ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

DEPARTMENT OF BIOCHEMISTRY

INVESTIGATION OF THE EFFECT OF COFFEE ON BODY WEIGHT, SERUM GLUCOSE, URIC ACID AND LIPID PROFILE LEVELS IN MALE ALBINO WISTAR RATS FEEDING ON HIGH-FRUCTOSE DIET

By: Teka Obsa

A Thesis Submitted to Addis Ababa University, School of Graduate Studies, Department of Biochemistry in Partial Fulfillment of the Requirement for the Degree of Master of Sciences in Medical Biochemistry.

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This is to clarify that thesis prepared by Teka Obsa entitled, "Investigation of the effect of coffee on body weight, serum glucose, uric acid and lipid profile levels in male albino Wistar rats feeding on high-fructose diet," is submitted in partial fulfillment of the Requirement for the Degree of Master of Sciences in Medical Biochemistry, complies with the regulations of the university and meets the accepted standards with respect to the originality and quality.

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List of abbreviations and acronyms

ANOVA	Analysis of Variance
ATP	Adenosine Triphosphate
BMI	Body Mass Index
BW	Body Weight
cAMP	Cyclic Adenosine Monophasphate
CGAs	Chlorogenic Acids
ChREBP	Carbohydrate Responsive Element–Binding Protein
DNL	De Novo Lipogenesis
F1P	Fructose-1- phosphate
FFA	Free Fatty Acid
HDL-C	High Density Lipoprotein Cholesterol
IDF	International Diabetes Federation
LDL-C	Low Density Lipoprotein Cholesterol
MetS	Metabolic Syndrome
NCEP ATP III	National Cholesterol Education Program Adult Treatment Panel III
SPSS	Statistical Package for Social Science
SREBP1c	Sterol Regulatory Element–Binding Protein 1c
T2DM	Type 2 Diabetes Mellitus
TC	Total Cholesterol
TG	Triglyceride
UA	Uric Acid
WHO	World Health Organization

Abstract

Background: High dietary intake of fructose is an important nutritional factor in the development of metabolic syndrome (MetS) and its associated complications. Consumption of coffee, functional foods such as vegetables and fruits are believed to have protective effects against MetS.

Objective: The main objective of the present study was to investigate the effect of coffee on body weight, serum glucose, uric acid and lipid profile levels in male albino Wistar rats feeding on high fructose diet.

Methodology: A post-test experimental study was conducted on a total of 30 (9-10 weeks old) male albino Wistar rats. The rats were divided into 6 groups: group I (normal control)-fed on standard chow and plain tap water only; group II (fructose control)-fed on standard chow and 20% of fructose solution; group III–VI (treatment groups)-fed on standard chow, 20% of fructose solution and treated with 71, 142, 213 and 284mg/kg body weight/day of coffee respectively for six weeks. At the end, diet consumption, body weight, serum glucose, uric acid and lipid profile levels were investigated. Data was entered by Epi-data v. 3.1 and analyzed by one way ANOVA followed by Tukey post hoc multiple comparison tests using SPSS V. 23.00. Statistical significance was considered at $p < 0.05$.

Results: Body weight, fasting serum glucose and uric acid levels significantly lowered in rats treated with 213 ($p = 0.047$; 0.049; 0.026) and 284 ($p = 0.035$; 0.029; 0.010) mg/kg body weight/day of coffee compared to fructose control group. Fasting serum triglycide (TG) and low density lipoprotein (LDL-C) levels showed significant reduction in rats treated with 284mg/kg body weight/day of coffee as compared to fructose control group ($p = 0.031$; 0.046) respectively. Statistically significant difference was not found in fasting serum levels of TC and HDL-C between coffee treatment groups and fructose control group ($p > 0.05$). Body weight was positively correlated with serum glucose ($r = 0.546$, $p = 0.002$), uric acid ($r = 0.560$, $p = 0.001$), TC ($r = 0.435$, $p = 0.016$), LDL-C ($r = 0.473$, $p = 0.008$) and TG ($r = 0.344$, $p = 0.063$) levels and negatively correlated with HDL-C level ($r = -0.216$, $p = 0.253$).

Conclusion: Treating rats with coffee decreased body weight, fasting serum glucose, uric acid, TC, TG and LDL-C, and increased HDL-C in a dose dependent manner in rats feeding on high fructose diet, suggesting that coffee consumption may be helpful in ameliorating metabolic syndrome.

Key words: Body weight, Coffee, Glucose, High-fructose diet, Lipid profiles, Uric acid

1. Introduction

1.1. Background

Fructose is a ketohexose monosaccharide, composed of six carbon atoms in simple covalent bonds, with a carbonyl group at the end of the chain (molecular formula, $C_6H_{12}O_6$), (Samuel, 2011). Sugar in the form of sucrose or high-fructose corn syrup, both of which are composed of nearly equal amounts of glucose and fructose, is added to numerous manufactured food products (Kit *et al*., 2013). Fructose has been claimed to be of health benefit, because it may aid glycemic control, but also has been claimed to be more harmful than other sugars (Kolderup and Svihus, 2015). Since fructose is less able to promote satiety and is more palatable, it will stimulate a higher consumption of food and alter the metabolism of lipids and carbohydrates thereby favoring the synthesis and accumulation of fat (Stanhope, 2016; Johnson *et al*., 2007). There is a growing body of evidence in both animal models and human studies suggesting that high dietary intake of fructose is an important nutritional factor in the development of metabolic syndrome (MetS) and its associated complications (Miller and Adeli, 2008).

Metabolic syndrome is a cluster of related metabolic abnormalities such as central obesity, hypertension, dyslipidemia, hyperglycemia and insulin resistance, and is associated with increased risk of type 2 diabetes mellitus (T2DM) and coronary heart disease (Srikanthan *et al*., 2016; Suwannaphet *et al*., 2010). The World Health Organization (WHO) estimates that more than half of a billion adults worldwide are classified as obese (Mendis, 2015) and the global prevalence of the MetS ranges from <10% to 84% between populations (Desroches & Lamarche, 2007). Lifestyle factors associated with an increased risk of MetS include a diet with a high refined grains, especially fructose, meat, and fried foods; low diet quality, i.e. high fat, low dietary fiber and vitamins and smoking (Srikanthan *et al*., 2016; Takami *et al*., 2013).

Consumption of coffee, functional foods such as vegetables, fruits and high levels of leisure-time physical activities are believed to have protective effects against MetS. Coffee is a sort of soft beverage prepared from dried and roasted seeds of coffee and consumed worldwide (Zhang *et al*., 2016; Bakuradze *et al*., 2014). The history of coffee began in 800 years A.D. in the Ethiopian highlands with the legend of Kaldi, the goat herder (Whayne, 2015).

There are hundreds of different species of coffee, however, commercial production mainly exploits the seeds of *Coffea arabica* (Arabica coffee), which represent about 70% of the world market, and *Coffea canephora* (Robusta coffee), which has a more bitter taste than arabica coffee, is used principally for instant and espresso coffee (Salomone *et al*., 2017). Coffee contains a multitude of substances, many of which are potentially biologically active. Although the main physiologic effects resulting from its consumption are usually ascribed to the presence of caffeine, coffee is also an extremely rich source of chlorogenic acids (CGA), melanoidins and diterpenes (Akash *et al*., 2014; Chen *et al*., 2011).

Coffee has several preparation methods, which significantly affect aroma, flavor and composition of coffee brews. Many of the coffee compounds are formed during the roasting process, which produce the unique taste and smell of coffee (Parliament and Stahl, 2005). On the other hand, the roasting process may cause degradation of many of other compounds, such as antioxidant polyphenols. Based on the brewing process, three main types of coffee can be distinguished: (i) boiled unfiltered coffee, (ii) filtered coffee and (iii) decaffeinated coffee (DC). Each of this type of coffee is related to specific granulation of coffee powder, water/coffee ratio, temperature and brewing time (Godos *et al*., 2014).

The numerous beneficial health effects of coffee consumption have received significant scientific attention over past few decades. The results of epidemiological and experimental studies suggest that drinking coffee regularly helps prevent several chronic diseases such as cardiovascular disease, T2DM, cancer, Parkinson disease, reproductive and inflammatory conditions (Penafort *et al*., 2016; Pourshahidi *et al*., 2016; Henry-Vitrac *et al*., 2010).

In general, worldwide, MetS has been diagnosed using several different sets of criteria such as those of the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) and the International Diabetes Federation (IDF). These definitions differ somewhat in terms of the prerequisite components and the cut-off levels used for each component (Takami, 2013). A progressive condition of MetS that encompasses a wide array of disorders with specific metabolic abnormalities presenting at different times can be detected and monitored via serum biomarkers (Srikanthan *et al*., 2016). Therefore, the present study was aimed at investigating the effects of coffee on body weight and serum biomarkers of metabolic syndromes such as glucose, uric acid and lipid profile levels in high fructose diet feeding male albino Wistar rats.

1.2. Literature review

1.2.1. Overview of fructose metabolism

Fructose is a monosaccharide having the same chemical formula as glucose ($C_6H_{12}O_6$), but its metabolism differs markedly from that of glucose due to its almost complete hepatic extraction and rapid hepatic conversion into glucose, glycogen, lactate, and fat (Nehal *et al*., 2016; Samuel, 2011). Fructose is up taken to cells via a transporter called GLUT5 and GLUT2 (to smaller extent) which accepts fructose to the liver cells. Whereas, glucose is up taken to most of the cells in the body for metabolism. It uses different types of transporters to be distributed to corresponding cells: GLUT1 (to all cells), GLUT2 (to gut, liver and pancreas), GLUT3 (to central nervous system and brain) and GLUT4 (to cells that need insulin such as skeletal muscles, fatty tissues and heart), (Jegatheesan and Bandt, 2017; Dourd and Ferraris, 2008).

In the liver, fructose is preferentially converted into fructose-1-phosphate (F1P) by fructokinase. Thereafter, phosphotrioses (dihydroxyacetone phosphate and glyceraldehyde-3-phosphate) produced from F1P through the action of aldolase B can be converted into glucose, lactate, and fatty acids (Tappy and Le, 2010; Ouyang *et al*., 2008). Fructokinase, which is induced by fructose, has a high affinity for fructose and is not controlled by insulin. Glucose metabolism depends on rate-limiting enzyme, phosphofructokinase, which is inhibited by adenosine monophosphate (AMP) and citrate, and limits the accumulation of carbon-rich intermediates entering Krebs cycle (Samuel, 2011; Tappy and Lê, 2010).

1.2.2. Metabolic effects of fructose

Feeding animals on large amounts of fructose can rapidly produce multiple features of the metabolic syndromes, including obesity, dyslipidemia, fatty liver, hypertension, insulin resistance, and diabetes (Sarah *et al*., 2018; Yeh *et al*., 2014). For instance, feeding fructose to rats causes rapid development of the metabolic syndrome (Nakagawa *et al*., 2005).

1.2.2.1. Effects of fructose on appetite and body weight

Fructose is the sweetest sugar, and generally enhances food palatability that may increase feeding behavior and encourage overeating (Avena, 2007). Fructose and sucrose can enhance food palatability and induce addiction-like behaviors such as binging and dependence in part by stimulating dopaminergic pathways (Tellez, 2016; Lane and Cha, 2009). Moreover, ingestion of fructose also decreases postprandial glucose levels and this lowers insulin and leptin levels, thereby predisposing the individual to eat continuously (Nakagawa, *et al*., 2005; Teff *et al*., 2004). Overconsumptions of sugar sweetened beverages and fructose are consistently associated with increased adiposity, which may attribute to increased caloric intake and body weight gain (Avena, 2007). Some studies in humans also documented that, a reduction in resting energy expenditure occurs in overweight and obese subjects fed fructose, but not glucose (Bawden *et al*., 2012; Cox *et al*., 2012).

1.2.2.2. Effects of fructose on lipid homeostasis

Excessive fructose consumption markedly enhances triglyceride synthesis, which increases intramyocellular TG content in the skeletal muscle and stimulates hepatic de novo lipogenesis (DNL) causing insulin resistance (Maarman *et al*., 2016; Nakagawa, *et al*., 2005). While the lipogenic pathway is quantitatively minor in physiological situations, it becomes very active immediately after an acute fructose load (Softic *et al*., 2016; Hudgins *et al*., 2011). The flux of fructose carbons into lipogenic precursors increases as the formation of F1P bypasses the glycolysis regulatory site of phosphofructokinase 1. This leads to an accumulation of glycolytic intermediates that can be converted to glycerol-3-phosphate and acetyl-CoA, which are used in TG synthesis (Jegatheesan and Bandt, 2017).

In addition to providing substrates for lipogenesis, chronic fructose consumption increases DNL by activating key transcription factors such as sterol regulatory element–binding protein 1c (SREBP1c) and carbohydrate responsive element–binding protein (ChREBP). SREBP1c promotes lipid synthesis and is regulated by nutrients and hormones, especially insulin. Chronic fructose ingestion can lead to hyperinsulinemia, which may increase hepatic SREBP1c expression and activation (Herman and Samuel, 2016; Li *et al*., 2010). Fructose may also activate SREBP1c independently of insulin (Koo *et al*., 2009).

ChREBP couples carbohydrate metabolites to lipid synthesis by inducing enzymes required for DNL. It is highly expressed in key metabolic tissues, including liver, adipose tissue, small intestine, pancreatic islets and kidney, where it regulates carbohydrate metabolism in an insulin-independent manner (Boergesen *et al*., 2011; Uyeda and Repa, 2006). Moreover, malonyl-CoA, generated via DNL, limits fatty acid oxidation by inhibiting carnitine palmitoyltransferase 1A (CPT1A), the enzyme required for translocation of fatty acids into the mitochondria (McGarry, 2002).

1.2.2.3. Effects of fructose on glucose homeostasis

Fructose does not directly stimulate pancreatic β-cells insulin secretion. However, high-fructose feeding readily induces hyperinsulinemia in animal models (Adams *et al*., 2008). Similarly, hypercaloric fructose feeding increases circulating insulin in human subjects. Fructose-induced hyperinsulinemia, often considered a proxy for insulin resistance, might be the result of insulin resistance in some combination of liver, muscle, and/or adipose tissue, which directly causes hyperglycemia (Ter *et al*., 2016). The mechanisms by which high-fructose feeding causes hyperinsulinemia and insulin resistance remain uncertain.

1.2.2.4. Effects of fructose on uric acid homeostasis

Fructose intake, particularly in the form of high fructose corn syrup has paralleled the rise in metabolic syndrome and hyperuricemia. Hyperuricemia may occur as a consequence of rapid fructose entry from portal blood into the liver, where it will reduce the total adenosine nucleotide (TAN) pool in liver cells (Buul *et al*., 2014). Once absorbed into the cell, unregulated phosphorylation of fructose by fructokinase leads to local ATP depletion and increased AMP production, which in turn increases uric acid formation via purine degradation pathway (Vieira et al., 2015; Johnson *et al*., 2009).

Uric acid is commonly associated with gout, an inflammatory condition, metabolic syndrome, including hypertension and diabetes. Proposed mechanisms of uric acid mediated metabolic syndrome include the inhibition of endothelial nitric oxide (NO) synthase leading to hypertension; inflammation and oxidative stress in adipocytes leading to insulin resistance; and increased endothelial and smooth muscle oxidative stress (Wang *et al*., 2012; Zhu and Pandya, 2011).

1.2.3. Ingredients of coffee and their mechanisms of action

1.2.3.1. Caffeine

Caffeine (1, 3, 7-trimethylxanthine) is a purine-like alkaloid with diverse biological actions and is the most commonly consumed psychoactive substance in the world (Popoli and Pepponi, 2012). It is also a potent stimulant and bronchodilator (O'Keefe *et al*., 2013). It is naturally found in more than 60 plants, including coffee beans, tea leaves, cola nuts and cocoa pods. Coffee accounts for almost all (80.6%) of the caffeine that adults consume. Tea and soft drinks account for 12.3% and 5.9% respectively (Messina *et al*., 2015). As literature indicates, the caffeine content of coffee varies according to its preparation (filtration, percolation, boiling, instant, espresso, etc.), which can differ in various parts of the world and as a result, the caffeine content in a cup of coffee varies considerably (Penafort *et al*., 2016).

The caffeine molecule is structurally similar to adenosine (fig.1), which is an endogenous neurotransmitter with mostly inhibitory effects in the central nervous system (CNS) when acting through high-affinity adenosine (A1) receptors. In general, adenosine inhibits adenylyl cyclase via A1 receptors and stimulates adenylyl cyclase via low-affinity (A2) receptors (Godos *et al*., 2014). Caffeine exerts its pharmacological effects primarily by acting as a competitive antagonist of adenosine-receptor in the cerebral cortex, peripheral blood, kidneys, heart, gastrointestinal and respiratory tracts, and enhances neuronal activity (Penafort *et al*., 2016).

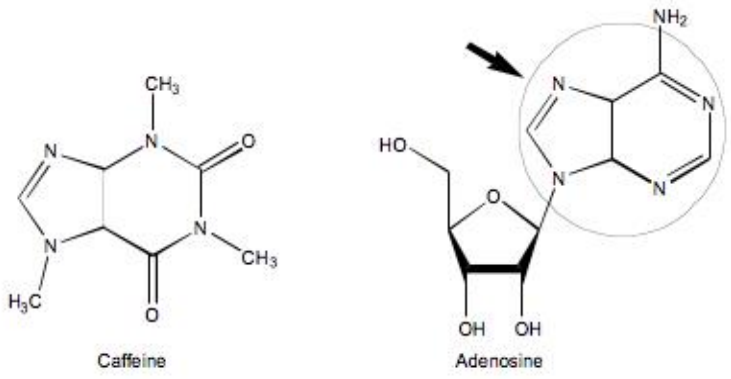

Figure 1. Chemical structure of caffeine and adenosine.

Caffeine crosses the blood-brain barrier easily, so that it produces stimulant effect on the CNS (Cakir *et al*, 2017; Popoli and Pepponi, 2012). Another effect of caffeine is the sympathetic stimulation, leading to a modest increase in blood pressure and lipolysis (Penafort *et al*., 2016). Caffeine is also known to inhibit gamma-aminobutyric acid (GABA) receptor signaling (Ribeiro and Sebastiao, 2010), and phosphodiesterase activity, preventing the breakdown of cAMP and stimulating lipolysis in fat cells (Gomes *et al*., 2013).

1. 2.3.2. Polyphenols

Coffee contains a complex mixture of polyphenols. Caffeic acid and its quinic acid-ester derivatives, chlorogenic acids (CGAs), are the major polyphenols in coffee. A typical cup of coffee can contain 70-300mg of CGAs (Zhao *et al*., 2012). In general, polyphenols have well-studied antioxidant activities in vitro (Wang and Ho, 2009). Chlorogenic acids reportedly benefit biological pathways related to risk of cognitive decline and dementia, including hypertension, inflammatory signaling, insulin signaling and glucose metabolism (Zhao *et al*., 2012).

It has been hypothesized that CGAs can protect against T2DM through its specific competitive inhibition of the glucose-6-phosphate translocase in rat liver microsomes. Glucose-6-phosphate translocase is an enzyme that catalyzes the terminal reactions in both glycogenolysis and gluconeogenesis, thus, it is highly involved in the regulation of homeostasis and blood glucose levels (Godos *et al*., 2014). It has also been reported that CGA activates adenosine monophosphate-activated protein kinase, a sensor and regulator of cellular energy balance, leading to beneficial metabolic effects such as suppression of hepatic glucose production and fatty acid synthesis (Ong *et al*., 2013).

Another proposed mechanism by which CGAs protect against diabetes mellitus is by inhibition of intestinal glucose absorption. Studies conducted on animals have shown that CGAs have reduced sodium-dependent glucose transport in brush border membrane vesicles isolated from rat small intestine. CGAs have also been reported to reduce intestinal absorption of glucose by inhibition of α-amylase (Narita & Inouye, 2009) and α-glucosidase activitities (Adisakwattana *et al*., 2009; Ishikawa *et al*., 2007).

1.2.3.3. Melanoidins and diterpenes

Melanoidins are formed as the final products of a non-enzymatic reaction between amino acids and reducing sugars that naturally occur in coffee beans and give coffee desirable flavor (Godos *et al*., 2014). Melanoidins can vary in content and bioactivity depending on the beans, roasting conditions, and decaffeination procedures (Chen *et al*., 2011). There is a wide evidence of the potential antioxidant and anti-inflammatory activity of melanoidins both in vitro and in vivo, despite the results are not univocal. The mechanism of the antioxidant action of coffee melanoidins is still unclear, but assumed to be based on their radical scavenging activity and/or their metal chelating capacity (Godos *et al*., 2014).

The two primary diterpenes in coffee are kahweol and cafestol, which reside in the lipid fraction (Cano-Marquina *et al*., 2013). Diterpenes can increase serum cholesterol depending 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 has a much lower concentrations of cafestol and kahweol (O'Keefe *et al*., 2013). Contents of major ingredients in coffee beans and beverages are summarized in table 1 below.

Table 1. Contents of major ingredients in coffee beans and beverages.

	Caffeine	Cafestol	Kahweol	Melanoidins	Chlorogenic acids
Green bean	3.85–4.29 mg/g	45–190 mg/kg	40-90 mg/kg	None	0.8–11.9%
Roasted bean	4.73–9.44mg/g	0.3–0.7% (arabica)	0.1–0.3% (Arabica)	7.2g/100g	Loss of 60.9–98%
Espereso (mg/50 mL)	97–330	0.06-0.17	0.09–0.18	116	160–185
Filtered (mg/150mL)	No data	0.02	0.02	270	165–285

Adapted from Godos *et al*. (2014).

1.2.4. Studies on health effects of coffee

Since coffee is one of the most commonly consumed beverages in the world, its beneficial effects on human health have been a subject of many studies (Tanaka *et al*., 2009). To date, numerous in vitro and in vivo studies, together with epidemiological and human trials have suggested beneficial health effects of coffee. Acute or habitual coffee consumption may reduce the risk of mortality, cardiovascular disease, T2DM, obesity, liver disease, cancer and a number of degenerative diseases such as Alzheimer's and Parkinson's (Cano-Marquina *et al*., 2013; Higdon and Frei, 2006). The probable mechanisms involved in prevention of T2DM by coffee consumption are shown in fig. 2 below.

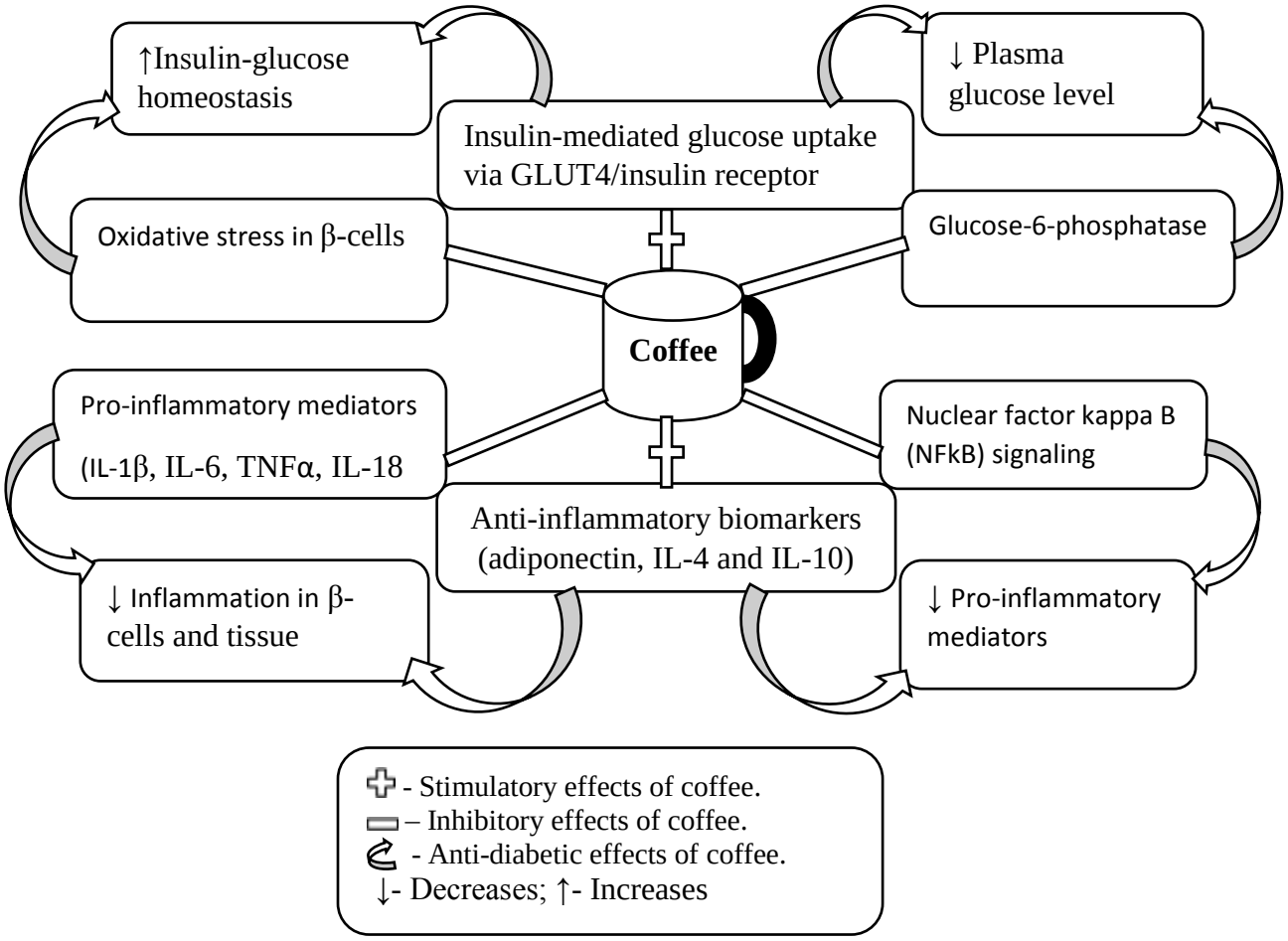

Figure 2. The probable mechanisms by which coffee consumption prevents T2DM. Adapted from Akash *et al*. (2014).

Coffee (caffeine) may cause weight loss by increasing physical activity. Caffeine has been found to increase motor activity in rodents at doses between 3 and 30 mg/kg body weight and to reduce motor activity at higher doses. Evidences from human subjects also indicate that ingestion of caffeine improves exercise performance, which could lead them to increase their level of physical activity (Greenberg *et al*., 2006). On the other hand, experimental study conducted to investigate the effects of coffee on dietary intake and appetite in normal-weight and overweight/obese individuals revealed no effect of coffee in normal weight individuals. However, coffee was found to significantly reduce energy intake in overweight/obese individuals (Gavrieli *et al*., 2013).

An experimental study conducted on male albino rats showed that rats fed different preparations of Turkish, Arabian and instant coffee had significantly smaller weight gain than control group. Group fed instant coffee lost considerable amount of body weight. Turkish coffee dark roasting was found to reduce serum TG, TC, LDL-C, VLDL-C, and total lipids (TL), whereas elevate HDL-C concentration significantly ($p < 0.05$), (Ismail *et al*., 2015). Another experimental study conducted on golden hamsters fed on high fat diet showed that eight weeks treatment with CGA has significantly lowered the levels of fasting serum TG, free fatty acid (FFA), TC, LDL-C, HDL-C, glucose and insulin. CGA also led to higher activity of hepatic lipase, lower contents of TG and FFA in liver, and lower activity of lipoprotein lipase in skeletal muscle (Li *et al*., 2009). However, the experimental study conducted on rats fed diet supplemented with low or high dose of coffee reported significant elevation of serum TL, TC, TG and LDL-C, while a significant decrease of HDL-C (Hanaa and Fattah, 2008).

In an experimental study conducted to investigate the effect of coffee on uric acid level, drinking of 2 cups of coffee/day in humans, which is equivalent to 0.72 mL/day in experimental rats, was able to decrease uric acid level as much as 15% in mice with hyperuricemia (Lelyana *et al*., 2015). Similar consecutive experimental study also reported that daily feeding of coffee (0.72 mL/200g/day) for 14 days decreased uric acid level in high carbohydrate fed obese Wistar rat models. It was also shown that coffee has anti-obesity activity and preventive action against metabolic syndrome diseases (Lelyana, 2016).

Another study conducted on human subjects showed that coffee consumption was significantly and inversely associated with fasting glucose, two-hour plasma glucose, and fasting insulin in both men and women (Bidel *et al*., 2006). Cross-sectional studies indicate that coffee consumers have lower body mass index (BMI) compared to individuals who do not consume coffee. Epidemiological evidence also indicates a negative association between coffee and/or caffeine consumption and body weight gain in the long term (Odegaard *et al.,* 2008*;* Wu *et al., 2008*).

In addition, a cross-sectional study conducted on human subjects in Japan reported that greater coffee consumption was associated with a significantly lower prevalence of metabolic syndrome, as defined by NCEP ATP III criteria (p = 0.03). The study results also showed participants who drank more coffee had a lower odds ratio (OR) for high serum triglycerides (p = 0.02), but not for increased waist circumference or high blood pressure. Moderate coffee consumption (1.5 to <3 cups/day) was associated with a significantly lower OR for high plasma glucose (Takami *et al*., 2013).

Moreover, study conducted to evaluate the effect of coffee indicated that regular coffee consumption is associated with beneficial health effects, including maintenance of DNA integrity, regulation of satiety, reductions of energy intake and increase of body fat catabolism (Bakuradze *et al*., 2014). A cross-sectional study conducted among Polish arm also showed that high coffee drinkers had lower BMI, waist circumference, systolic and diastolic blood pressure, TG and higher HDL-C than those drinking less than 1cup/day (Grosso *et al*., 2015).

In general, the relationship between coffee consumption and MetS has not yet been well-investigated, and results of previous epidemiological studies are contrasting. Several studies conducted on Japanese population and Southern European region (Mediterranean area) showed that coffee consumption was inversely associated with MetS. On the other hand, studies conducted in central and north European countries reported no relation between coffee consumption and the development of MetS or its components (Grosso *et al*., 2015). Further mover, to the best of our knowledge, most of the studies conducted so far to investigate the effect of coffee on metabolic syndromes were based on obese rat models. Therefore, the present study was aimed at investigating the protective effects of coffee against metabolic syndromes by exploring its effects on body weight, serum glucose, uric acid and lipid profile levels in high fructose diet feeding male albino Wistar rats.

1.3. Statement of the problem

The prevalence of MetS has been dramatically increasing worldwide due to a modern lifestyle and an increase in the consumption of high-sugar diets, especially fructose (Suwannaphet *et al*., 2010). The IDF estimates that 25% of the world's adult population is affected by MetS (Zimmet, 2010). Consumption of sugar sweetened beverages and processed foods, which has been associated with the prevalence of diet-induced obesity and T2DM, has increased in the last decade in developed countries and on the rise in developing countries, particularly in the African continent (Maarman *et al*., 2016). As reviewed by researchers, over the last decade, the life style of the Ethiopian population is changing due to urbanization and demographic transition (Alemseged *et al*., 2012). Urbanization brings about increases in consumption of refined sugars and animal fats, usually coupled with a more sedentary lifestyle, which promote obesity (Tebekaw *et al*., 2014).

Recent research findings indicated that metabolic syndrome induced by fructose feeding in rats is correlated to hyperglycemia, dyslipidemia and insulin resistance (Shahraki *et al*., 2017). Experts agree that the best way forward MetS is to focus not on treatment but rather on prevention, specifically on obesity prevention (Tuñón-Pablos and Dreby, 2016). A protective effect is attributable, at least in part, to the contents of plant-derived foods and bioactive phytochemicals in the diet (Grosso *et al*., 2015). The numerous beneficial health effects of coffee consumption have received significant scientific attention, because the results of epidemiological and experimental studies suggest that drinking coffee regularly helps prevent several chronic diseases, especially metabolic disorders, such as T2DM (Henry-Vitrac *et al*., 2010).

1.4. Significance of the study

In Ethiopia, there is a lack of information about the effects of coffee on health problems, including metabolic syndrome in both human subjects and animal models. But high fructose diet intake is recently increasing mainly due to the consumption of processed foods and soft drinks in which fructose is added as sweetener. Therefore, the present study is expected to provide an important information on the protective effect of coffee against metabolic syndrome. In addition , the findings of the study will provide a base line data for further researches in the future. Moreover, it will also be helpful in pointing out the research gap regarding to the effects of coffee on health.

1.5. Hypothesis of the study

Alternative hypothesis (HA): Coffee has a decreasing effects on body weight, serum glucose, uric acid and lipid profile levels in high fructose diet feeding male albino Wistar rats.

2. Objective

2.1. General objective

- To investigate the effect of coffee on body weight, serum glucose, uric acid and lipid profile levels in male albino Wistar rats feeding on high-fructose diet.

2.2. Specific objectives

- To assess the effect of coffee on body weight of the rats.
- To determine the effect of coffee on serum glucose level of the rats.
- To evaluate the effect of coffee on serum uric acid level of the rats.
- To determine the effect of coffee on serum lipid profile levels of the rats.
- To correlate serum glucose, uric acid and lipid profile levels with body weight of the rats.

3. Methods and materials

3.1. Study area

The study was carried out at Biochemistry Department Laboratory, Addis Ababa University, Addis Ababa, Ethiopia.

3.2. Study period and duration

The study was undertaken for a period of 6 months from July 2017 to January 2018.

3.3. Study design

A post-test experimental study was conducted on rat model in order to investigate the effect coffee on body weight, fasting serum glucose, uric acid and lipid profile levels.

3.4. Study variables

(A) Independent variable

- ✓ Coffee administration

(B) Dependent variables

- ✓ Food and liquid consumption
- ✓ Body weight
- ✓ Serum glucose, uric acid and lipid profile levels.

3.5. Sample size determination

Sample size determination was based on WHO standard, which recommends that each treatment group of experimental animal should be at least 5 animals (Lelyana, 2016). The study was conducted on 6 groups of rats. Therefore, the total sample size was 6 groups × 5 rats= 30 rats.

3.6. Inclusion and Exclusion criteria

3.6.1. Inclusion criteria

- ✓ Age (9-10 weeks old), normal body weight (≤ 250g) and physically normal rats.

3.6.2. Exclusion criteria

Rats suffering from diarrhea, or any physical abnormality before treatment were excluded from the experiment.

3.7. Acute oral toxicity test

The acute oral toxicity (limit) test was conducted on 5 animals. Test doses of coffee were calculated in relation to the body weight of every fasted animal and administered via oral gavage at 2000mg/kg body weight/day (OECD, 2016).The animals were regularly and individually observed for behavioral changes and general toxicity signs after dosing for the first 24 h, with special attention being given during the first 4 h. Thereafter, observation was continued daily for a total of 14 days. No sign of toxicity or mortality was observed within 24h and over two weeks respectively.

3.8. Experimental animals

A total of 30 male albino Wistar rats of 6-7 weeks were obtained from Addis Ababa University, department of Pharmacology. The experimental rats were placed in a plastic cage with stainless steel cover (5-rats/cage) and housed in animal laboratory with optimum temperature (24$\pm$1 °C), relative humidity, optimum ventilation and 12-hour light-dark cycle. They were standardized for their feeding behavior and observed continuously for three weeks. The cages were kept clean throughout the experiment. Until the initiation of the experiment, all rats were provided with free access to standard chow and plain tap water. Female rats were excluded from the study because of their cyclic hormonal variations that can affect the biochemical levels.

3.9. Experimental protocol

When the rats became 9-10 weeks old (adult), they were randomly assigned into six groups. Each rat in the given group was identified by giving a number on its tail by permanent marker. Prior to the initiation of experiment, their body weight was measured by triple beam balance and ranged from 205g to 245g, with mean value of 225.4±11.5g. Then the rats were grouped and treated as follows:

Group I (Normal control group): fed on standard chow and plain tap water only.

Group II (Fructose control group): fed on standard chow and 20% (w/v) of fructose solution

Group III (Treatment group): fed on standard chow and 20% (w/v) of fructose solution, and treated with 71mg/kg BW/day of coffee.

Group IV (Treatment group): fed on standard chow and 20% (w/v) of fructose solution, and treated with 142mg/kg BW/day of coffee.

Group V (Treatment group): fed on standard chow and 20% (w/v) of fructose solution, and treated with 213mg/kg BW/day of coffee.

Group VI (Treatment group): fed on standard chow and 20% (w/v) of fructose solution, and treated with 284mg/kg BW/day of coffee.

3.10. Preparation of fructose solution and coffee

3.10.1. Preparation of fructose solution

Fructose used in this experiment was pure crystalline, SIGMA-ALDRICH, USA, purchased from India. Twenty percent (20%, W/V) of fructose solution (He *et al*., 2017) was prepared on daily basis (appendix-II) and substituted for tap water in five groups of the rats (group II to group VI). The net consumption of each group was recorded in mL/day.

3.10.2. Preparation of coffee and dosage calculation

Coffee used as a treatment in the present study was *Coffea arabica* (TO.MO.CA Coffee packet), purchased from coffee shop in Addis Ababa. TO.MO.CA is an abbreviated name of the company, which stands for Torrefazione Moderna Cafe (Italian), translated directly as modern coffee roasting. Taking into account the coffee brewing process in Ethiopia, boiled, unfiltered coffee was prepared following the instruction written on the packet (by adding 10g of coffee powder into 180mL of hot water (appendix-I). To estimate the amount of dissolved coffee powder, indirect method of measurement was used (the residue of the coffee powder was measured and subtracted from the total added powder). Accordingly, the volume of the coffee solution after boiling and decantation was 120mL. The amount of undissolved air-dried residue of coffee powder was 5.2g, so the dissolved amount was calculated as 10g-5.2g =4.8g.

The volume of 1 cup of coffee was as recently used by Lelyana (2016) and Lelyana et al. (2015), (0.36mL/200g body weight/day) in rats, which is equivalent 125mL/70kg body weight/day of coffee in humans. In our study, the average body weight (BW) of the experimental rats was 225g. Therefore, the volume of coffee was extrapolated as follows:

125mL = 70,000g x = (225mL*225g)/70,000g

x = 225g x = 0.4mL

Combining the above assumptions, the approximate amount coffee powder in 0.40mL of boiled coffee was calculated as follows:

4800mg (4.8g) = 120mL x = (4800mg*0.4mL)/120mL

x = 0.4mL x = 16mg

Accordingly, 16mg/225g BW/day of coffee was considered as a single dose of coffee. Similarly, 0.8, 1.2 and 1.6mL/225g BW/day (32mg, 48mg and 64mg/225g BW/day) were considered as a double, triple and quadruple doses of coffee. For the seek convenience, these doses were converted to standard unit (mg/kg) as 71,142,213 and 284mg/kg BW/day respectively.

The total volume of coffee administered to treatment groups and clear warm water to normal and fructose control group by oral gavage was 2mL. The oral gavage was performed by the principal investigator (appendix-III) between 09:00 and 10:00 a.m. every day. The experiment was conducted for a period of 6 weeks. No rat died as a result of the treatment or other causes throughout the experiment.

3.11. Data collection

3.11.1. Assessment of food and liquid consumption

Food (g/group/day) and liquids (mL/group/day) were measured and given to the rats according to their assigned group every day. 100g of standard chow for each group; 200mL of 20% fructose solution for fructose control and coffee treatment groups and 200mL of tap water for normal control group. To assess chow and liquid intake, consumption was calculated as the difference between the amount of chow and fructose or water provided and the amount remaining in 24-hrs period daily during the entire experimental period.

3.11.2. Measurement of body weight

Body weight of the experimental rats was measured by triple beam balance capable of measuring 610±0.1g at initial and weekly during the experiment and recorded to the corresponding code of each rat in the group. However, due to the fluctuation of body weight between weeks, only the initial and final body weights were considered for final statistical test.

3.11.3. Blood sample collection, serum preparation and storage

At the end of the sixth week, the rats were fasted overnight and anesthetized with diethyl ether. Blood sample was collected by cardiac puncture and the rats were killed by exsanguination. To prepare serum, the blood sample was transferred into serum separator tube (SST) and left to clot at room temperature for 30 min immediately following collection. Subsequently, the clotted blood sample was centrifuged at 2000 rpm for 15 min. Finally, the serum was transferred into necked tube and stored at -80 °C (Cannizzaro *et al*., 2017) until the analyses were performed.

3.11.4. Laboratory tests

Serum glucose, uric acid, TC, HDL-C and TG were determined by an enzymatic colorimetric methods using fully automated analyzer (Mindary BS-200E); LDL-C was calculated using Frieldwald's formula.

3.11.4.1. Determination of serum glucose level

Fasting serum glucose level was measured with the glucose oxidase method. It is highly specific for β-D-glucose and does not act on α-D-glucose.

Principle of Glucose oxidase method: Glucose oxidase catalyzes the oxidation of β- D- glucose to D- glucono-1, 5-lactone with the formation of hydrogen peroxide (H_2O_2). The lactone is then slowly hydrolyzed to D-Gluconic acid. The H_2O_2 produced is then broken down to oxygen and water by peroxidase enzyme. Then oxygen reacts with an oxygen accepter such as O-dianisidine and results in the formation of a coloured compound that can be measured. The concentration of glucose is determined by calculating change in absorbance at 460nm (Dohnal *et al*.,2010). The reactions are summarized on the next page.

$$\text{B-D-glucose} + H_2O + O_2 \xrightarrow{\text{Glucose Oxidase}} \text{Gluconic acid} + H_2O_2$$

$$\text{O-dianisidine (Colourless)} + H_2O_2 \xrightarrow{\text{Peroxidase}} \text{Oxidised O – dianisidine (coloured)} + H_2O$$

3.11.4.2. Determination of serum uric acid level

Fasting serum uric acid level was measured with the uricase method**.**

Principle of the test: Uricase converts uric acid to allantoin and H_2O_2. The H_2O_2 formed further reacts with a phenolic compound and 4-aminoantipyrine by the catalytic action of peroxidase to form a red coloured quinonimine dye complex. The intensity of the colour formed is measured at 520nm and directly proportional to the concentration of uric acid present in the sample (*Zhao et* al., 2009). The test reactions are:

$$\text{Uric acid} + H_2O \xrightarrow{\text{Uricase}} \text{allanto} + H_2O_2 + CO_2$$

$$H_2O_2 + \text{4-aminoantipyrine} + \text{phenolic compound} \xrightarrow{\text{Peroxidase}} \text{quinonimine (red)} + H_2O$$

3.11.4.3. Determination of lipid profile levels

TC, TG and HDL-C were determined by standard enzymatic method (Hopkins, 2004).

(A) Determination of TC

Principle: Total Cholesterol is measured enzymatically in serum or plasma in a series of coupled reactions that hydrolyze cholesteryl esters and oxidize the 3-OH group of cholesterol. One of the reaction byproducts, H_2O_2 is measured quantitatively in a peroxidase catalyzed reaction that produces a color. The intensity of the color formed is measured at 500 nm (500-550nm) and proportional to the cholesterol concentration in the sample. The reactions are described as follows:

Cholesterol Esters+ H_2O $\xrightarrow{\text{Cholesterol Esterase}}$ Cholesterol + Fatty acids

Cholesterol + O_2 $\xrightarrow{\text{Cholesterol Oxidase}}$ 4 – Cholesterol + H_2O_2

$2H_2O_2$ +Phenol + 4-AP $\xrightarrow{\text{Peroxidase}}$ Quinonimine + 4 H_2O

(B) Determination of TG

Principle: Sample triglycerides incubated lipoprotein lipase (LPL), liberate glycerol and free fatty acids. Glycerol is converted to giycarol-3 phosphate (G3P) and adenosine-5- diphosphate (ADP) by glycerol kinase and ATP. Glycerol-3-phosphate (G3P) is then converted to dihydroxyacetone phosphate (DAP) and hydrogen peroxide (H_2O_2) by glycerol phosphate dehydrogenase (GPO).

In the last reaction, H_2O_2 reacts with 4-aminophenazone (4-AP) and P-chlorophenol in presence of peroxidase (POD) to give a red colored dye whose intensity is measured at 500nm and directly proportional to the triglycerides concentration in present the sample. The reactions are summarized as follows.

Triglycerides + H_2O $\xrightarrow{\text{LPL}}$ Glycerol + free fatty acids

Glycerol + ATP $\xrightarrow{\text{Glycerol kinase}}$ G3P + ADP

G3P+ O_2 $\xrightarrow{\text{GPO}}$ DAP + H2O2

H_2O_2 + 4 – AP + p- Chlorophenol $\xrightarrow{\text{POD}}$ Quinone +$2H_2O$

(C) Determination of HDL-C

Principle: HDL-C level is measured directly in the serum without the need for any pre-treatment. The apoB containing lipoproteins in the specimen are reacted with a blocking reagent that renders them non-reactive with the enzymatic cholesterol reagent under conditions of the assay. The apoB containing lipoproteins are thus effectively excluded from the assay and only HDL-C is detected under the assay conditions. The absorbance of the dye is measured at 600 nm and directly proportional to the concentration HDL-c present in the sample. The sequences of reactions are summarized as follows:

ApoB containing lipoproteins + α-cyclodextrin + Mg^{+2} + dextran $\xrightarrow{SO_4}$ soluble non-reactive complexes with apoB-containing lipoproteins.

HDL-cholesteryl esters $\xrightarrow{\text{PEG- cholesteryl esterase}}$ HDL-Unesterified cholesterol + fatty acid

Unesterified cholesterol + O_2 $\xrightarrow{\text{PEG-cholesterol oxidase}}$ cholestenone + H_2O_2.

H2O2 + 5-aminophenazone + N-ethyl-N-(3-methylphenyl)-N'-succinyl ethylene diamine+ H2O+ H^+ $\xrightarrow{\text{peroxidase}}$ qunoneimine dye + H_2O.

(D) Determination of LDL-C: The LDL-C was calculated from measured values of TC, HDL-C and TG using Frieldwald's formula (Sharma *et al*., 2016) as follows:

[LDL-C] = [TC] - [HDL-C] - [TG]/5.

3.12. Data entry and analysis

All data were entered and cleared by epi-data software version 3.1 and exported to SPSS (statistical Package for Social Science) software version 23.0 for statistical analysis. Normality distributions were assessed by Shapiro-Wilks test and plots (stem-and-leaf and histogram). One-way analysis of variance (ANOVA) was done to determine statistical differences among all groups of the study. Pairwise comparisons were conducted by Tukey post hoc multiple comparison tests. In addition, Pearson bivariate correlation was used to find the relationship of body weight with serum glucose, uric and lipid profile levels. The results of the data were presented as mean±standard deviation (SD). The *p*-values < 0.05 were considered statistically significant.

3.13. Quality assurance

Fructose solution and coffee were freshly prepared by the principal investigator on daily basis. All chemical and reagents used in this experiment were of analytical grade. Each biochemical test was performed by experienced laboratory technologists following standard operational procedures (SOPs). Negative and positive control samples were run for serum glucose, uric acid and lipid profiles to assure the validity of the test results. In addition, data entry and clearance were performed by Epi-data prior to analysis by SPSS.

3.14. Ethical consideration

The ethical clearance was obtained from Department of Biochemistry Research and Ethical Review Committee (DRERC), by approval letter with Ref. No. of SOM/BCHM/154/2009, meeting No. of DRERC 02/17 and protocol No. of M.Sc. 11/17 issued on March 2/2017. All experimental activities were carried out in accordance with recommendations from the declaration of nationally and internationally conventional standards for the employment of experimental animals, and code of ethics of animal experiments, which comply with scientific and ethical guidelines.

3.15. Utilization and dissemination of the results

The results of the present study will be communicated to different stakeholders in different formats, including face-to-face presentation, publishing in a peer-reviewed journals and presentation at scientific meetings.

4. Results

4.1. Effect of coffee on food and liquid consumption

The rats treated with 284mg/kg BW/day of coffee consumed lower amount of chow compared to fructose control group (57.5 g/day vs 68.5g/day) and normal control (57.5g/day vs 95.5g/day). The results also shows that the mean liquid intake in rats treated with 284mg/kg BW/day of coffee was lower when compared to fructose control group (174.5mL/day vs 187mL/day).

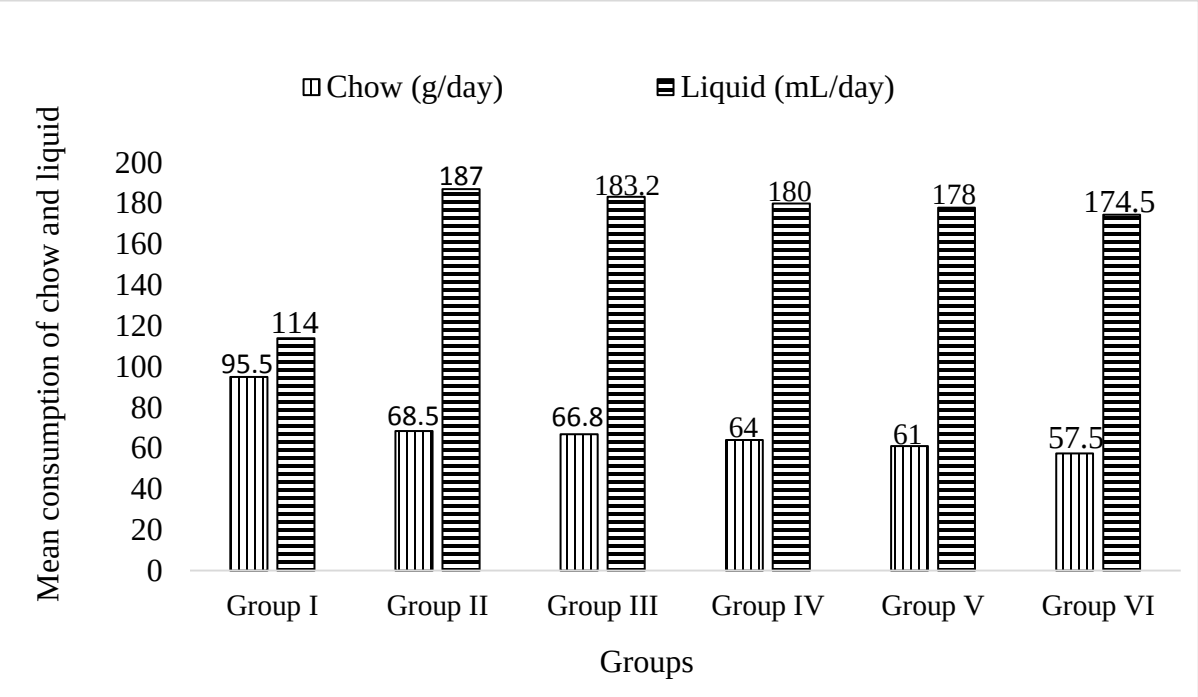

Figure 3. The mean consumption of chow and liquid in the rats. The values are expressed as mean. Sample size (n) is 5 for each group. Liquid - refers to fructose solution for all groups, except for group I where it refers to plain tap water. Group I – Normal control group; Group II-Fructose control group; Group (III- VI) – Treatment groups (received 71, 142, 213 and 284mg/kg BW/day of coffee) respectively.

4.2. Effect of coffee on body weight

Initially, the body weight of the rats was statistically similar among all groups (p = 0.96). At end of the sixth week, the body weights of rats treated with 213 and 284mg/kg BW/day of coffee significantly reduced compared to fructose control group (p = 0.047; 0.035) respectively. Treating the rats with 71 and 142mg/kg BW/day of coffee did not bring significant difference in body weight as compared to fructose control group (p > 0.05). Statistically significant differences in body weight were not found between rats treated with any dose of coffee and normal control group (p > 0.05). However, body weight of fructose control group was significantly higher compared to normal control group (p = 0.020).

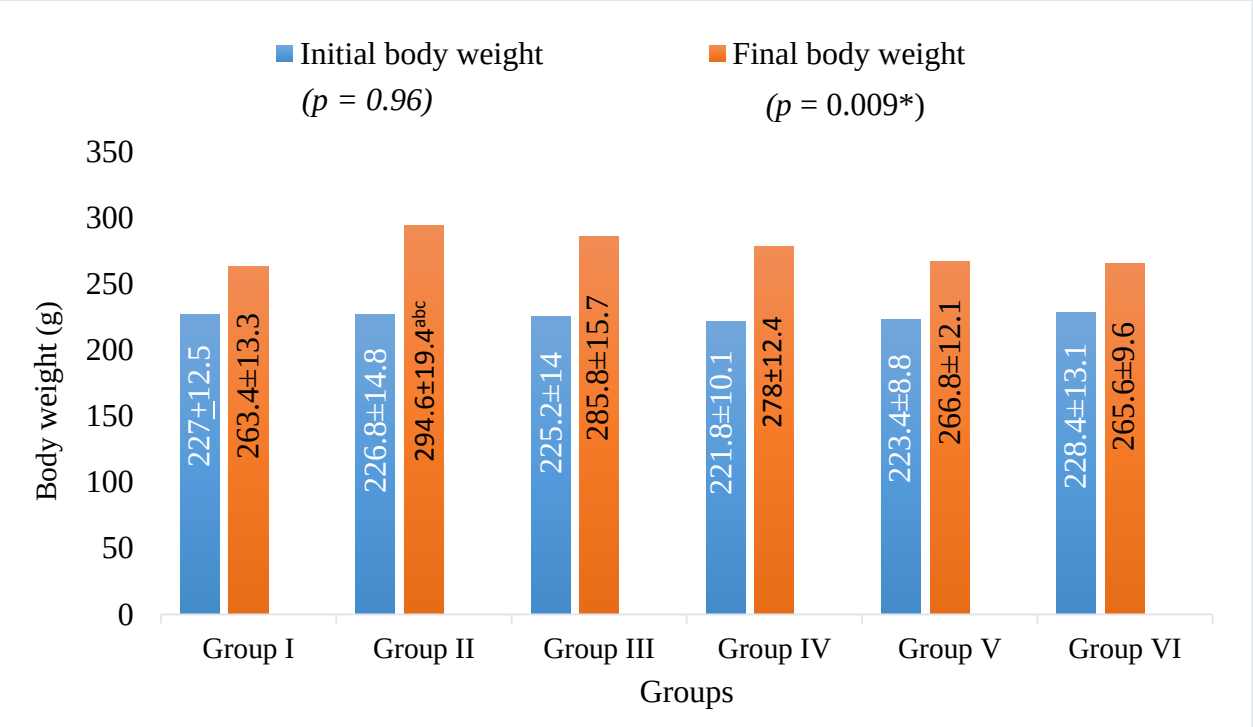

Figure 4. Initial and final body weight of the rats. Values are expressed as mean±SD. Sample size (n) is 5 for each group. I- Normal control group; II-Fructose control group; III-VI -Treatment groups (received 71, 142, 213 and 284mg/kg BW/day of coffee) respectively. *- Indicates significant difference among all groups at p < 0.05 as tested by one-way ANOVA. Superscript letters (a, b and c) indicate significant differences compared to group I, V and VI respectively at p < 0.05 as tested by Tukey post hoc multiple comparisons.

4.3. Effect of coffee on serum glucose and uric acid levels

Treating the rats with 213 and 284mg/kg BW/day of coffee significantly decreased fasting serum glucose (p = 0.049; 0.029) and uric acid (p = 0.026; 0.010) levels compared to fructose control group. On the other hand, 71 and 213mg/kg BW/day of coffee were not found to bring significant changes in fasting serum glucose and uric acid levels as compared to fructose control group (p > 0.05). In comparison to normal control group, fasting serum glucose and uric acid levels did not show significant differences in coffee treatment groups (p > 0.05). However, fasting serum glucose and uric acid levels were significantly increased in fructose control group when compared to normal control (p = 0.007, 0.002) respectively.

Table 2. Fasting serum glucose and uric acid levels of the rats.

Variable (mg/dL)	Groups						F	*P*
	I	II	III	IV	V	VI		
Glucose	74.2±8	94.4±9.6^{abc}	88.8±9	81±5.9	78.4±8.4	77.2±7.5	4.5	0.005*
Uric acid	1.32±0.3	2.18±0.3^{abc}	1.7±0.4	1.64±0.3	1.52±0.26	1.44±0.3	4.7	0.004*

Values are expressed as mean±SD. I- Normal control group; II-Fructose control group; III-VI-Treatment groups (received 71, 142, 213 and 284mg/kg BW/day of coffee) respectively. Sample size (n) is 5 for each group. *-Indicates significant differences among all groups at p < 0.05 as tested by one-way ANOVA. Superscript letters (a, b and c) indicate significant differences compared to group I, V and VI respectively at p < 0.05 as tested by Tukey post hoc multiple comparisons.

4.4. Effect of coffee on serum lipid profile levels

Fasting serum TG and LDL-C levels were significantly lower in rats treated with 284mg/kg BW/day of coffee compared to fructose control group (p = 0.031; 0.046) respectively. Similarly, significant elevation of fasting serum TG (p = 0.013) and LDL-C (p = 0.007) levels were also found in fructose control group when compared to normal control group. Significantly increased fasting serum LDL-C level was also observed in rats treated with 71mg/kg BW/day of coffee as compared to normal control group (p = 0.046). Fasting serum TC and HDL-C levels did not indicate statistically significant changes in all coffee treatment groups when compared to fructose control and normal control groups (p > 0.05).

Table 3. Fasting serum lipid profile levels of the experimental rats.

Variable (mg/dL)	Groups						F	*P*
	I	II	III	IV	V	VI		
TC	101.4 ±13.2	120.2 ±12.4	118.6 ±8.8	113.2±10.2	106.6±9.4	103.8±10 .7	2.6	0.051
HDL-C	44±5.5	33.4±5.5	37.6 ±4.5	39±4.3	39.8±6.8	41±7.3	2	0.132
TG	116.8 ±11	147.8 ±17.8[ab]	142.6 ±13.2	135±15.4	125.4±10.6	120±9.8	4.5	0.005*
LDL-C	34±9.5	57.2 ±10.4[ab]	52.5 ±4.4[a]	47.2 ±13.5	41.7±6.7	38.8±8.8	4.4	0.006*

Values are expressed as mean±SD. HDL-C- High density lipoprotein cholesterol; LDL-C- Low density lipoprotein cholesterol; TC- Total cholesterol; TG- Triglycerides. Sample size (n) is 5 for each group.

* -Indicates significant difference among all groups at p < 0.05 as tested by one-way ANOVA. Superscript letters (a) - indicates significant differences compared to group I; (b) - indicates significant differences compared to group VI at p < 0.05 as tested by Tukey post hoc multiple comparisons.

4.5. Correlation of serum glucose level and body weight

Serum glucose level showed moderate, significant positive correlation with body weight ($r = 0.546$, $p = 0.002$).

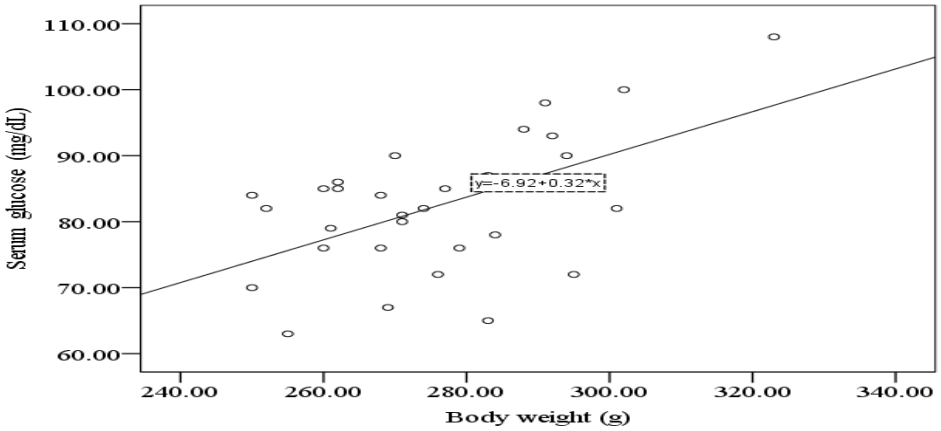

Figure 5. Pearson correlation of serum glucose level and body weight of the rats. Moderate correlation refers to *r* - values closest to ± 0.5.

4.6. Correlation of serum uric acid level and body weight

Moderate, significant positive correlation was found between serum uric acid level and body weight of the rats ($r = 0.560$, $p = 0.001$).

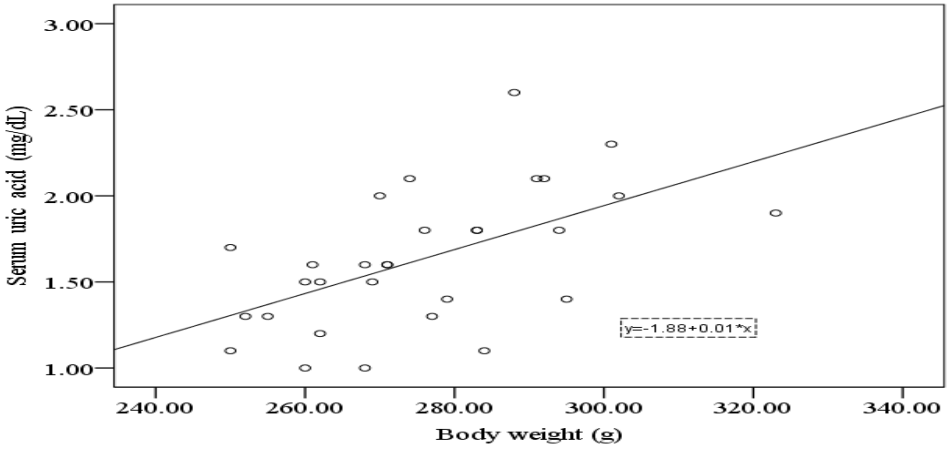

Figure 6. Pearson correlation of serum uric acid level and body weight of the rats.

4.7. Correlation of serum TC level and body weight

Serum total cholesterol level was associated with the body weight of the rats through weak, significant positive correlation ($r = 0.435$, $p = 0.016$).

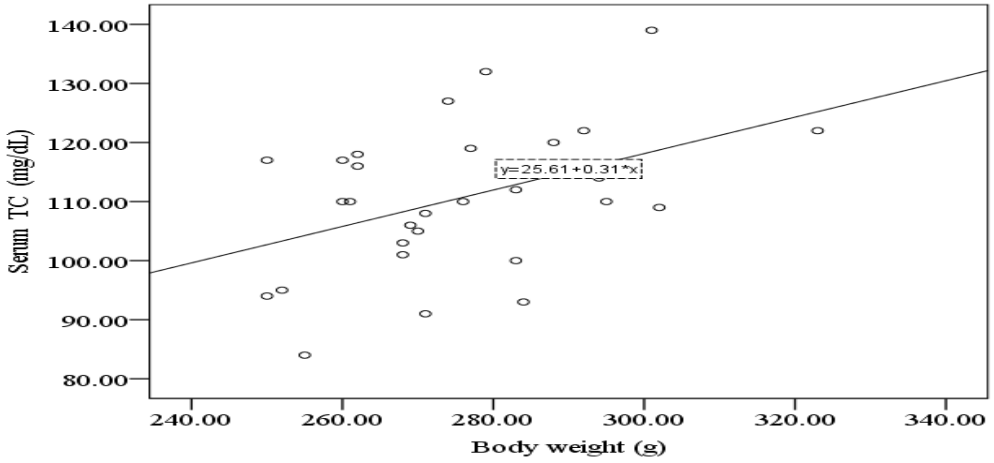

Figure 7. Pearson correlation of serum TC level and body weight of the rats. Weak correlation refers to *r* - values closest to ± 0.3.

4.8. Correlation of serum TG level and body weight

Pearson correlation revealed that serum triglyceride showed weak, non-significant positive correlation with body weight of the rats although not significant ($r = 0.344$, $p = 0.063$).

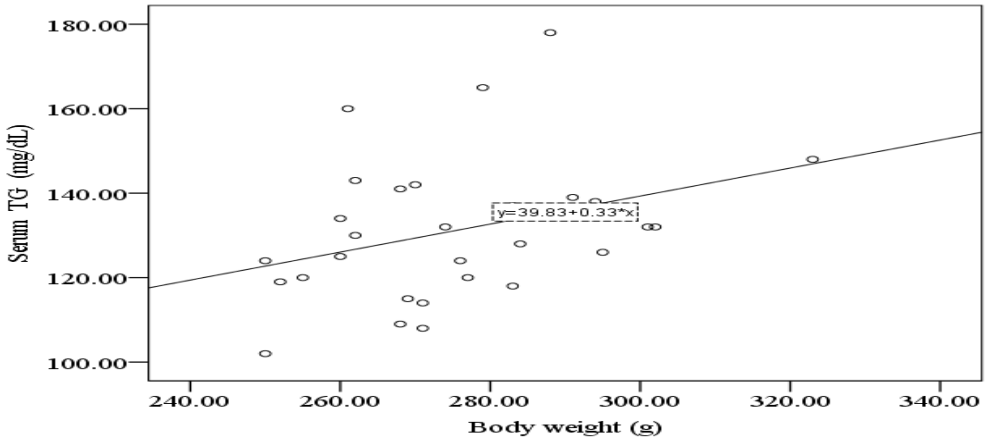

Figure 8. Pearson correlation of serum TG level and body weight of the rats.

4.9. Correlation of LDL-C level and body weight

Serum LDL-C and body weight of the rats were associated through moderate, significant positive correlation at (r = 0.473, p = 0.008).

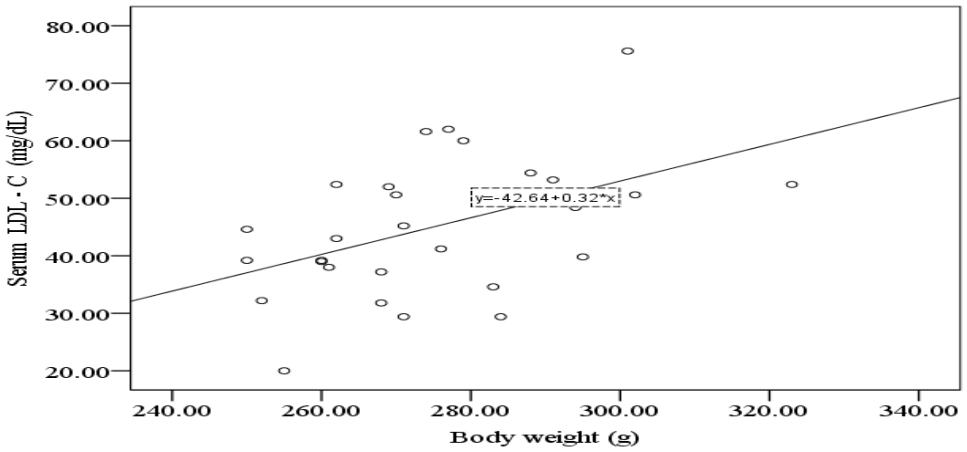

Figure 9. Pearson correlation of serum LDL-C level and body weight of the rats.

4.10. Correlation of serum HDL-C level and body weight

Pearson correlation indicated that serum HDL-C showed weak, non-significant negative correlation with body weight of the rats (r = -0.216, p = 0.253).

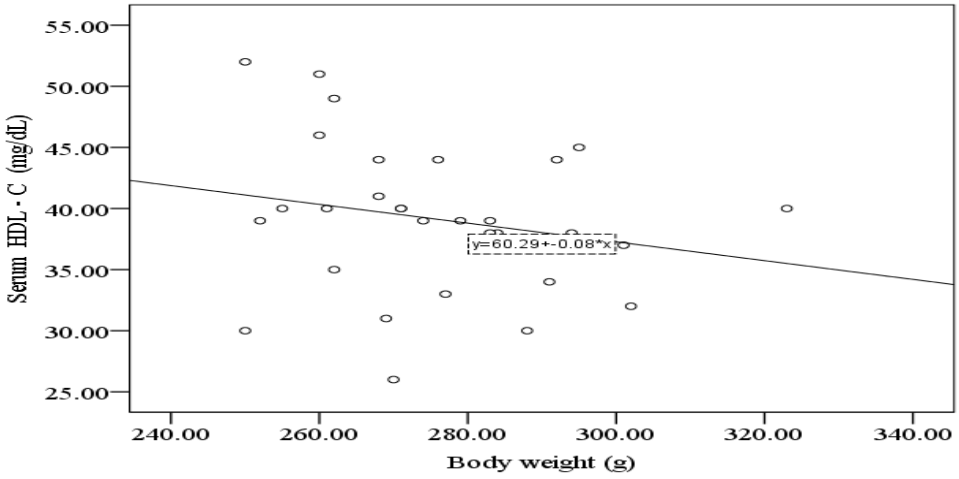

Figure 10. Pearson correlation of serum HDL-C level and body weight of the rats.

5. Discussion

Dietary fructose, which induces metabolic disorders including body weight gain, hyperglycemia, hyperinsulinemia, dyslipidemia and hyperuricemia, can lead to pathophysiologic importance for the development of T2DM in both humans and animal models. Hepatic de novo lipogenesis and lipotoxicity, oxidative stress, and hyperuricemia have been proposed as mechanisms responsible for adverse effects of fructose (Nehal *et al*., 2016; Sandeva *et al*., 2015). Intake of certain beverages such as coffee and functional foods has been shown to be effective at the suppression or reduction of metabolic syndromes. Coffee has received significant scientific attention due to various epidemiological and experimental studies that point to its therapeutic effects (Tanaka *et al*., 2009). Therefore, in the present study, the effects of coffee on food and liquid consumptions, body weight, fasting serum glucose, uric acid and lipid profile levels were investigated in male albino Wistar rats fed on 20% fructose solution for six weeks.

Assessment of food and liquid consumption showed that rats treated with 284mg/kg BW/day of coffee consumed lower amount of chow (57.5g/day) and fructose solution (174.5mL/day) compared to fructose control group, which consumed 68.5g/day of chow and 187mL/day of fructose solution respectively. The possible mechanism by which coffee (caffeine) decreased diet consumption might be by increasing satiety, because the animals were fed ad libitum.

The obtained results indicated that significantly decreased body weight gain was recorded in rats treated with 213 and 284mg/kg BW/day of coffee at the end of sixth week as compared to fructose control group ($p = 0.047$; 0.035) respectively. These results suggest that coffee may have negative effect on body weight. In line with this finding, Ismail *et al.* (2015) reported that male albino rats fed on different preparations of Turkish, Arabian and instant coffee had significantly smaller weight gain than normal control. Similar finding was also reported by Mohmoud *et al.* (2013), in which feeding on different doses of Arabic coffee for 30 days lowered body weight in rats fed on basal diet.

On the other hand, treating the rats with 71 and 142mg/kg BW/day of coffee did not significantly lowered body weight as compared to fructose control group ($p > 0.05$), suggesting that the effect of coffee on body weight loss is a dose dependent. It was also found that, rats treated with different doses of coffee had not shown stastically significant differences in mean body weights as compared to normal control group ($p > 0.05$). The possible mechanism by which coffee prevented higher body weight gain in the present study could be by increasing lipolysis via catecholamines (Lopez *et al*., 2006; Kobayashi-Hattori *et al.,* 2005). Caffeine might also have caused body weight loss by increasing physical activity. In addition, coffee had decreased diet consumptions, which might in turn had led to decreased in the body weight gain.

In comparison with normal control group, fructose control group had statistically significant body weight gain ($p = 0.020$). This result is in line with Tanaka *et al.'s (*2009), in which feeding on 20% fructose solution in tap water for 8 weeks had brought statistically significant body weight gain in both sexes of rats. As demonstrated in both short-term and long-term studies, fructose consumption results in decreased circulating levels of insulin and leptin and increased caloric intake (Stanephone and Havel, 2008).

Treating rats with 213 and 284mg/kg BW/day of coffee had significantly reduced fasting serum glucose levels as compared to fructose control group ($p = 0.049$; 0.029) respectively. Consistent with these findings, Li *et al*. (2009) reported that CGA significantly lowered fasting glucose levels in golden hamsters. In addition, Takami *et al*. (2013) reported moderate coffee consumption in human subjects was associated with a significantly lower odds ratio for high plasma glucose. In rats treated with 71 and 142mg/kg BW/day of coffee, serum glucose levels were non-significantly lower (table 2) when compared to fructose control group. The overall effects of coffee on glucose level in the present study revealed good implication to support the hypothesis that states, "Heavy coffee consumption has been associated with a lower risk of diabetes," (Urzúa *et al*., 2012).

Most probably, coffee prevented increase in glucose level due to its main component, caffeine, which inhibits adenosine receptors that stimulates hepatic glucose production through the activation of A2B adenosine receptors (Abd *et al*., 2009). Caffeine might also have stimulated glucose transport through activation of cyclic AMP-dependent protein kinase α-1 (Urzua *et al*., 2012). Another mechanism could be via selective inhibition of hepatic glucose-6-phosphatase, a rate-limiting enzyme of gluconeogenesis, by CGA of coffee (Khojah, 2016; Akash *et al*., 2014).

Fasting serum glucose was significantly higher in fructose control group than in normal control group (p = 0.007). This finding is in agreement with Sandeva *et al.'s* (2015) who reported that serum glucose level was significantly increased in male and female rats fed on 20% of fructose solution in drinking water for 8 weeks.

It was found that, fasting serum uric acid levels were significantly lower in rats treated with 213 and 284mg/kg BW/day of coffee compared to fructose control group (p = 0.026; 0.010) respectively. Similar studies by Lelyana (2016) and Lelyana *et al*. (2015) reported that coffee had non-significantly reduced serum uric acid in obese rats and mice with hyperuricemia respectively. In addition, Mahmoud *et al*. (2013) reported that different doses of Arabic coffee had lowered serum uric acid levels in experimental rats fed on basal diet. In comparison with mentioned findings, the significant decrease in fasting serum uric acid found in the present study might be due to variation in the doses of coffee, study design and/or duration.

The result also indicated that 71 and 142mg/kg BW/day of coffee treatments showed non-significant lowering effect on fasting serum uric acid levels when compared to fructose control group (table 2). Fasting serum uric acid level did not show statistically significant difference between normal control and treatment group received any dose of coffee (p > 0.05). Coffee consumption may lower serum uric acid levels and risk of gout via various mechanisms. The main mechanism is via competitive inhibition of xanthine oxidase by caffeine (1,3,7-trimethyl-xanthine), (Rho *et al*., 2011). The other mechanism is probably by a diuretic action of caffeine that might affect serum uric acid concentration through increased excretion in urine (Abd *et al*., 2009).

Evaluation of fasting serum lipid profiles showed that treating rats with 284mg/kg BW/day of coffee had significantly lowered fasting serum levels of TG and LDL-C when compared to fructose control group (p = 0.031; 0.046) respectively. Consistent with these findings, Gomes *et al.* (2013) reported that, feeding of 7.2mL/kg of body weight for 41 days significantly lowered lipid percentage in hyperlipidemic diet feeding rats. In addition, Li *et al*. (2009) reported CGA significantly lowered the levels of fasting serum TG, FFA, TC, and LDL-C in golden hamsters.

Fasting serum levels of TC and HDL-C were non-significantly lower and higher respectively, in rats treated with 213 and 284mg/kg BW/day of coffee compared to fructose control group (table 3). These results are in agreement with Karabudak *et al.*'s (2015) that reported Turkish and instant coffee consumption did not significantly affect serum levels of TC among Turkish subjects. On the other hand, in Mohmoud *et al*.'s (2013) study, Arabic coffee had significantly decreased serum TC levels in experimental rats fed on basal diet. In contradiction to our findings, Hanaa and Fattah's (2008) reported significant elevation of serum TL, TC, TG and LDL-C, while a significant decrease of HDL-C in rats fed diet supplemented with low or high dose of coffee. The causes for these variations are unclear. However, it may be due to the variations in the mode of coffee administration. Lipid lowering effects of coffee found in the present study could be related to caffeine, (methylxanthine) which stimulates lipolysis by increasing cellular levels of cAMP through antagonism adenosine receptors (A1) and inhibition of phosphodiesterase activity (Gomes *et al*., 2013; Acheson *et al*., 2004).

Feeding rats on 20% of fructose solution for six weeks significantly increased fasting serum levels of TG in fructose control ($p = 0.013$); LDL-C in fructose control ($p = 0.007$) and 71mg/kg BW/day of coffee treated ($p = 0.046$) groups compared to normal control group. The fasting serum levels of TC ($p = 0.106$) and HDL-C ($p = 0.073$) were non-significantly higher and lower, respectively in fructose control group than in normal control group (table 3). Fasting serum levels of TC, TG, and LDL-C were possibly increased in fructose control group as metabolism of fructose by bypasses the enzyme phosphofructokinase and unregulated the flow of fructose-derived substrates (glycerol-3- phosphate and acetyl-CoA) into lipogenesis (Rutledge and Adeli, 2007).

Pearson bivariate correlation analysis indicated that serum glucose and uric acid levels showed moderate, significant positive correlations with body weight ($r = 0.546$, 0.560 and $p = 0.002$, 0.001) respectively. There is a lack of study that correlates serum glucose and uric acid levels with body weight in rat models. However, in line with these results, Agrawal *et al*. (2017) reported positive correlation between serum glucose and BMI among Jharkhand population. The positive correlation found in the present study between body weight and glucose level of the rats could imply that increase in body weight may trigger changes to the body's metabolism and cause development of insulin resistance (Gatineau *et al*., 2014).

Similarly, Duan *et al*. (2015) reported that serum uric acid level was positively correlated with overweight and obesity among university students of China. Increase in serum uric acid levels as body weight increased might be due to increased synthesis of triglycerides that increases uric acid concentrations and arterial hypertension that lowers uric acid excretion.

Body weight had moderate positive correlation with the serum fasting levels of LDL-C ($r = 0.0473$) and weak, positive correlation with TC ($r = 0.435$). However, body weight was significantly correlated with both serum LDL-C ($p = 0.008$) and TC ($p = 0.016$) levels. Fasting serum TG and HDL-C levels showed weak, non-significant positive and negative correlations with body weight ($r = 0.344$, - 0.216 and $p = 0.063$, 0.253) respectively. Similar finding that correlates body weight and lipid profile levels in rat model is limited. However, consistent with our findings, Omotoye and Fadupin (2016) reported positive correlation of LDL-C, TC and TG levels with overweight and negative correlation between HDL-C levels and BMI among T2D patients of Nigeria. In the present study, increased body weight perhaps led to increased TC, TG and LDL-C levels by increasing hepatic lipogenesis. On the contrary, decreasing HDL-C levels with increasing body weight could be due to increased rate of clearance and decreased rate of production of HDL-C.

6. Conclusions

Treating rats with coffee was found to significantly reduce body weight, fasting serum glucose, uric acid, TG and LDL-C levels in a dose dependent manner in male albino Wistar rats fed on 20% of fructose solution for six weeks. Notably, 213 and 284mg/kg BW/day of coffee significantly reduced body weight, fasting serum glucose and uric acid levels. In addition, 284mg/kg BW/day of coffee significantly decreased fasting serum TG and LDL-C levels. Therefore, the alternative hypothesis proposed in the present study has been tested and accepted. Coffee treatment also lowered the fasting serum levels of TC and increased HDL-C although not significant. Generally, the overall findings of the present study suggest that coffee consumption may be helpful in ameliorating metabolic syndromes and its associated complications such as obesity, diabetes, inflammation and cardiovascular diseases.

7. Strength and limitations of the study

7.1. Strength of the study

- ✓ Both positive and negative controls were used as reference comparison to investigate the effect of coffee in treatment groups.
- ✓ Four different doses of coffee were evaluated to determine the dose dependent effect of coffee.

7.2. Limitations of the study

- ✓ Minimum sample size was used, so that the statistical power of the study was somewhat limited.
- ✓ The rats were maintained as 5-rats/cage, as a result, the amount of chow and fructose consumption of individual rat was not known.
- ✓ The mechanisms by which coffee suppresses metabolic disorders were not demonstrated (based on published data).

8. Recommendations

Based on the present study findings, the following recommendations are forwarded:

- ✓ Long-term experiment should be conducted to determine the effect of coffee on body weight, blood glucose, uric acid and lipid profile levels.
- ✓ Further experimental research is required to compare the effect of coffee and standard drugs on MetS induced by overconsumption of high caloric diet.
- ✓ The effect of different modes of coffee preparation and extractions should be investigated to determine the best effective form and ingredient at prevention or reduction of MetS.
- ✓ The effect of coffee drinking on health should clinically be investigated in Ethiopian.

9. References

Abd E., Afify M.R., Faten, M, Abou E., Alia A. and Hassan A. (2009). Short and Long Term Effect of Caffeine on Liver, Kidney as well as Glucose, Insulin, Triglycerides and Cholesterol on Normal Rats. *Aust. J. Basic & Appl. Sci.* 3(4): 3259-3265.

Acheson J. Germaud G., Meirim I., Montigon F.and Krebs Y. (2004). Metabolic effect of caffeine in humans:Lipid Oxdation or Futile Cycling?. *Am J Clin Nutr*. 79(1):40-46.

Adams S.H., Stanhope K.L., Grant R.W., Cummings B.P., and Havel P.J. (2008). Metabolic and endocrine profiles in response to systemic infusion of fructose and glucose in rhesus macaques. *Endocrinology*. 149(6):3002–3008.

Adisakwattana S., Chantarasinlapin P., Thammarat H., Yibchok-Anun S. (2009). A series of cinnamic acid derivatives and their inhibitory activity on intestinal alpha-glucosidase. *J Enzyme Inhib Med Chem*. 24:1194–1200.

Agrawal N., Agrawal M.K., Kumari T., and Kumar S. (2017). Correlation between Body Mass Index and Blood Glucose Levels in Jharkhand Population. *Int J Cont Med Res.*4(8):1633-1636.

Akash M.S.H., Kanwal Rehman K., and Chen S. (2014). Effects of coffee on type 2 diabetes mellitus. *Nutrition*. 30:755-763.

Alemseged F., Haileamlak A., Tegegn A., Tessema T., Woldemichael K., Asefa M. et al. (2012). Risk Factors for Chronic Non-Communicable Diseases at Gilgel Gibe Field Research Center, Southwest Ethiopia: Population Based Study. *Ethiop J Health Sci.* 22:19-28.

Avena N.M. (2007). Examining the addictive-like properties of binge eating using an animal model of sugar dependence. *Exp Clin Psychopharmacol*. 15(5):481–491.

Bakuradze T., Parra G.A.M., Riedel A., Somoza V., Lang R., Dieminger N. et al. (2014). Four - week coffee consumption affects intake, satiety regulation, body fat, and protects DNA integrity. *Food Res Inter.* 63:420–427

Bawden S.J., Stephenson M.C., Marciani L., Aithal G.P., Macdonald I.A., Gowland P. et al. (2012). Investigating alterations in hepatic ATP levels following fructose and fructose+glucose ingestion: a simple non-invasive technique to assess liver function using 31P MRS. *Proc Intl Soc Magn Reson Med Sci Meet Exhib.* 20:1369-1375.

Bidel S., Hu G., Sundvall J., Kaprio J., and Tuomilehto J. (2006). Effects of Coffee Consumption on Glucose Tolerance, Serum Glucose and Insulin Levels-A Cross sectional Analysis. *Horm Metab Res.* 38: 38-43.

Boergesen M., Poulsen L., Schmidt SF., Frigerio F., Maechler P., and Mandrup S.(2011). ChREBP mediates glucose repression of peroxisome proliferator activated receptor alpha expression in pancreatic beta-cells. *J Biol Chem*. 286(15):13214–13225.

Buul V.J. V., Tappy L. and Brouns F.J.P.H. (2014). Misconceptions about fructose-containing sugars and their role in the obesity epidemic. *Nutr Res Rev.* 27:119–130.

Çakir O.K., Ellek N., Salehin N., Hamamci R., Keleş H., Kayali D.G. et al. (2017). Protective effect of low dose caffeine on psychological stress and cognition function. *Physiology & Behavior*. 168:1–10.

Cannizzaro L., Rossoni G., Savi F., Altomare A., Marinello C., Saethang T et al. (2017). Regulatory landscape of AGE-RAGE oxidative stress axis and its modulation by PPARγ activation in high fructose diet induced metabolic syndrome. *Nutr & Metabol.* 14(5):1-13.

Cano-Marquina A., Tarin J.J., and Cano A. (2013). The impact of coffee on health. *Maturitas.* 75:7-21.

Chen Y., Brown P.H., Hu K., Black R.M., Prior R.L., Ou B. and Chu YF. (2011). Supercritical CO2 decaffeination of unroasted coffee beans produces melanoidins with distinct NF kappaB inhibitory activity. J *Food Sci.*76:182-186.

Cox C.L., Stanhope K.L., and Schwarz J.M. (2012). Consumption of fructose sweetened beverages for 10 weeks reduces net fat oxidation and energy expenditure in overweight/obese men and women. *Eur J Clin Nutr*. 66:201–208.

Desroches, S. and Lamarche B. (2007). The evolving definitions and increasing prevalence of the metabolic syndrome. *Applied Physiolo, Nutr, and Metabol*. 32 (1): 23-32.

Dohnal L., Kalousová M. and Zima T. (2010). Comparison of Three Methods for Determination of Glucose. *Prague Medical Report*. 111 (1):42–54.

Douard V. and Ferraris R.P. (2008). Regulation of fructose transporter GLUT5 in health and disease. *Am J Physiol Indocrinol Metab.* 295(2): E227-E237.

Duan Y., Liang W., Zhu L., Zhang T., Wang L., Nie Z. et al. (2015). Association between serum uric acid levels and obesity among university students (China). *Nutr Hosp.*31(6):2407-2411.

Gatineau M., Hancock C., Holman N., Outhwaite H., Oldridge L., Christie A. et al., (2014). Adult obesity and type 2 diabetes. *Eng. Pub. Health*. 1:1-39.

Gavrieli1 A., Karfopoulou1 E., Kardatou1 E., Spyreli1 E., Fragopoulou1 E., Mantzoros C.S. et al. (2013). Effect of Different Amounts of Coffee on Dietary Intake and Appetite of NormalWeight and Overweight/Obese Individuals. *Obesity*. 21:1127-1132.

Godos J., Pluchinotta F.R., Marventano S., Buscemi S.,Volti G.L., Galvano F. et al. (2014). Coffee components and cardiovascular risk: beneficial & detrimental effects. *Int J Food Sci Nutr.*1-12.

Gomes J.A.S., Faria B.J, Silva V.O., Zangerônimo M.G. and Miranda J.R. (2013). Influence of Integral and Decaffeinated Coffee Brews on Metabolic Parameters of Rats Fed with Hiperlipidemic Diets. *Braz. Arch. Biol. Technol.* 56(5): 829-836.

Greenberg J.A., Boozer C.N., and Allan Geliebter A. (2006). Coffee, diabetes, and weight control. *Am J Clin Nutr.* 84:682–93.

Grosso G., Stepaniak U., Micek A., Topor-Madry R., Pikhart H., Szafraniec K. et al. (2015). Association of daily coffee and tea consumption and metabolic syndrome: results from the Polish arm of the HAPIEE study. *Eur J Nutr.* 54:1129–1137.

Hanaa, M. and Fattah A. (2008). Nutritional Interaction Effect of Zinc and Coffee on Serum Lipid Profile and Copper in Rats. *J Hosp Med.* 33: 492– 502.

He C., Guo X., Fan J., and Xu Z. (2017). Different dietary contribution to hepatic inflammatory and lipogenic factor mRNA expression. *Int J Clin Exp Med.* 10(1):325-333.

Henry-Vitrac C., Ibarra A., Roller M., Merillon J-M. and Xavier Vitrac X. (2010). Contribution of Chlorogenic Acids to the Inhibition of Human Hepatic Glucose-6-phosphatase Activity in Vitro by Svetol, a Standardized Decaffeinated Green Coffee Extract. *J. Agric. Food Chem.* 58:4141–4144.

Herman, M.A and Samuel V.T. (2016). The sweet path to metabolic demise: Fructose and lipid synthesis. *Trends Endocrinol. Metab.* 27:719–730.

Higdon J.V and Frei B. (2006). Coffee and health: a review of recent human research. *Crit Rev Food Sci Nutr*. 46: 101-123.

Hopkins J. (2004). Laboratory Procedure Manual. Total Cholesterol, HDL-Cholesterol, Triglyserides, and LDL Cholesterol. 1-23.

Hudgins L.C., Parker T.S., Levine D.M., Hellerstein MK. (2011). A dual sugar challenge test for lipogenic sensitivity to dietary fructose. *J Clin Endocrinol Metab*. 96(3):861–868.

Ihedioha J.I., Noel-Uneke O.A. and Ihedioha T.E. (2013). Reference values for the serum lipid profile of albino rats (Rattus norvegicus) of varied ages and sexes. *Comparative Clinical Pathology*. 22 (1): 93 – 99.

Ishikawa A., Yamashita H., Hiemori M., Inagaki E., Kimoto M., Okamoto M. et al. (2007). Characterization of inhibitors of postprandial hyperglycemia from the leaves of Nerium indicum. *J Nutr Sci Vitaminol*. 53:166–173.

Ismail M.S., Saad H.H., Elmaadawy A.A. and Al-Qahiz N.M. (2015). Different Preparations of Coffee Have Varied Effects on Body Weight and Blood Lipids in Experimental Rats. Med Aromat Plants 4(4):1-5.

Jegatheesan P. and Bandt D.J. (2017). Fructose and NAFLD: The Multifaceted Aspects of Fructose Metabolism. *Nutrients.* 9:230-242.

Johnson R.J., Perez-Pozo S.E, Sautin Y.Y., Manitius J., Sanchez-Lozada L.G., Feig D.I. et al. (2009). Hypothesis: could excessive fructose intake and uric acid cause type 2 diabetes? *Endocr Rev.* 30:96–116.

Johnson, R.J., Segal, M.S., Sautin, Y., Nakagawa T., Feig, D.I., Kang, D. et al. (2007). Potential role of sugar (fructose) in the epidemic of hypertension, obesity and the metabolic syndrome, diabetes, kidney disease, and cardiovascular disease. *Am. J. Clin. Nutr.* 86:899–906.

Jonsson M., Jestoi M., Nathanail, A.V., Kokkonen, U.M., Anttila M., Koivisto, P. et al. (2013). Application of OECD Guideline 423 in assessing the acute oral toxicity of moniliformin. *Food Chem. Toxicol.* 53: 27–32.

Karabudak E., Turkozu D., and Koksal E. (2015). Association between coffee consumption and serum lipid profile. *Exp Ther Med.* 9(5):1841-1846.

Khojah E.Y. (2016). Effect of Arabic and Green Coffee Beans on Lowering Lipid Profile Parameters in Male Rats. *Aust J Basic A Sc.* 10(18): 310-317.

Kit B.K., Fakhouri T.H., Park S., Nielsen S.J., and Ogden C.L. (2013). Trends in sugar-sweetened beverage consumption among youth and adults in the United States: *Am J Clin Nutr*. 98(1):180–188.

Kobayashi-Hattori K., Mogi A., Matsumoto Y. and Takita T. (2005). Effect of Caffeine on Body fat and Lipid Metabolism of Rats Fead on a High–fat Diet. *Biosci Biotechnol Biochem*. 69(11):2219-2223.

Kolderup A. and Svihus B. (2015). Fructose Metabolism and Relation to Atherosclerosis, Type 2 Diabetes, and Obesity. *J Nut and Metabol.* 1-12.

Koo H.Y., Miyashita M., Cho B.H., and Nakamura M.T. (2009). Replacing dietary glucose with fructose increases ChREBP activity and SREBP-1 protein in rat liver nucleus. *Biochem Biophys Res Commun*. 390(2):285–289.

Lane M.D. and Cha S.H. (2009). Effect of glucose and fructose on food intake via malonyl-CoA signaling in the brain. *Biochem Biophys Res Commun.* 382:1–5.

Lelyana R. (2016). Effect of Coffee Daily Consumption on Uric Acid Level and Body Weight to Prevent Metabolic Syndrome. *J Nanomed Nanotechnol.*7 (5):1-5.

Lelyana R., Wijayahadi N., Kusmiyati D.K., Puwanto A.P. and Hardian N. (2015). The influence of coffee consumption to decrease uric acid level an experimental study on hyperuricemia Wistar strain rats. *Int J Current Res.* 7: 19147-19153.

Li S., Brown M.S., and Goldstein J.L. (2010). Bifurcation of insulin signaling pathway in rat liver: mTORC1 required for stimulation of lipogenesis, but not inhibition of gluconeogenesis. *Proc Natl Acad Sci U S A.*107(8):3441–3446.

Li S., Chang C., Ma F. and Yu C. (2009). Modulating Effects of Chlorogenic Acid on Lipids and Glucose Metabolism and Expression of Hepatic Peroxisome Proliferator-activated Receptor-α in Golden Hamsters Fed on High Fat Diet. *Biomed Envint Sci.* 22:122-129.

Lopez-Garcia E., Van V.M. and Rajpathak M.S. (2006). Changes in caffeine intake and long-term weight change in men and women. *Am J Clin Nutr.* 83:674–680.

Maarman G.J., Mendham A.E., Madlala H.P. and Ojuka E.O.(2016). Excessive consumption of fructose-containing sugars: An emerging threat for developing nations? *J. Afr. Ass. Physiol. Sci.* 4(2): 71-80.

Mahmoud O.L., Al-Qahiz N.M., and Ismail M.S. (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.

McGarry J.D. (2002). Dysregulation of fatty acid metabolism in the etiology of type 2 diabetes. *Diabetes*. 51(1):7–18.

Mendis, S. (2015). Global status report on noncommunicable diseases: World Health Organization.

Messina G., Zannella C., Monda V., et al. (2015). The Beneficial Effects of Coffee in Human Nutrition. *Biol Med (Aligarh).* 7(4):1-5.

Miller A. and Adeli K. (2008). Dietary fructose and the metabolic syndrome. *Current Opinion Gastroenterol.* 24:204–209.

Nakagawa T., Tuttle K.R., Short R.A. and Johnson R.J. (2005). Hypothesis: fructose-induced hyperuricemia as a causal mechanism for the epidemic of the metabolic syndrome. *Nat Clinic Pract Nephrol*. 1(2):80-86.

Narita Y. and Inouye K. (2009). Kinetic analysis and mechanism on the inhibition of chlorogenic acid and its components against porcine pancreas alpha-amylase isozymes I and II. *J Agric Food Chem*. 57:9218–9225.

Nehal A. Ramadan A.A., Afifi N.A., Emad Y., Erian E.Y., Dalia O. et al. (2016). Beneficial Effect of Trigonelline on the Metabolic Changes Associated With Insulin Resistance in Rats. *J Clin Nutr*. 5(2):1238-1250.

O'Keefe J.H., Bhatti S.K., Patil H.R., James J. DiNicolantonio, J.J., Lucan S.C. et al. (2013). Effects of Habitual Coffee Consumption on Cardiometabolic Disease, Cardiovascular Health, and All-Cause Mortality. *JACC* 62(12):1043-105.

Odegaard A.O., Pereira M.A., Koh W.P. (2008). Coffee, tea, and incident type 2 diabetes: the Singapore Chinese Health Study. *Am J Clin Nutr*. 88:979-985.

OECD, (2016). Guideline for Testing of Chemicals. Avian cute oral toxicity test. 1-28.

Omotoye F.E. and, Fadupin G.T. (2016). Effect of Body Mass Index on Lipid Profile of Type 2 Diabetic Patients at An Urban Tertiary Hospital In Nigeria. *IOSR-JDMS* 15(9):65- 70.

Ong K.W., Hsu A. and Tan B.K. (2013). Anti-diabetic and anti-lipidemic effects of chlorogenic acid are mediated by ampk activation. *Biochem Pharmacol*. 85:1341–1351.

Ouyang X., Cirillo P., Sautin Y., McCall S., Bruchette J.L., Diehl A.M. et al. (2008). Fructose consumption as a risk factor for non-alcoholic fatty liver disease. *J. Hepatol.* 48:993–999.

Parliament T.H. and Stahl H.B. (2005). What makes the coffee smell so good? *Chem Tech* 25:38–47.

Penafort, A.G., Carneiro, I.B.P., Carioca, A.A.F., Sabry, M.O.D., Pinto, F.J.M. and de Carvalho H.A. (2016). Coffee and Caffeine Intake among Students of the Brazilian Northeast. *Food and Nutr Sc.* 7:30-36.

Popoli P. and Pepponi R. (2012). Potential therapeutic relevance of adenosine A2B and A2A receptors in the central nervous system. *CNS Neurol Disord Drug Targets*. 11:664-674.

Pourshahidi K.L., Navarini L., Petracco M. and Strain J.J. (2016). A Comprehensive Overview of the Risks and Benefits of Coffee Consumption. *Compr Rev Food Scie Food Safety*.15:674-684.

Rho Y.H., Shu Y. and Choi H.K. (2011). The Epidemiology of Uric Acid and Fructose. *Semin Nephrol.* 31(5):410-419.

Ribeiro J.A and Sebastiao A.M. (2010). Caffeine and adenosine. *J Alzheimers Dis*. 20:3-15.

Rutledge A.C, and Adeli K. (2007). Fructose and the metabolic syndrome: pathophysiology and molecular mechanisms. *Nutr Rev*. 65:S13–S23.

Salomone F., Galvano F., and Volti G.L. (2017). Molecular Bases Underlying the Hepatoprotective Effects of Coffee. *Nutr. Rev.* 9:1-13.

Samuel V.T. (2011). Fructose induced lipogenesis: from sugar to fat to insulin resistance. *Trends Endocrinol Metab*.22:60-65.

Sandeva, R.V, Mihaylova S.M., Sandeva G.N., Trifonova K.Y. and Popova-Katsarova R.D. (2015). Effect Of High-Fructose Solution on Body Weight, Body Fat, Blood Glucose and Triglyceride Levels in Rats. *J Biomed Clin Res.*8(1):1-8.

Sarah A., Danielle E., Nicola M., and Mark A. (2018). Fructose metabolism and metabolic disease. *J Clin Invest*. 128(2):545-555.

Shahraki M.R., Mirshekari H., Samadi Z., Shahraki A.R. and Shahraki E. (2017). Effects of Artemisia Dracunculus Aqueous Extract on Blood Sugar, Serum Insulin, Triglyceride and Liver Enzymes In Fructose Drinking Water Male Rats. *Zahedan J Res Med Sci.* In Press e4402.

Sharma M., Batra H.S., Banerjee M., Bhattarai A.M., Agarwa D. (2016). Clinical Significance of Low Density Lipoprotein Cholesterol Measurements: Direct Versus Indirect Method. *MJSBH.* 15(1):17-25.

Softic S., Cohen D.E. and Kahn C.R. (2016). Role of dietary fructose and hepatic de novo lipogenesis in fatty liver disease. *Dig. Dis. Sci.* 61:1282–1293.

Srikanthaan K., Fehy A., Visweshwar H., Shapiro J.I. and Sodhi K. (2016). Systemic review of metabolic syndrome biomarkers: A panel for early detection, management, and risk stratification in the West Virginian population. *Int J Med Sci.* 13(1):25-38.

Stanhope K.L. and Havel M. (2008). Endocrine and metabolic effects of consuming beverages sweetened with fructose, glucose, sucrose, or high fructose corn syrup. *Am J Clin Nutr.* 88(6): 1733S–1737S.

Stanhope K.L. (2016). Sugar consumption, metabolic disease and obesity: The state of the controversy. *Crit. Rev. Clin. Lab. Sci.* 53:52–67.

Suwannaphet W., Meeprom A., Yibchok-Anun S. and Adisakwattana S. (2010). Preventive effect of grape seed extract against high-fructose diet-induced insulin resistance and oxidative stress in rats. *Food chem toxicol.* 48:1853–1857.

Takami H., Nakamoto M., Uemura H., Katsuura S., Yamaguchi M., Hiyoshi M. et al. (2013). Inverse Correlation between Coffee Consumption and Prevalence of Metabolic Syndrome. *J Epidemiol.* 23(1):12-20.

Tanaka K., Shoko N., Shizuka T., Mihoko K. and Hiroshi S. (2009). Anti-obesity and hypotriglyceridemic properties of coffee bean extract in SD rats. *Food Sci. Technol. Res*. 15: 147-152.

Tappy L. and Lê K.A. (2010). Metabolic effects of fructose and the world wide increase in obesity. *Physiol. Rev.* 90: 23–46.

Tebekaw Y., Teller C. and Colón-Ramos U. (2014). The burden of underweight and overweight among women in Addis Ababa, Ethiopia. *BMC Public Health.* 14:1126-1136.

Teff K.L., Elliott S.S., and Tschöp M. (2004). Dietary fructose reduces circulating insulin and leptin, attenuates postprandial suppression of ghrelin, and increases triglycerides in women. *J Clin Endocrinol Metab* .89:2963– 2972.

Tellez L.A. (2016). Separate circuitries encode the hedonic and nutritional values of sugar. *Nat Neurosci*. 19(3):465–470.

Ter K.W., Schene M.R., Holman R., Romijn J.A., and Serlie M.J. (2016). Effect of fructose consumption on insulin sensitivity in nondiabetic subjects: a systematic review and meta-analysis of diet-intervention trials. *Am J Clin Nutr*.104(6):1562–1576.

Tuñón-Pablos E. and Dreby J. (2016). Risk Factors for Overweight and Obesity among Mexican Children in New York. *Inter J Popul Res.*1-8. 130.

Urzúa Z., Trujillo X., Huerta M., Trujillo-Hernández B., Ríos-Silva M., Onetti C. et al. (2012). Administration on Blood Glucose Levels and on Glucose Tolerance in Healthy and Diabetic Rats. *J Int Med Res*.40: 2220 – 2230.

Uyeda K. and Repa J.J. (2006) Carbohydrate response elemen binding protein, ChREBP, a transcription factor coupling hepatic glucose utilization and lipid synthesis. *Cell Metab*. 4(2):107–110.

Vieira F.O., Leal V.O., Stockler-Pinto M.B., Barros A.F., Borges N.A., Lobo J.C. et al. (2015). Fructose intake: is there an association with uric acid levels in nondialysis-dependent chronic kidney disease patients? *Nutr Hosp.* 31(2):772-777

Wang D.D. Sievenpiper J.L. de Souza, R.J. Chiavaroli L. and Ha V. (2012). The Effects of Fructose Intake on Serum Uric Acid Vary among Controlled Dietary Trials. *J.Nutr.916-923.*

Wang Y. and Ho C.T. (2009). Polyphenolic chemistry of tea and coffee: a century of progress. *J Agric Food Chem.* 57:8109-8114.

Whayne T.F. (2015). A Selected Overview of Beneficial or Harmful Effects on the Cardiovascular System? *Int Med Faculty Pub.* 1-15.

Wu T., Willett W.C. and Hankinson S.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*. 28:1390-1396.

Yeh T., Liu C., Cheng W., Chen B., Lu P., Cheng P. et al. (2014). Caffeine Intake Improves Fructose-Induced Hypertension and Insulin Resistance by Enhancing Central Insulin Signaling. *Hypertension*. 63:535-541.

Zhang Y., Yang T., Zeng C., Wei J., Li H., Xiong Y.et al. (2016). Is coffee consumption associated with a lower risk of hyperuricaemia or gout? A systematic review and meta-analysis. *BMJ Open.* 6:1-10.

Zhao Y., Wang J., Ballevre O., Luo H., and Zhang W. (2012). Antihypertensive effects and mechanisms of chlorogenic acids. *Hypertens Res*.35:370-374.

Zhao Y., Yang X., Lu W., Liao H. and Lia F. (2009). Uricase based methods for determination of uric acid in serum. *Microchim Acta*. 164:1–6

Zhu Y., Pandya B.J., and Choi H.K. (2011). Prevalence of gout and hyperuricemia in the US general population: the National Health and Nutrition Examination Survey 2007–2008. *Arthritis Rheum.*63:3136–41.

Zimmet, P. Z. (2010). International Diabetes Federation: The IDF consensus worldwide definition of the metabolic syndrome. Available at: *http://www.idf.org/metabolic-syndrom*.

Appendices

Appendix-I: Procedure of coffee preparation

Boiled unfiltered coffee was prepared daily without adding sugar or any other sweetener as follows.

1. 180mL of water was heated in traditional clay coffee pot
2. 10g of coffee powder was added into the hot water
3. The mixture was boiled for 10 minutes.
4. Boiled coffee was allowed to settle for 15 minutes.
5. The settled coffee was poured into thermos flask.
6. Before administration, the coffee was poured into coffee cup

Appendix II: Procedure of fructose solution preparation

20% (W/V) of fructose solution (200mL) was prepared as follows:

1. 40g of fructose was weighed out on analytical balance.
2. The weighed out fructose was transferred to a clean, dry graduated cylinder.
3. Water was added up to two-thirds of 200mL graduated cylinder and stirred until the fructose is dissolved.
4. After the solution stopped moving, water was gradually added until the solution reached the 200mL mark.
5. The solution was stirred once more to finish mixing.
6. The solution was transferred into aluminum foil covered water bottle.

Appendix - III: Procedure of oral gavage

1. Weigh the rat to determine the appropriate dosing volume. The volume should be 10- 20 ml/kg (never exceed the 20 ml/kg).

2. Measure the tube for the correct length by placing it along the outside of the animal, so that the ball tip is at the last rib and the other end of tube is by the animal's nose.

3. Mark the tube at the point where it reaches the tip of animal's nose. Do not pass the tube farther than this mark or you risk perforation of the stomach.

4. Restrain the rat in an upright position using standard restraint technique (extend the head and neck keep the nose, head and spine aligned so that the esophagus is straight).

5. Insert the bulb into the comer of the mouth and reposition the needle toward the center,

6. Slide the tip gently past the back of the tongue and run the bulb along the roof of the mouth and down the esophagus.

7. Once the gavage tube is properly placed, slowly administer the material. If animal has trouble breathing, struggles violently, or coughs, you may be in the trachea. Stop administration immediately and remove the gavage tube!

10. After administration, slowly remove the tube from esophagus.

11. Return animal to its cage.

12. Observe animal for 5-10 minutes for any signs of pain or distress, and again 12-24 hours later.

Declaration

I, the undersigned, hereby declare that this MSc thesis is my original work and has not been presented for a degree in any other university, and that all sources of materials used for the thesis have been duly acknowledged.

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