EFFECT OF CAFFEINE ON BLOOD GLUCOSE AND LIPID PROFILE IN ALLOXAN-INDUCED DIABETIC WISTAR RATS

By

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AUGUST, 2014

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By

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(MSc/MED/2435/2010-2011)

A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, AHMADU BELLO UNIVERSITY, ZARIA, IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF A DEGREE OF MASTERS OF SCIENCE IN HUMAN PHYSIOLOGY

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AUGUST, 2014.

DECLARATION

I declare that the work in this thesis entitled "EFFECT OF CAFFEINE ON BLOOD GLUCOSE AND LIPID PROFILE IN ALLOXAN-INDUCED DIABETIC WISTAR RATS" was performed by me in the Department of Human Physiology, Ahmadu Bello University, Zaria under the supervision of Dr. Aliyu Mohammad and Dr. Yusuf Tanko. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this project was previously presented for another degree or diploma at any university.

SUNDAY ADEGOKE OKOOSI ---------- ----------

Signature Date

CERTIFICATION

This thesis entitled "EFFECT OF CAFFEINE ON BLOOD GLUCOSE AND LIPID PROFILE IN ALLOXAN-INDUCED DIABETIC WISTAR RATS" by Sunday Adegoke OKOOSI meets the regulations governing the award of the degree of MSc. Human Physiology of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to the loving memory of my late father, Pa. Samuel Ajayi Okoosi, who encouraged me to undergo this programme. And who unfortunately did not live to witness its completion. Daddy, you were such a loving dad and a wonderful friend. You will always be remembered. May your soul continue to rest in perfect peace.

ACKNOWLEDGEMENT

Firstly, I wish to sincerely express my profound gratitude to the Almighty God, for His infinite mercy, support, favour, divine provision and for making everything worthwhile.

I am greatly indebted to my Supervisors, Dr Aliyu Muhammad and Dr Yusuf Tanko, for your determination to get this study done against all odds. I will always cherish your encouragement and support.

I thank the Head of Human Physiology Department, Dr Aliyu Mohammad for his fatherly disposition. I owe thanks to all academic and non-academic members of staff of the department, all of whom have been of help to me in many ways. I am indebted to Dr Yusuf Tanko for his untiring encouragement and for providing me with the Alloxan. I am thankful to Messrs Jimoh Abdulazeez and Eze Ejike for helping me out during the stage of induction of diabetes.

I owe thanks to the departmental Chief Technologist, Mr Bambe, for teaching me the rudiments in carrying out cardiac puncturing. I am grateful to my students, Adesoji, Kayode and Habila, who all assisted me at the difficult stages of drug administration and evaluation of blood glucose particularly on the very first day. And of course, Mallam Ya'u, who was responsible for taking care of the animals before and during the experiment. I am thankful to Dr John Bugau and Rahanat Otaru for typesetting and editing this work several times.

I am thankful to my mother, Mrs R. A. Okoosi, my brothers Abiodun and Babatunde Okoosi; my sisters Adenike Adams and Folasade Abbah, for your encouragement and support.

I am grateful to Professor J.O. Ogunwole for the role he played especially at the very beginning of this programme and for his constant encouragement. I am sincerely thankful to Dr. K.V. Olorunshola and Mrs Nene Avidime.

I will not forget my classmates- Abdul-Akeem, Dr Innocent, Haruna, Sheba, Aisha, Richard, Busayo, Adams, Dr Hassan, Abubakar Musa and my Class-captain, Musa. You are all wonderful people.

I remember two members of my class who lost their lives in the course of this programme- Peter Tumba and Abubakar Shehu, coincidentally, I fondly called the two of you Prof. I pray that your souls rest in perfect peace.

I cannot but mention Bashir, whom I fondly call Bash. You were my room-mate at least, that was how we first met. You subsequently became a friend and offered me a place to stay when I needed it most. You are a friend indeed.

I am thankful to the Management of the Ahmadu Bello University Teaching Hospital, Zaria, for approving Time-off for me to undertake this programme. I am most grateful.

Lastly, I am most grateful to my wife, Adunni and my son, Alexander, for the enormous support and encouragement I received during the course of this programme. I am overwhelmed by your display of love, mutual understanding and most importantly by your prayers. You are simply wonderful.

Abstract

Diabetes mellitus is characterized by hyperglycemia. Caffeine has been suggested to be capable of disrupting glucose metabolism. The effects of caffeine on blood glucose level and on lipid profile of alloxan induced diabetic Wistar rats were studied. The effect of caffeine by determining the blood glucose levels at intervals of 30mins, 1hour and 2hours respectively following administration of caffeine. Caffeine at a dose of 25 mg/kg and 100 mg/kg produced significant ($P<0.05$) increases in the blood glucose levels 2 hours after administration on comparison with control animals. After the 2 weeks duration that the experiment lasted, caffeine at a dose of 25mg/kg was found to significantly ($P<0.01$) increase blood glucose level of the rats. There was a significant ($P<0.05$) increase in the level of triglycerides in animal that received 100 mg/kg caffeine after 2 weeks of caffeine administration compared to those in all other groups. All other lipid profile indices showed no significant difference (P□0.05) compared with the control group. Caffeine was found to produce no significant (P□ 0.05) changes in the body weights of animals in all groups in comparison with those of the control. It can therefore be concluded that caffeine administration causes increases in blood glucose and blood triglyceride levels of diabetic Wistar rats, although the results suggest that these increases may be dose dependent.

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LIST OF ABBREVIATIONS

A2AR	Adinosine 2A Receptor
ADP	Adenosine Di Phosphate
AMPK	Adenosine Monophosphate Kinase
ANOVA	Analysis of Variance
ATP	Adenosine TriPhosphate
BBB	Blood Brain Barrier
cAMP	Cyclic Adenosine Monophosphate
CM	Chylomicron
CNS	Central Nervous System
CNS	Central Nervous System
CYPE1	Cytochrome E1 P450
EE	Energy Expenditure
EGCG	Epigallocatechin-3-gallate
FFA	Free Fatty Acid
GK	Glycerol Kinase
Glut-4	Glucose Transporter 4
GPO	Glycerol Phosphate Oxidase
HDL	High Density Lipoproteins
IDDM	Insulin Dependent Diabetes Mellitus
LDL	Low Density Lipoproteins
MPTP	1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine
mRNA	Messenger Ribonucleic Acid
NIDDM	Non-Insulin Dependent Diabetes Mellitus
PD	Parkinson’s Disease
SEM	Standard Error of Mean
T2DM	Type2 Diabetes Mellitus
TAG	Triacylglycerol
TC	Total Cholesterol
TG	Triglyceride
TNF-α	Tumor Necrosis Factor- alpha
VLDL	Very Low Density Lipoproteins
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Diabetes Mellitus

Excessive urine excretion is one of the major symptoms of diabetes. The most common form of diabetes is diabetes mellitus, a metabolic disorder in which there is an inability to oxidize carbohydrate due to disturbances in insulin function. Diabetes mellitus is characterized by elevated glucose in the plasma and episodic ketoacidosis. Additional symptoms of diabetes mellitus include polydipsia, glucosuria, polyuria, lipemia and hunger (King *et al*., 1998; WCPD, 2012).

Diabetes mellitus is a heterogeneous clinical disorder with numerous causes. Two main classifications of diabetes mellitus exist, idiopathic and secondary. Idiopathic diabetes is divided into Type 1 diabetes (IDDM) and Type 2 diabetes (NIDDM) (WHO, 2003).

Type 2 diabetes is associated with a cluster of interrelated plasma lipid and lipoprotein abnormalities, including reduced HDL cholesterol, a predominance of small dense LDL particles, and elevated triglycerides. These abnormalities occur in many patients despite normal LDL cholesterol levels. These changes are also a feature of the insulin resistance syndrome (also known as the metabolic syndrome), which underlies many cases of type 2 diabetes. In fact, pre-diabetic individuals often exhibit an atherogenic pattern of risk factors that includes higher levels of total cholesterol, LDL cholesterol, and triglycerides and lower levels of HDL cholesterol than individuals who do not develop diabetes (Krauss, 2004).

World Health Organization (WHO) estimated that approximately 120-140 million people were globally affected by diabetes mellitus in 1999 (WHO, 1990). In 2000, this figure increased to more than 177 million (Kalda *et al*., 2008) and is projected to

increase to 221 million by 2010 and to double by the year 2025 (King *et al*., 1998 and Emerson *et al*., 2009).

1.2 Alloxan (2, 4, 5-6-pyrimidinetetrone)

Alloxan and streptozotocin are widely used to induce experimental diabetes in animals. The mechanism of their action in β cells of the pancreas has been intensively investigated and now is quite well understood. The cytotoxic action of both of these diabetogenic agents is mediated by reactive oxygen species. However, the source of their generation is different. Alloxan and the product of its reduction, dialuric acid, establish a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide. Thereafter highly reactive hydroxyl radicals are formed by the Fenton reaction. The action of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of β cells (Szkudelski, 2001).

1.3 Caffeine

Caffeine is a bitter, white crystalline xanthine alkaloid that acts as a stimulant drug and an acetyl cholinesterase inhibitor (Cardoso-Lopes *et al.,* 2009). It is found in varying quantities in the seeds, leaves, and fruits of some plants, where it acts as natural pesticides that paralysis and kills certain insects feeding on the plants. It is most commonly consumed by humans in infusions extracted from the seeds of the coffee plant and the leaves of the tea bush, as well as from the various foods and drinks containing products derived from the Kola nut. In humans, caffeine acts as a central nervous system stimulant, temporarily warding off drowsiness and restoring alertness. It is the world most widely consumed psychoactive drug, but unlike many other

psychoactive substances, it is both legal and unregulated in nearly all parts of the world. Beverages containing caffeine such as coffee, tea, soft drinks, and energy drinks, enjoy great popularity; and about 90% of adults consume caffeine daily (Lovett, 2005).

1.3.1 Caffeine and diabetes

Coffee is the most widely consumed beverage in the world and heavy coffee consumption has been associated with a lower risk of diabetes, but little is known about the mechanisms responsible for this association. Caffeine is one of the active biological components in coffee and is the principal source of the suggested benefits of coffee consumption. The effect of caffeine on glucose tolerance is still controversial, however (Urzua *et al.,* 2012). Caffeine had been reported to somewhat reduce the risk of type 2 diabetes (Van Dam, 2008). A growing body of research suggests that caffeine disrupts glucose metabolism and may contribute not only to the development of but also the control of type 2 diabetes, a major public health problem (Lane, 2011). It has also been reported that caffeine may increase the effectiveness of some medications (Gilmore *et al*., 2011).

1.4 Metformin

Metformin is a hypoglycemic biguanide diabetic medication which acts by reducing the release of glucose from the liver and increasing glucose uptake by the muscles (Klip and Leiter, 1990). Metformin has been used for over 40 years as an effective glucose lowering agent in type 2 (non-insulin dependent) diabetes mellitus. It is an oral biguanide that ameliorates hyperglycemia by improving peripheral sensitivity to insulin, and reducing gastrointestinal glucose absorption and hepatic glucose production (Muharnniad *et al*., 2000).

1.5 Statement of the Research Problem.

There is growing concern that diabetes will assume epidemic proportions affecting the developing world in Asia and Africa more than the developed world (Amos *et al*., 1997; Rheeder, 2006). Diabetes mellitus is a risk factor for cardiovascular disease, stroke, amputation and blindness (Gong *et al*., 2009; Duze *et al*., 2012). Findings have indicated that caffeine intake influences glucose metabolism (Lane *et al*., 2004) while caffeine and coffee have been shown to have exaggerating effects on postprandial hyperglycemia, a condition that can worsen type 2 diabetes mellitus (Lane *et al*., 2007).

1.6 Justification for the Study

While it is expedient that we are certain about the effects of caffeine on diabetes mellitus since it is popularly consumed in the Nigerian society in various forms, there is the need to also clear the seeming controversy over the effect of caffeine the active ingredient in kolanut, coffee, tea, chocolate and cola drinks on diabetes mellitus.

The reports of certain previous studies on caffeine were contradictory. While some indicated that caffeine exerts a protective effect against the onset of diabetes mellitus (Shearer *et al*., 2003; Van Dam *et al.,* 2006; Shearer *et al*., 2007), recent studies have shown that it actually increases insulin resistance, a factor, in the development of type 2 diabetes (Van Dam, 2006; Lane, 2011). These recent studies conflict with past studies about coffee which have consistently found that coffee drinkers have a lower risk of developing type 2 diabetes mellitus and that the consumption of coffee may ameliorate the effects of the disease.

1.7 Aim and Objectives

The aim of this study is to determine the effects of caffeine on blood glucose and lipid profile in alloxan-induced diabetic Wistar rats.

1.7.1 The specific objectives of this study are as follow:

i. To study the acute and sub-chronic effects of caffeine on blood glucose level in alloxan- induced diabetic rats.
ii. To investigate the effect of caffeine on lipid profile in alloxan-induced diabetic rats.
iii. To investigate the effect of caffeine on the body weight of alloxan-induced diabetic Wistar rats.

1.8 Hypothesis.

Caffeine has no significant effects on blood glucose level and lipid profile in alloxan-induced diabetic Wistar rats.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Caffeine and the Body

2.1.1 Caffeine and the nervous system

Caffeine stimulates the central nervous system first at the higher levels, resulting in increased alertness and wakefulness, faster and clearer flow of thought, increased focus, and better general body coordination, and later at the spinal cord level at higher doses (Karau *et al.*, 2010). This stimulatory activity is achieved through several mechanisms including increased ATP production through cyclic adenosine monophosphate (cAMP) accumulation and inhibition of dopamine re-uptake (Huang *et al*., 2005).

Parkinson's disease (PD) is the degeneration of dopamine producing neurons. Dopamine producing neurons stimulate the motor cortex effecting motor control. Loss of dopamine produces loss of motor control skills, cognitive function, and autonomic nervous system. Epidemiological studies have shown that the risk of developing PD decreases with increasing levels of caffeine intake. Caffeine binds to the adenosine receptor (A2aR) and indirectly prevents MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine,an experimental neurotoxin known to cause PD) from destroying dopamine producing neural cells in the brain and the substantia nigra, thus preventing neural degeneration and loss of motor control (Jiang-Fan *et al*., 2001).

Duarte *et al.* (2012) using an animal model of diabetes type 2 that closely mimics the human form of the disease – suggested that diabetes affects memory by causing synaptic degeneration, astrogliosis and increased levels of A2AR. The study indicated as well that chronic consumption of caffeine can prevent the neuro-degeneration and

the memory impairment. And this not only in diabetes, since synaptic degeneration and astrogliosis are both part of a cascade of events common to several neuro-degenerative diseases, suggesting that caffeine (or similar drugs) could help them too through the same mechanisms.

2.1.2 Caffeine and muscles

Caffeine improves endurance exercise by sparing muscle glycogen through increased fat oxidation (Essig *et al*, 1980). Studies have found that caffeine ingestion does not always spare muscle glycogen, increase plasma free fatty acid or reduce the respiratory exchange ratio during exercise (Arogyasami *et al*., 1989).

Caffeine increases the rate of glucose transport in the absence of insulin in rodent skeletal muscle, and enhances glucose transporter-4 (Glut-4) mRNA and protein levels in cultured myotubes. In a rat model, caffeine predominantly activates the cyclic AMP-dependent protein kinase α-1isoform (α-1 cAMP) by means of an insulin independent mechanism, which increases AMP kinase-1 to promote glucose transport. In addition, the up-regulation of Glut-4 is dependent on the caffeine concentration. An alternative mechanism could be that caffeine acts as an antagonist of adenosine A1 receptors, which are present in skeletal muscle. Antagonism of these receptors has been shown to improve glucose tolerance in rats (Urzua *et al.*, 2012).

2.1.3 Caffeine and the cardiovascular system

Caffeine increases cardiac arrhythmia by increasing stress hormone secretions. It has been shown there is an increase in brachial diastolic blood pressure, but not in brachial systolic blood pressure. However, both aortic systolic and diastolic blood pressures

increase significantly during caffeine consumption. It has been noted that long term consumption leads to increasing aortic systolic pressure which leads to chronic arterial stiffness (Bracco *et al*., 2001; Mahmud *et al.,* 2001).

Terry *et al.,* (2000) demonstrated that caffeine affects persons to a progressively greater degree according to their blood pressure classification. They further demonstrated that the higher the risk classification, the more likely are BPs in the hypertensive range 45 to 60 minutes after consumption of a dietary dose of caffeine and while resting.

2.1.4 Caffeine and urinary system

Caffeine intake increases renal excretion of sodium and water. This is caused by both slightly increasing the glomerular filteration rate and inhibiting the tubular reabsorption of sodium and water. Although the ability for caffeine and theophylline to induce diuresis and natriuresis is well established, the mechanisms behind them are not well understood (Milon *et al.*, 1988; Rieg *et al*., 2004). Because of its diuretic effect, some authorities have recommended that athletes or airline passengers avoid caffeine in order to reduce the risk of dehydration through stimulation of urinary output (Maughan *et al*., 2003).

2.1.5 Caffeine and the pancreas

The antioxidant effect of caffeine has also been described. It has been proposed that caffeine could contribute to the protection of β pancreatic cells from free radicals produced by oxidative stress, prevent membrane damage and preserve the β pancreatic cell (Urzua *et al.,* 2012).

Caffeinated and decaffeinated coffee, caffeine, and an unidentified substance in coffee other than caffeine were associated with increased inter-digestive exocrine pancreatic trypsin secretion. In addition, Coffey *et al.* (1989) also confirmed that coffee and caffeine stimulated gastric acid secretion and decaffeinated coffee raised serum gastrin concentrations.

2.1.6 Caffeine and the liver

Research has shown there is a strong negative correlation between coffee consumption and liver cirrhosis (Klatsky *et al*., 2006; Muriel *et al*., 2010). However, there is a lack of corresponding evidence for other beverages that contain caffeine (Corrao *et al*., 2001).

Caffeine is metabolized in the liver into three primary metabolites: paraxanthine (84%), theobromine (12%), and theophylline (4%) (Julien, 2005).

In addition, there is other research that shows caffeine to have suppressive effects on Tumor necrosis factor-alpha (TNF-α)-induced hepatitis by suppressing the TNF-α production, and by suppressing the TNF-α-induced apoptosis (Sugiyama *et al*., 2001).

2.1.7 Effect of caffeine on pregnancy

Russell (2007) reported that recent studies have shown that consuming more than 300 milligrams of caffeine a day will increase one's chances of a miscarriage, and based on studies on animals, high levels of caffeine may also cause birth defects, preterm delivery, reduced fertility and low birth weight.

There is also a link between consuming even more caffeine (more than 500 mg a day) and faster heart and breathing rates in babies in the first few days after birth.

Caffeine passes through the placenta and is absorbed by the baby. In pregnant women, caffeine is active in the body for up to 11 hours, but up to 100 hours in the fetus because the developing babies still lack the necessary enzymes to detoxify and break it down.

2.2 Metabolism of Caffeine

Caffeine is metabolized in the liver into three primary metabolites: paraxanthine (84%), theobromine (12%), and theophylline (4%), each of which has its own effects on the body:

- Paraxanthine: increases lipolysis, leading to elevated glycerol and free fatty acid levels in the blood plasma.
- Theobromine: Dilates blood vessels and increase urine volume.
- Theophylline: Relaxes smooth muscles of the bronchi, and is used to treat asthma (Julien, 2005).

A study on biomarkers showed that caffeine metabolites (isoprenoids) persist in the bloodstream for 24 hours and are not easily eliminated following daily coffee and caffeine intake for15 days or more. This could partially explain the long-term effects of caffeine (Urzau *et al.*, 2012).

2.3 Caffeine and Blood Glucose Level

2.3.1 Caffeine and blood glucose level in non-diabetics

According to the work done by Gerben *et al.,* (2002), caffeine was found to be capable of decreasing insulin sensitivity in humans. Caffeine, in a dose that equals moderate consumption, decreased insulin sensitivity in healthy volunteers. The decrease in insulin sensitivity that was reported as result of caffeine ingestion is close to the magnitude of the increase in insulin sensitivity that can be achieved with glucose-lowering agents, such as metformin and thiazolidinedione derivatives, and is therefore clinically relevant. This finding may have serious health implications, especially when superimposed on already-disturbed glucose tolerance or established diabetes mellitus Type-2. They explained that caffeine can decrease insulin sensitivity in healthy humans, possibly as a result of elevated plasma epinephrine levels. Because dipyridamole did not affect glucose uptake, peripheral adenosine receptor antagonism does not appear to contribute to this effect. This is contrary to the view of others who argued strongly in favour of adenosine antagonism as the possible way by which caffeine can reduce insulin sensitivity. It was however concluded that further investigation is required to elucidate whether this effect persists over time with chronic use of caffeine.

Another work revealed that acute caffeine ingestion does not affect endogenous glucose production and that caffeine's attenuation of insulin-mediated, whole-body glucose disposal is not attributed to the small increase in adrenaline concentration alone. Additional mechanisms, possibly adenosine receptor antagonism, must be involved (Battram *et al*., 2005).

Again, caffeine intake was found to reduce the risk of type 2 diabetes (Heather *et al*., 2004; Hiroyasu *et al*., 2006). It was also reported that caffeine exerts a protective effect against the onset of diabetes mellitus (Shearer *et al*., 2003; Van Dam *et al.,* 2006; Shearer *et al*., 2007). It was found that caffeine intake of 416mg/day reduced the risk by 33%. These findings are consistent with that of Eduado *et al.,* (2004). Additional laboratory studies showed that caffeine may be one of the most effective anti-diabetic compounds in coffee (American Chemical Society, 2010).

2.3.2 Caffeine and blood glucose level in diabetics

Acute administration of caffeine impaired postprandial glucose metabolism in diabetic patients. Human subjects were reported to have demonstrated exaggerations of both glucose and insulin responses when caffeine was ingested with carbohydrates. These results raised concerns about the potential hazards of caffeine for patients with type 2 diabetes and possibly for individuals who are glucose intolerant or "pre-diabetic." Repeated exaggerations of postprandial glucose, resulting from daily consumption of caffeinated beverages with meals, could produce higher average glucose levels that increase the risk of diabetes complications (Lane *et al.*, 2004).

Caffeine intake has also been proposed to increase symptomatic warning signs of hypoglycemia in patients with type 1 diabetes and elevate blood glucose levels in patients with type 2 diabetes (Dessi *et al.*, 2013).

Urzúa *et al.*, (2012) observed and reported that blood glucose levels decreased, and glucose tolerance improved, in diabetic rats administered increasing doses of caffeine.

2.4 Coffee and Blood Glucose Level

2.4.1 Coffee and blood glucose level in non-diabetics

Recent scientific evidence has consistently linked regular, moderate coffee consumption with a possible reduced risk of developing type2 diabetes (WCPD, 2012). Coffee contains numerous substances; among them, caffeine, chlorogenic acid, quinides, and magnesium have been shown to affect glucose metabolism in animal (Robinson *et al*., 2004; Andrade-Cetto *et al*., 2001). Van Dam *et al*., (2002) reported that coffee is a major source of caffeine, which has been shown to acutely reduce sensitivity to insulin, but also has potentially beneficial effects. They found that coffee consumption was associated with a substantially lower risk of clinical type 2 diabetes. In a work to find out the relationship between Green tea and total caffeine intake and the risk for self-reported Type2 diabetes, Hiroyasu *et al*. (2006) reported that consumption of green tea, coffee, and total caffeine was associated with a reduced risk for type 2 diabetes.

The ingestion of caffeinated coffee with either a high or low glycemic index meal significantly impairs acute blood glucose management and insulin sensitivity compared with ingestion of decaffeinated coffee (Moisey *et al*., 2008).

The work of Aramesh and Marshall (2003) on the other hand provided no evidence for a relationship between coffee consumption and risk of type 2 diabetes.

Findings indicated that consumers who drink at least three or more cups of either caffeinated or decaffeinated coffee every day show a decrease in the risk of type II diabetes. It was found that caffeine-containing beverages were associated with a

decreased risk of type II diabetes. The mechanism responsible for this inverse relationship between caffeine containing beverage and the risk of diabetes includes basal energy expenditure, fat oxidation stimulation, glycogen mobilization in muscles, and stimulation of increased lipolysis from peripheral tissues (Hiroyasu *et al.,* 2006).

Van Dam *et al.*,(2004) presented results which indicated that tolerance to the adverse effects of high coffee consumption on insulin-glucose homeostasis does not develop within a 4-week period that their work last, they therefore suggested that it is premature to advocate high coffee consumption as a means to lower risk for type 2 diabetes. Long-term trials of coffee consumption that include detailed measures of insulin sensitivity and glucose metabolism are warranted to elucidate the apparent discrepancy with studies that observed an inverse association between habitual coffee consumption and risk for type 2 diabetes.

2.4.2 Coffee and blood glucose level in diabetics

Although coffee has been associated with a lowered risk of developing type 2 diabetes, it has also been claimed to have negative effects on diabetes patients, because the caffeine in regular coffee is capable of reducing insulin sensitivity and makes it difficult for patients with diabetes to control their blood glucose levels. Studies have shown that caffeine consumption increases blood glucose, but diabetes medication pills decrease blood glucose levels showing that the two counter-interact. Diabetes patients have trouble controlling their sugar level in their blood due to lack of insulin resistance hormone. Once in the human body, caffeine produces alloxan and either produces diabetes' conditions or worsens the existing diabetes (DeNoon, 2008).

Sadeek *et al.* (2010) found a significant decrease in serum glucose and insulin following administration of green, roasted and decaffeinated coffee, with the lowest glucose value observed with green coffee and with decaffeinated coffee for insulin.

2.5 Caffeine and Lipid Profile

2.5.1 Caffeine and lipid profile in non-diabetics.

Onuegbu *et al.* (2001) found significant increases in the mean total serum cholesterol concentration and LDL- cholesterol concentration were observed in healthy human subjects after regular administration of caffeine. No significant differences were obtained in the mean HDL cholesterol concentration and in the mean serum triglyceride levels.

Amany *et al.* (2012) found that green tea extract, epigallocatechin-3-gallate (EGCG) and caffeine induced different beneficial effects on lipid disorders in obese rats. Caffeine increased the effect of EGCG and green tea extract on lipid profile. It was observed that supplementation of caffeine in combination with EGCG or green tea extract enhance their effect on serum levels of lipid profile by decreasing total cholesterol, triglycerides and decrease LDL-cholesterol with concurrent increase in HDL-cholesterol level in obese rats.

Grzegorzewska *et al.* (2009) reported that plasma concentration of HDL cholesterol was significantly higher in patients who were drinkers of caffeinated coffee compared to those who were nondrinkers. Also, the plasma concentration of LDL cholesterol was significantly lower in coffee drinkers than in nondrinkers of coffee. As a percentage of total cholesterol, HDL cholesterol was higher in coffee drinker. Serum total cholesterol

and serum triglycerides were not significantly different between the groups. Mahmoud *et al.* (2013) found that coffee consumption reduced total cholesterol and LDL-cholesterol in rats and therefore concluded that it may be consumed in considerable amounts for reducing cholesterol problems.

Lipolysis is another indicator of lipid metabolism, and greater lipolysis has frequently been observed after caffeine or coffee intake by human subjects. Investigators have used either plasma free fatty acids (FFA) or glycerol assays as indicators of lipolysis, and glycerol is considered the more reliable indicator. Several human studies found that an acute increase in lipolysis resulted from ingestion of caffeine, ground caffeinated coffee and instant caffeinated coffee. No increase in lipolysis was found after ingestion of decaffeinated coffee, instant decaffeinated coffee or ground decaffeinated coffee; these results derive from only one human study for each type of decaffeinated coffee (Greenberg *et al.,* 2006).

2.5.2 Caffeine and lipid profile in diabetics

Sadeek *et al.,* (2010) found that caffeine produced a significant increase in serum total cholesterol(TC); triacylglycerol (TAG); LDL-cholesterol; VLDL-cholesterol and in LDL\ HDL ratio and TC \ HDL ratio, however a significant decrease in serum HDL-Cholesterol was observed in diabetic rats compared to normal control .

Green, roasted and decaffeinated coffee resulted in a significant decrease in triacylglycerol (TAG); LDL-cholesterol; VLDL-Cholesterol and in LDL\HDL ratio and TC \ HDL ratio. On the other hand a significant increase in serum HDL-cholesterol is observed in green, roasted and decaffeinated coffee groups compared to diabetic rats

with the highest value for green coffee. Non-significant effect on serum total cholesterol (TC) was reported in this study.

2.6 Metformin and Blood Glucose Level in the Diabetics

2.6.1 Metformin and blood glucose level in diabetics

Ripudaman *et al.* (2000) reported that there was no significant change in body weight or plasma insulin levels in diabetes mellitus type 2 patients that were treated with metformin. It reduces fasting plasma glucose concentrations by reducing rates of hepatic glucose production, its effect on the relative contributions of hepatic glycogenolysis and gluconeogenesis remains controversial. Some studies conclude that metformin works mostly by reducing rates of gluconeogenesis; others, that it works by reducing rates of hepatic glycogenolysis.

Marc *et al.* (2010) found that metformin inhibited cumulative glucose production stimulated by the cAMP analog dibutyryl-cAMP (Bt2-cAMP) in mouse primary hepatocytes, consistent with previous findings. Interestingly, the ability of metformin to suppress Bt2-cAMP–stimulated glucose production was maintained in primary hepatocytes lacking both AMPKα1 and -α2 catalytic subunits (AMPKα1α2-null hepatocytes) regardless of the gluconeogenic substrates used (lactate/pyruvate or dihydroxyacetone).

Muharnniad *et al.,* (2000) reported that metformin therapy significantly decreased fasting blood glucose levels in all type 2 diabetic patients. This result was explained by stating that Metformin alleviates hyperglycemia of type 2 diabetes by inhibiting hepatic

glucose production and improving peripheral insulin sensitivity. Metformin interferes with several processes linked to hepatic glucose production (gluconeogenesis, glycogenolysis and their regulatory mechanisms), lowering glucose production and re-sensitizing the liver to insulin. The hepatic drug effect is largely favored by prevailing glycemia. In peripheral tissues, metformin potentiates the effects of both hyperglycemia and hyperinsulinemia. Increase in glucose—mediated glucose transport is mainly mediated by an improvement in the glucose transporter's intrinsic activity. Potentiation of the hormone effect relates to an increase in insulin receptor tyrosine kinase activity. Both mechanisms (insulin signaling and glucose transport) result in the activation of glycogen synthase, a limiting enzyme in the causal defects of type-2 diabetes. This finding indicates that at metformin certainly has positive effect on these physiological mechanisms.

Mourão-Júnior *et al.,* (2006), found that glycemic control significantly improved after the addition of metformin to the treatment regimen of type 2 diabetes patients who were already on insulin. Kassem *et al.,* (2009) stated that metformin lowers plasma glucose levels by suppressing hepatic gluconeogenesis and glycogenolysis, while increasing peripheral sensitivity to insulin. Its beneficial effects on glucose metabolism are not accompanied by weight gain, a clear advantage over other commonly used oral hypoglycemic agents.

2.7 Metformin and Lipid Profile in Diabetics

2.7.1 Metformin and lipid profile in diabetics

Studies suggest that controlling hyperglycemia, LDL-cholesterol and blood pressure are important to protect the diabetic from atherosclerosis. Low—density lipoprotein (LDL) cholesterol is a critically important factor in the development of atherosclerosis. Low-density lipoprotein (LDL) oxidation has been suggested to play a key role in the pathogenesis of atherosclerosis, a major complication of diabetes mellitus. High-density lipoproteins (HDLs) appear to exert the greatest influence independently of other lipoproteins, with low-density lipoproteins (LDLs) having a weaker, though still significant independent relation with coronary heart disease (Muharnniad *et al*., 2000).

Metformin treatment resulted in a 24% reduction in cholesterol and a 30% reduction in free fatty acid concentrations in plasma (Ripudaman, 2000). According to Salih *et al.,* (2011), metformin produces a non-significant favorable effect on all lipids profile parameters.

Muharnniad *et al*., (2000) reported that metformin decreased serum total cholesterol marginally. Serum total triglycerides concentration also decreased as well as Low—density lipoproteins, and very-low density lipoprotein cholesterol. Whereas, high-density lipoprotein cholesterol tended to increase while no significant changes occurred in body weight and body mass index.

There was a statistically significant reduction of total cholesterol, waist circumference, and daily necessity of insulin. No changes were detected in HDL-cholesterol or blood pressure following simultaneous administration of metformin and insulin (Mourão-Júnior *et al*., 2006).

Rojas *et al.* (2013) reported that metformin is associated with improvements in lipoprotein metabolism, including decreases in LDL-C, fasting and postprandial TGs, and free fatty acids. Metformin was also reported to probably have a neutral effect on body weight of patients with T2DM when compared to diet or may limit or decrease the weight gain experienced with sulfonylureas and insulin. The mechanisms by which metformin contributes to weight loss may be explained through the reduction in gastrointestinal absorption of carbohydrates and insulin resistance, reduction of leptin and ghrelin levels after glucose overload, and by induction of a lipolytic and anoretic effect by acting on glucagon–like peptide 1(Rojas *et al.*, 2013). The effect of metformin on lipid profile is favorable. It significantly reduces plasma triglyceride levels, a result related to improved glucose levels. Modest reduction in LDL levels was demonstrated with metformin therapy. However, analysis of 29 trials failed to demonstrate significant elevation in HDL levels with metformin (Kassem *et al.*, 2009).

2.8 Mechanism of Action of Caffeine

Caffeine acts through various mechanisms of action. According to Davis (2003), caffeine manages to affect the body by stimulating the central nervous system (CNS). Its lipophilic properties grant caffeine entry through the blood brain barrier (BBB), where it interferes with adenosine receptors. There is a similarity between the molecular structures of caffeine and adenosine, a naturally occurring substance in the brain.

This similarity allows caffeine to bind to the same receptor sites as adenosine. However, the binding of caffeine to an adenosine receptor site does not produce the

same effect as binding of adenosine to the receptor. Thus, caffeine is classified as an adenosine antagonist; a drug that attaches to a receptor and blocks the action of any other molecule trying to bind to the receptor site (Julien, 2005). The other mechanisms by which caffeine acts include mobilization of intracellular calcium and inhibition of specific phosphodiesterases both of which only occur at high non-physiological concentrations of caffeine.

2.9 Mechanism of Action of Metformin

Metformin is effective only in the presence of insulin, and its major effect is to decrease hepatic glucose output (Bailey, 1992; Bailey *et al.,* 1996). In addition, metformin increases insulin-mediated glucose utilization in peripheral tissues (such as muscle and liver), particularly after meals, and has an anti-lipolytic effect that lowers serum free fatty acid concentrations, thereby reducing substrate availability for gluconeogenesis (Bailey *et al*., 1996). As a result of the improvement in glycemic control, serum insulin concentrations decline slightly. Metformin also increases intestinal glucose utilization via non-oxidative metabolism, at least in experimental animals (Bailey *et al.,* 1996). The lactate produced by this process is largely metabolized in the liver as a substrate for gluconeogenesis (Bailey, 1992). The latter effect could protect against hypoglycemia.

The molecular mechanisms of metformin action are not fully known. Activation of the enzyme AMP-activated protein kinase (AMPK) appears to be the mechanism by which metformin lowers serum lipid and blood glucose concentrations (Zhou *et al.,* 2001). AMPK-dependent inhibitory phosphorylation of acetyl-coA carboxylases Acc1 and Acc2 then suppresses lipogenesis and lowers cellular fatty acid synthesis in liver and

muscle, which in turn improves insulin sensitivity and reduces blood glucose levels (Fullerton *et al*., Shaw 2013).

2.10 Caffeine and Body Weight

2.10.1 Effect of caffeine on body weight

Urzúa *et al.* (2012) observed a weight loss in all the groups during the first 15 days of their study and over the 60 days after diabetes induction and administration of various dosages of caffeine, although there was no significant difference in weight loss over the course of the study, or between the caffeine-treated groups and the control group, at 15 or 60 days. Lopez-Garcia *et al.,* (2006) on the other hand, reported that increases in caffeine intake may lead to a small reduction in long-term weight gain. According to them, it seems possible that long-term coffee consumption may have a weak ability to help persons achieve weight loss, and that this weight loss may contribute toward reducing the risk of diabetes. Coffee was found to reduce body weight and may be beneficial for obese persons, it also decreases liver enzymes and this may be helpful in improving health status of hepatic patients (Mahmoud *et al.*, 2013).

Considerable evidence from studies in humans supports the hypothesis that coffee consumption induces weight loss by increasing thermogenesis. It has been estimated that a habitual consumption of 6 cups coffee (i.e, 600 mg caffeine/d) causes an increase in energy expenditure (EE) of ≈100 kcal/d, which could indeed lead to significant weight loss. This estimate does not apply equally to all types of coffee. EE has been found to be elevated for several hours after either injection of caffeine or ingestion of caffeine, ground caffeinated coffee, or instant caffeinated coffee. There is evidence that the increase in energy consumption is related to the dose of caffeine consumed. In addition, 24-h EE has been found to be raised by the ingestion of caffeine and instant-

caffeinated coffee. Similarly, it has been shown that the thermic effect of food is increased by the ingestion of caffeine and instant-caffeinated coffee (Greenberg *et al*., 2006).

It has been suggested that caffeine and coffee consumption could help individuals lose weight by reducing body fat, possibly by increasing lipid metabolism. Using a lowered respiratory exchange ratio as an indicator of greater fat oxidation in human subjects, some investigators concluded that fat oxidation is increased in humans by consumption of caffeine and caffeinated coffee (Greenberg *et al.,* 2006).

2.11 Pathophysiology of Diabetes Mellitus

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both (WHO, 2003). In type 2 diabetes, the body either produces inadequate amounts of insulin to meet the demands of the body or insulin resistance has developed. Insulin resistance refers to when cells of the body such as the muscle, liver and fat cells fail to respond to insulin, even when levels are high. In fat cells, triglycerides are instead broken down to produce free fatty acids for energy; muscle cells are deprived of an energy source and liver cells fail to build up glycogen stores. This also leads to an overall rise in the level of glucose in the blood. Glycogen stores become markedly reduced and there is less glucose available for release when it may be needed (Ananya, 2014).

In a healthy person, insulin is produced in response to the increased level of glucose in the bloodstream, and its major role is to control glucose concentration in the blood. What insulin does is, allowing the body cells and tissues to use glucose as a main energy source. Also, this hormone is responsible for conversion of glucose to glycogen for storage in the muscles and liver cells. This way, sugar level is maintained at a near stable amount (Ananya, 2014).

In a diabetic person, there is an abnormal metabolism of insulin hormone. The actual reason for this malfunction differs according to the type of diabetes. Whatever the cause is, the body cells and tissues do not make use of glucose from the blood, resulting in elevated blood glucose (a typical symptom of diabetes called hyperglycemia). This condition is also exacerbated by the conversion of stored glycogen to glucose, i.e., increased hepatic glucose production. Over a period of time, high glucose level in the bloodstream can lead to severe complications, such as eye disorders, cardiovascular diseases, kidney damage, and nerve problems (Ananya, 2014).

In Type 1 diabetes, the pancreas cannot synthesize enough amounts of insulin as required by the body. The pathophysiology of Type 1 diabetes mellitus suggests that it is an autoimmune disease, wherein the body's own immune system generates secretion of substances that attack the beta cells of the pancreas. Consequently, the pancreas secretes little or no insulin. Type 1 diabetes is more common among children and young adults (around 20 years). Since it is common among young individuals and insulin hormone is used for treatment, Type 1 diabetes is also referred to as Juvenile Diabetes or Insulin Dependent Diabetes Mellitus (IDDM) (WHO, 2003).

In case of Type 2 diabetes mellitus, the insulin hormone secreted by the beta cells is normal or slightly lower than the ideal amount. However, the body cells are not responding to insulin as they do in a healthy person. Since the body cells and tissues are resistant to insulin, they do not absorb glucose instead it remains in the bloodstream. Thus, the Type 2 diabetes is also characterized by elevated blood sugar. It is commonly manifested by middle-aged adults (above 40 years). As insulin is not necessary for treatment of Type 2 diabetes, it is known as Non-insulin Dependent Diabetes Mellitus (NIIDM) or Adult Onset Diabetes (Ningthouian, 2011).

The third type of diabetes is called Gestational diabetes. As the term clearly suggests, it is exhibited by pregnant women. Over here, high level of blood glucose is caused by hormonal fluctuations during pregnancy. Usually, the sugar concentration returns to normal after the baby is born. However, there are also instances, in which it remains high even after childbirth. This is an indication for increased risks of developing diabetes in the near future.

As already mentioned, the symptoms and effects of all the three forms of diabetes are similar. The noticeable symptoms include increased thirst (polydipsia), increased urination (polyuria), and increased appetite (polyphagia). Other diabetes signs and symptoms include excessive fatigue, presence of sugar in the urine (glycosuria), body irritation, unexplained weight loss, and dehydration. Elevated blood sugar and glycosuria are interrelated; when sugar amount in the blood is abnormally high, the reabsorption by proximal convoluted tubule is reduced, thereby retaining some glucose in the urine (Ningthouian, 2011).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Animals

A total of twenty five (25) male Wistar rats weighing between 140 – 180 grams were used for this research. The animals were purchased from the Animal House, Department of Human Physiology, Faculty of Medicine, Ahmadu Bello University, Zaria, Nigeria. The rats were housed in the Animal House of the Department of Human Physiology, Faculty of Medicine, Ahmadu Bello University, Zaria- Nigeria. The animals were kept in cages under normal environmental temperature and were fed with standard pellet diet and water given *ad libitum*. The rats were allowed to acclimatize to the laboratory environment for two weeks before the experiment commenced.

3.2 Chemicals and Drugs

The Caffeine, Metformin, Chloroform and Alloxan used for this study were all of analytical grades. The caffeine was manufactured by BDH Chemical Ltd, England. Metformin Hydrochloride was manufactured by Theon Pharmaceuticals Ltd, Vill. Saini Majra, Tehsil Nalagarh, India. The Alloxan was manufactured by Sigma Chemical Company St. Louis, U.S.A.

3.3 Experimental Design

3.3.1 Induction of experimental diabetes mellitus

The induction of diabetes mellitus was carried out as described by Katsumata *et al.*, (1999). The animals were fasted for 16 hours with free access to water prior to the induction. Induction of diabetes was then carried out by single intra-peritoneal injection of alloxan monohydrate (Sigma St Louis, M.O., USA) dissolved in 0.9%v/v cold

normal saline solution at a dose of 150 mg/kg body weight. Rats were provided with 5% glucose solution in place of water to counter alloxan- induced hypoglycemia due to insulin leakage from damaged beta cells (Adaramoye *et al.*, 2006; Dhandapani *et al*., 2002). Since alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release, rats were kept for the next 24hours on 5% glucose solution to prevent hypoglycemia. The rats were assessed for diabetes mellitus by determining the blood glucose concentration 72 hours after injection of alloxan, using blood from the tail by the glucose oxidase method and a portable glucometer (Accutrend Plus). All animals with blood glucose level above 200mg/dl were considered diabetic and therefore used for this study.

3.3.2 Animal grouping

After the induction of diabetes mellitus in the Wistar rats, the animals were randomly allotted into five groups consisting of experimental and control groups. Each group consisted of five diabetic Wistar rats.

Group 1 (5):

Diabetic control Wistar rats that received distilled water only.

Group 2 (5):

Diabetic Wistar rats that were administered 25mg/kg caffeine.

Group 3 (n=5):

Diabetic Wistar rats that were administered 50mg/kg caffeine.

Group 4 (n=5):

Diabetic Wistar rats administered with 100mg/kg caffeine (modification of Silverman, 1978).

Group 5 (n=5):

Diabetic Wistar rats administered with 300mg/kg metformin (positive control, Duze *et al*., 2012).

3.4 Determination of Weights

The weights of animals were measured on the first day of the experiment after induction of diabetes and subsequently at an interval of 1and 2 week after commencement of treatment, using a digital spring balance.

3.5 Sample Collection

Blood glucose levels of each group was determined after single oral dose of caffeine (50mg/kg, modification of Silverman's,1978), metformin (300mg/kg, Duze, 2012), and distilled water at 0, 0.5, 1 and 2 hour post-treatment by glucose oxidase method, using a portable glucometer, with blood obtained via tail vein.

For the long term effects of caffeine, diabetic rats were orally treated daily with either distilled water, 300mg/kg metformin, 25mg/kg, 50mg/kg or 100mg/kg caffeine for a total period of two weeks (Silverman, 1978, modified). All treatments were given by oral gavage using a 16G feeding needle.

On the final day, all animals were weighed and blood glucose levels determined using a portable glucometer and blood obtained via tail vein. Animals were then deeply anesthetized by chloroform inhalation and blood collected into tubes for biochemical tests by cardiac puncture. Fasting blood glucose levels and weight of each group were determined weekly for the two weeks.

3.6 Determination of Blood Glucose Levels

For the acute study, blood samples for the determination of glucose levels were collected from the tail of the rats at an interval of 0, 0.5, 1 and 2 hr. Also, using the same method, blood glucose levels were measured at an interval of 0, 1 and 2 week, for the chronic study. The glucose-oxidase principle was used (Beach and Turner, 1958), using the portable digital glucometer (Accutrend Plus ®). The results were expressed in mg/dl (Katsumata *et al*., 1999).

3.7 Determination of Lipid Profile

3.7.1 Evaluation of serum total cholesterol (TC)

The plasma cholesterol was determined by the hydrolysis of cholesterol esters to free cholesterol. The free cholesterol was then oxidized by cholesterol oxidase to cholesten-4 en-3-one with the simultaneous production of hydrogen peroxide. The hydrogen peroxide reacts with 4-aminoantipyrine and phenolic compound in the presence of peroxidase to yield a coloured complex which was read at 505nm. The intensity of colour produced is directly proportional to the concentration of total cholesterol in the plasma (Allain *et al*., 1974).

3.7.2 Evaluation of serum triglycerate (TG)

The triglycerol (TG) was determined after enzymatic hydrolysis with lipases. Triacylglycerols are hydrolysed by lipases to glycerol and free fatty acids (FFA). The glycerol reacts with adenosine triphosphate (ATP) to form glycerol-3-phosphaate and adenosine diphosphate (ADP) under the influence of glycerol kinase (GK). Glycerol-3-phosphate is oxidized by glycerol phosphate oxidase (GPO) to dihydroxyacetone and hydrogen peroxide. Hydrogen peroxide then combined with 4-aminoantipyrine to form quinoeimine (coloured compound). The coloured complex was read at a wavelength of 500nm (Tietz, 1994).

3.7.3 Evaluation of high density lipoprotein (HDL)

HDL-L reagent is produced by using a combination of detergents and phosphorous compounds which specifically bind LDL, VLDL and chylomicron (CM) but not HDL. The combination protects LDL, VLDL and CM from the reaction by cholesterol esterase and cholesterol oxidase. Consequently HDL-cholesterol was selectively exposed to react with both enzymes.

3.8 Data Analysis

Data obtained from the study were expressed as mean ± SEM. The differences between the groups were analyzed by one-way analysis of variance (ANOVA) followed by post hoc multiple comparison tests of Tukey using SPSS statistical tool version 19. Values of $p < 0.05$ were considered significant.

CHAPTER FOUR

4.0 RESULT

4.1 Physical Observations

Following the induction of diabetes mellitus, it was observed that the frequency of urination by the rats increased and the beddings in the cages had to be changed at a more frequent rate. It was also observed that the animals drank water more frequently too.

4.2 Effect of Caffeine on Blood Glucose

4.2.1 Acute and chronic effects of caffeine on blood glucose level in diabetic rats

Table 4.1.1 shows that caffeine given at a dose of 25 mg/kg significantly ($p<0.05$) increased the blood glucose levels in alloxan-induced diabetic Wistar rats 2 hours following administration. Also, 2 hours after the administration, caffeine at a dosage of 100mg/kg produced a significant ($p< 0.05$) increase in blood glucose level when compared with the negative control group that was given distilled water. The increases observed 0.5 hour and 1 hour after the administration of caffeine were not statistically significant ($p < 0.05$). No significant changes were observed between the various caffeine groups and the control group 0.5 hour and 1 hour following administration. Caffeine at 50 mg/kg produced no statistically significant ($p \square 0.05$) changes at all intervals. Metformin on the other hand produced a significant ($p< 0.05$) mean decrease in blood glucose level after 2 hours of administration compared with the control.

For the sub-chronic study, caffeine at a dose of 25 mg/kg produced a significant ($p<0.01$) increase in blood glucose level after 2 weeks of daily administration. The mean changes seen in other dosages of 50 mg/kg and 100 mg/kg respectively were found not to be statistically significant ($p \square 0.05$) following administration for 2 weeks. However, there was a significant ($p<0.05$) difference between the mean increases in

blood glucose levels of rats that received 25 mg/kg and those that received 50 mg/kg caffeine. See appendix 1 on page 65 for the chart which shows the trend and fluctuations in blood glucose over the course of the first 2 hours and the 2 weeks duration that the experiment lasted.

Again, metformin at a dose of 300 mg/kg was found to produce a significant ($p<0.001$) decrease in mean difference in the blood glucose levels when compared with the control group and with diabetic rats that received 25 mg/kg caffeine.

Tab 4.2.1: Acute and sub-chronic effects of caffeine on blood glucose level in diabetic Wistar rats.

	MEAN ± SEM					
Groups	0hr	0.5hr	1hr	2hr	1week	2weeks
Control	228.40 ± 2.89	229.20 ± 3.51	223.40 ± 3.86	222.00 ± 4.96	201.80 ± 3.57	206.00 ± 2.39
Metformin	227.20 ± 2.85	216.60 ± 2.94	218.00 ± 8.87	198.20 ± 4.89□	203.60 ± 5.06	183.40 ± 3.89□
Caffeine (25mg/kg)	227.60 ± 6.46	232.00 ± 17.56	229.80 ± 13.75	239.80 ± 16.96 □	205.60 ± 3.26	240.80±17.28 □
Caffeine (50mg/kg)	228.80 ± 2.52	231.20 ± 8.54	245.80 ± 12.66	231.40 ± 6.74	210.00 ± 7.79	205.00 ± 3.36
Caffeine (100mg/k)	233.80 ± 7.25	237.40 ± 13.84	233.40 ± 13.00	247.20 ± 11.93 □	208.20 ± 7.20	218.00 ± 4.24

Where * = significance (p<0.05). and n= 5 (SPSS V.19)

4.3 Effect of Caffeine on Lipid Profile

4.3.1 Triglycerides (TG)

Figure 4.3.1 shows the levels of triglyceride in the control, metformin and caffeine groups. The results indicated that only animals in the 100 mg/kg group showed statistical significant ($p < 0.05$) difference in triglyceride levels when compared with those in the control group. All other groups did not show significant ($p \square 0.05$) difference in the mean triglyceride levels on comparison with the control.

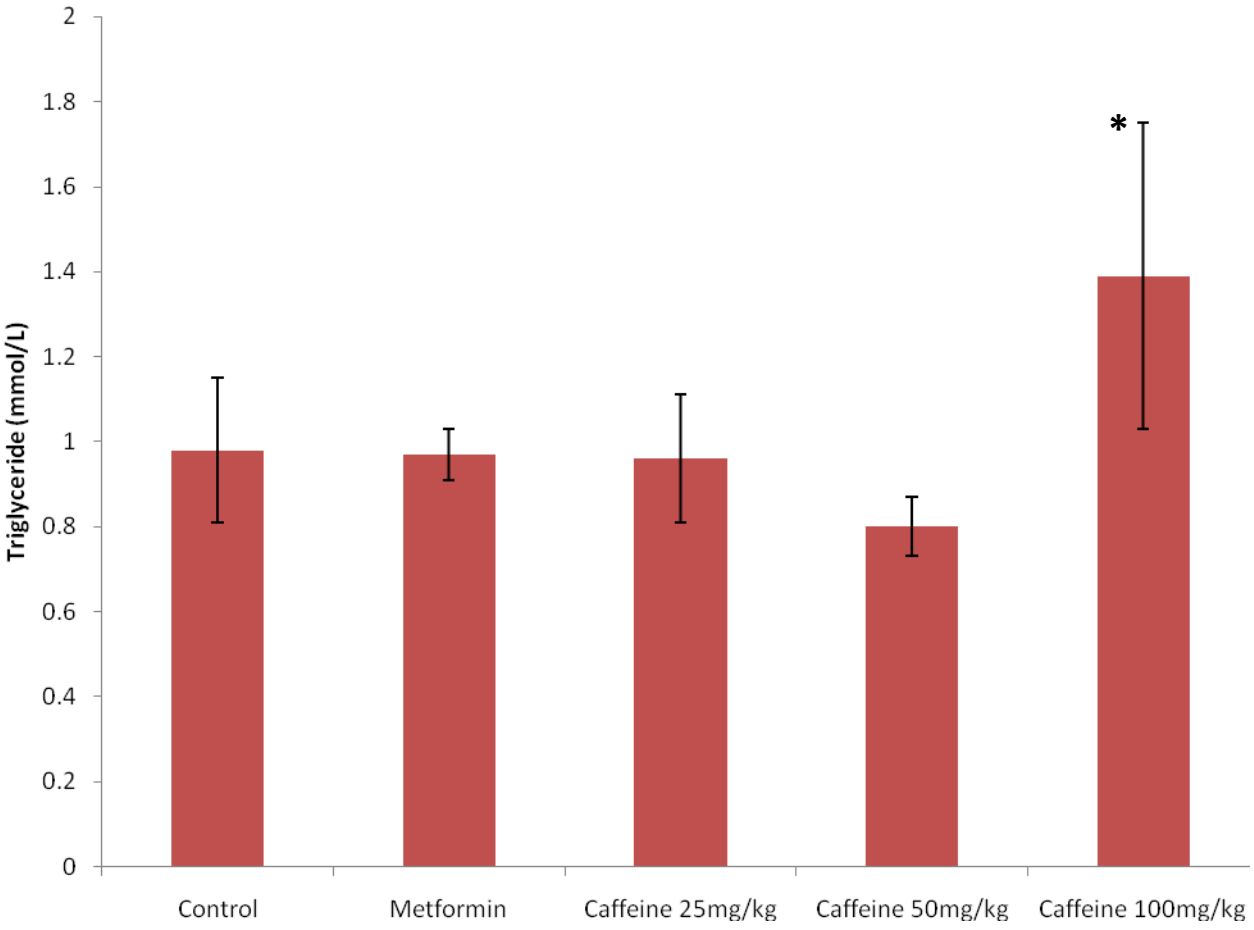


Figure 4.3.1 Effect of caffeine on triglyceride in alloxan-induced diabetic Wistar rats at the end of 2 weeks of administration.

4.3.2 Total Cholesterol

Figure 4.3.2 indicated that metformin produced a significant ($p < 0.05$) reduction in blood cholesterol levels when compared with the control group. Also, caffeine, at a dose of 25 mg/kg body weight was found to reduce blood cholesterol levels when compared with the results from the control group. This reduction was however not statistically significant ($p \square 0.05$). Caffeine, at a dose of 100 mg/kg body weight, however, was found to have produced an increase in the levels of cholesterol significantly ($p < 0.05$) on comparison with the group that received metformin.

The difference between the cholesterol levels in rats which received different dosages of caffeine was found not to be statistically significant ($p \square 0.05$). That is, when the cholesterol levels in animals that received caffeine at 50 mg/kg were compared with those that received caffeine at 100 mg/kg body weight, the levels of cholesterol were not different.

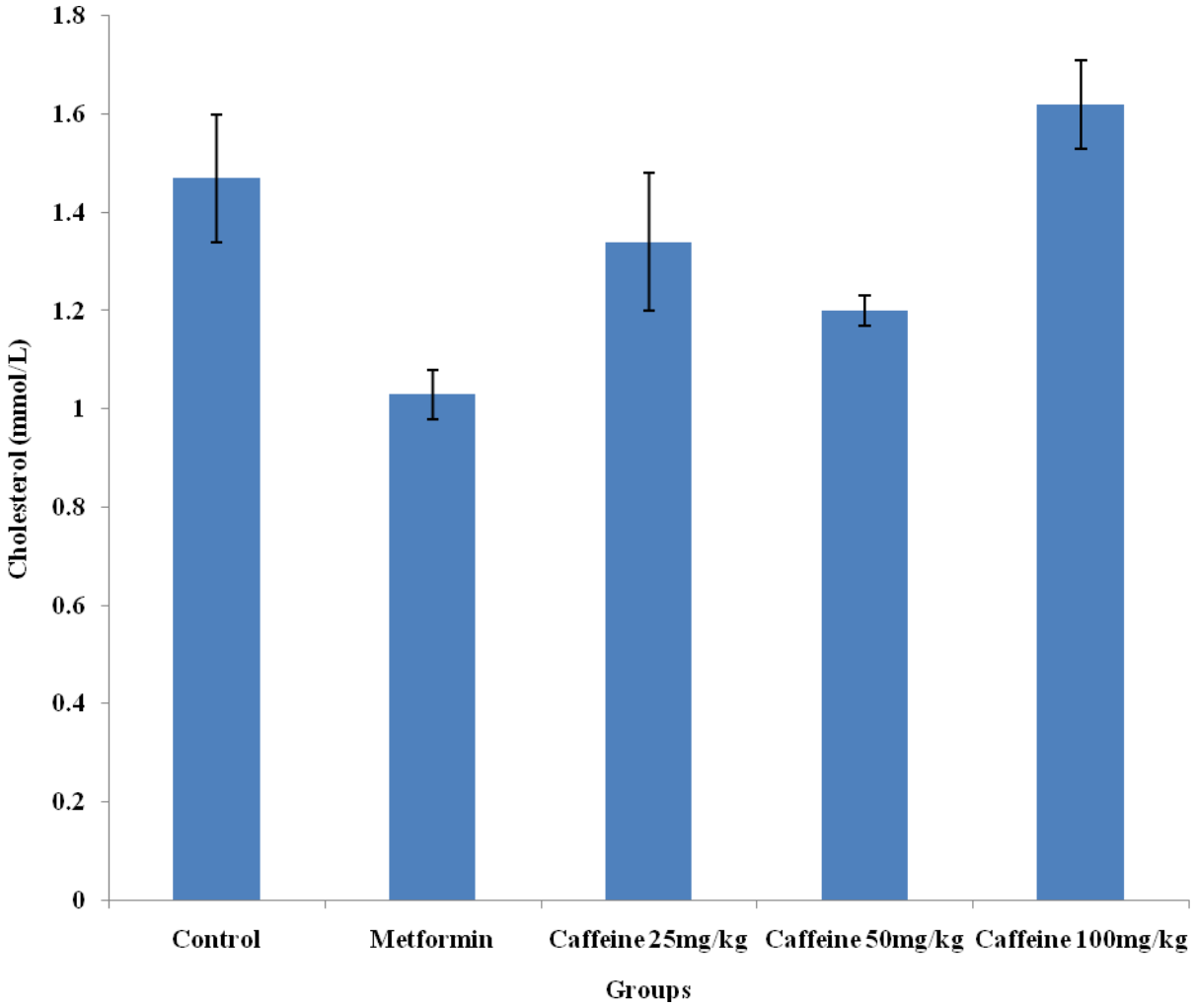


Figure 4.3.2 Effect of caffeine on cholesterol in alloxan-induced diabetic Wistar rats at the end of 2 weeks of administration

4.3.3 High density lipoproteins (HDL)

Figure 4.3.3 show that caffeine at the various dosages of 25mg/kg (0.21 ± 0.01), 50mg/kg (0.25 ± 0.02) and 100mg/kg (0.18 ± 0.02) lowered HDL for animals in all the groups as compared with those of the animals in the control group (0.26 ± 0.02). These reductions were not significant, however. Metformin on the other hand produced a reduction in the levels of HDL when compared with the control group. Again, these reductions were found not to be statistically significant (p□ 0.05).

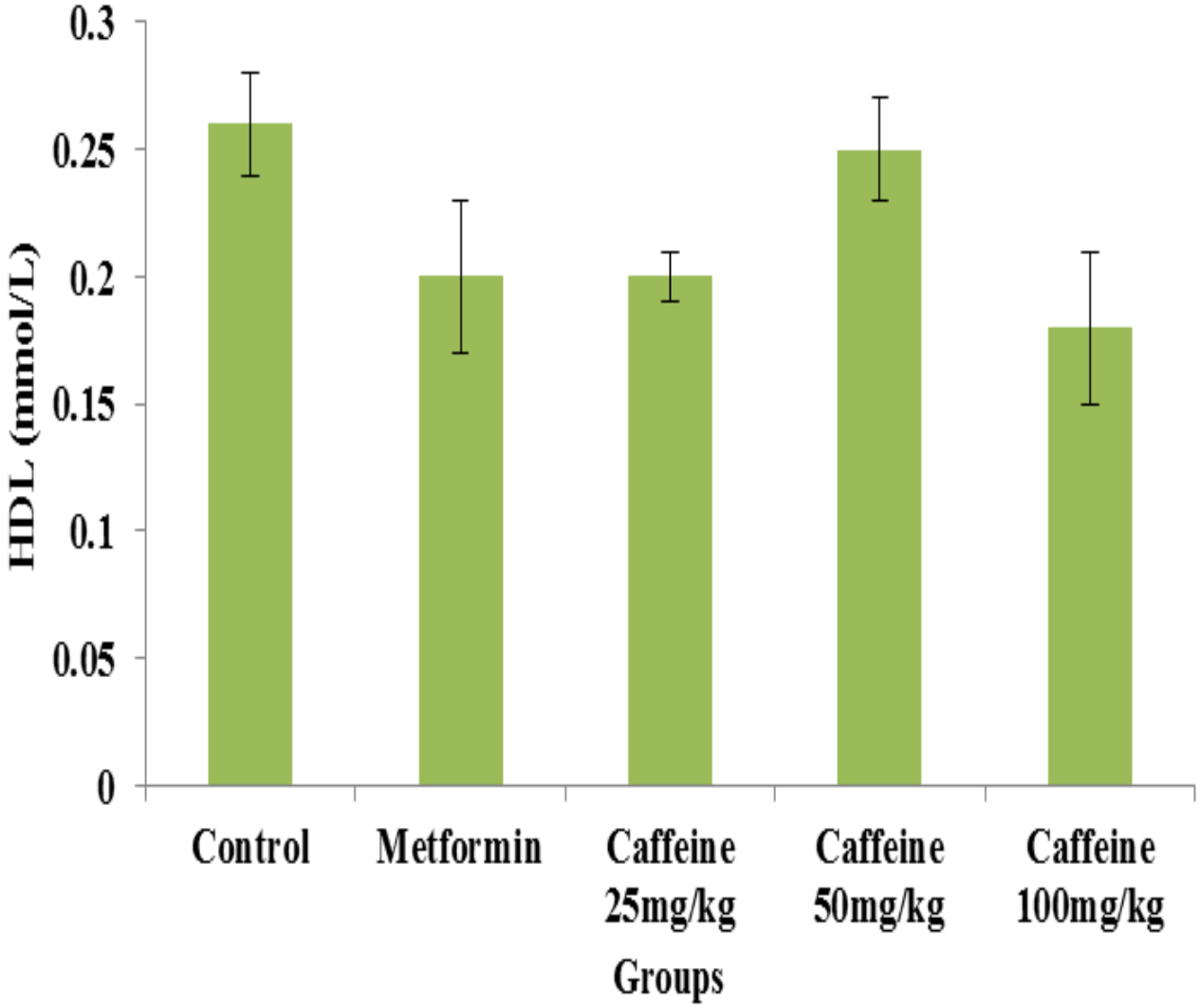


Figure 4.3.3 Effect of caffeine on HDL in alloxan-induced diabetic Wistar rats at the end of 2 weeks of administration.

4.3.4 Lower density lipoprotein (LDL)

Figure 4.2.4 indicate that caffeine at higher dosages of 50 mg/kg and 100 mg/kg body weight, produced slight increases in the levels of LDL when compared with the control group. Metformin on the other hand produced a significant ($p < 0.05$) reduction in the LDL levels when the results of the animals that received metformin were compared with those that received distilled water. Similarly, caffeine administered at 25mg/kg also produced a non-significant (p 0.05) reduction in the LDL levels as compared with those that received distilled water.

See appendix 2 on page 66 for details on the effect of caffeine on lipid profile of diabetic rats at the end of two weeks of administration.

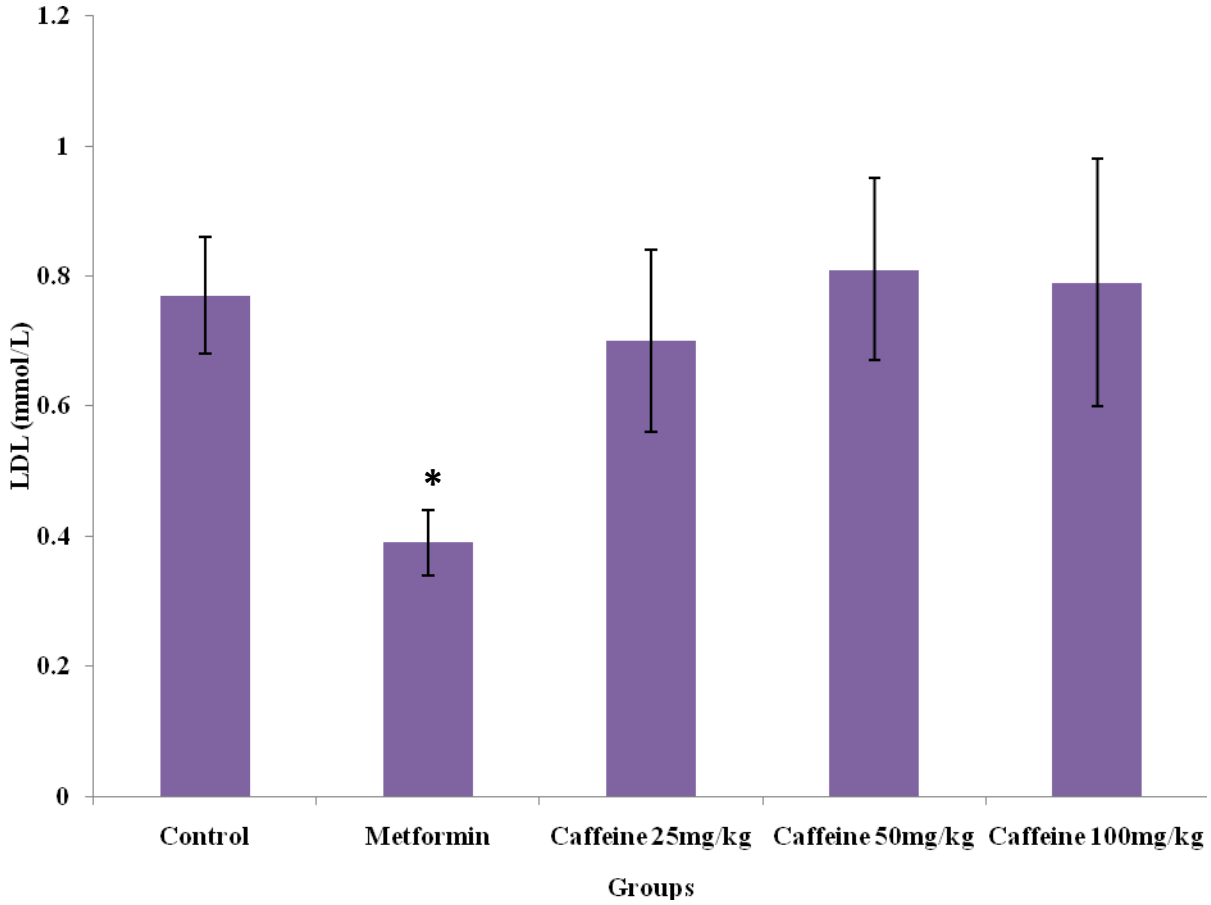


Figure 4.3.4 Effect of caffeine on LDL in alloxan-induced diabetic Wistar rats at the end of 2 weeks of administration.

4.4 Effect of caffeine on body weight

Fig 4.4 below indicates that the average changes in weights of the animals in each group and between the various groups were not statistically significant (p□ 0.05) in comparison with the control.

Appendix 3 on page 67 shows variations in weight of diabetic rats after the 2 weeks of caffeine administration.

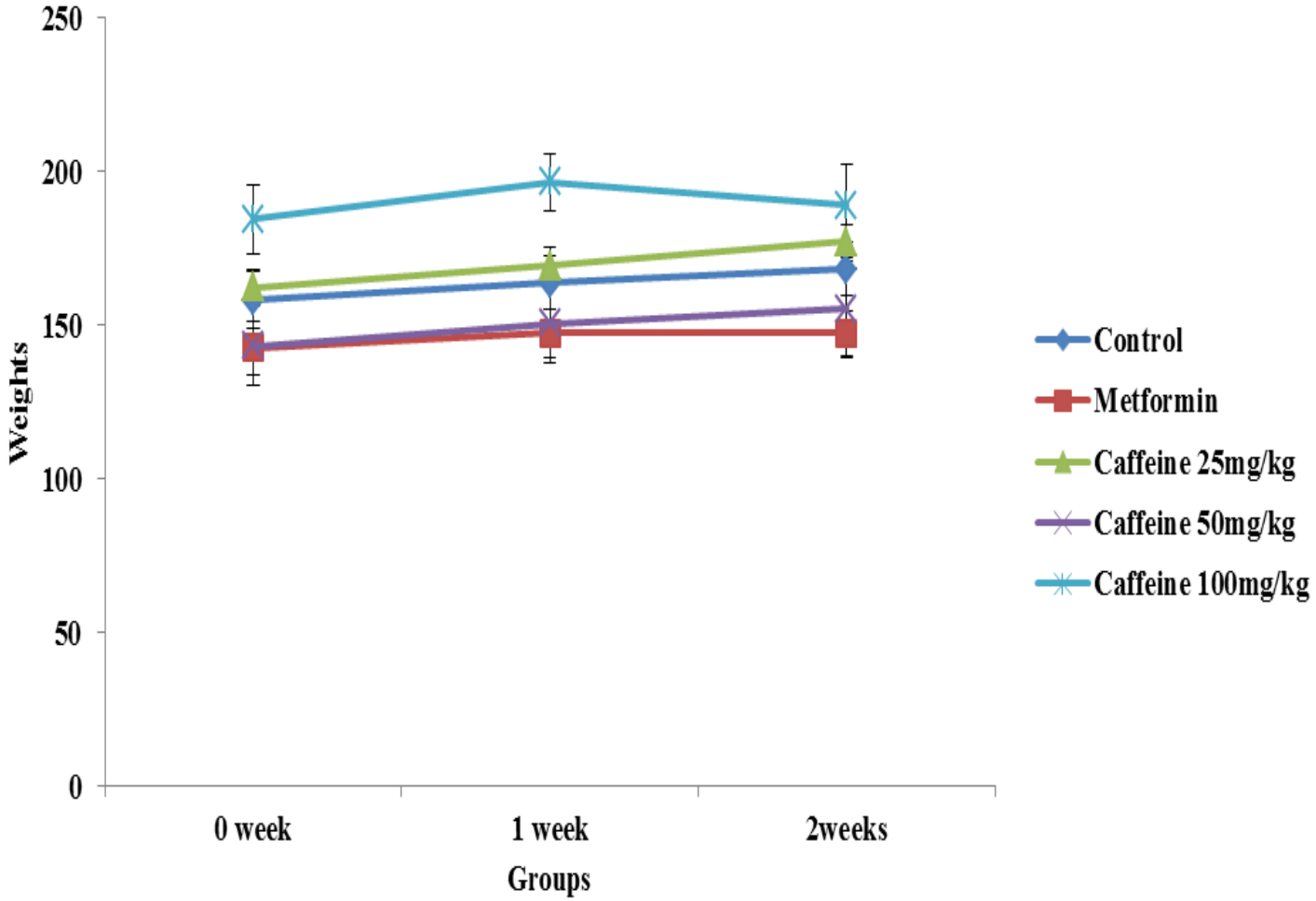


Figure 4.4 Effect of caffeine on body weight of alloxan-induced diabetic Wistar rats over an interval of 2 weeks

CHAPTER FIVE

5.0 DISCUSSION

The results indicated that caffeine given at a dose of 25 mg/kg significantly increased the blood glucose levels in alloxan-induced diabetic Wistar rats 2 hours following administration on comparison with the control. Also, 2 hours after the administration, caffeine at a dosage of 100mg/kg produced a significant increase in blood glucose level when compared with the negative control group that was given distilled water. This finding was consistent with that of Lane *et al*. (2004) who reported that acute administration of caffeine impaired postprandial glucose metabolism in diabetic patients. They concluded that daily consumption of caffeinated beverages with meals could produce higher average glucose levels that increase the risk of diabetes complications.

Again, caffeine intake has also been proposed to increase symptomatic warning signs of hypoglycemia in patients with type 1 diabetes and elevate blood glucose levels in patients with type 2 diabetes (Dessi *et al.,* 2013). The significant elevation observed in this study is in line with the findings of Dessi *et al.* (2013) in human subjects. Gerben *et al.,* (2002) explained that caffeine can decrease insulin sensitivity in healthy humans, possibly as a result of elevated plasma epinephrine levels. Caffeine can directly antagonize adenosine receptors in many tissues including tissues in the central nervous and cardiovascular systems and skeletal muscle and adipose tissue. This could result in a multitude of responses including adrenaline secretion, altered blood flow and lipolysis (Terry *et al*., 2000). Contrary to this, Battram *et al.,* (2005) believed that acute

caffeine ingestion does not affect endogenous glucose production and that caffeine's attenuation of insulin-mediated, whole-body glucose disposal is not attributed to the small increase in adrenaline concentration alone. Additional mechanisms, possibly adenosine receptor antagonism, must be involved.

Previous works have shown that caffeine is capable of increasing the rate of glucose transport in the absence of insulin in rodent skeletal muscle using a rat model. Caffeine is believed to alter blood glucose levels by enhancing glucose tranporter-4 (Glu-4) mRNA. Caffeine is thought to predominantly activate the cyclic AMP-dependent protein kinase α-1isoform (α-1cAMP) by means of an insulin independent mechanism. An alternative mechanism could be that caffeine acts as an antagonist of adenosine A1 receptors. Antagonism of these receptors has been shown to improve glucose tolerance in rats (Urzua *et al*., 2012).

The result of this study also indicated non- significant changes in blood glucose levels between the various caffeine groups and the control group 0.5 hour and 1 hour following administration. This may be explained to be due to the time interval between administration, absorption as well as onset of action of caffeine after oral administration.

Contrary to the increases observed in this study, Urzúa *et al.* (2012) observed and reported that blood glucose levels decreased, and glucose tolerance improved, in diabetic rats administered increasing doses of caffeine.

Metformin on the other hand produced a significant ($p < 0.05$) mean decrease in blood glucose level after 2 hours of administration compared with the control. This result is consistent with the expected outcome since metformin is a standard hypoglycemic agent.This result was explained by stating that Metformin alleviates hyperglycemia of type 2 diabetes by inhibiting hepatic glucose production and improving peripheral insulin sensitivity. Metformin interferes with several processes linked to hepatic glucose production (gluconeogenesis, glycogenolysis and their regulatory mechanisms), lowering glucose production and re-sensitizing the liver to insulin (Muharnniad *et al.*, 2000).

In the long term, caffeine at a dose of 25 mg/kg produced a significant increase in blood glucose level after 2 weeks of daily administration when compared with the control group. The mean increases seen in other dosages of 50 mg/kg and 100 mg/kg respectively were found not to be statistically significant following administration for 2 weeks. After 2 weeks of administration, there was a significant difference in the mean increases in blood glucose levels of rats that received 25 mg/kg and those that received 50 mg/kg caffeine.

The results of this study indicated that animals in the control group showed no significant difference in triglyceride levels when compared with 25 mg/kg and 50 mg/kg caffeine groups. 100 mg/kg caffeine however produced a statistical significant increase in the level of triglyceride.

From these results, it appears like the level of triglyceride is influenced by the dose of caffeine. Higher dosages tend to produce higher levels of triglyceride. This is more so

as the levels of triglyceride and free fatty acids have been closely associated with level of lipolysis, a process which caffeine has been reported to promote (Greenberg *et al.,* 2006). The increased level of triglyceride seen in this study is consistent with the finding of Sadeek *et al.,* (2010). Sadeek *et al.,* (2010) found that caffeine produced a significant increase in serum total cholesterol as well as triacylglycerol in diabetic rats compared to normal control.

On the contrary to the above, Onuegbu *et al.,* (2001) reported no significant differences in the mean serum triglyceride levels in healthy humans, again this difference can be attributed to variation in species used.

Amany *et al.,* (2012) found that green tea extract, epigallocatechin-3-gallate (EGCG) and caffeine induced different beneficial effects on lipid disorders in obese rats. Caffeine increased the effect of EGCG and green tea extract on lipid profile. It was observed that supplementation of caffeine in combination with EGCG or green tea extract enhance their effect on serum levels of lipid profile by decreasing triglycerides in obese rats.

The results of this study revealed that caffeine, at a dose of 25 mg/kg was found to produce a non-significant reduction in blood total cholesterol levels when compared with the results from the control group.

Mahmoud *et al.,* (2013) found that coffee consumption reduced total cholesterol and LDL-cholesterol in non-diabetic rats and therefore concluded that it may be consumed

in considerable amounts for reducing cholesterol problems. This probably explains why the reduction observed in this study, though not statistically significant, was seen in the considerable dosage of 25mg/kg but not in other higher dosages.

On the contrary however, Sadeek *et al.,* (2010) found that caffeine produced a significant increase in serum total cholesterol (TC) in diabetic rats. Also, Onuegbu *et al.,* (2001) found significant increases in the mean total serum cholesterol concentration and LDL- cholesterol concentration in healthy human subjects.

Certain previous studies have also reported that serum total cholesterol and serum triglycerides were not significantly different between drinkers and non-drinkers of caffeinated coffee (Grzegorzewska *et al.,* 2009, Salih *et al*., 2011). Metformin on the other hand, produced a significant reduction in serum cholesterol levels when compared with the control group.

The results of this study show that caffeine at the various dosages of 25mg/kg, 50mg/kg and 100mg/kg lowered HDL for animals in all the groups as compared with those of the animals in the control group. These reductions were not significant, however**.**

On the contrary, previous study by Sadeek *et al.,* (2010) however indicated a significant decrease in serum HDL-Cholesterol in diabetic rats compared to normal control. This finding is significant in that these results are similar and the slight deviation may be attributed to the duration of administration which was longer (4 weeks) in the previous study compared with the present (2 weeks).

Onuegbu *et al.,* (2001) on the other hand, in another study, reported that no significant differences were seen in the mean HDL cholesterol concentration when healthy humans were administered caffeine over a period of time.

Grzegorzewska *et al.* (2009) reported that plasma concentration of HDL cholesterol was significantly higher in patients who were drinkers of caffeinated coffee compared to those who were nondrinkers.

Metformin on the other hand produced a reduction in the levels of HDL when compared with the control group. Again, these reductions were found not to be significant. This finding is similar and consistent with that of Mourão-Júnior *et al.,* (2006). They reported that there was a statistically significant reduction of total cholesterol, waist circumference, and daily necessity of insulin. No changes were detected in HDL-cholesterol or blood pressure following simultaneous administration of metformin and insulin (Mourão-Júnior *et al.*, 2006). Also, Salih *et al.,* (2011) reported that metformin produces a non-significant favorable effect on all lipids profile parameters affirming the finding of the current study.

The results of the current study indicates that caffeine at higher dosages of 50 mg/kg and 100 mg/kg body weight, produced slight increases in the levels of LDL when compared with the control group.

Metformin on the other hand produced a significant ($p< 0.05$) reduction in the LDL levels when the results of the animals that received metformin were compared with those that received distilled water. Again, the current finding is in line with that of Sadeek *et al.,* (2010) who found that caffeine produced a significant increase in serum

LDL-cholesterol in diabetic rats. Although the increases seen in the current study were not significant, the discrepancies may be attributed to dosages used and the length of duration over which caffeine was administered. Similarly, Onuegbu *et al.,* (2001) found significant increases in the mean total serum cholesterol concentration and LDL-cholesterol concentration were observed in healthy human subjects after regular administration of caffeine.

On the contrary, coffee consumption has been consistently revealed a reduction in LDL-cholesterol in rats and in humans alike (Mahmoud *et al*., 2013; Grzegorzewska *et al*., 2009).

Rojas *et al.,* (2013) reported that metformin is associated with improvements in lipoprotein metabolism, including decreases in LDL-C, fasting and postprandial TGs, and free fatty acids. This is in consonance with what was observed in the current study. Low-density lipoprotein (LDL) oxidation has been suggested to play a key role in the pathogenesis of atherosclerosis, a major complication of diabetes mellitus. High-density lipoproteins (HDLs) appear to exert the greatest influence independently of other lipoproteins, with low-density lipoproteins (LDLs) having a weaker, though still significant independent relation with coronary heart disease (Muharnniad *et al*., 2000).

After two weeks of administration, the results of the effect of caffeine on the weight of diabetic Wistar rats in the current study indicated that the changes observed in the weights of the animals across and between the groups are not statistically significant (p□ 0.05). Although 100mg/kg caffeine produced a non-significant slight decrease in weights between the first and the second week of the study, this finding is in agreement

with that of Urzúa *et al.,* (2012) who also observed a weight loss in all the groups during the first 15 days of their study and over the 60 days after diabetes induction and administration of various dosages of caffeine, there was no significant difference in weight loss over the course of the study too. In healthy individuals, considerable evidence from studies in humans supports the hypothesis that coffee consumption induces weight loss by increasing thermogenesis. It has been estimated that a habitual consumption of about 600 mg caffeine/day causes an increase in energy expenditure, which could indeed lead to significant weight loss.

The result of the current study is consistent with earlier study done by Ripudaman *et al.,* (2000) who reported that there was no significant change in body weight in diabetes mellitus type 2 patients that were treated with metformin. Kassem *et al.,* (2009) also concurred by stating that metformin's beneficial effects on glucose metabolism are not accompanied by weight gain, and that this appears to be a clear advantage over other commonly used oral hypoglycemic agents. Again, Muharnniad *et al*., (2000) reported that metformin produced no significant changes in body weight and body mass index. Metformin was also reported to probably have a neutral effect on body weight of patients with T2DM when compared to diet or may limit or decrease the weight gain experienced with sulfonylureas and insulin.

The mechanisms by which metformin contributes to weight loss may be explained through the reduction in gastrointestinal absorption of carbohydrates and insulin resistance, reduction of leptin and ghrelin levels after glucose overload, and by

induction of a lipolytic and anoretic effect by acting on glucagon–like peptide 1 (Rojas *et al.*, 2013).

The increases in the frequency of urination with the associated polydipsia are consistent symptoms associated with diabetes mellitus. The noticeable symptoms include increased thirst (polydipsia), increased urination (polyuria), and increased appetite (polyphagia). Other diabetes signs and symptoms include excessive fatigue, presence of sugar in the urine (glycosuria), body irritation, unexplained weight loss, and dehydration (Ningthouian, 2011).

Again, the increased frequency in urination observed in this study may also be attributed to the ability of caffeine to increase the glomerular filtration rate and inhibit the tubular reabsorption of sodium and water thereby resulting into diuresis and natriuresis (Milon *et al.*, 1988; Maughan *et al*; 2003; Rieg *et al*., 2004).

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary and Conclusion

In summary, the results of this work revealed that alloxan is capable of inducing diabetes mellitus by destroying insulin producing β cells in Wistar rats.

This study shows that acute administration of caffeine is capable of increasing blood glucose levels in diabetic rats. On the long term effect, caffeine consumption at moderate quantities (25 mg/kg), raised blood glucose levels in diabetic rats. The results indicated possibility of habituation at higher dosages over a prolong period of time. The mechanism(s) by which this elevation in blood glucose is achieved is possibly by elevation in epinephrine levels activation of the cyclic AMP-dependent protein kinase α-1isoform (α-1cAMP) by means of an insulin independent mechanism, or by antagonism of adenosine receptors or even through the combination of these.

The results of this study also revealed that caffeine has variable effects on the lipid profile of diabetic rats. It shows that caffeine at higher dosages of 50 mg/kg and 100 mg/kg produced increases in plasma triglyceride levels. At moderate (considerable) dosages, caffeine decreased serum cholesterol levels. At all the dosages in this study, caffeine lowered blood HDL levels. Caffeine, at dosages of 50 mg/kg and 100 mg/kg, produced increases in the serum LDL levels.

This study shows that caffeine has no effect on the body weight of diabetic rats after daily administration over the course of two weeks.

6.2 Recommendations

Based on the findings of this study, the following recommendations are therefore suggested:

- Other doses of caffeine should be used for similar study for a longer duration to better assess its long term effects.
- Future similar study on the effect of caffeine on blood glucose levels should include measurement of insulin levels following administration of different doses.
- Other future similar study on the effect of caffeine on blood glucose levels and lipid profile should be done on normo-glycemic rats.
- Similar future study should include the effect of caffeine on important organs such as the liver that are capable of upsetting blood glucose levels.

REFERENCES

Adaramoye, O A and Adeyemi, E O (2006).Hypoglycaemic and hypolipidaemic effects of fractions from kolaviron, a biflavonoid complex from Garcinia kola in streptozotocin induced diabetes mellitus rats. *Journal of Pharmacy and Pharmacology*. 58:121-128.

Allain, C., Poon, L.S., Chan, C.S., Richmond, W. and Fu, F.C. (1974). Enzymatic determination of total serum cholesterol. *Clininical Chemistry*. 20 (4): 470-475

Amany, S E., Elwakkad, D B., Alazhary, S M., S R. Elzaya, S R and Mohamed, A.H (2012): The enhancement effect of administration of caffeine in combination with Green tea and its component on lipid profile elements in obese rats. *New York Science Journal*. 5(6)

American Chemical Society (2010). New evidence that drinking coffee may reduce the risk of diabetes. *ScienceDaily*. 58(9)

Amos, A F., Mcarty, D J. and Zimmet, P (1997). The rising global burden of diabetes and its implications: Estimates and projections to the year 2010. *Diabetology of Medicine*. 14 5: S1-S85

Ananya, M., (2014). Diabetes mellitus type 2 pathophysiology. *Medical News*. www.news-medical.net/health.

Arogyasami, J., Yang H T and Winder, W W (1989)**.** Effect of caffeine on glycogenolysis during exercise in endurance trained rats. *Medicine & Science in Sports & Exercise.* 21: 173-177.

Aramesh, S., Marshall, T R and William, C K (2003). *Coffee* consumption and the incidence of type 2 diabetes. *Diabetes Care* 26 (7):2211-2212

Andrade-Cetto, A., Wiedenfeld, H., Bailey, C J (2001). Hypoglycemic effect of Cecropia obtusifolia on streptozotocin diabetic rats. *Journal of Ethnopharmacology.* 78,2-3, 145-149.

Bailey, C J. and Turner, R C (1996). Metformin. *New England Journal of Medicine*; 334:574.

Bailey, C J (1992). Biguanide and NIDDM. *Diabetes Care*; 15 (6); 755-772

Bashandy, S A., Alhazza, I M., El-Desoky, G E and Al-Othman, Z. A.(2011). Hepatoprotective and hypolipidemic effects of Spirulinaplatensis in rats

administered mercuric chloride. *African Journal of Pharmacy and Pharmacology*. 5(2), pp. 175-182.

Battram, D S., Graham, T E., Richter, E A and Flemming, D (2005).The effect of caffeine on glucose kinetics in humans – influence of adrenaline. The Journal of Physiology, 569(Pt 1): 347-355.

Beach, E F. and Turner, J J (1958). An enzymatic method for glucose determination in body fluids. *Clinical Chemistry*, 4(6): 462-475.

Bracco, D., Ferrarra, J M., Arnaud, M J., Jéquier, E. and Schutz, Y. (2001). "Effects of caffeine on energy metabolism, heart rate, and methylxanthine metabolism in lean and obese women". .*American Journal of Physiology* **269** (4): *145-149 PubMed.*

Cardoso-Lopes, E M., Bentes de Paula, D M., Barbo, F E., Souza, A., Terumi, C (2009). Chemical composition, acetyl cholinesterase inhibitory and antifungal activities of Pera glabrata (Schott) baill. *Brazilian Journal of Botany* (Sao Paulo) 32(4).

Coffey, R J., Go, V L., Zinsmeister A R and DiMagno, E P (1989): The acute effects of coffee and caffeine on human interdigestive exocrine pancreatic secretion. *Pancreas.* 1(1):55-61**.**

Corrao, G., Zambon, A., Bagnardi, V., d'Amicis, A., Klatsky, A, Collaborative Sidecir Group. (2001). "Coffee, caffeine, and the risk of liver cirrhosis".*Annals of Epidemiology* **11** (7): 458–65.

Davis, J M. and Bailey, S P (2003). "Central nervous system effects of caffeine and adenosine on fatigue." *American Journal of Physiology*: Regulatory, Integretative, and Comparative Physiology 284.2: 399-404.

DeNoon, D J (2008). "Caffeine Bad for Diabetes".WebMD. Wikipedia, the free encyclopedia. Retrieved 2010-11-13.

Dessi, P., Zaharieva, M C. and Riddel, C (2013). Caffeine and glucose homeostasis during rest and exercise in diabetes mellitus. *Applied Physiology, Nutrition, and Metabolism*. 38(8):

Dhandapani, S., Ramasamy, S V., Rajagopal, S. and Namasivayam, N. (2002): Hypolipidemic effect of Cuminumcyminum L. on alloxan-induced diabetic rats. *Pharmacological Research*, 46 (3): 251-255.

Duarte, J M N., Agostinho, P M., Carvalho, R A. and Cunha, R A (2012). Caffeine consumption prevents diabetes-induced memory impairment and synaptotoxicity in the hippocampus of NONcZNO10/LTJ mice. *PLoS ONE* 7(4): e21899. doi:10.1371/journal.pone.0021899.

Duze, B N., Sewanui-Rusike, C R and Nkeh-Chungag, B N (2012): Effects of an ethanolic extract of Garcinia kola on glucose and lipid levels in streptozotocin-induced diabetic rats*; African Journal of Biotechnology*. 11 (33): 8309-8315

Eduardo, S M., Willett, W C., Alberto, A., Manson, J E., Leitzmann, M F., Stampfer, M J and Frank, B H (2004): Coffee consumption and risk for type 2 diabetes mellitus. *Annals of internal medicine.*140 (1): 1-8.

Emerson, P., Van Haeften, T W., Pimenta, W., Plummer, E., Woerle, H J., Mitrakou, A., Szoke, E., Grerich, J. and Meyer, C (2009). Different pathophysiology of impaired glucose tolerance in first-degree relatives of individuals with type 2 diabetes mellitus. *Metabolism.* 58(5): 602- 607.

Essig, D., Costill, D L and Van Handel P J (1980)**.** Effects of caffeine ingestion on utilization of muscle glycogen and lipid during leg ergometer cycling. *International Journal of Sports Medicine* 1: 86-90.

Fullerton, M D., Galic, S., Marcinko, K., Pulinikunnil, T., Chen, Z P and O'Neil H M. (2013). Single phosphorylation sites in Acc1 and Acc2 regulate lipid homeostasis and the insulin-sensitizing effects of metformin. *Nature Medicine*; 19 (12):1649-54

Gerben, B Keijzers., De Galan, B E., Tack C J. and Smits, P. (2002). Caffeine Can Decrease Insulin Sensitivity in Humans *Diabetes Care* 259(2):364-369; doi:10.2337/diacare.25.2.364

Gilmore, B. and Michael, M (2011). Treatment of acute maigraine headache. *American family physician* 83 (3): 271- 280.

Gong, W., Lu, B., Yang, Z., Ye, W., Du, Y., Wang, M and Cheng, Y. (2009). Early-stage atherosclerosis in newly diagnosed, untreated type 2 diabetes mellitus and impaired glucose tolerance. *Diabetes and Metabolic syndrome* 35(6): 458-462

Greenberg, J A., Boozer, C N and Geliebter, A (2006). Coffee, diabetes, and weight control, *American Journal of Clinical Nutrition*, 84 (4): 682-693.

Grzegorzewska, A E., Michalska, M M and Wobszal, P (2009). Does ingestion of regular coffee influence serum lipid profile in dialysis patients? *Advances in Peritoneal Dialysis*, Vol. 25, 200-9.

Heather, J P., Chown, S E., Belfie, L M., Duncan, A M., McLaren, D H., Julie A Conquer, J A and Graham, T E. (2004). Caffeine ingestion increases the insulin response to an oral-glucose-tolerance test in obese men before and after weight loss. *The American Journal of Clinical Nutrition.*80(1): 22-28.

Hiroyasu, I., Chigusa, D., Kenji, W., Akiko, T and the JACC Study Group.(2006). The relationship between green tea and total caffeine intake and risk for self-reported type 2 diabetes among Japanese adults. *Annals of Internal Medicine*.144(8): 554-562.

Huang, Z L., Qu, W M., Eguchi, N., Chen, J F., Schwarzschild, M A., Fredholm, B B., Urade, Y. and Hayaishi, O. (2005). "Adenosine A2A, but not A1, receptors mediate the arousal effect of caffeine". *Nature Neuroscience* **8**(7): 858–9.

Jiang-Fan C., Xu, K., Petzer, J P., Staal, R., Xu, Y H., Beilstein, M., Sonsalla, P K. and Castagnoli, K. (2001). "Neuroprotection by caffeine and A2A adenosine receptor inactivation in a model of Parkinson's disease". *The Journal of Neuroscience* **21**(10): RC143.

Julien, R.M. (2005). A Primer of drug action. 10th ed. New York: Worth Publishers.- Medical- 690 pages.

Kalda, R., Ratsep, A. and Lember, M (2008). Predictors of the quality of life of patients with type 2 diabetes. Patients preferences and adherence. 2: 21- 26.

Karau, M., Kihunyu, J., Kathenya, N., Wangai, L., Kariuki, D. and Kibet, R (2010). "Determination of caffeine content in non-alcoholic beverages and energy drinks using Hplc-Uv method". .*African Journal of Drug and Alcohol Studies* (Centre for Research and Information on Substance Abuse). **9**(1): 15–21.

Kassem, S A and Itamar, Raz (2009).Is there evidence that oral hypoglycemic agents reduce cardiovascular morbidity or mortality? No. *Diabetes Care* 32(2): S337-S341.

Katsumata, K Y., Katsumata, T O and Katsumata, K. (1999): Potentiating effects of combined usage of three sulfonylurea drugs on the occurrence of alloxan-induced diabetes in rats. *Hormone and Metabolic Research.* 25: 125-126.

King, H., Albert, R E and Herman, W H (1998). Global Burden of Diabetes 1995-2025; *Diabetes care*, 21(9): 1414-1431

.

Klatsky, A L., Morton, C., Udaltsova, N. and Friedman, G D (2006)."Coffee, cirrhosis, and transaminase enzymes".*Archives of Internal Medicine* **166**(11): 1190–5.

Klip, A. and Leiter, L A (1990). Cellular mechanism of action of metformin. *Diabetes Care* 13(6): 696-704.

Krauss, R M (2004); Lipids and Lipoproteins in Patients with type 2 diabetes. *Diabetes Care* 27 (6): 1496-1504.

Lane, J D., Barkaus, C E., Surwit, R S. and Feinglos, M N (2004). Caffeine impairs glucose metabolism in type 2 diabetes. doi:10.2337/*diacare*.27.8.2047 *Diabetes Care*. 27(8): 2047-2048.

Lane, J D and Hwang, A L (2007). Exaggeration of postprandial hyperglycemia in patients with type 2 diabetes by administration of caffeine in coffee. *Endocrine. Practice*. 13(3): 239-243.

Lane, J D (2011). Caffeine, glucose metabolism and type 2 diabetes. *Journal of caffeine research*. 1(1): 23-28

Lopez-Garcia, E., Rob, M D., Swapnil, R., Walter, C W., Manson, J E and Hu, F B (2006): Changes in caffeine intake and long-term weight change in men and women .*American Journal of Clinical Nutrition,* 83(3): 674-680.

Lovett, R (2005). Coffee: The demon drink? *New scientist* (magazine issue 2518).

Mahmoud, O N., 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 DOI: 10.5923/j.fph.20130304.07

Mahmud, A. and Feely, J. (2001)."Acute effect of caffeine on arterial stiffness and aortic pressure waveform". *Hypertension* **38** (2): 227–31.

Marc, F., Hébrard1, S., Leclerc, J., Zarrinpashneh, E., Soty, M., Mithieux, G., Sakamoto, K., Andreelli, F and Benoit, (2010). Metformin inhibits hepatic gluconeogenesis in mice independently of the LKB1/AMPK pathway via a decrease in hepatic energy state: *Journal of Clinical Investigation.*120 (7): 2355–2369. doi:10.1172/JCI40671. Copyright © 2010, American Society for Clinical Investigation

Maughan, R J and Griffin J (2003). "Caffeine ingestion and fluid balance: a review*"*. *Journal of Human Nutrition and Dietetics.* 16(6): 411-20.

Milon, H., Guidoux, R. and Antonioli, J A. (1988)."Physiological effects of coffee and its components". In Clarke, R. J.; Macrae, R. *Coffee: Physiology*. Berlin: Springer. pp. 81–122.

Moisey, L L., Kacker, S., Bickerton, A C., Robinson, L E. and Graham, T E (2008). Caffeinated coffee consumption impairs blood glucose homeostasis in response to high and low glycemic index meals in healthy men. *American Journal of Clinical Nutrition*. 87(5):1254-61.

Mourão-Júnior, C A., Sá, J R., Guedes, O M S. and Dib, S.A (2006): Effects of metformin on the glycemic control, lipid profile, and arterial blood pressure of type 2 diabetic patients with metabolic syndrome already on insulin. *Brazilian Journal of Medical and Biological Research* 39: 489-494.

Muharnniad, A M., Muhammad, J W., Muhammad, M., Muhammad, Y M. and Mehar, A (2000): The Effect of Metformin on glycemic control, serum lipids and lipoproteins in diet alone and sulfonylurea-treated type 2 diabetic patients with sub-optimal metabolic control. *Journal P Medical Association* 50(28):381-385.

Muriel, P. and Arauz, J (2010). Coffee and liver diserases. *Fitoterapia,* 81(5): 297-305.

Ningthouian,S. (2011).Diabetes management. *Buzzle*. www.buzzle.com/articles/pathophysiology of diabetes mellitus

Onuegbu, A J and Agbedana, E O (2001).The effects of coffee consumption on serum lipids and lipoprotein in healthy individuals. *African Journal of Medicine and Medical Science*, 30(1-2):43-5.*Source:*Department of Chemical Pathology, University of Ibadan, Ibadan, Nigeria.

Rheeder, P (2006). Type 2 diabetes: The emerging epidemic. *South African Family Practice*. 48 (10): 20-24

Rieg, T., Steigele, H., Schnermann, J., Richter, K., Osswald, H and Vallon, V. (2004). "Requirement of intact adenosine A1 receptors for the diuretic and natriuretic action of the methylxanthines theophylline and caffeine". *Journal of Pharmacology and Experimental Therapeutics* 313(1): 403–409.

Ripudaman, S., Hundal, M K., Dufour, S., Laurent, D., Lebon, V., Chandramouli, V., Inzucchi, S E., Schumann, W C., Petersen, K F., Landau, B R and Shulman, G I (2000): Mechanism by which metformin reduces glucose production in type 2 diabetes. *Diabetes*. 49(12): 2063–2069.

Robinson, L E., Savani, S., Battram, D S., McLaren, D H., Sathasivam, P and Graham, T E. (2004). Caffeine ingestion before an oral glucose tolerance test impairs blood glucose management in men with type 2 diabetes. *Journal of Nutrition*.134(10):2528-2533 PubMed.

Rojas, L B A and Gomes, M B (2013). Metformin: an old but still the best treatment for type 2 diabetes: *Diabetology & Metabolic Syndrome.* 5(6) doi:10.1186/1758-5996-5-6

Russell, S. (2007). Caffeine consumption in pregnant women and its effects on infant health .*Health Psychology Home Page.*

Sadeek, E A., Hala, A A. and Waffa, S A (2010). The Effect of green, roasted and decaffeinated coffee on serum glucose, insulin and serum lipid profile in diabetic rat models. *Journal of American Science* 6 (10): 968-977.

Salih, K and Abdullah, A (2011): Comparative effect of metformin and glibenclamide on Lipid profile in type 2 diabetic patients, *Tikrit Journal of Pharmaceutical Sciences* 27(1):

Shaw, R J.(2013). Metformin trims fats to restore insulin sensitivity. *Nat Med.* 19 (12):1570-2. Doi:10. 1038/nm.3414

Shearer, J., Farah, A. and de Paulis, T (2003). Quinides of roasted coffee enhance insulin action in conscious rats. *Journal of Nutrition*. 133: 3529-3532

Shearer, J., Sellars, E A. and Graham T E (2007). Effects of chronic coffee consumption on glucose kinetics in the conscious rats. *Canadian Journal of Physiology and Pharmacology.* 85: 823-830.

Silverman, A. P (1978); Animal Behaviour in the laboratory. Behavioural tests and their interpretation illustrated mainly by Psychopharmacology in the rat. Published by Chapman and Hall.Pp.118, 323

.

Sugiyama, K., Noda, Y and He, P (2001). Suppressive effect of caffeine on hepatitis and apoptosis induced by tumor necrosis factor-alpha, but not by the anti-Fas antibody, in mice. *Bioscience, Biotechnology, and Biochemistry,* 65(3): 674–677.

Szkudelski, T. (2001). The mechanism of alloxan and streptozotocin action on β cells of the rat pancreas. *Physiological Research,* 50(6): 537-546

Terry. E G., Jorn W H., David, A M., Bente, K and Erik, A R (2000). Caffeine ingestion does not alter carbohydrate or fat metabolism in human skeletal muscle during exercise. *The Journal of Physiology,* 529: 837-847.

Terry, R., Hartley, B H S., Gwendolyn, A., Pincomb, T L., Whitsett, M F. and Wilson, W R L (2000). Hypertension risk status and effect of caffeine on blood pressure. *Hypertension.* 36: 137-141 doi: 10.1161/01.HYP.36.1.137

Tietz, N W., Prude, E L. and Sirgard- Anderson, O (1994).Tietz Textbook of clinical chemistry.2nd edition,W.B. Saunders Company, London, pp.1354-1374.

Urzúa, Z X., Huerta, T M., Trujillo-Hernández, B., Ríos-Silva, M., Onetti, C., Ortiz-Mesina, M and Sánchez-Pastor, E (2012) : Effects of chronic caffeine administration on blood glucose levels and on glucose tolerance in healthy and diabetic rats. *The Journal of International Medical Research.* 40: 2220 – 2230

Van Dam R.M (2008). Coffee consumption and the risk of diabetes, cardiovascular diseases, and cancer. *Applied Physiology, Nutrition and Metabolism*. 33(6): 1269-1283.

Van Dam, R M. and Willett, W C (2006). Effect of coffee consumption on fasting blood glucose of 28812 post-menopausal women. *Archive of Medicine*, 166: 1311-1316.

Van Dam, R.M., Wilrike J. Pasman, and Petra Verhoef (2004). Effects of Coffee Consumption on Fasting Blood Glucose and Insulin Concentrations Randomized controlled trials in healthy volunteers: *Diabetes Care* December, 27(12): 2990-2992.

Van Dam, R.M., Feskens, E J M (2002). Coffee consumption and risk of type 2 diabetes mellitus: *Lancet,* 360(9344): 1477–1478.

World Congress on Prevention of Diabetes and Its Complications (WCPD), 2012 Report. European Science Foundation Press Release.

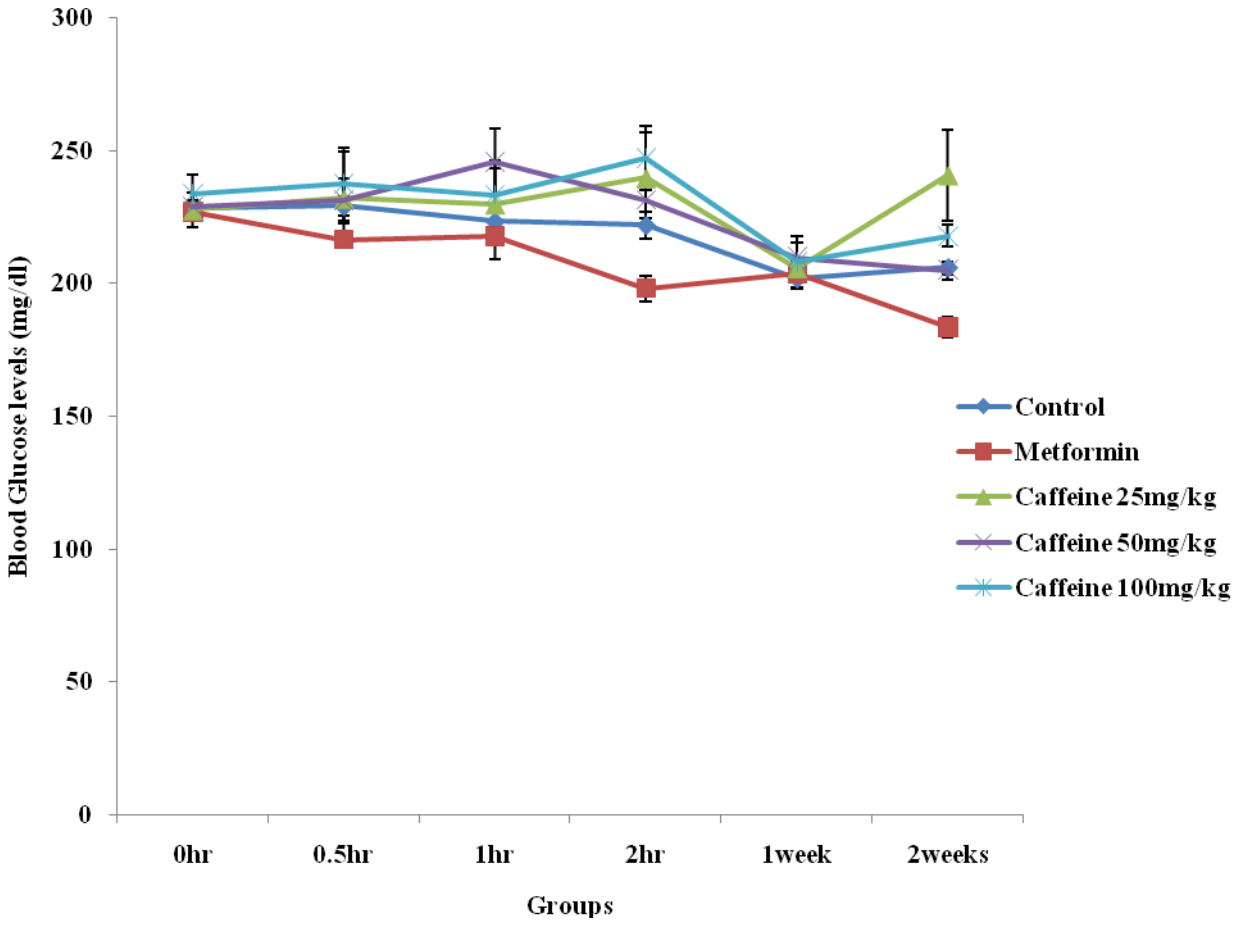
World Health Organization Expert committee on diabetes mellitus (1990): Second report, Technical report series 646, WHO, Geneva.

World Health Organization (2003). Screening for type 2 diabetes. Department of non-communicable disease management.pg1

Zhou, G., Myers, R and Li, Y (2001). Role of AMP-activated protein kinase in mechanism of metformin action. *Journal of Clinical Investigation.* 108:1167.

APPENDICES

Appendix 1: Effect on caffeine on blood glucose levels in the course of first two hours and at the end of each week for a period of two weeks



Appendix 2: Effect of caffeine on lipid profile of diabetic rats at the end of two weeks of administration

Groups	MEAN ± SEAM			
	CHOL	**TRIG**	**HDL**	**LDL**
Control	1.47 ± 0.13	0.98 ± 0.17	0.26 ± 0.02	0.77 ± 0.09
Metformin	1.03 ± 0.52*	0.97 ± 0.06	0.20 ± 0.03	0.39 ± 0.05
Caffeine (25mg/kg)	1.34 ± 0.14	0.96 ± 0.15	0.21 ± 0.01	0.70 ± 0.14
Caffeine (50mg/kg)	1.20 ± 0.31	0.80 ± 0.07	0.25 ± 0.02	0.81 ± 0.14

Caffeine (100 mg/kg)	1.62 ± 0.85	1.39 ± 0.36	0.18 ± 0.02	0.79 ± 0.19

Appendix 3: Effect of caffeine on body weight of diabetic rats after two weeks of administration Weights

Groups	MEAN ± SEAM		
	0 week	**1 week**	**2weeks**
Control	158.20 ± 9.27	163.80 ± 8.63	168.40 ± 8.80
Metformin	142.60 ± 8.85	147.40 ± 8.00	147.60 ± 7.28
Caffeine (25mg/kg)	162.40 ± 6.02	169.60 ± 6.15	177.40 ± 5.17
Caffeine (50mg/kg)	143.40 ± 12.81	150.60 ± 12.67	155.40 ± 15.70
Caffeine(100 mg/kg)	184.60 ± 11.27	196.60 ± 9.29	189.20 ± 13.50