KAMPALA INTERNATIONAL UNIVERSITY

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EFFECT OF A MIXTURE OF *Allium cepa* AND *Camellia sinensis* EXTRACTS ON HYPERLIPIDEMIC MALE WISTAR RATS

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DECLARATION

This is to certify that this dissertation was prepared by me under the guidance of my supervisors.

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Above all I thank God for giving me good life and all I needed to accomplish this research. May His name be glorified forever. Amen.

APPROVAL BY SUPERVISORS

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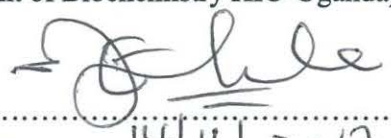

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LIST OF ABBREVIATIONS

ACE	*Allium cepa* extract
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
AST	Aspartate aminotransferase
CSE	*Camellia sinensis* extract
CVD(s)	Cardio vascular disease (s)
DPPH	2, 2-diphenyl-1-picrylhydrazyl
GP	Group
HDL	High density lipoprotein
HFD	High fat diet
IC_{50}	Inhibitory concentration 50%
LDL	Low density lipoprotein
MX	Mixture (of onion extract, OE and tea extract, TE)
ND	Normal diet
P value	Degree of freedom
SD	Standard deviation
TC	Total cholesterol
TG	Triglycerides
VLDL	Very low density lipoprotein
WHO	World Health Organization

ABSTRACT

The burden of hyperlipidemia is on the rise globally especially in many low income countries like Uganda. Management of this metabolic disorder mainly involves dietary and behavioral therapies, which are often met with poor results as they require time and discipline from the patients. The chemotherapeutic options available are expensive, are associated with many side effects and are not readily available to the average citizen. Thus, an alternative effective remedy which is readily available and cheap is needed to combat the problem of hyperlipidemia. This study sought to establish the combination of *Allium cepa* and *Camellia sinensis* extracts with the highest antioxidant activity and evaluated the effect of this mixture on the plasma lipid profile of the male wistar rats. It also assessed the toxic effect of the mixture on the liver. The mixture of *Allium cepa* and *Camellia sinensis* at a ratio of 3:7 had the highest antioxidant activity. It reduced body weight, reduced total cholesterol, increased HDL and had no toxicity to the liver. It has thus been recommended as a potential therapy for hyperlipidemia and its associated complication of liver toxicity. A further study on the kinetics of the interaction of the antioxidants in the mixture has also been recommended.

CHAPTER ONE

BACKGROUND

1.1 Theoretical background

Hyperlipidemia is a metabolic disorder, associated with high serum lipid levels, which include total cholesterol, triglycerides, and phospholipids. The causes of hyperlipidemia include genetic (primary hyperlipidemia) factors, dietary, or secondary factors as a result of alcoholism, obesity, side effects of hormonal (steroids) therapy, kidney disease, diabetes, hypothyroidism and pregnancy among others (Nelson *et al.*, 2013; Nair *et al.*, 2014; Kathleen, 2015). Lipids are transported in blood by lipoproteins (low density lipoproteins (LDL), high density lipoproteins (HDL), very low density lipoproteins (VLDL) and chylomicrons) which accumulate in hyperlipidemia (Jung *et al.*, 2015). The LDL-bound cholesterol is primarily responsible for the atherosclerotic buildup of fatty deposits on the blood vessel walls, while HDL particles may actually reduce or retard such atherosclerotic buildups and are thus beneficial to health (Kathlene, 2015; Visavadiya *et al.*, 2011). Often elevated levels of both LDL and triglycerides result in hyperlipidemia (Visavadiya *et al.*, 2011).

An INTERHEART study by Steyn *et al.*, (2005), observed that hyperlipidemia was the leading risk factor for ischemic heart disease and several other chronic diseases like obesity, diabetes, and others. This could be due to the oxidative reactions on lipids accumulating in blood resulting in production of free radicals such as reactive oxygen species (ROS) (Daniela, 2016). The ROS in turn oxidize the circulating LDL making it to be trapped in the artery walls leading to the formation of atherosclerotic plaques and

development of coronary and vascular diseases (Steyn *et al.*, 2005; Mahmoud *et al.*, 2015; Peer *et al.*, 2016).

1.2 Conceptual background

According to the report by WHO, 2014a, there is a rising burden of hyperlipidemia in many low income countries and it is a major risk factor for cardiovascular diseases (CVDs), which cause up to 4 million deaths per year globally. Hyperlipidemia and the associated non communicable diseases (NCDs) such as CVDs and diabetes are the leading cause of death in majority of developed and developing countries (Capingana *et al.*, 2013; WHO, 2014a; WHO, 2014b). While they were responsible for less than 10% of all deaths a century ago, it is estimated that today they account for approximately 30% of deaths worldwide, accounting for nearly 40% in high-income countries and approximately28% in low-and middle-income countries (Gershim *et al.*, 2015; WHO, 2014a). It is predicted that global trend in deaths from NCDs associated with hyperlipidemia especially CVD will be at an estimated rate of 32% for the year 2020, with a greater contribution from middle-and low-income countries compared with high-income countries (WHO, 2014a; Capingana *et at.*, 2013).This estimation is largely attributed to the shift towards diets dominated by higher intakes of animal and partially hydrogenated fats, lower intakes of fibre, sedentary life style which results in lack of exercise and reduced mobility due to modern technological advancements all of which are predisposing factors that lead to hyperlipidemia and its associated diseases (Peer *et al.*, 2016).

Studies on hyperlipidemia and its risk factors in some parts of rural Africa have generally shown a low but rising prevalence. However, there are major differences across the continent regarding ethnicities and dietary practices that may modify risk profiles across various populations (Gershim *et al*, 2015; Oladapo *et al.*, 2010; WHO, 2014a).

In Uganda a recent study conducted by Gershim *et al.*, (2015) reported an estimated 31,700 deaths per year due to cardiovascular diseases such as stroke, heart attack and other risk factors of hyperlipidemia and this figure is projected to rise. WHO, (2014b) also reported that 9% deaths of the total 353,000 deaths per year in Uganda are attributable to hyperlipidemia and the associated diseases.

1.3 Contextual background

The present synthetic hypolipidemic agents available such as statins, fibrates, resins and nicotinic acid are capable of efficiently reducing plasma total cholesterol levels, but LDL does not undergo any significant alteration. These effects are through the inducement of the hepatic LDL receptor resulting in increased lipid catabolism (Jung *et al.*, 2015).They also have one or more side effects such as nausea, vomiting, diarrhea, gastric irritation and others, and are unable to increase HDL levels (Nidhi *et al.*, 2013; Shrank *et al.*, 2015).

A number of plants such as vegetables fruits, green tea and onion among others have been found to contain phytonutrients or phytochemicals that possess antioxidant properties (Haruno *et al.*, 2015). Therefore, despite the progress in conventional chemistry and pharmacology in producing effective drugs, the plant kingdom still provides useful sources of new medicines. Plants produce an amazing variety of metabolites such as isoflavones, phytosterols, saponins, fibers, polyphenols, flavonoids, and ascorbic acid, and these have aroused much interest in therapeutics (Visavadiya *et al.*, 2011). A recent study has shown that medicinal plants can be used to treat hyperlipidemia thereby reducing the risk of atherosclerosis and hypertension (Mahmoud *et al.*, 2015). Furthermore, foods and plants such as *Solanum melongena, Allium sativum* L, *Brassica napus*, *Solanum lycopersicum, Brassica Oleraceae Italica, Camellia sinensis, Allium cepa,* among others are rich in

antioxidants and have been shown to be potential therapies for hyperlipidemia (Kateregga *et al.*, 2015; Mahmoud *et al.*, 2015).

Previous studies by Habauzit *et al.*, 2012; Haruno *et al.*, 2015; Shogo *et al.*, 2013 have shown that both *Camellia sinensis* and *Allium cepa* are good sources of antioxidant phytochemicals; catechins and quercetin respectively with hypolipidemic activity.

1.4 PROBLEM STATEMENT

Health complications such as CVDs, hypertension, diabetes, certain cancers, obesity and others resulting from hyperlipidemia are becoming a cause of concern in developing nations (Gershim *et al.*, 2015). For instance, in Uganda, there are an estimated 2.7 % of total deaths due to cardiovascular disease among other health complications due to hyperlipidemia and the age adjusted death rate is 67.64 per 100,000 of population, ranking Uganda 126th in the world (WHO, 2014b).

In spite of this, management of hyperlipidemia mainly involves dietary and behavioral therapies such as increased physical exercise and reduced cholesterol in diet which are often met with poor results as they require time and discipline from the patients. The synthetic hypolipidemic agents available such as statins, fibrates, resins and nicotinic acid are capable of efficiently reducing plasma total cholesterol levels, but LDL does not undergo any significant alteration. They also have side effects and are unable to increase HDL levels, on top of being expensive and not readily available to the average citizen (Nidhi *et al.*, 2013; Shrank *et al.*, 2015). Therefore, there is a need for an alternative solution that is effective, readily available and cheap for the community. Earlier studies have shown that each of the plants, *Allium cepa* and *Camellia sinensis* have hypolipidemic effects (Orie *et al.*, 2015; Haruno *et al.*, 2015). However, the

hypolipidemic effect *Allium cepa* is lower compared to *Camellia sinensis* due to less antioxidant content of the plant (Mahmoud, 2015; Peer *et al.*, 2016). Therefore, this study aimed at utilizing the possible synergistic combinatorial effect of the two plants readily grown and available in Western Uganda, thus obtaining a more effective remedy than individual plants for combating the problem of hyperlipidemia.

1.5 General objective

To evaluate the effect of a mixture of the aqueous extracts of *Allium cepa* and *Camellia sinensis* on high fat-induced hyperlipidemia in male wistar rats.

1.5.1 Specific objectives

1. To establish the ratio of *Allium cepa* to *Camellia sinensis aqueous* extract mixture with the highest antioxidant activity.
2. To evaluate the effect of a mixture of *Camellia sinensis* and *Allium cepa* aqueous extracts on the plasma lipid profile and body weight of hyperlipidemic male wistar rats.
3. To assess the toxicity of a mixture of *Allium cepa* and *Camellia sinensis* on the liver of hyperlipidemic male wistar rats.

1.6 Research question

1. Which ratio of the aqueous extract mixture of *Allium cepa* and *Camellia sinensis* has the highest antioxidant activity?

1.7 Null Hypotheses

1. A mixture of *Allium cepa* and *Camellia sinensis* aqueous extracts has no effect on the plasma lipid profile and body weight of hyperlipidemic male Wistar rats.

2. A mixture of *Allium cepa* and *Camellia sinensis* aqueous extracts has no effect on the liver of hyperlipidemic male wistar rats.

1.8 Significance of the study

A safe mixture with hypolipidemic effects will be of pharmaceutical value; a strategy that could improve health with minimal costs. The study contributes to the growing body of information on the use of foods as therapeutic agents. The study will avail the community with the local and cheap solution to hyperlipidemia, which would increase commercialization of these crops as they have more needs thus increasing income of the citizens.

1.9 Conceptual framework

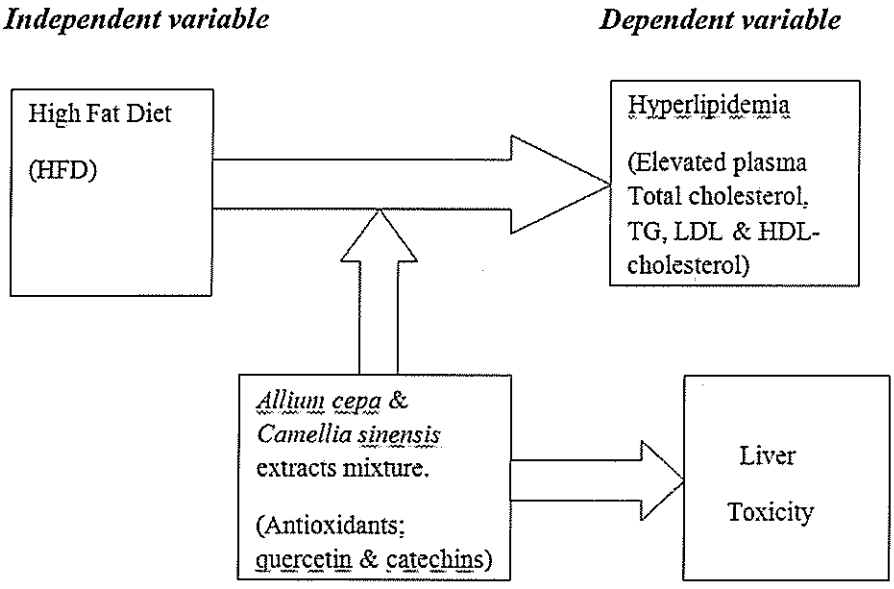

Fig1. Conceptual Framework

Feeding on a high fat diet (HFD) is the leading cause of hyperlipidemia (Olfa *et al.*, 2016). *Allium cepa* and *Camellia sinensis* contain high amounts of antioxidants; quercetin and catechins respectively. These antioxidants are known to lower serum lipids thus reducing the risks of acquiring the co morbidities of hyperlipidemia such as CVD, artherosclerosis, and others. A mixture of these two plants could have a higher hypolipidemic effect and thus a better therapy for hyperlipidemia. However, such a mixture may lead to liver toxicity thus the need to assay liver function.

1.10 Scope

The study focused on the effect of the antioxidants in the aqueous extract mixture of *Allium cepa* and *Camellia sinensis* on dietary induced hyperlipidemia in male wistar rats. The weight of the liver as well as an assay of ALT and AST was conducted to assess the possible toxicity of the aqueous extract mixture on the liver. Plant materials were sourced from the local community in Bushenyi district.

CHAPTER TWO

LITERATURE REVIEW

2.1 Experimental plants

Onion (*Allium cepa*), which is also known as the bulb onion or common onion and locally known as Obutungulu in Luganda and Runyankole, is a bulbous herb belonging to the Alliaceae family and commercially cultivated worldwide (Mete *et al.*, 2016). Several studies have shown that extracts from the outer scales of onions have potent radical scavenging activities in vitro (Nuutila *et al.*, 2003; Kateregga *et al.*, 2015; Mahmoud *et al.*, 2015). In addition, onion oil has been shown to increase the activities of antioxidant enzymes such as superoxide dismutase and glutathione peroxidase in a variety of tissues (Mete *et al.*, 2016). On the other hand, Tea (*Camellia sinensis*) locally known as Amajaani in Luganda and Runyankole is cultivated in more than 30 countries. The global average consumption of tea is about 0.5 kg *per capita*, though amounts (in kg *per capita*) are greater where tea drinking is common such as India (0.73), China (0.95), Japan (0.96), Ireland (1.90), United Kingdom (1.97), Turkey (2.04), and Libya (1.90). By the fourth century, tea was an important part of Chinese life because of its perceived value as a medicine for the treatment of a variety of ailments. Today, after water, tea is the most widely consumed beverage in the world (Prisk, 2014; Blumberg *et al.*, 2015).

2.2 Antioxidant activity of plants

An antioxidant is a compound that scavenges "free radicals." These are very reactive molecules that set off chain reactions in tissues like cell membranes and DNA, and cause significant damage if left unchecked (Prisk, 2014). These oxidation reactions are thought to lead to cancers, degenerative brain diseases and cardiovascular disease. Healthy antioxidant

levels can be maintained via endogenous antioxidants produced by the healthy body or consumption of vegetable - rich diets which are rich in exogenous antioxidants such as polyphenols and vitamins C and E (Prisk, 2014).

Plants produce an amazing variety of metabolites such as isoflavones, phytosterols, saponins, fibers, polyphenols, flavonoids, and ascorbic acid, which have aroused much interest for their role in lipid and antioxidant metabolism (Bulku *et al.*, 2010). In one study that involved combination of cinnamon and cocoa extracts to evaluate the antioxidant activity of their combinations in different ratios, the results indicated that the addition of the cinnamon extract significantly increased the antioxidant activity of the cocoa extract (Dimas *et al.*, 2017). The interaction ranged from synergetic to antagonistic. It was less synergetic when cinnamon extract was added in higher proportion. The interaction of their constituents substantially influenced the antioxidant activity of the mixture and was dependent on the ratio (Dimas *et al.*, 2017).

The use of herbal combinations is not new and several have been experimentally tested for the treatment of diabetes, allergic rhinitis, atherosclerosis, rheumatoid arthritis, and antimicrobial activity (Mothana *et al.*, 2010). An example is the experimental combination of rhizomes of ginger, and young mulberry leaves which was administered to non-insulin dependent diabetes mellitus human subjects. It caused a significant reduction in blood glucose, total cholesterol, triglycerides, LDL cholesterol and VLDL cholesterol (Visavadiya *et al.*, 2011).

Combination of *Allium cepa* and *Camellia sinensis* could have a similar effect on hyperlipidemia as both have been shown to have high antioxidants. Extracts from the outer scales of onions have potent radical scavenging effects, while onion oil increases the activity of antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase in a

variety of tissues (Krishnakumar *et al.*, 2000; Nuutila *et al.*, 2003; Blumberg *et al.*, 2015). In addition, chemical content analysis of *Allium cepa* extract showed that an *Allium cepa* extract weight gain control composition includes 20 to 40% by weight of polyphenols belonging to the flavonol family of antioxidants (Mete *et al.*, 2016). The same study indicates that the antioxidants composition of *Allium cepa* extract based on the dry extract total weight is; from 60 to 90% of the total flavonols in the extract comprises of glycosylated polyphenol antioxidants while from 85 to 98% comprises quercetin in free form (aglycone).

Regular consumption of tea has been found to be associated with an array of bioactivities, including antibacterial, antioxidant, anti-inflammatory, antiviral, glucoregulatory, and immune-stimulant actions, and putative health benefits, including a reduced risk of chronic conditions like arthritis, some forms of cancer, cardiovascular disease, dental caries, type 2 diabetes mellitus, dyslipidemia, and neurodegenerative diseases (Blumberg *et al.*, 2015). This diversity of actions is largely related to the flavonoid constituents of green tea which may result directly or indirectly into the antioxidant capacity *in vivo* to reduce oxidative stress and associated DNA damage and lipid peroxidation (Blumberg *et al.*, 2015; Guan *et al.*, 2015). Furthermore, CSE is one of the most popular herbal extracts on the market today, found in over 100 supplements (Prisk, 2014).

However, natural antioxidants especially polyphenols present efficacy up to a certain level above which they are found ineffective or as pro-oxidant by revoking their own beneficial effects (Peer *et al.*, 2016). Therefore, the current study assessed the ratio of aqueous extracts of both *Allium cepa* and *Camellia sinesis* with the highest antioxidant activity, hence the highest synergism.

2.3 Plasma lipids

Lipids can be formally defined as substances such as a fat, oil or wax that dissolves in alcohol but not in water. When a lipid is conjugated with protein a group of substances called lipoprotein forms. These occur in both soluble complexes as in egg yolk and mammalian blood plasma and insoluble ones, as in cell membranes. The lipoproteins in blood transport cholesterol through the bloodstream and lymphatic fluid (Kathleen, 2015). There are several types of lipoproteins of which two types; LDL and HDL are of clinical significance. LDLs transport cholesterol from its site of synthesis in the liver to the body's cells, where the cholesterol is separated from the LDL and is then used by the cells for various purposes. HDLs transport excess or unused cholesterol from the body's tissues back to the liver, where the cholesterol is broken down to bile acids and is then excreted (Kathlene, 2015; Nelson *et al.*, 2013). About 70 percent of all cholesterol in the blood is carried by LDL particles, and most of the remainder is carried by HDLs. HDL is the good lipoprotein because it carries extra cholesterol back to the liver where it can be eliminated. LDL is bad, as it builds up excess cholesterol in the blood (Kathlene, 2015; Peer *et al.*, 2016).

The causes of hyperlipidemia are either genetic (familial or primary hyperlipidemia) or from a poor diet (especially high fat diet) and other specific factors (secondary hyperlipidemia) (Kathlene, 2015; Devlin *et al.*, 2005). In familial hyperlipidemia, the high cholesterol has nothing to do with poor habits but is caused by a genetic disorder. A mutated gene passed down from either the mother or father causes a missing or malfunctioning LDL receptor. The LDL accumulates to dangerous amounts in the blood (Jung *et al.*, 2015; Kathlene, 2015). Other causes of hyperlipidemia may include excessive drinking of alcohol, obesity, side effects of medications such as hormones or

steroids, diabetes, kidney disease, underactive thyroid gland, and pregnancy (Nidhi *et al.*, 2013; Kathlene, 2015; Shrank *et al.*, 2015).

Disease conditions caused by hyperlipidemia can be improved by consumption of certain foods, such as onions, which have been reported to lower serum lipid levels and enhance excretion of fecal sterols (Vidyavati *et al.*, 2010). Onion oil has been reported to decrease lipid levels in experimental animals (Jung *et al.*, 2015). These effects are through the inducement of the hepatic LDL receptor resulting in increased lipid catabolism (Jung *et al.*, 2015). CSE has been shown to improve fat burning via the inhibition of fat cell division, reduced fat absorption from food, increased sympathetic nervous system activity, increased energy expenditure (thermogenesis) and improved utilization of fat (Phung *et al.*, 2010). Studies have also shown that regular drinking of green tea over 10 years correlates with lower body fat percentage (Prisk, 2014). Natural antioxidants from plants are able to prevent lipid mobilization from tissues and the consequent rise in plasma TG and free fatty acids (FFA) levels. They also increase the plasma HDL levels (Peer *et al.*, 2016).

The current study assessed the efficacy of combinational therapy comprising of a mixture of natural antioxidants in aqueous extracts of both *Allium cepa* and *Camellia sinensis* leaves on plasma lipid levels.

2.4 Liver function and toxicity

The liver is an essential organ that has many functions in the body, including making proteins and blood clotting factors, manufacturing triglycerides and cholesterol, glycogen synthesis, bile production, and detoxification (Wedro *et al.*, 2015; Nelson *et al.*, 2013). Many different disease processes can occur in the liver, including infections such as

hepatitis, cirrhosis (scarring), cancers, and damage by medications or toxins. The liver plays an important role in detoxifying the body by converting ammonia, a bi - product of metabolism in the body, into urea that is excreted in the urine by the kidneys. The liver also breaks down medications and drugs, including alcohol, and is responsible for breaking down insulin and other hormones in the body (Huins *et al.*, 2015; Nelson *et al.*, 2013).

The imbalance between production of reactive oxygen species and the system's defense represents oxidative stress which is one of the essential pathogenic factors in numerous liver diseases including inflammatory, metabolic and proliferative ones (Guan *et al.*, 2015). Hence almost all chronic liver diseases including hepatotoxicity are under the background of elevated oxidative stress. Antioxidants, rich in many food plants can be used as prevention and treatment of such diseases.

As the liver performs its various functions, it uses enzymes that may pass into the bloodstream. Damage to the hepatocytes as it occurs in various liver disorders such as hepatitis, causes a leakage of these enzymes in blood. Those usually measured include Alanine transaminase (ALT), Aspartate aminotransferase (AST) amongst others (Devlin *et al.*, 2005; Wedro *et al.*, 2015).

Highly purified flavan-3-ols from either green tea or onion have been observed to induce hepatotoxicity in rodents at lower doses than CSE (Blumberg *et al.*, 2015; Schmidt *et al.*, 2005). For example, in an acute study using male mice, Lambert *et al.*, (2010) administered a single oral dose of Epigallocatechin – 3 – gallate (EGCG) at 1500mg/kg body weight and observed liver injury and death. Consistent with an apparent greater sensitivity of mice to flavan-3-ol toxicity, Lambert *et al.*, (2010) fed male and female rats a semi-purified CSE (81% polyphenols) at 5% (w/w) of the diet for 90 days and observed

only an increase in plasma alkaline phosphatase and aspartate transaminase but no histo-pathological changes in the liver or other tissues.

Rarely, moderate consumption of aqueous *Camellia sinensis* infusions, aqueous CSE, and hydro-ethanolic CSE have been associated with liver injury in humans but confounding factors and effect modifiers make it impossible to assign a direct causal relationship to individual or combined tea flavonoids or related polyphenols (Yarnell *et al.*, 2014).

On the other hand, reports show that when the toxic effects of oral and intraperitoneal administration of onion extracts on lung and liver tissue of rats were investigated, oral or intraperitoneal administration of low doses of onion (50 mg/kg) to rats had little effect on lung and liver tissues when compared to control animals (Martha *et al.*, 1998). In contrast, administration of high doses of onion (500 mg/kg) resulted in apparent histological changes in lung and liver tissues of rats. Intraperitoneal administration of the high dose of onion was more damaging to lung and liver tissue than oral administration and resulted in a 25% rate of mortality in this treatment group. These results suggest that low doses of onion are non-toxic and may be administered with few ill effects (Martha *et al.*, 1998).

Various studies both in vitro and in vivo have been published reporting that *Allium*-genus plants (including *Allium cepa*) and other plants like *Camellia sinensis* have potent hepatoprotective activity and distinct effects on various liver conditions such as hypercholesterolemia-induced oxidative stress, cadmium liver accumulation, liver fibrosis, liver fluke, and alcoholic fatty liver since they are a rich source of natural antioxidants (Guan *et al.*, 2015).

CHAPTER THREE

Materials and Methods

3. 1 Study design and setting

This was an experimental laboratory-based study with all laboratory experiments conducted at the Department of Biochemistry laboratory and the Institute of Biomedical Research (IBR) laboratory of Kampala International University – western campus.

3.2 Materials

3.2.1 Reagents

The following reagents were used in this study; 2, 2-diphenyl-1-picrylhydrazyl(DPPH), methanol, vitamin C (standard antioxidant), Assay kits for total cholesterol, HDL-cholesterol, LDL-cholesterol, Triacylglycerol, Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) by (Cypress diagnostics, UK).

3.2.2 Equipment

Equipment that were used included; weighing balance (model: CS 200, USA), spectrophotometer (Wagtech, model 722, UK;), centrifuge (Eppendorf, Germany), water bath (Memmert, model: WB 14, Germany), laboratory oven (BTI Bio – Tech model, Germany), syringes (Euroject – II, Germany), gloves (Supermax, Malaysia), micropipettes; 0.5 - 10µl and 100 - 1000µl (Eppendorf, Germany) and a deep freezer (Toshiba, Japan).

3.2.3 Glassware

These included; beakers (500ml and 250ml), test tubes, cuvettes, vacutainers, measuring cylinder (100ml).

3. 2.4 Animals

The study was done using male wistar rats aged six weeks old, weighing between 120 – 200g which were purchased from the Animal Facility at Mbarara University of Science and Technology.

3.2. 5 Plant materials and collection

Fresh *Allium cepa* (10kg) were purchased from the local market in Bushenyi while fresh *Camellia sinensis* leaves were obtained from Igara tea estate-Bushenyi where 10kg of leaves were picked from different tea plants in different parts of the plantation. Both plant samples were collected in the month of November. Both plants were then identified with the help of a botanist; Dr. Namaganda Juliet from Makerere University, Department of Botany. Voucher specimens were deposited in the herbarium of the university and both specimens were assigned voucher numbers K21017 (for *Allium cepa*) and K22017 (for *Camellia sinensis*).

3.3 Processing of the plant materials (extracts)

Allium cepa extract (ACE) was prepared according to the method described by Okoro *et al.*, (2007) and Mete *et al.*, (2016). Briefly, fresh onion bulbs were rinsed thoroughly in clean, sterile, distilled water, air-dried for 1 hour and then the outer coverings were manually peeled off. Two hundred grams (200g) of onion was then blended in 1000ml of distilled water. The resulting juice was allowed to stand for 24 hours in a clean 1000ml glass beaker after which it was filtered and stored at 4^0C. Likewise, fresh *Camellia sinensis* leaves were dried under shade at room temperature (25^0C), and then fine dried in the oven at 20^0C until they attained constant dry weight, 200g of the dry *Camellia sinensis* leaves were soaked in 1000ml of water and allowed to stand for 24 hours in a

clean 1000ml glass beaker. The juice was then filtered and *Camellia sinensis* extract (CSE) was stored at 4^0C.

3.4 Preparation of the mixture of aqueous extracts

A mixture of aqueous extracts was prepared following the ratios as shown in the table 1.

Mixture	Aqueous Extracts	
	***Allium cepa* (ml)**	***Camellia sinensis* (ml)**
1	0	100
2	10	90
3	20	80
4	30	70
5	40	60
6	50	50
7	60	40
8	70	30
9	80	20
10	90	10
11	100	0

Table 1: Mixtures of aqueous extracts *Allium cepa* and *Camellia sinensis*

3.5 Determination of Antioxidant activity

The antioxidant activity of all the mixtures of aqueous extracts of *Allium cepa* and *Camellia sinensis* was determined according to the method described by Perere *et al.*,

(2016). Briefly, 5µL of the extract mixture in 750µL of final volume adjusted by methanol (99 %) was reacted with 300µL of 0.1mM DPPH. The absorbance was measured at 517 nm in a spectrophotometer against a blank containing methanol (750µL) and DPPH (300µL), and a control of distilled water (750µL) and DPPH (300µL). The final absorbance for antioxidant activity was determined as shown in the formula below.

$$\textbf{DPPH scavenging \% } (A^0) = \frac{\text{(Absorbance control – Absorbance sample)}}{\text{Absorbance Control}} \textbf{ X 100}$$

The ratio of the plant extract mixture with the highest antioxidant activity was used in subsequent experiments.

3.6 Induction of hyperlipidemia

A total of 48 male wistar rats aged six weeks, weighing between 120 – 200g were randomly divided into 8 experimental groups of 6 rats each. Twenty four rats in Groups 1-4, with each group consisting of 6 rats were fed on standard ND (protein, fats and fibers each up to 12 %, carbohydrates 65 % and minerals 2–5 %). The rest (Group 5-8) were on high fat diet (induction of hyperlipidemia) as described by (Olfa *et al.*, 2016). Baseline lipid levels for each rat were measured before introduction to the two diets. Subsequently the rats were fed on a feed formulation (Appendix 10) with high fat diet (HFD) and were observed for 4-6 weeks, with periodical assessment of serum for lipid levels, until they attained a level of hyperlipidemia. The table 2 shows the hyperlipidemia that was observed in a group of rats fed on HFD as in the study by Olfa *et al.*, (2016) and which were used as reference values in this study.

Lipid profile	Average reference range (mg/dl)
Total cholesterol (TC)	3.06 ± 0.10
Triacylglycerol (TG)	2.65 ± 0.01
HDL-Cholesterol	0.34 ± 0.02
LDL-Cholesterol	0.97 ± 0.06

Table 2: Reference lipid profile for rats with hyperlipidemia (Olfa *et al.*, 2016)

3.7 Administration of the extracts

Following acclimatization rats were randomly put in 8 groups, 4 on ND and the other 4 on HFD and aqueous ACE, CSE and the MX were administered to the rats orally using a safe and clean cannular fitted on a syringe. The rats in different experimental groups were administered with the above extracts as shown in table 3.

Group	Diet	Treatment (kg/body weight)
1	ND	Control (No treatment)
2	ND	ACE
3	ND	CSE
4	ND	ACE + CSE (3:7)
5	HFD	Control (No treatment)
6	HFD	ACE
7	HFD	CSE
8	HFD	ACE + CSE (3:7)

Table 3: Treatment of experiment groups

On 28th day post treatment 0.5-1 ml of blood was collected in ethylenediaminetetracetic acid (EDTA) free tubes from all the rats (all groups) between 7: 00 and 9:00am after 12hours of fasting. One milliliter of blood per animal was kept at room temperature for approximately 30 minutes and then centrifuged at 4000 r/min for 10 minutes to get serum for lipid profiling and liver enzyme function assay. Serum samples in triplets for all the groups were kept in tubes at room temperature and then serum lipid levels were measured between 10:00 am and 12:00pm.

3.8 Lipid profile analysis

For the lipid profile assays, total cholesterol (TC), triglycerides (TG) and high density lipoproteins (HDL-Cholesterol) were determined enzymatically using enzymatic assay kits from Cypress diagnostic, Belgium.

To determine Total cholesterol (TC) and triglycerides (TG), a similar procedure was followed but done independently whereby 10 µl of the prepared serum free of hemolysis was pipetted and added to 1ml of the working reagent (composed of buffer pH 7, Phenol, Cholesterol esterase, Cholesterol oxidase, Peroxidase, and 4-Aminoantipyrine) in a cuvette. It was based on the principle that cholesterol and its esters are released from lipoproteins by detergents, cholesterol esterase hydrolyses the esters and H_2O_2 is formed in the subsequent enzymatic oxidation of cholesterol by cholesterol-oxidase. In the last reaction a red dye quinonimine dye is formed of which the intensity is proportional to the cholesterol concentration (Young, 2001).

On the other hand, the triglycerides are enzymatically hydrolysed to glycerol and free fatty acids. The liberated glycerol is phosphorylated, resulting in Glycerol-3-Phosphate by Glycerol Kinase and then oxidized yielding H_2O_2 by Glycerol-3- Phosphate Oxidase. The

H_2O_2 concentration is determined through the Trinder's reaction (H_2O_2 + 4 – Aminophenazone + p-chlorophenol) which results in a red coloured dye. The intensity of the color formed is proportional to the triglyceride concentration in the sample. Absorbance (Abs) for both Total cholesterol and triglycerides was measured against blank using a spectrophometer at 505nm wavelength for maximum absorbance (Young, 2001). The obtained results of absorbance were then used to calculate either Total cholesterol (TC) or triglycerides (TG) in the serum sample using the expression;

$$\textbf{Total cholesterol/ TG (mg/dl)} = \frac{\textbf{Abs of sample} - \textbf{Abs of blank}}{\textbf{Abs of standard} - \textbf{Abs of blank}} \times \textbf{200 (stand. conc.)}$$

Conversion factor: mg/dl x 0.0258 = mmol/l for total cholesterol and mg/dl x 0.0113 = mmol/l for TG were also used.

To determine serum HDL- Cholesterol, 1ml of prepared serum, free of hemolysis was added to 100 µl of the working reagent (or precipitation reagent) in a centrifuge tube, mixed and allowed to stand for 10 minutes at room temperature. LDL and VLDL were specifically precipitated by phosphotungstic acid and magnesium ions and were then separated by centrifugation at 4000 revolutions per minute for 20 minutes and then the supernatant was collected and tested for HDL-Cholesterol following a similar procedure for Total cholesterol described above (Young, 2001).

The serum HDL- Cholesterol was then calculated following the expression;

$$\textbf{HDL- Cholesterol (mg/dl)} = \frac{\textbf{Abs of sample}}{\textbf{Abs of standard}} \times \textbf{50 (standard concentration)}$$

Low density lipoproteins (LDL-Cholesterol) was calculated according to the following formula (Friedewald *et al.*, 1997; Peer *et al.*, 2016).

$$\textbf{LDL-Cholesterol} = \textbf{Total cholesterol} - \frac{\textbf{TG}}{5} - \textbf{HDL-cholesterol}$$

3.9 Assessment of liver function

3.9.1 Measurement of AST and ALT levels

To determine serum ALT and AST, a similar procedure was followed but each was done independently. It was also based on a similar biochemical principle, whereby ALT or AST in the test sample catalyzes a reaction that leads to conversion of α–ketoglutarate and L–Alanine to Glutamate and Pyruvate. This is followed by LDH converting Pyruvate and NADH to Lactate and NAD^+. Finally, the rate of NADH consumption is determined photometrically and is directly proportional to the ALT or AST activity in the sample. Briefly, 0.1ml of the prepared serum free of hemolysis was pipetted and added to 1ml of the working reagent (composed of TRIS buffer pH 7.8, L–Alanine, NADH, LDH and α–ketoglutarate) in a cuvette. The contents were allowed to stand for 1 minute at 37^0C. Initial absorbance (Abs) of the serum sample was measured using a spectrophometer with wavelength set at 340nm for maximum absorbance and the instrument was adjusted to zero with distilled water. A stopwatch was started and absorbance was read every minute for 3 min. The difference between the absorbance and the average absorbance differences per minute (Δabs./min) were calculated.

The obtained results of average absorbance differences per minute were then used to calculate either AST or ALT in the serum sample using the expression by (Young, 2001).

$$\textbf{AST or ALT (U/l)} = \Delta\textbf{Abs./min} \times 1750$$

Normal serum AST and ALT levels (100 – 120 U/L and 70 – 75 U/L respectively) in adult male wistar rats were used as reference range (Raghunath, 2016).

3.9.2 Liver histopathology

On the 28th day all the rats were sacrificed following standard procedures and the liver aseptically removed and weighed. For the histopathological examination under light microscope, fresh liver samples cut from three lobes were sectioned and fixed using formalin 10%. Following two days of fixation, the specimens were washed and dehydrated through a graded series of ethanol. Then, they were embedded in paraffin wax. Blocks were made and sectioned at 4 mm thickness using a rotary microtome. Sections were rehydrated in distilled water and stained with hematoxylin–eosin and then examined under a light microscope (Mete *et al.*, 2016). Micrographs of different liver lobes were taken under light microscope (Nikon Eclipse Ci, 104C type) having a mounted digital camera (Nikon digital sight DS, Fi 1) connected to a computer with software (NIS-Elements F3.00, SP7; Build 547) for photography and data collection. Micrographs were taken in triplet for each group and there after interpreted with help of a histologist for the extent of pathology that would have been due to the plant extracts administered to rats fed on ND and HFD.

3.10 Statistical Analysis

Results were expressed as mean values of different animal groups and the mean values were presented with standard deviation. The difference between the means was statistically established using one way analysis of variance (ANOVA) and Turkey's pairwise test, and $p \leq 0.05$ were considered significant. For the Null hypotheses one tail

analysis was used and they were rejected if the P value was less ≤ 0.05. The open source software PAST 3 and Microsoft Excel 2010 were used to analyze data.

3.11 Ethical consideration

The rats were housed in clean rat cages (6 rats per cage) in a research laboratory at a temperature of 23±1^{0}C with 12/12hr light/ dark cycles and 45±5% humidity. Rats were allowed free access to filtrated tap water and standard laboratory rat feed. The institutional research and ethics committee was consulted for consent and permission prior to research and the number of animals per experimental group was kept to a minimum acceptable. All animals received humane care according to the criteria outlined in the ''Guide for the Care and Use of Laboratory Animals''.

3.12 Limitations and delimitations

Failure of animals to consume the aqueous extraction mixed with food. This was solved by oral administration of the aqueous extracts by the researcher using a clean cannular, daily between 4:00pm and 6:00pm for all the experimental period.

Absence of necessary laboratory facilities for carrying out histopathological examinations, which was solved by transporting the preserved liver samples to the Central Diagnostic Laboratory (CDL) at the College of Veterinary Medicine and Biosafety (CoVB), Makerere University-Kampala, Uganda.

CHAPTER FOUR

RESULTS

4. 1 Ratio of ACE to CSE with the highest antioxidant activity

The CSE showed higher antioxidant activity (92.33± 2.34% DPPH scavenging) than ACE (60.91 ± 4.61% DPPH scavenging) (Fig 2 point A & K). An equal combination F (1:1) of both aqueous extracts showed much lower antioxidant activity (20.12 ± 26.81% DPPH scavenging) than individual plant extracts (Fig 2 point F). A combination of both extracts in the ratio of 30:70ml (3:7; ACE to CSE) showed a high antioxidant activity ($p = 0.02$) from the antioxidant activities of individual aqueous extracts on analysis by Turkey's Pairwise test (Fig 2 point D), hence chosen for treatment of six rats per group in the subsequent experiments with rats on ND and HFD. Generally there was observed gradual decrease in antioxidant activities of the combinations as the composition of ACE increased as portrayed in Fig 2 (E-J).The mixtures with the highest antioxidant activity had at least 70 mls of CSE (Fig. 2 point D).

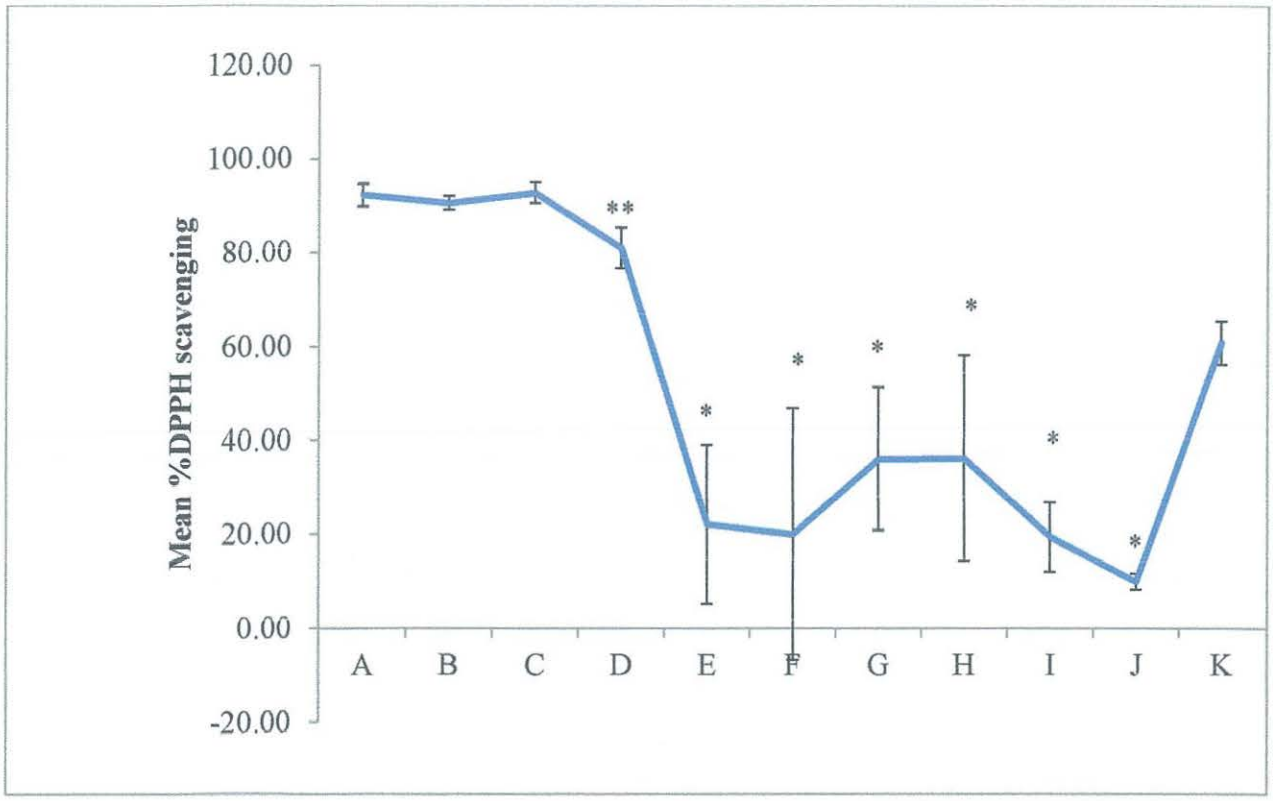

Fig2: Antioxidant activities of different ratios of ACE and CSE.

KEY

ACE = *Allium cepa* extract, CSE = *Camellia sinensis* extract, * Significantly lower than antioxidant activity of CSE and ACE, ** significantly high and different from CSE and ACE, **A** = CSE, **B** = 10ml ACE and 90ml CSE, **C**=20ml ACE and 80ml CSE, **D**=30ml ACE and 70ml CSE, **E** = 40ml ACE and 60ml CSE, **F**=50ml ACE and 50ml CSE, **G** = 60ml ACE and 40ml CSE, **H**=70ml ACE and 30ml CSE, **I** = 80ml ACE and 20ml CSE, **J** = 90ml ACE and 10ml CSE, **K**= 100ml ACE.

4.2 Lipid profile and body weight

4.2.1 Lipid profile

Generally ACE, CSE and the mixture caused significant reduction ($p = 0.01$) in the serum lipids of rats fed on ND and HFD on analysis by a one way ANOVA (Fig. 3 and 4).

Rats fed on HFD had the highest (3.04 ± 0.01 mg/dl) total cholesterol. Following intervention the mixture caused the greatest reduction in serum Total cholesterol (0.72± 0.01mg/dl) of rats fed on HFD. Both ACE and CSE caused reduction in the Total cholesterol (1.43±0.30mg/dl and 1.26±0.79mg/dl respectively) of rats fed on ND and HFD though this reduction was not significantly different. Of note, the mixture did not cause a significant reduction ($p = 2.62$) of the Total cholesterol of the rats fed on normal diet when analyzed by Turkey's Pairwise test (Fig. 3).

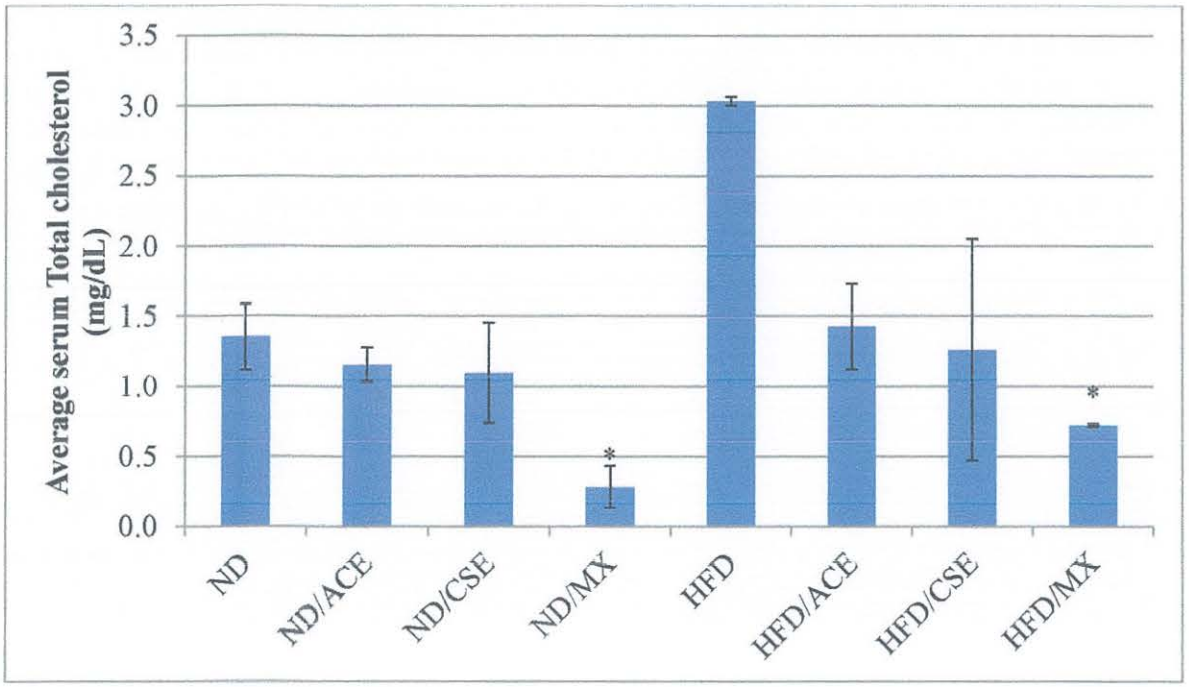

Fig3: Serum Total cholesterol of rats fed on ND and HFD treated with the extracts

KEY

* differs significantly from ND, ND = Normal diet, HFD = High Fat Diet, ACE = *Allium cepa* Extract, CSE = *Camellia sinensis* Extract, MX = Mixture of ACE and CSE (3:7)

Serum triglycerides (TG) levels for the control groups on ND and HFD were 1.36± 0.1mg/dl and 1.10± 0.31mg/dl respectively. The mixture caused a significant reduction ($p = 0.02$) in the TG (0.12±0.02mg/dl and 0.65± 0.28mg/dl respectively) in rats fed on both ND and HFD when analyzed by a one way ANOVA (Fig. 4). All the extracts caused an increase in serum HDL levels. However, the increment caused by the mixture was significantly ($p = 0.002$) higher especially in the rats fed on HFD revealed using Turkey's Pairwise test. All the extracts caused a reduction in serum LDL levels. However, the reduction caused by the mixture was significantly higher in the rats fed on HFD.

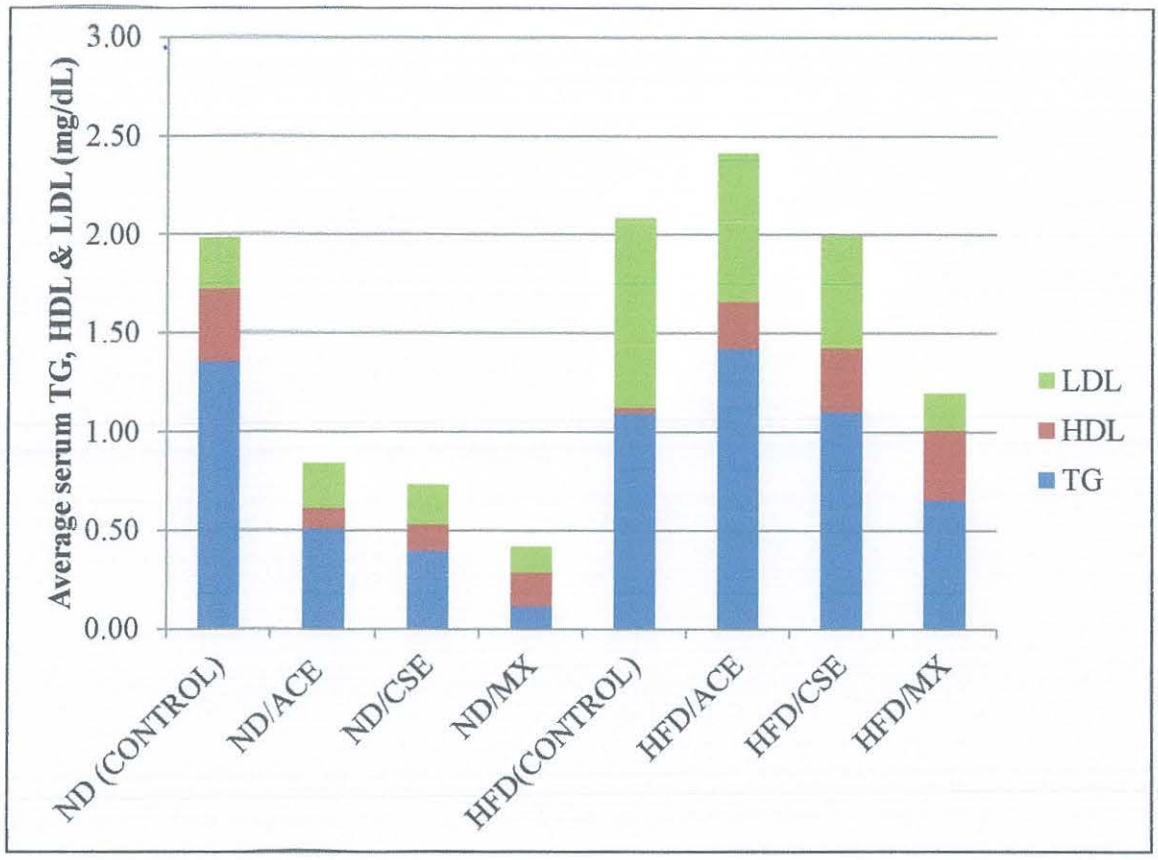

Fig4: Serum levels of triglycerides (TG), HDL and LDL of rats fed on ND and HFD and treated with the extracts

KEY

ND = Normal diet, HFD = High fat diet, ACE = *Allium cepa* extract, CSE = *Camellia sinensis* extract and MX = Mixture of ACE and CSE (3:7)

4.2.2 Assessment of the effects of a mixture of ACE and CSE on the body weight of the male wistar rats.

Generally, there was a continued loss of body weight in groups of rats fed on both ND and HFD treated with all the extracts. Rats fed on the HFD were the heaviest (349 ± 7.3g) compared to those fed on normal diet (245.9 ± 11.2g). Treating rats on HFD with all the extracts generally caused significant ($p = 0.0002$) reductions in the baseline average body weights of the rats to 265.9 ± 12g, 238.3 ± 5.3g and 210.1 ± 11.2g for ACE, CSE and

MX respectively by day 28 on analysis by a one way ANOVA. However, the MX showed a much significant (p = 0.0001) reduction in body weight than ACE and CSE both in ND and HFD (Figure 5).

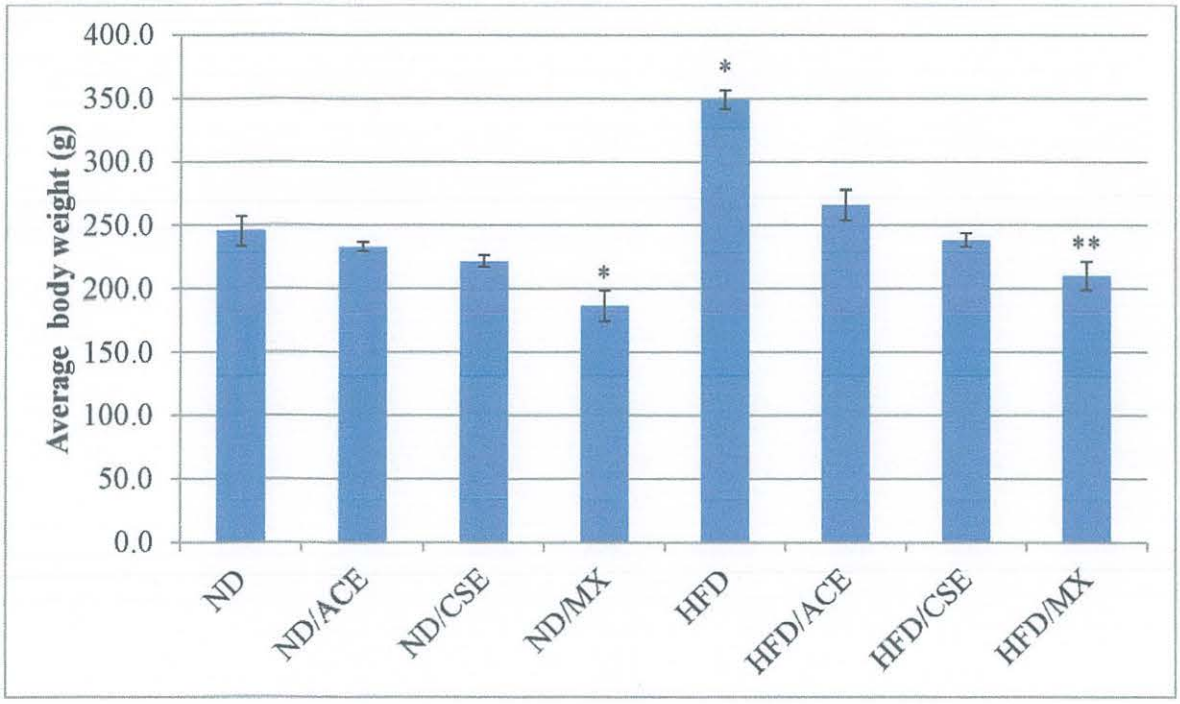

Fig5: Body weights of rats fed on ND and HFD treated with the extracts.

KEY

* differs significantly from ND, ** differs significantly from HFD, ND = Normal diet, HFD = High fat diet, ACE = *Allium cepa* extract, CSE = *Camellia sinensis* extract and MX = Mixture of ACE and CSE (3:7)

4.3 Assessing the toxicity of the mixture on the liver

4.3.1 The effect of the mixture on serum AST and ALT levels

The average serum AST and ALT levels for the control group on HFD (baseline level) after induction of hyperlipidemia were 118.0 ± 2.82U/L and 77.4 ± 2.4 U/L while the control group rats on ND the levels were 114.5 ± 5.6 U/L and 72.7 ± 2.3U/L respectively.

Generally rats on HFD treated with individual ACE and CSE for 28 days indicated significant (p = 0.003) decreases in average serum AST levels and ALT levels. However, the group of rats on HFD treated with the MX for the same experimental period showed a much significant decrease (p = 0.002) in average plasma AST and ALT levels (100.7 ± 2.11U/L and 65.6 ± 7.1 U/L respectively) than individual ACE and CSE in HFD in reference to the levels of both enzymes in the control group on HFD only, when analyzed by Turkey's Pairwise test (Figure 6).

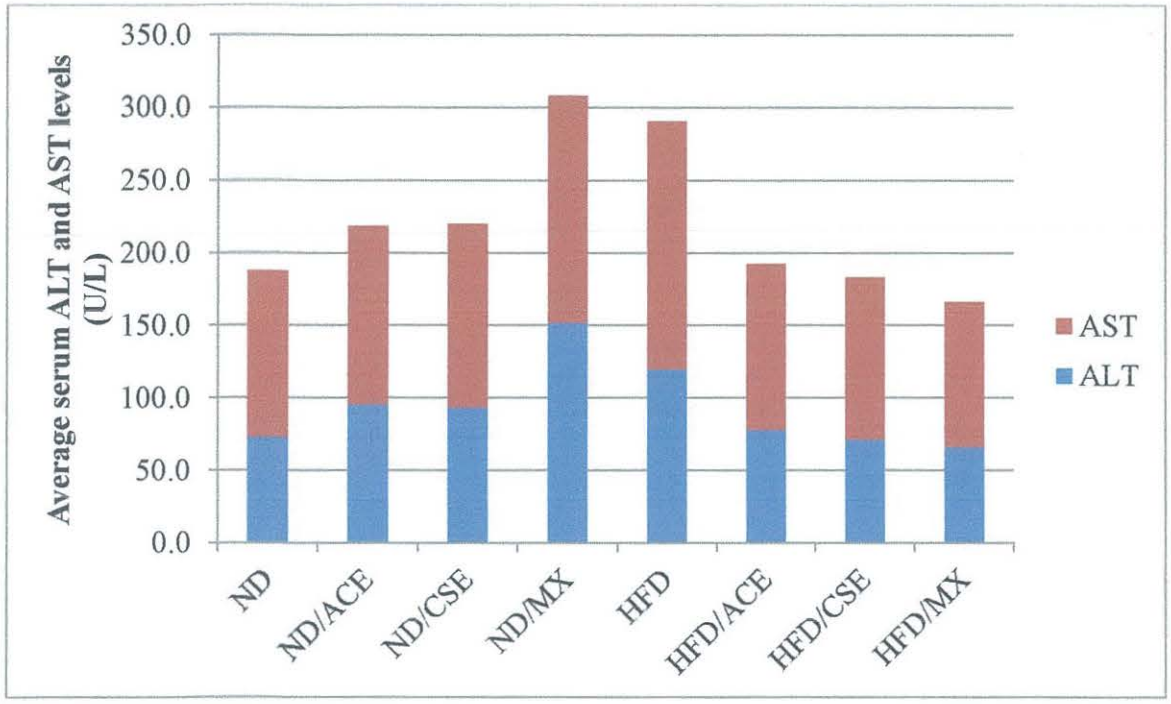

Fig6: Serum AST and ALT levels of rats fed on ND and HFD treated with the extracts

KEY

ND = Normal diet, HFD = High fat diet, ACE = *Allium cepa* extract, CSE = *Camellia sinensis* extract, MX = Mixture of ACE and CSE (3:7)

4.3.2 Effect of a mixture of CSE and ACE on liver weight of male wistar rats

The average liver weight for rats in a control group on ND without treatment with extracts was 9.1 ± 0.5g while the control group on HFD without treatment with extracts was 12.2 ± 0.5g. Analysis of results by a one way ANOVA indicated that rats on HFD treated with individual ACE and CSE for 28 days showed significant ($p = 0.02$) reduction in the liver weight. However, the rats on HFD treated with the MX showed much reduction ($p = 0.002$) in the liver weight (8.8 ± 0.6g) as shown in Figure 7.

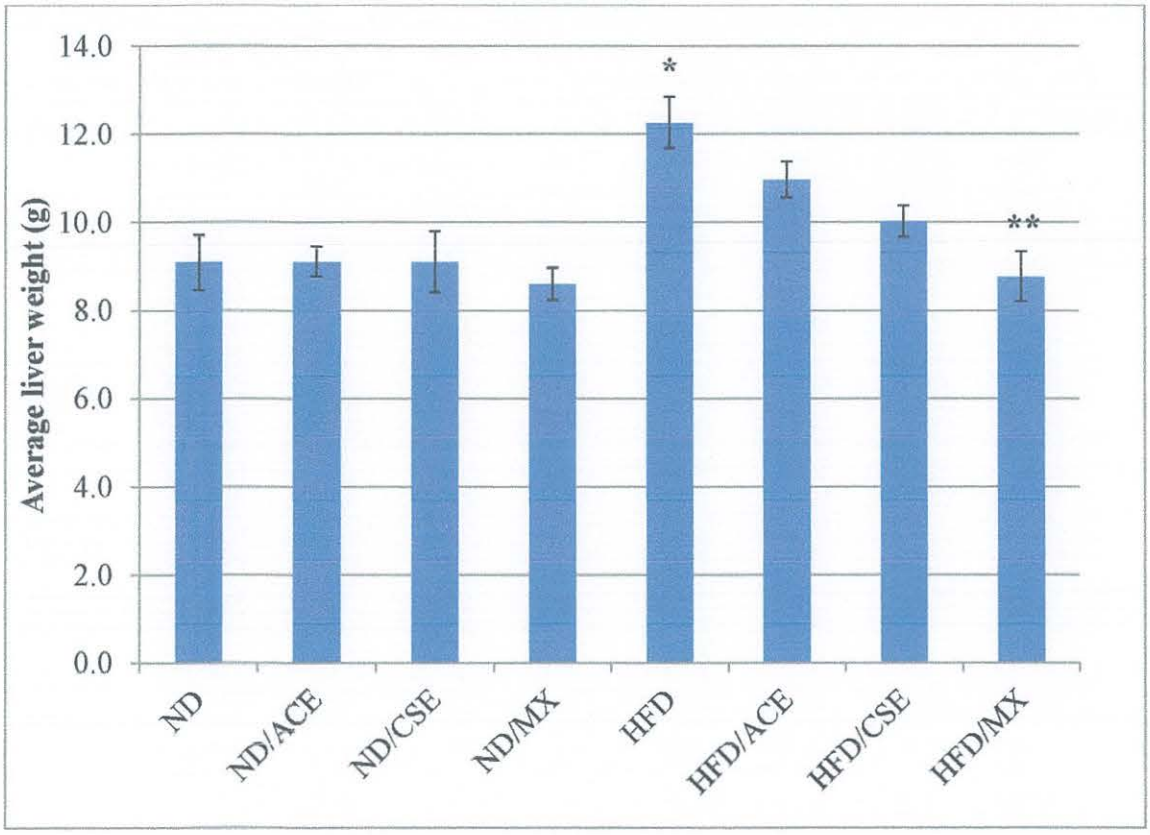

Fig7: Liver weights of rats fed on ND and HFD treated with the extracts

KEY

* differs significantly from ND, ** differs significantly from HFD, ND = Normal diet, HFD – High fat diet, ACE = *Allium cepa* extract, CSE = *Camellia sinensis* extract, MX = Mixture of ACE and CSE (3:7)

4.3.3 Histopathology results

The control group on HFD without treatment showed loss in normal liver cell architecture with severe vacoulations in the hepatocytes, nuclei being centrally retained and numerous lipid droplets throughout the tissue. The droplets were large and densely distributed as shown (Fig 8 panel B). However, cell architecture, the size and number of lipid droplets were enormously reduced in MX treated rats (Fig. 8 panel C).

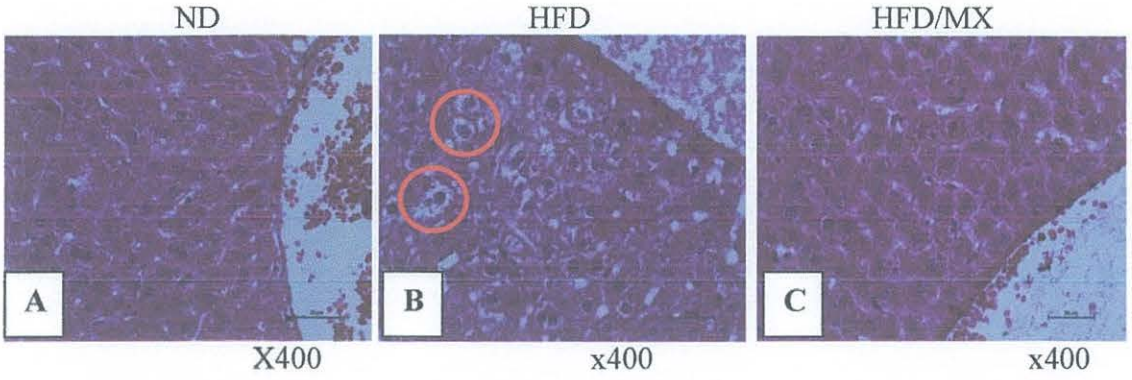

Fig8: Histopathological micrographs for the liver lobes of male Wister rats in control groups ND, HFD without treatment and experimental group on HFD treated with the mixture of ACE and CSE (3:7)

KEY

ND = Normal diet, HFD = High fat diet, ACE = *Allium cepa* extract, CSE = *Camellia sinensis* extract, MX = Mixture of ACE and CSE (3:7)

Panel A and C showed no significant pathological lesions in liver cells, but panel B showed a centrally place nucleus, vacuolation and numerous lipid droplets in the liver cells, see red circle in panel B.

CHAPTER FIVE

Discussion, Conclusion and Recommendations

5.1 Discussion

Hyperlipidemia, a leading cause of the metabolic syndrome, is a disorder of high serum lipid levels, including total cholesterol, triglycerides and phospholipids. The current study focused on establishing a mixture of both plants *Allium cepa* and *Camellia sinensis* with the highest antioxidant activity, evaluated the effect of this MX on the serum lipid profile, and finally assessed the effect of the MX on liver cells in male wistar rats. Findings obtained indicated that a mixture of *Allium cepa* and *Camellia sinensis* in the ratio of 30ml to 70ml, respectively, had the highest antioxidant activity, the MX significantly reduced body weight and improved serum lipid levels and showed no significant lesions in the liver parenchyma of the wistar rats.

The addition of the CSE to ACE significantly affected the antioxidant activity in the mixture with the ration of 3:7; the other mixtures varied in their antioxidant activities, which could virtually indicate synergy or antagonistic responses of the interaction of antioxidant polyphenol phytochemicals. The interaction was less synergetic when ACE was added in higher proportion and the combinations with lower proportion of ACE gave higher antioxidant activity because the interaction of antioxidants was much synergistic. The interaction of their constituent antioxidants substantially influenced the antioxidant activity of the mixture and was dependent on the ratio, which concurred with the study conducted by Dimas *et al.*, (2017) on evaluation of antioxidant activity of combination of cinnamon and cocoa extracts in different ratios. There results were also in line with Peer *et al.*, (2016)

whose findings indicated that combinations of antioxidants present enhanced effects at various doses due to the preventive behavior for one another. Furthermore, findings show that natural antioxidants in different ratios present efficacy/ synergism up to certain level above which they are found ineffective or antagonistic, revoking their own beneficial effect (Dimas *et al.*, 2017; Peer *et al.*, 2016).

The significant increase in body weight of experimental rats in the group on high fat diet was comparable to the study conducted by Prisk, (2014) and could be due to the elevation in percentage of body fat especially triacylglycerides. ACE decreases lipid levels in experimental animals, through the inducement of the hepatic LDL receptor resulting in increased lipid catabolism (Jung *et al.*, 2015). It has been suggested that CSE may be acting through various mechanisms associated with lowering hyperlipidemia which include; improved fat burning via the inhibition of fat cell division, reduced fat absorption from food, increased sympathetic nervous system activity, increased energy expenditure (thermogenesis) and improved utilization of fat, all of which are in line with a study by Phung *et al.*, (2010). The combination of natural antioxidants from *Allium cepa* and *Camellia sinensis* in the ratio 3:7 respectively, provided more potent anti-hyperlipidemic agents than individual extracts. The results were in line with Peer *et al.*, (2016) whose study indicated that the mixture of herbal extracts employs a combination therapy that involves different natural antioxidants delivering different anti-hyperlipidemic mechanisms for the prevention of lipid mobilization from tissues and the consequent rise in serum triglycerides (TG) and free fatty acids (FFA) levels. It was also responsible for the significantly increased serum HDL levels and the significantly decreased levels of serum total cholesterol portrayed in results from groups of rats on ND and HFD under treatment with the MX (3:7, ACE :

CSE) compared to less significant effect of individual ACE and CSE. The significantly reduced serum total cholesterol levels and increased serum HDL-cholesterol levels (good cholesterol) in rats on HFD supplemented by MX was also comparable to the study by Jung *et al.*, (2015) with similar findings when high cholesterol-fed rats were treated with an aqueous ACE.

The liver is an essential organ that has many functions in the body, including manufacturing triglycerides and cholesterol among others (Devlin *et al.*, 2005; Wedro *et al.*, 2015; Kathlene, 2015). Thus, the significant elevation in the liver weight in a group of rats on HFD was comparable to the studies by Jung *et al.*, (2015) and Kathlene, (2015) and it could be due to the enlarged fat – storing cells in the pericental regions of the liver, resulting in cholesterol overload induced pericentral liver fibrosis, the end product being hepatic fibrosis and fatty liver. Liver fibrosis can occur due to alcohol consumption or abnormal lipid accumulation such as feeding on HFD (Jung *et al.*, 2015).

Serum levels of AST and ALT are among the clinically used biomarkers for the functioning of the major organs and tissues. For example in chronic liver, kidney, heart and skeletal muscle damage and inflammation, the levels of the two enzymes in blood become differently elevated. However, elevation in the serum levels of both AST and ALT specifically indicates liver damage (Devlin *et al.*, 2005; Nelson *et al.*, 2013).

Data from this study indicated significantly elevated levels of serum AST and ALT in ND treated with the MX compared to the low levels in ND with individual ACE and CSE which could be due to the liver cells or cells of other organs such the heart and kidneys being injured or inflamed. The results were in line with the study by Huins *et al.*, (2015) where findings indicate that liver disorders such as hepatotoxicity alter the blood level of these

enzymes. Hence, treatment with the mixture of *Allium cepa* and *Cemellia sinensis* (MX) in ND could have caused damage to the liver cells leading to elevation in serum levels of ALT and or other organs like the heart and kidneys resulting in the elevated serum levels of AST.

The significantly elevated serum levels of AST and ALT in a group of rats on HFD may be due to the fact that in normal metabolic processes; reactive oxygen species (ROS) are released as by-products. They act not only as essential part of immunity, in signaling transduction and other vital processes but also responsible for detrimental effects through peroxidation of lipid bilayers (Devlin *et al.*, 2005; Peer *et al.*, 2016). Therefore to maintain the physiological redox environment, the excess radicals get scavenged by blood antioxidants. Therefore hyperlipidemia due to HFD disrupts this redox balance and causes abnormal and uncontrolled release of free radicals leading to in accelerated atherogenic events developing a state of oxidative stress, resulting in hepatotoxicity hence the elevation AST and ALT levels in blood (Peer *et al.*, 2016). The antioxidants present in blood and organs like the liver fight against the progression of oxidative stress to regain the redox homeostasis but gets depleted in chronic conditions like hyperlipidemia. In such circumstances interventions with natural antioxidants supplements were found effective in normalizing lipid profile, blood antioxidant levels and serum levels of AST and ALT as reported previously by Peer *et al.*, (2016).

The detected significant loss of normal cellular architecture and the numerous large and densely distributed lipid droplets present throughout the tissue in HFD fed rats was comparable to the study of Jung *et al.*, (2015) where similar histopathological results were obtained when samples of liver from high cholesterol diet (HCD) rats were examined. The

size and number of lipid droplets in the liver were reduced in the group of rats fed on HFD treated with the MX thus improved accumulation of fat in the liver and it showed no significant lesion. This data therefore indicates that the plant extract used treated the pathological effects of high fat diet in the liver.

5.2 Conclusions

a) The combination of *Allium cepa* and *Camellia sinensis* in the ratio 3:7 respectively has the highest significant antioxidant activity compared to antioxidant activities of the individual extracts.

b) The mixture of *Allium cepa* and *Camellia sinensis* can significantly improve the lipid profile in hyperlipidemic condition through decreasing the plasma total cholesterol, triacylglycerides, LDL-cholesterol and increasing HDL- cholesterol levels. In addition, the mixture is a potential body weight reducing agent.

c) The mixture of *Allium cepa* and *Camellia sinensis* in the ratio of 3:7 has therapeutic effects on the toxic effect of HFD.

5.3 Recommendations

a) The mixture of ACE and CSE could be used in combating hyperlipidemia and the associated diseases in communities of Uganda since the two crops are widely grown and readily available in many parts of the country.

b) A pharmaco-kinetic study regarding the interaction of antioxidants for combinations of *Allium cepa* and *Camellia sinensis* extracts in different ratios should be conducted to understand the cause of synergism and antagonism in the different ratios of both extracts.

c) It is also desired that a study be conducted to assess the effect of the mixture of *Allium cepa* and *Camellia sinensis* extracts with the significant high antioxidant activity on other organs especially the heart and kidneys.

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APPENDICES

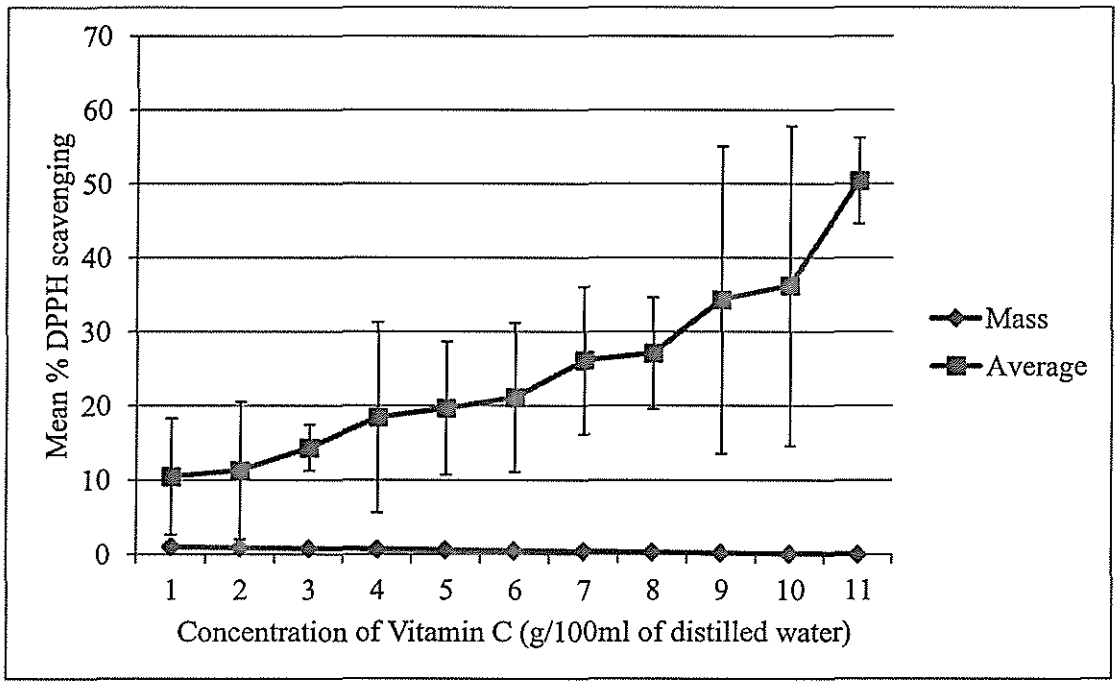
Appendix1: Average antioxidant activities of different mixtures of ACE and CSE.

SN	Extract mixtures (ml)		Tests (% DPPH Scavenging)			Mean % DPPH scavenging
	A. cepa	*C. sinensis*	Test1	Test2	Test3	
A	0	100	89.69072	94.15808	93.12715	92.33 ± 2.34
B	10	90	91.66667	91.16162	88.88889	90.57 ± 1.48
C	20	80	90.7489	92.51101	95.15419	92.80 ± 2.22
D	30	70	85.43689	81.06796	76.69903	81.07 ± 4.37
E	40	60	25.44987	3.856041	37.27506	22.19 ± 16.95
F	50	50	7.719298	1.754386	50.87719	20.12 ± 26.81
G	60	40	33.14607	22.47191	52.52809	36.05 ± 15.24
H	70	30	23.11688	24.15584	61.55844	36.28 ± 21.90
I	80	20	17.98107	27.44479	12.93375	19.45 ± 7.37
J	90	10	8.301887	10.18868	11.69811	10.06 ± 1.70
K	100	0	55.90909	61.81818	65	60.91 ± 4.61

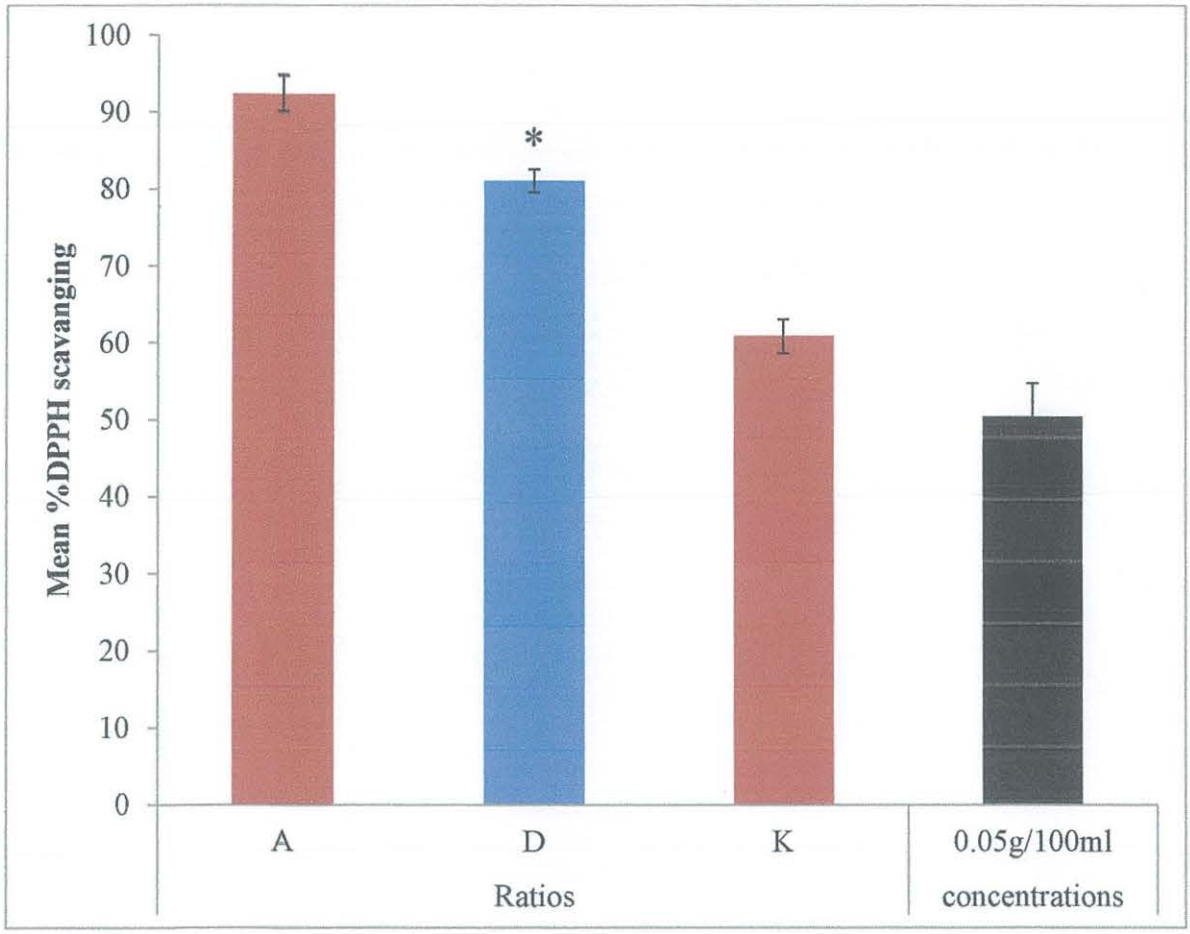
Appendix 2: Determination of antioxidant activity of vitamin C

Concentration of Vitamin C (g/100ml of distilled H_2O)	Tests (% DPPH Scavenging)			Average % DPPH scavenging
	Test1	Test2	Test3	
1.0	12.17949	17.30769	1.923077	10.470086 ± 7.83
0.9	1.538463	12.30769	20	11.282051 ± 9.27
0.8	15.34653	10.89109	16.83168	14.356433 ± 3.09
0.7	15.64246	7.26257	32.40223	18.435753 ± 12.80
0.6	24.84472	9.31677	24.84472	19.668737 ± 8.97
0.5	10.55556	22.22222	30.55556	21.111113 ± 10.05
0.4	14.62264	31.13208	32.54717	26.10063 ± 9.97
0.3	35.68075	23.94366	21.59624	27.07355 ± 7.55
0.2	12.54613	36.53137	53.87454	34.317347 ± 20.75
0.1	11.2782	48.1203	49.24812	36.21554 ± 21.60
0.05	53.78151	53.78151	43.69748	50.420167 ± 5.82

Appendix3: Antioxidant activities of different concentrations of vitamin C expressed as Percentage DPPH scavenging

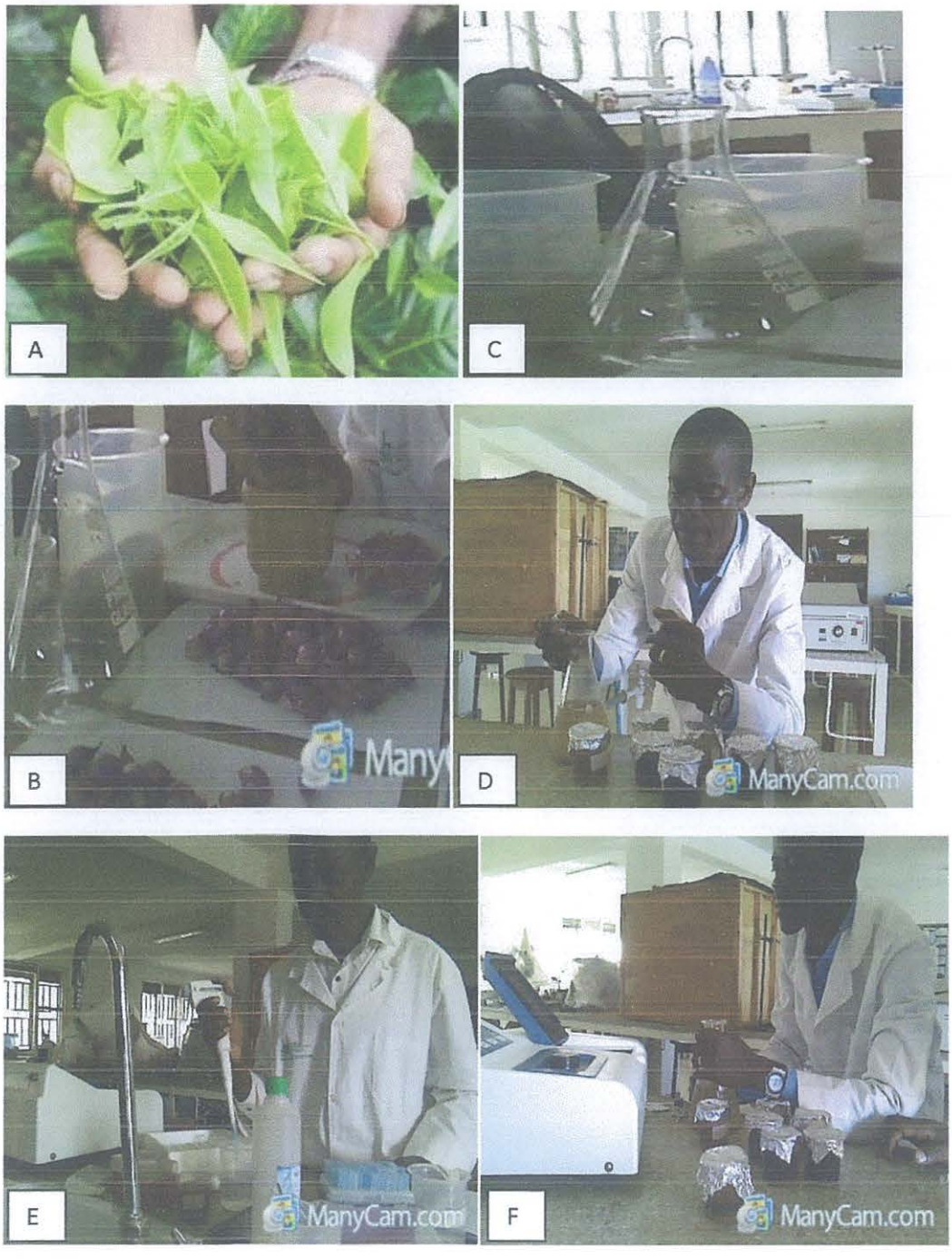


Appendix4: Comparing antioxidant activities of selected ACE and CSE ratios with vitamin C solution of the highest percentage DPPH scavenging (A = *Camellia sinensis*, K = *Allium cepa* and D = 30:70 ml ACE to CSE).

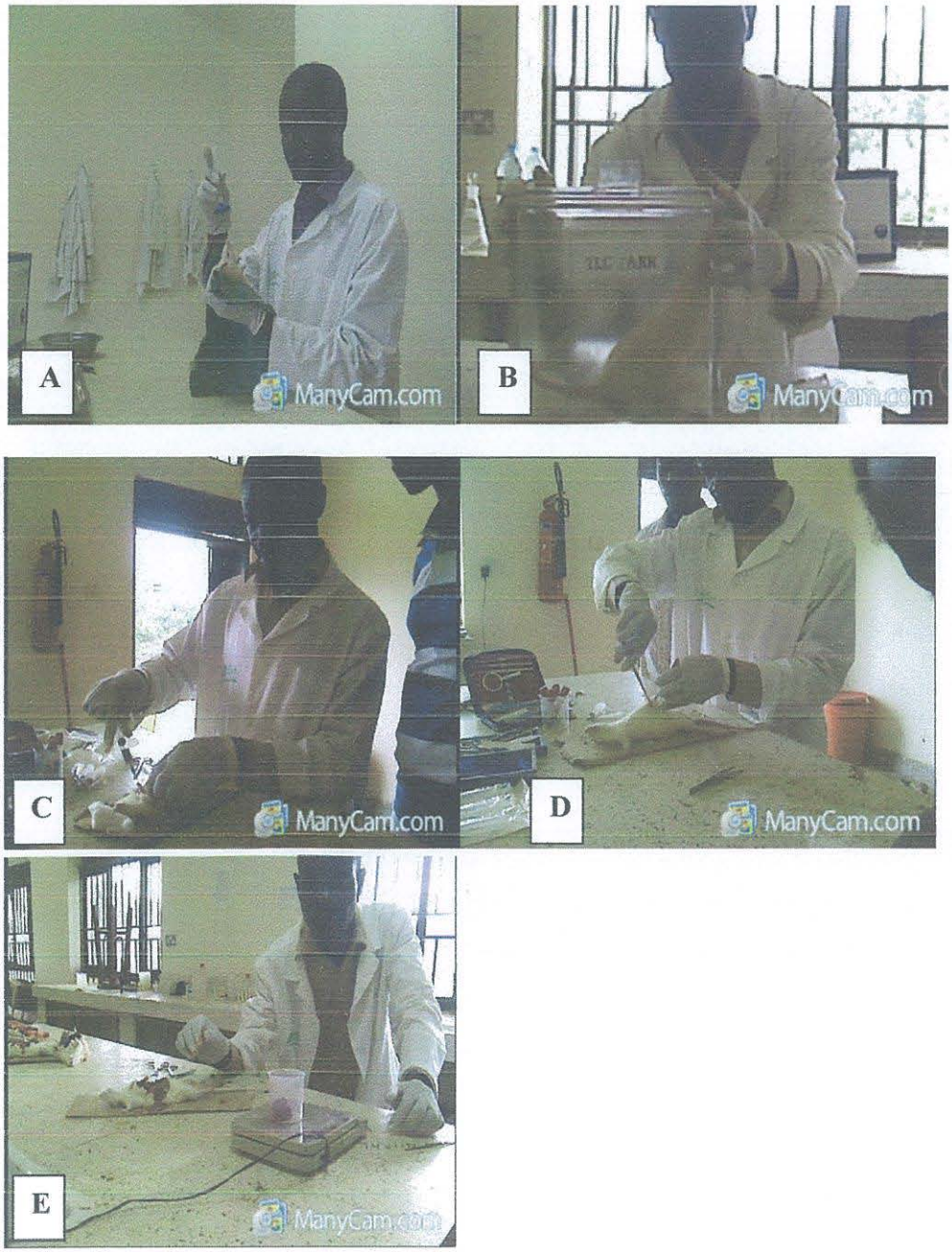


*** denotes significance.**

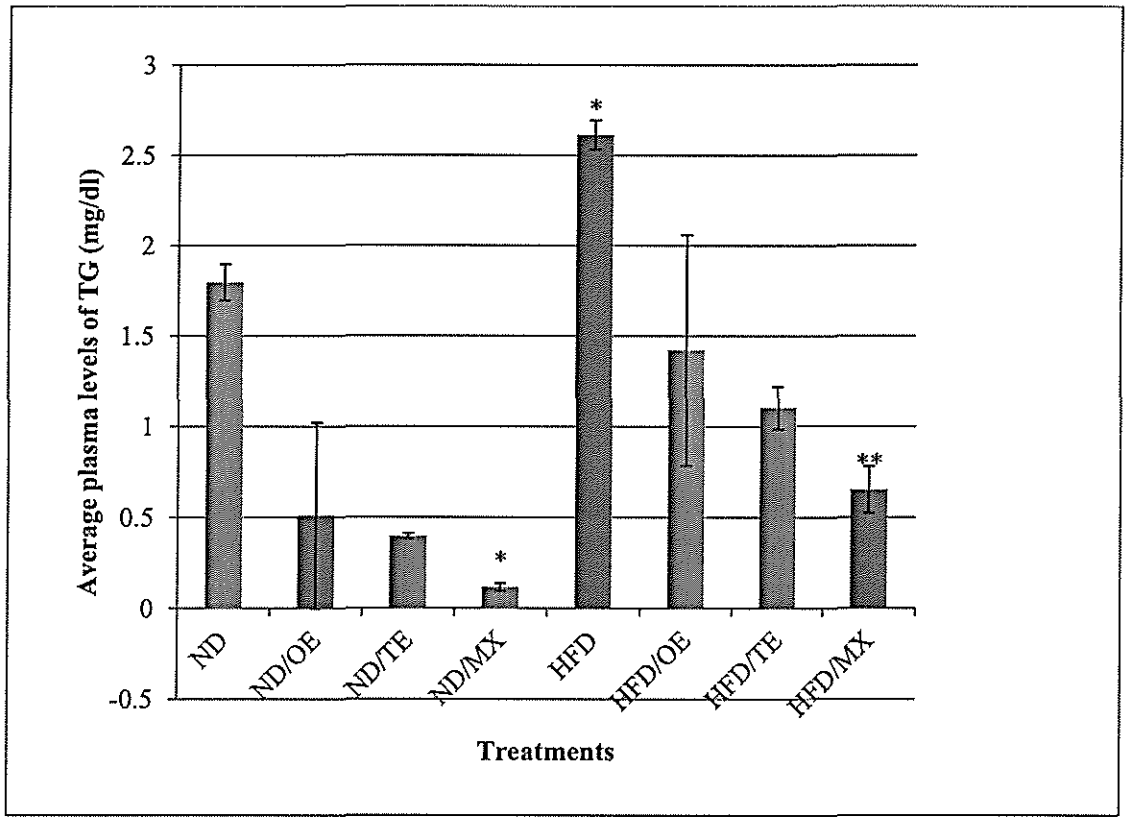
Appendix5: Fresh Tea (*Camellia sinensis*) leaves (A) and Onion (*Allium cepa*) (B), prepared tea leaves for extraction (C), prepared extracts (D), determining antioxidant activity (E and F).



Appendix6: Administering extracts to rats (A), preparing rats for sacrifice (B), dissecting the rat to obtain blood for serum preparation (C and D), and taking fresh liver weight (E).

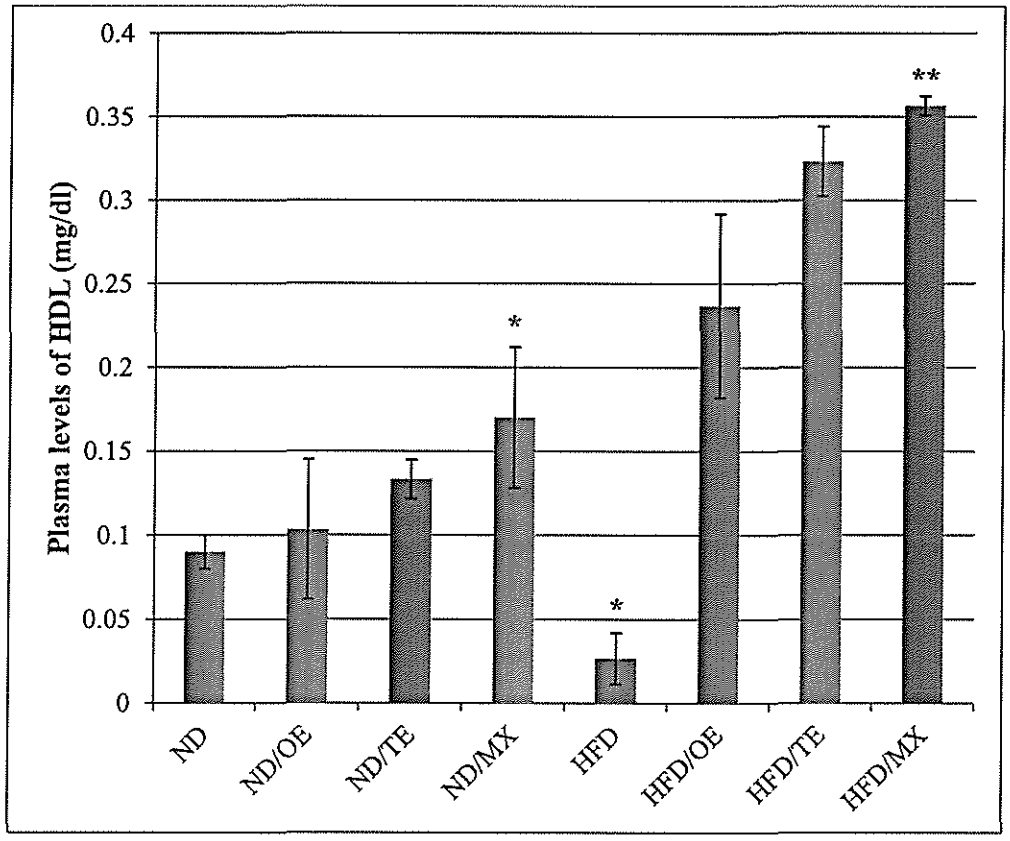


Appendix 7: Serum triglyceride levels of rats fed on ND HFD treated with the extracts on day 28



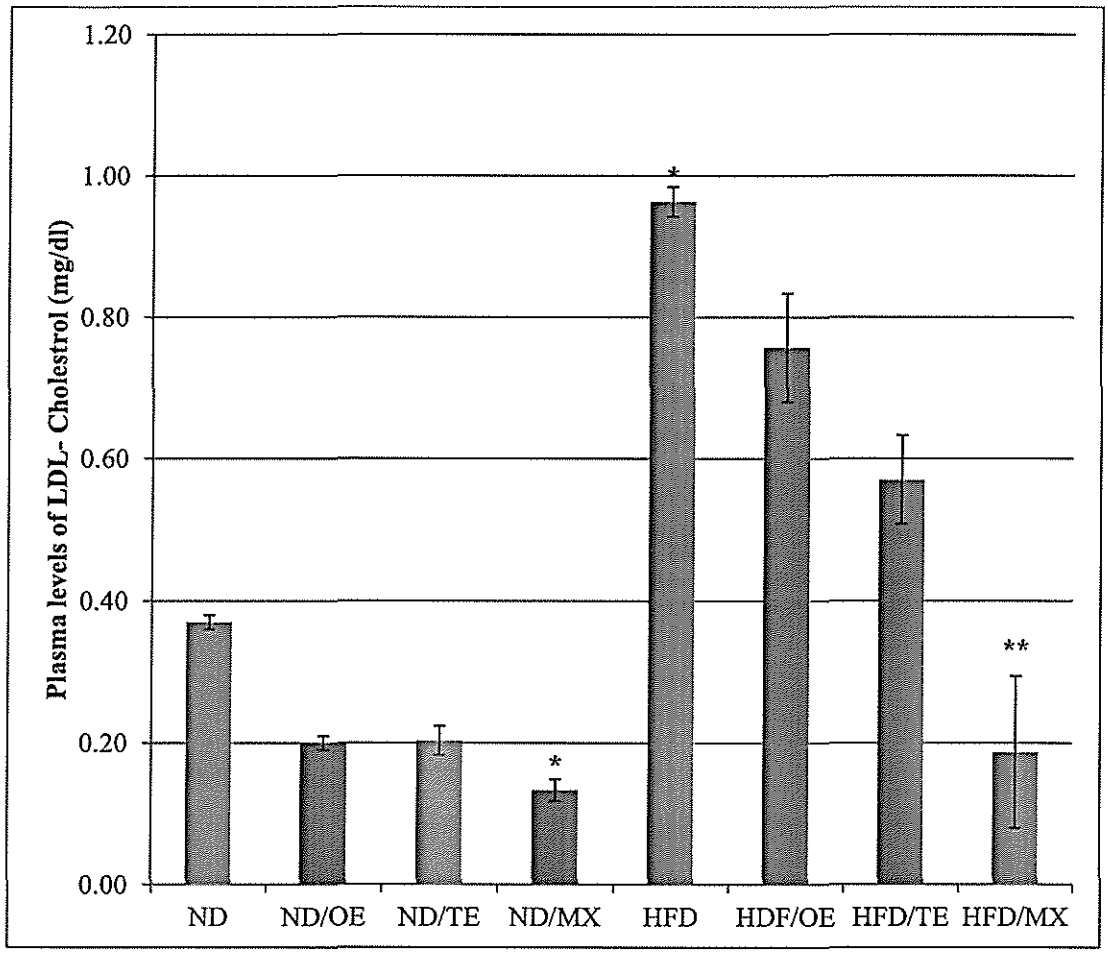
* differs significantly from ND, ** differs significantly from HFD

Appendix 8: Serum HDL-Cholesterol levels of rats fed on ND HFD treated with the extracts on day 28



* differs significantly from ND, ** differs significantly from HFD

Appendix 9: Serum LDL-Cholesterol levels of rats fed on ND HFD treated with the extracts on day 28



* differs significantly from ND, ** differs significantly from HFD

Appendix10: Composition of high fat die (HFD) used to induce hyperlipidemia in the experimental animals (male wistar rats)

HFD was a mixture of the food stuffs below in the ratio of 2:4:4 respectively.

1. Maize bran
2. Cotton seed cake
3. Sun flower seed cake