium.

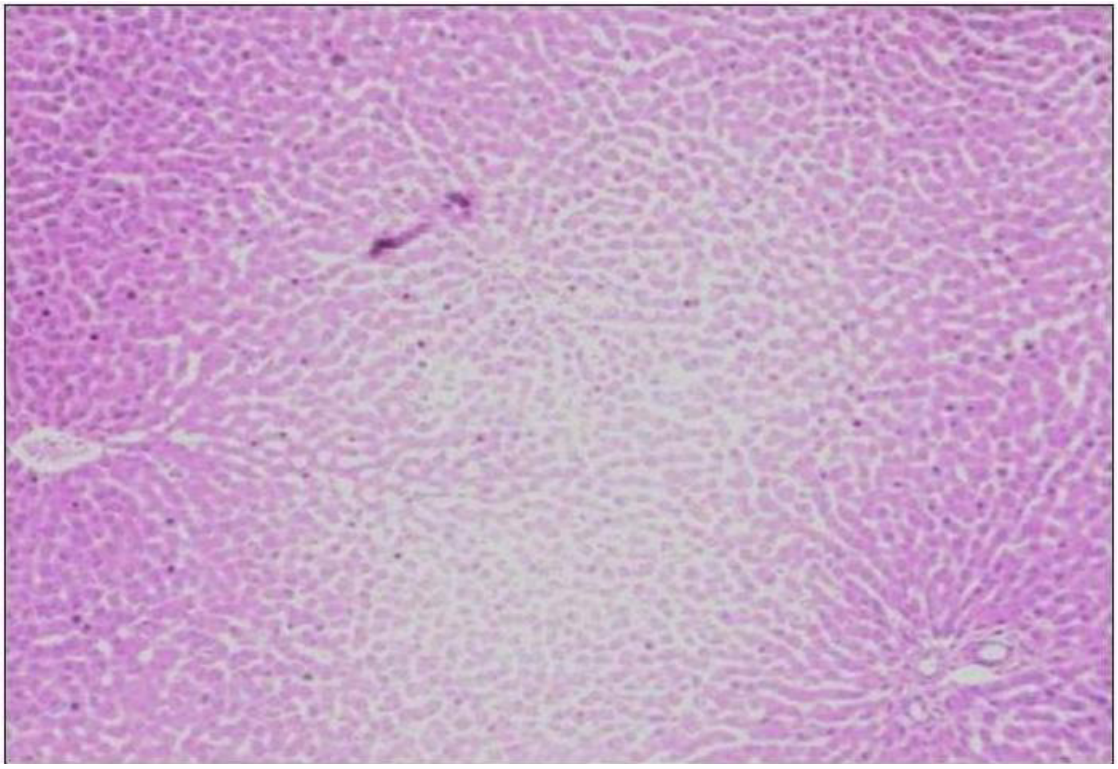


Figure 4. Liver of Nescafe group. As shown, there are medium degenerative changes of the hepatocytes.

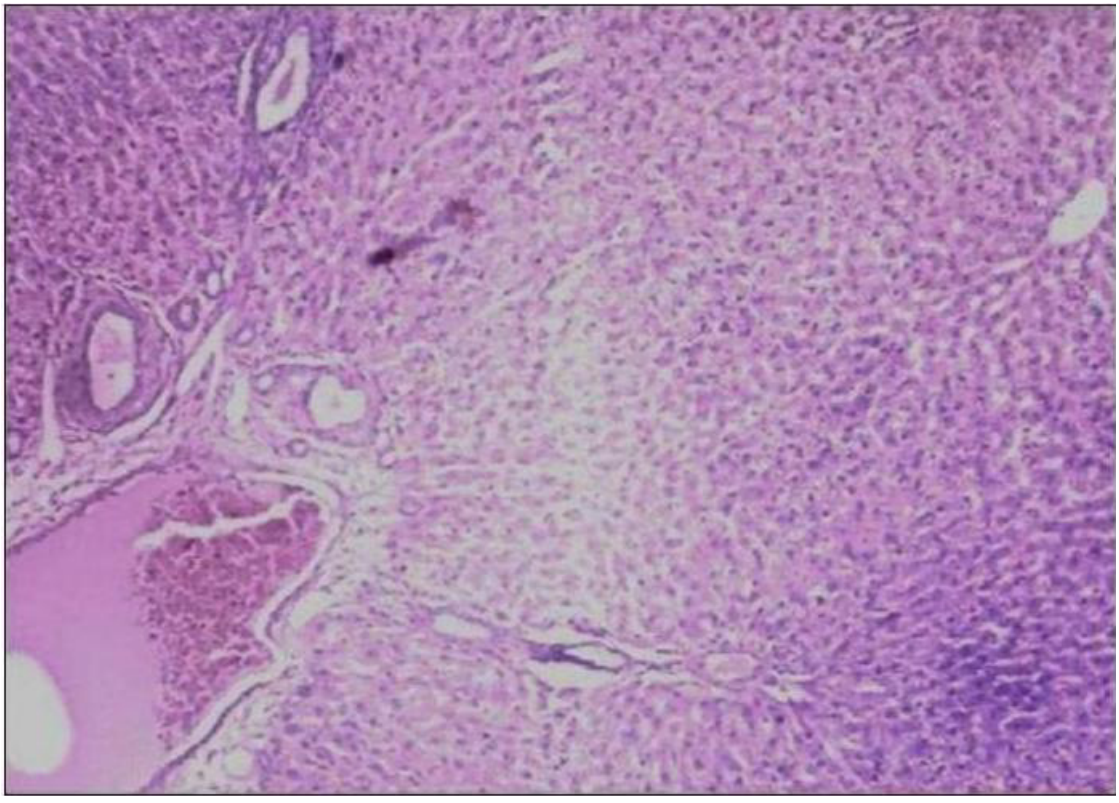


Figure 5. Liver of Arabic coffee group (AC). As shown, there are mild degenerative changes of hepatocytes.

DISCUSSION

When compared with control group, the results showed that rats fed different preparations of coffee had significantly smaller weight gain than control group who gained more and significant body weight. On the other hand, group fed Nescafe lost considerable amount of body weight, which in turn mean that drinking Nescafe had negative effect on body weight. In addition, we noticed that the food intake by Nescafe group was the lowest among studied groups. Lopez-Garcia et al. (2006) found that coffee consumption had adverse effects on body weight, and they attributed that effect to caffeine intakes. Some studies explained the relationship between coffee consumption and body weight. Some of these studies suggested that caffeine has several important metabolic effects and work as an adenosine-receptor antagonist (Van Soeren and Graham, 1998), and all tissues with adenosine receptors can be affected by caffeine exposure. Astrup et al. (1990) observed a dose-dependent increase in BMR with caffeine intake in healthy subjects who had moderate habitual caffeine consumption. The researchers attributed this effect to an increase in lactate and triacylglycerol production and increased vascular smooth muscle tone. Acheson et al. (2004) suggested that caffeine might stimulate thermogenesis by increasing lipid turnover. All the above mechanisms suggest a beneficial effect of caffeine on energy metabolism.

The results of this study showed that different preparations of coffee especially Turkish coffee dark roasting, Nescafe and Arabic coffee reduced serum uric acid. In agreement with our findings, a cross-sectional study of Japanese, Kiyohara et al. (1999) found that coffee consumption was negatively associated with serum uric acid, and the mean serum level in individuals consuming more than five cups of coffee daily was lower than that in individuals consuming one cup by 0.4 mg/dl. In concordance, a study of US men and women (Choi and Curhan, 2007) found that serum uric acid level decreased significantly with increasing coffee intake, and there was no association with total caffeine intake. These findings suggest that there are some components of coffee other than caffeine contribute to the observed inverse effect of coffee on uric acid levels. In this line, some studies tried to test whether caffeine intake affect serum uric acid or no? Moreover, one study found that coffee itself (caffeinated and decaffeinated coffee) not caffeine decreased insulin resistance and subsequently decreased serum uric acid level (Wu et al., 2005). Because there is an evident positive correlation between serum insulin resistance and elevated serum uric acid – the higher the insulin resistance, the higher the serum uric acid level– (Fam 2002, and Choi et al., 2005), decreased insulin resistance and insulin levels associated with coffee consumption may lead to lower uric acid levels (Choi and Curhan, 2007).

Other studies suggest that coffee is the major source of chlorogenic acid, which is a strong antioxidant (Van Dam and Hu, 2005 and Wu et al.,2004). Earlier studies have found that plasma glucose concentrations are reduced by chlorogenic acid (Arion et al.,1997 and Wu et al., 2004), which may combine with other antioxidants in coffee to decrease oxidative stress. Nevertheless, antioxidants may improve insulin sensitivity and decrease its levels in rats (Thirunavukkarasu and 2004, and Wu et al., 2004). Chlorogenic acid also acts as a competitive inhibitor of glucose absorption in the intestine (Clifford, 2000 and Van Dam and Hu 2005). Furthermore, the effect of caffeine may also depend on other components of coffee. It has also been speculated that noncaffeine xanthines contained in coffee may inhibit xanthine oxidase, thus contributing to lowering serum uric acid levels (Kiyohara et al., 1999).

The results showed that in comparison with control and other groups, rats fed Turkish coffee medium roasting had significantly ($P<0.05$) the lowest values of AST and ALT, then the group fed Nescafe but the values for Nescafe group were not significant. Most of studies obtained similar results, where earlier studies found that liver enzymes (gamma-glutamyl transferase, alanine-amino transferase, and alkaline phosphatase) and serum bilirubin in coffee drinkers were lower than in non-coffee-drinking subjects. The researchers proposed that liver enzymes are a target for caffeine contained in coffee (Casiglia et al.,1993). Also, the results of Japanese study (Noriyuki et al., 2000) suggest that coffee may inhibit the elevation of serum AST and/or ALT levels and that its effect is more pronounced in the protection of the development of higher serum AST and/or ALT levels. More recently Ruhl and Everhart (2005) found that coffee and tea drinking decreases the risk of clinically significant chronic liver disease and also they attributed those effects to caffeine, they concluded that persons at high risk for liver injury, consumption of coffee and especially caffeine was associated with lower risk of elevated ALT activity. Finally, the findings of Costentin et al. (2011) supported potential hepatoprotective properties of caffeine in chronic liver diseases. Other studies attributed the hepatoprotective properties of coffee to chlorogenic acid. According to Moon et al. (2009) chlorogenic acid, which is a polyphenol present in larger amounts in green coffee beans than in roasted coffee, and this may explain why Turkish coffee (medium roasting) in our study decreased significantly ALT and AST than other coffees. However, we think that although coffee consumption decrease serum liver enzyme but the mechanism is still not fully understood.

CONCLUSION

In conclusion, moderate amounts of Nescafe have the most favorable effects on body weight, serum uric acid,

and liver enzymes. In addition, Turkish coffee, and Arabic coffee were found to reduce body weight. Turkish coffee (medium roasting) decreased liver enzymes more than other experimental groups and could therefore be helpful in improving health status of persons who suffering from elevated liver enzymes.

REFERENCES

Acheson KJ, Gremaud G, MeirimI (2004). Metabolic effects of caffeine in humans: lipid oxidation or futile cycling? Am. J. Clin.Nutr. 79: 40–6.

Arion WJ, Canfield WK, Ramos FC, Schindler PW, Burger HJ, Hemmerle H (1997). Chlorogenic acid and hydroxynitro-benzaldehyde: new inhibitors of hepatic glucose 6-phosphatase. Arch. Biochem. Biophys. 339:315–22.

Astrup A, Toubro S, Cannon S, Hein P, Breum L, Madsen J (1990). Caffeine: a double-blind, placebo-controlled study of its thermogenic, metabolic, and cardiovascular effects in healthy volunteers. Am. J. Clin. Nutr. 51:759–67.

Bak AA, Grobbee DE (1989). The effect on serum cholesterol levels of coffee brewed by filtering or boiling. N. Engl. J. Med. 321:1432–7.

Baylin A, Hernandez-Diaz S, Kabagambe EK, Siles X, Campos H (2006). Transient exposure to coffee as a trigger of a first nonfatal myocardial infarction. Epidemiology 17: 506–11.

Bonita JS, Mandarano M, Shuta D, Vinson J (2007). Coffee and cardiovascular disease: in vitro, cellular, animal, and human studies. Pharmacol. Res. 55:187–98.

Buchanan J, Isdale C, Rose S (1965). Serum uric acid estimation chemical and enzymatic methods compared. Ann. Rheum. dis. 24:285–288.

Butt MS, Sultan MT (2011). Coffee and its consumption: benefits and risks. Crit. Rev. Food Sci. Nutr. 51(4), 363–373.

Casiglia E, Spolaore P, Ginocchio G, and Ambrosio GB (1993). Unexpected effects of coffee consumption on liver enzymes. Eur. J. Epidemiol. 9(3):293-7.

Chen Zhang, Robert Linforth, Ian D. Fisk (2012). Cafestol extraction yield from different coffee brew mechanisms. Food Res. Int. 49: 27–31.

Choi HK, Curhan G (2007). Coffee, Tea, and Caffeine Consumption and Serum Uric Acid Level: The Third National Health and Nutrition Examination Survey. Arthritis and Rheumatism (Arthritis Care & Research). 57 (5): pp 816–821.

Choi HK, Mount DB, Reginato AM (2005). The American College of Physicians, and the American Physiological Society. Pathogenesis of gout. Ann Intern Med 143:499–516.

Chown S, Petrie H, Duncan A, Battram DB, Belfie L, Conquer J, Graham TE(2001) Caffeine increases the insulin/glucose response to an OGTT in obese, resting males. Can. J. Appl. Physiol. 26 (suppl.): S249.

Clifford MN (2000). Chlorogenic acid and other cinnamates: nature, occurrence, dietary burden, absorption and metabolism. J Sci Food Agric 80:1033–43.

Costentin CE, Roudot-Thoraval F, Zafrani ES, Medkour F, Pawlotsky JM, Mallat A, Hézode C (2011). Association of caffeine intake and histological features of chronic hepatitis C. J Hepatol54 (6):1123-9.

de Roos B, Caslake MJ, Stalenhoef AF, Bedford D, Demacker PN, Katan MB, Packard CJ (2001). The coffee diterpene cafestol increases plasma triacylglycerol by increasing the production rate of large VLDL apolipoprotein B in healthy normolipidemic subjects. Am J ClinNutr. 73:45–52.

Fam AG (2002). Gout, diet, and the insulin resistance syndrome. J Rheumatol29:1350–5.

Habeeb S, James P (2010). From the Lands of Figs and Olives. I.B.Tauris Publishers. pp. 232–233.

Jee H, He J, Whelton K, Suh I, Klag J (1999). The effect of chronic coffee drinking on blood pressure: a meta-analysis of controlled clinical trials. Hypertension 33:647–52.

Keijzers GB, De Galan BE, Bastiaan E, Tack CJ, Smits P (2002). Caffeine can decrease insulin sensitivity in humans. Diabetes Care 25: 364–369.

Kempf K, Herder C, Erlund I, Kolb H, Martin S, Carstensen M (2010). Effects of coffee consumption on subclinical inflammation and other risk factors for type 2 diabetes: a clinical trial. Am J ClinNutr 91:950–7.

Kiyohara C, Kono S, Honjo S, Todoroki I, Sakurai Y, Nishiwaki M (1999). Inverse association between coffee drinking and serum uric acid concentrations in middle-aged Japanese males. Br J Nutr 82:125–30.

Lopez-GarciaE, Rob M van Dam, SwapnilRajpathak, Walter C Willett, JoAnn E Manson, Frank B Hu (2006). Changes in caffeine intake and long-term weight change in men and Women. Am J ClinNutr 83:674–80.

Mahmoud ON, Al-Qahiz NM, Ismail MS (2013). Different doses of Arabic coffee improve serum lipid profile, uric acid and liver enzymes of experimental rats. Food and Public Health, 3(4): 228-233.

Medina-Remon A, Zamora-Ros R, Rotches-Ribalta M, Andres-Lacueva C, Martınez-Gonzalez MA, Covas MI (2010). Total polyphenol excretion and blood pressure in subjects at high cardiovascular risk. NutrMetabolCardiovasc Dis 21:323–31.

Moon JK, Yoo HS, Shibamoto T (2009). Role of roasting conditions in the level of chlorogenic acid content in coffee beans: correlation with coffee acidity. J Agric Food Chem 57:5365–9.

Mostofsky E, Schlaug G, Mukamal KJ, Rosamond WD, Mittleman MA (2010). Coffee and acute ischemic stroke onset: the Stroke Onset Study. Neurology; 75: 1583–8.

Noriyuki N, Koji N, Kenji S, Kozo T (2000). Effects of Coffee Consumption against the Development of Liver Dysfunction: A 4-Year Follow-Up Study of Middle-Aged Japanese Male Office Workers. Industrial Health 38, 99–102

Ranheim T, Halvorsen B (2005). Coffee consumption and human healthdbeneficial or detrimental?dMechanisms for effects of coffee consumption on different risk factors for cardiovascular disease and type 2 diabetes mellitus. MolNutr Food Res 49:274–84.

Rej R (1984). Measurement of amino-transferases: Part I, aspartate amino-trasferase. Crit. Rev. Clin. Lab. Sci. 21: 99-186.

Ruhl CE, Everhart JE (2005). Coffee and caffeine consumption reduce the risk of elevated serum alanine aminotransferase activity in the United States. Gastroenterology 128: 24–32.

Saab S, Mallam D, Cox GA (2014).Impact of coffee on liver diseases: a systematic review. Liver Int. 34(4): 495–504.

Salazar-Martinez E, Willett W, Ascherio A, Leitzmann M, Manson J, Hu FB (2003). Coffee consumption and risk of type 2 diabetes in men and women. Diabetes 52: A72 (abs.).

Sunil KP, Hemant P, Jennifer W, Lindsay B (2012). Coffee Extract Attenuates Changes in Cardiovascular and Hepatic Structure and Function without Decreasing Obesity in High-Carbohydrate, High-Fat Diet-Fed Male Rats. J. Nutr. 142: 690–697.

Thirunavukkarasu V, Anuradha CV (2004). Influence of alpha-lipoic acid on lipid peroxidation and antioxidant defence system in blood of insulin-resistant rats. Diabetes ObesMetab 6.

Tietz NW (1970). Fundamentals of Clinical Chemistry. W. B. Saunders Co. Philadelphia. USA.

Tofovic SP, Kost CK Jr, Jackson EK, Bastacky SI (2002). Long-term caffeine consumption exacerbates renal failure in obese, diabetic, ZSF1 (fa-fa (cp)) rats. Kidney Int. 61:1433–44.

Urgert R, Essed N, Van der Weg G, Kosmeijer-Schuil T, Katan M (1997). Separate effect of the coffee diterpenes cafestol and kainweol on serum lipids and liver aminotransferascs. Am. J. Clin. Nutr; Vol., 65, pp. 519-524.

Urgert Rob, Angela GM Schulz, Martijn B Katan (1995). Effects of cafestol and kahweol from coffee grounds on serum lipids and serum liver enzymes in humans. Am J ClinNutr 61:149-54.

van Dam RM, Feskens EJM (2002). Coffee consumption and risk of type 2 diabetes mellitus. Lancet 360: 1477–1478.

Van Dam RM, Hu FB (2005). Coffee consumption and risk of type 2 diabetes: a systematic review. JAMA 294:97–104.

Van Dieren S, Uiterwaal CSPM, Van der Schouw YT, van der ADL, Boer JMA, Spijkerman A (2009). Coffee and tea consumption and

risk of type 2 diabetes. Diabetologia 52:2561–9.
Van Soeren MH, Graham TE (1998). Effect of caffeine on metabolism, exercise endurance, and catecholamine responses after withdrawal. J ApplPhysiol 85:1493–501.
Wu T, Giovannucci E, Pischon T, Hankinson SE, Ma J, Rifai N (2004). Fructose, glycemic load, and quantity and quality of carbohydrate in relation to plasma C-peptide concentrations in US women. Am J ClinNutr 80:1043–9.
Wu T, Willett WC, Hankinson SE, Giovannucci E (2005). Caffeinated coffee, decaffeinated coffee, and caffeine in relation to plasma C-peptide levels, a marker of insulin secretion, in US women. Diabetes Care 28:1390–6.
Yamaguchi T, Chikama A, Mori K, Watanabe T, Shioya Y, Katsuragi Y (2008). Hydroxyhydroquinone-free coffee: a double-blind, randomized controlled dose-response study of blood pressure. Nutr. Metabol. Cardiovasc. Dis. 18:408–14.