www.ajbrui.org

Afr. J. Biomed. Res. Vol. 24 (May, 2021); 281- 289

Research Article

Lipid - Lowering Effect of a Mixture of *Allium cepa* bulb and *Camellia sinensis* Leaf Extracts in Rats fed on High fat Diet

Kimanje K R.[1] , Kinyi1 H.[3], Ssekatawa K.[1,2], Tirwomwe M.[1,3] and Wampande E.[2]

[1]*Department of Biochemistry, Faculty of Biomedical Sciences, Kampala International University-Western Campus P. O. Box 71, Bushenyi*

[2]*College of Veterinary Medicine Animal Resources and Biosecurity, Makerere University P. O. Box 7062, Kampala*

[3]*Department of Biochemistry, School of medicine, Kabale University P.O Box 317, Kabale*

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, have many side effects and are rarely 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 effect of the mixture of *Allium cepa* extract and *Camellia sinensis* extract on the serum lipid profile of the male Wistar rats. *Allium cepa* and *Camellia sinensis* mixture in a ratio of 3:7 had the highest antioxidant activity. It reduced body weight, total cholesterol, triacylglycerides, LDL-cholesterol and increased HDL, and in addition it had no toxicity to the liver of the animal models used. It has thus been recommended as a potential therapy for hyperlipidemia and its associated complication of liver toxicity. 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.

Keywords: *Allium cepa, Camellia sinensis, Antioxidant activity, Hyperlipidemia, Lipid profile, High fat diet*

Author for correspondence: Email: eenwanna@futa.edu.ng; kimanje.ronald@kiu.ac.ug;* Tel: *+256-775213050*

Received: June 2020; Accepted: January, 2020

INTRODUCTION

Hyperlipidemia is a metabolic disorder, associated with elevated levels of total cholesterol, phospholipids and triglycerides in serum. 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). High density lipoproteins (HDL), low density lipoproteins (LDL), chylomicrons and very low-density lipoproteins (VLDL) are the lipoproteins that transport lipids in blood and these accumulate in hyperlipidemia (Jung *et al*., 2015). The atherosclerotic buildup of fatty deposits on the blood vessel walls is majorly due to the LDL-bound cholesterol, while HDL- bound cholesterol is vital to health because it reduces or even retards buildup of such condition (Kathleen, 2015; Visavadiya *et al*., 2011). Often elevated levels of both LDL and triglycerides result in hyperlipidemia (Visavadiya *et al*., 2011).

A INTERHEART study by Steyn *et al*., (2005), observed that hyperlipidemia was the leading cause of diseases like ischemic heart disease and several other non-communicable illnesses such as obesity, diabetes, and others. This could be due to the oxidative reactions on lipids accumulating in blood where free radicals such as reactive oxygen species (ROS) are part of the products (Daniela, 2016). The free radicals in turn oxidize the circulating LDL - cholesterol which results in the building up of atherosclerotic plaques in the walls of the arteries and development of coronary and vascular diseases (Steyn *et al*., 2005; Mahmoud *et al*., 2015; Peer *et al*., 2016). According to the report by (WHO, 2014a), there is a rising burden of hyperlipidemia in many countries with low income and is a major cause of 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 in many developed and developing countries are the major cause of death (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 worldwide they are responsible for nearly 30% of deaths, accounting for approximately 40% in countries with high-income and nearly 28% in countries of low-and middle-income (WHO, 2014a; Gershim *et al*., 2015). It is predicted that by the year 2020 the global trend in deaths from NCDs

associated with hyperlipidemia especially CVD will be nearly at a rate of 32%, with low and middle income countries contributing greater than those with high-income (Capingana *et al*., 2013; WHO, 2014a). This estimation is largely attributed to the shift towards diets rich in lipids, lower fibre intake, 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 have indicated an increasing prevalence of the condition both in urban and many rural regions of Africa (Oladapo *et al*., 2010; WHO, 2014a; Gershim *et al*., 2015).

Furthermore, a recent study in Uganda conducted by (Gershim *et al*., 2015) reported that 31,700 deaths occur per year caused by CVDs such as stroke, heart attack and other risk factors of hyperlipidemia and the figure is predicted to continue rising. (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.

The present synthetic hypolipidemic agents available which include; fibrates, nicotinic acid, statins, and resins have been reported effectively lower plasma total cholesterol levels, but serum levels of LDL –cholesterol do not change significantly. 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).

Several plants (for instance green tea, vegetables fruits and onion among other) have been found to contain phytonutrients or phytochemicals that possess antioxidant properties (Haruno *et al*., 2015). Therefore, apart from advanced researches about the pharmacology of the plants in being sources of effective drugs, several recent medicines are provided by the plant kingdom. Plants are a source of a number of metabolites likes saponins, polyphenols, flavonoids, isoflavones, ascorbic acid, phytosterols, and fibers, and they have become of great importance in therapeutics (Visavadiya *et al*., 2011). A recent study has shown that plants with medicinal value can be used to in management of 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 (Mahmoud *et al*., 2015; Kateregga *et al*., 2015).

Previous studies have shown that both *Camellia sinensis* and *Allium cepa* are good sources of bioactive antioxidant polyphenol phytochemicals; catechins and quercetin respectively with hypolipidemic activity (Habauzit *et al*., 2012; Shogo *et al*., 2013; Shashank *et al*., 2013; Haruno *et al*., 2015). Flavonoids; catechins and quercetins inhibit LDL cholesterol (bad cholesterol) oxidation via a mechanism that involves free radical scavenging (Shashank *et al*., 2013). These polyphenolic compounds also improve serum lipid profile through the inducement of the hepatic LDL receptors resulting in increased lipid uptake and catabolism and consequential increase in the plasma HDL levels (Jung *et al*., 2015). In addition, fat burning is improved by CSE via fat cell division inhibition, decreased fat absorption from food, elevated activity of sympathetic nervous system, proper usage of fat and raised thermogenesis (Phung *et al*., 2010). Furthermore, natural antioxidants from plants reduce levels of serum free fatty acids and TG by being able to prevent lipid mobilization from body tissues (Peer *et al*., 2016).

The current study aimed at evaluating the effect of a mixture of aqueous extracts of *Allium cepa* and *Camellia sinensis* on male Wistar rats with high fat-induced hyperlipidemia.

Plate 1
Pictures of Camellia sinensis plant (A) and Allium cepa bulbs (B)

MATERIALS AND METHODS

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.

Experimental animals: The study was done using male Wistar rats aged six weeks, weighing between 120 – 200g obtained from the Animal Facility at Mbarara University of Science and Technology.

Plant materials and collection: Fresh *Allium cepa bulbs* (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*).

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.

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

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

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*

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.

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

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

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 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.

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 2.

Table 2:
Treatment of experiment groups

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)*

On 28th day post treatment, 0.5-1 ml of blood was collected in ethylene diaminetetracetic 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.

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 10µlof 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 *et al*., 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 spectrophotometer at 505nm wavelength for maximum absorbance (Young *et al*., 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;

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

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 100µl 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 *et al*., 2001).
The serum HDL- Cholesterol was then calculated following the expression;

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

Low density lipoproteins (LDL-Cholesterol) was calculated according to the following formula.

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

Assessment of liver function

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 spectrophotometrically 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 spectrophotometer 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 (Friedewald *et al*., 1972).

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

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 (Friedewald *et al*., 1972).

Liver histology: 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 (Okoro *et al*., 2007). 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.

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.

Ethical considerations: The rats were housed in clean rat cages (6 rats per cage) in a research laboratory at a temperature of 23±10C with 12/12hr light/dark cycles and 45±5% humidity. Rats were allowed free access to filtrated tape water and standard laboratory rat feed. This was in consent with the Institutional Research and Ethics Committee (IREC) of Kampala International University. All animals received human care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" of the above committee. After an experimental period of 28 days, the rats were fasted for 3hours with access to water, and blood samples were collected in non – heparinized vacutainers by cardiac puncture under Ketamine plus xylazine anesthesia at a dose of 40 – 90 mg/kg and xylazine of 5 – 10 mg/kg respectively by intraperitoneal route for 45 – 90 minutes.

RESULTS

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 1 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 1 point F). A combination of both extracts in the ratio of 30:70ml (3:7; ACE to CSE) showed a significantly high antioxidant activity (p = 0.02) from the antioxidant activities of individual aqueous extracts on analysis by Turkey's Pairwise test (Fig 1 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 1. (E-J). The mixtures with the highest antioxidant activity had at least 70 ml of CSE (Fig. 1-point D).

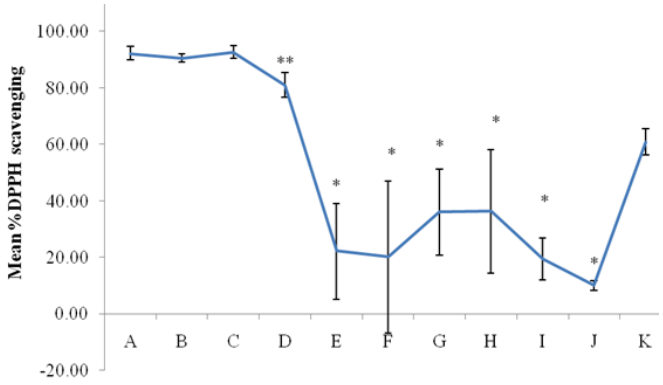

Figure 1
ACE = *Allium cepa* extract, CSE = *Camellia sinensis* extract, * Significantly lower than antioxidant activity of CSE and ACE, ** significantly high and different from CSE and ACE, **A** = CSE, **B** = 10ml ACE and 90ml CSE, **C** = 20ml ACE and 80ml CSE, **D** = 30ml ACE and 70ml CSE, **E** = 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.

Lipid profile and body weight

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. 2 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. 2).

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 the 6/6 rats fed on both ND and HFD when analyzed by a one-way ANOVA (Fig. 3). However, the reduction caused by the mixture was significantly higher in the rats fed on HFD.

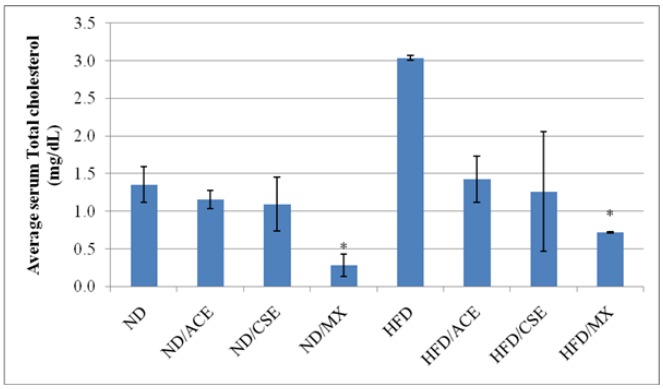

Figure 2
Antioxidant activities of different ratios of ACE and CSE
** differs significantly from ND, ND = Normal diet, HFD = High Fat Diet, ACE = Allium cepa Extract, CSE = Camellia sinensis Extract, MX = Mixture of ACE and CSE (3:7)*

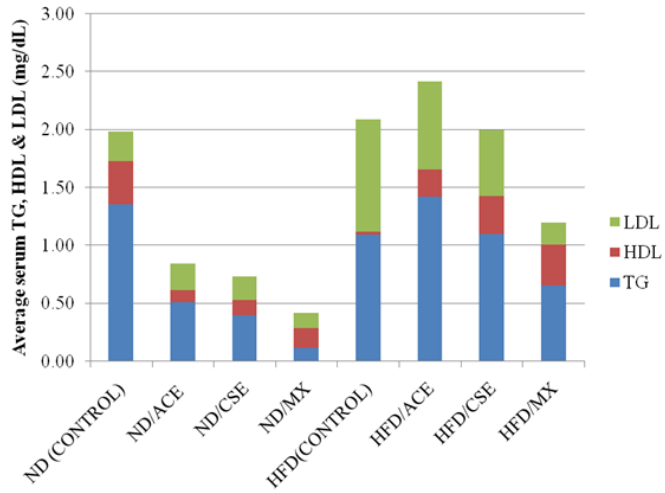

Figure 3
Serum Total cholesterol of rats fed on ND and HFD treated with the extracts
ND = Normal diet, HFD = High fat diet, ACE = Allium cepa extract, CSE = Camellia sinensis extract and MX = Mixture of ACE and CSE (3:7)

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 all the 6/6 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 (Fig.4).

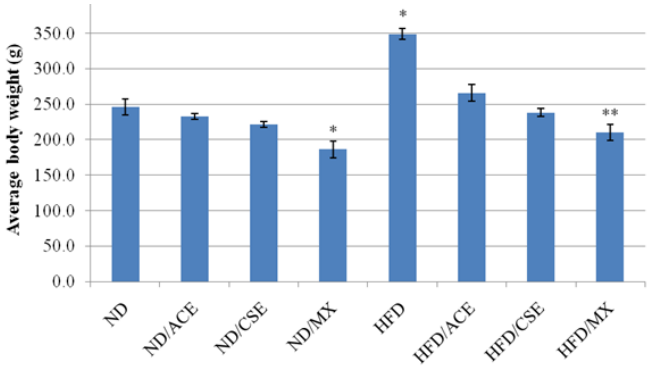

Figure 4

Body weights of rats fed on ND and HFD treated with the extracts.
** 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)*

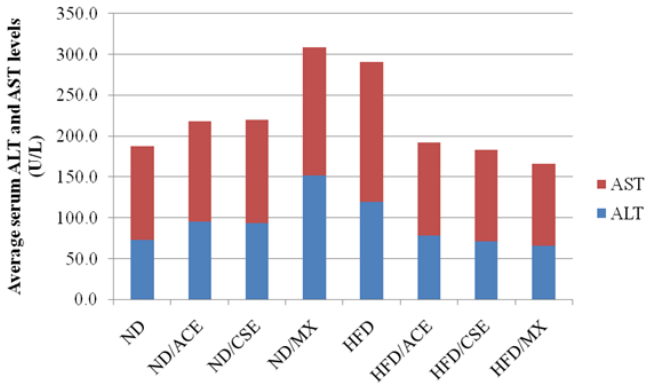

Figure 5

Serum AST and ALT levels of rats fed on ND and HFD treated with the extracts.
ND = Normal diet, HFD = High fat diet, ACE = Allium cepa extract, CSE = Camellia sinensis extract, MX = Mixture of ACE and CSE (3:7)

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 6/6 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).

Effect of a mixture of CSE and ACE on liver weight of male Wistar rats: The average liver weight for 6/6 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 6.

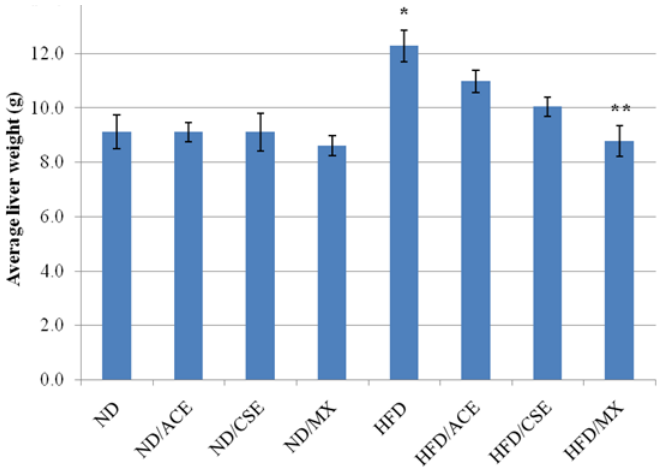

Figure 6

Liver weights of rats fed on ND and HFD treated with the extracts
** 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)*

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 (Plate 2 panel B).

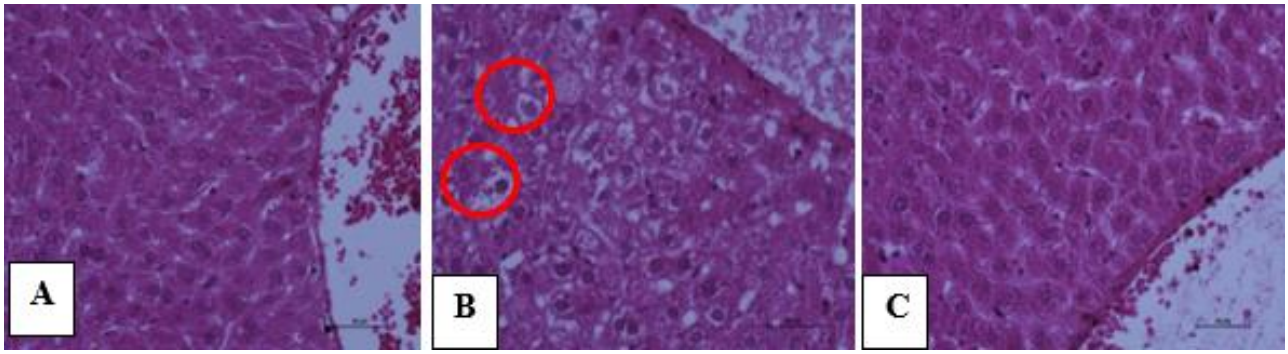

Plate 2

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

However, cell architecture, the size and number of lipid droplets were enormously reduced in the 6/6 MX treated rats (Plate 2 panel **C**). 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.

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 (Peer et al., 2016; Dimas et al., 2017).

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 mobilization of lipids from body tissues which results in the rise in levels of serum free fatty acids and triglycerides. This was responsible for significantly increased serum HDL-cholesterol levels plus 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 resultant low levels of serum total cholesterol and elevated serum levels of HDL-cholesterol (good cholesterol) in serum of rats on HFD supplemented by MX was 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; Kathleen, 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 (Kathleen, 2015) and it could be due to the enlarged fat store in fat cells within the liver especially the pericental regions, 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, elevated AST and ALT levels in serum specifically indicate liver damage (Devlin et al., 2005; Nelson et al., 2013).

Data from this study indicated significantly elevated AST and ALT levels in serum of the rats on ND under treatment 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 raised AST levels in serum.

The AST and ALT serum levels being elevated in rats on HFD may be due to the fact that oxidizing agents such as the ROS are produced normally as by-products in metabolic processes; (ROS). These are not only important in contributing to the immunity, transduction of signals and other essential processes; they also result in lipid bilayer peroxidation and hence detrimental effects (Devlin et al., 2005; Peer et al., 2016). Hence, blood antioxidants scavenge the excess oxidizing agents (free radicals) in order to balance

the redox environment in the body. Therefore, high serum lipids due to HFD causes free radicals to be released uncontrollably as a result of a disrupted redox balance, which leads to increased atherogenicity and 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 to maintain the physiological redox balance but this eventually fails in such conditions elevated serum lipids. Supplementing with natural antioxidants from plants is a potential remedy intervening elevated serum lipids and normalizing lipid profile 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.

In conclusion, the combination of *Allium cepa* and *Camellia sinensis* in the ratio 3:7 respectively had the highest significant antioxidant activity compared to individual extracts antioxidant activities. 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, to being a potential body weight reducing agent. Furthermore, the mixture of *Allium cepa* and *Camellia sinensis* in the ratio of 3:7 has therapeutic effects on the toxic effect of HFD.

Authors' contributions: *This work was carried out in collaboration between all authors. KRK, EW and HK conceptualized this project and drafted the experimental design. KRK performed the literature search. KRK, KS, MT and set up all the laboratory experiments, collected the raw data and also drafted the first manuscript. KRK managed manuscript revisions. KRK, HK, KS, MT and performed data analysis. EW, HK, KRK, KS, and MT participated in final manuscript writing and revisions. All authors read and approved the final manuscript.*

Acknowledgements: *We are thankful to the Directorate of Post graduate Studies and Research, Kampala International University – Western Campus for partly funding this research. We also extend our thanks to the administration of Central Diagnostic Laboratory, College of Veterinary Medicine, Animal Resources and Biosafety (CoVAB), Makerere University for offering us laboratory space and other materials needed to perform histopathologic experiments.*

REFERENCES

Capingana D.P, Pedro Magalhaes, Amilcar Silva BT, Sergio RodriguesL. , Maver AA Goncalves, Marcelo Baldo P., Cristovao Simoes C.F., Albarno Ferreira, and Jose Mill G., (2013): Prevalence of cardiovascular risk factors and socioeconomic levels among public sector workers in Angola. *BMC Public Health*, 13, 732.

Daniela Pellegrino, (2016): Antioxidant and Cardiovascular Risk Factors. *BMC Public Health*, 13, 732.

Devlin T. M., & Harwood, J. L. (2005): Textbook of biochemistry with clinical correlations, sixth Edition. *Trends in Biochemical Sciences-Library Compendium, 18*, 62-62.

Dimas Rahadian, Aji Muhammad, Danar Praseptiangga, Davy Van deWalle and KoenDewettinck, (2017): Interaction between natural antioxidants derived from cinnamon and cocoa in binary and complex mixtures. *Food Chemistry*, 231, 356–364.

Friedewald WT, Levy RI, Fedrickson DS, (1972): Estimation of the concentration of Low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. *Clinical Chemistry*, 18, 499-502.

GershimAsiki, Georgina A. V. Murphy, Kathy Baisley, Rebecca N. Nsubuga, Alex Karabarinde, Robert Newton, Janet Seeley, Elizabeth H. Young, Anatoli Kamali, ManjinderS. Sandhu, (2015). Prevalence of Dyslipidaemia and Associated Risk Factors in a Rural Population in South-Western Uganda: A Community Based Survey. *PloS one*, *10*(5), e0126166.

Haruno Nishimuro, Hirofumi Ohnishi, Midori Sato, Mayumi Ohnishi-Kameyama, Izumi Matsunaga, Shigehiro Naito, Katsunari Ippoushi, Hideaki Oike, Tadahiro Nagata, Hiroshi Akasaka, Shigeyuki Saitoh, Kazuaki Shimamoto and Masuko Kobori, (2015). Estimated Daily Intake and Seasonal Food Sources of Quercetin in Japan. *Nutrients,7*(4), 2345-2358.

Habauzit Vèronique and Christine Morand, (2012). Evidence for a protective effect of polyphenols-containing foods on cardiovascular health: *Therapeutic Advances in Chronic Disease*, 3 (2),87-106.

Huins Helen, Tim Kenny and Louise Newson, (2015): Liver Function Tests. Available at; patient.info/health/liver-function tests. Accessed 23rd October 2015.

Jung Hyun Jung, Ji-Hyang Wee, Kyung Mi Kim, HaeMi Sung, and HyunKyung Shin, (2015): Effect of Onion (*Allium cepa*) Ultra-high pressure Processing and Hot water Extracts on the Serum Cholesterol Levels in High Cholesterol-fed Rats. *Food Sci. Biotechnology*, *24(*1), 287-294.

Kateregga, J N; Sooka, S; Ndukui, J G. (2015). Evaluation of the Anti-hyperlipidemic Activity of Ethanolic and Aqueous Extracts of Solanum melongena in Wistar Rats. *British Journal of Pharmaceutical Research*, *5*(2), 90.

Kathleen Davis, (2015): Hyperlipidemia: Causes, Diagnosis and Treatments. Available at: http://www.medicalnewstoday.com/knowledge-center. (Accessed Thursday 18 June 2015).

Mahmoud Bahmani, (2015*):* Contrasting actions of various antioxidants on hyperlipidemia: *Scholars Research Library, Der Pharmacia Lettre*, 7 (12), 81-88.

Mete Rafet, Mustafa Oran, Birol Topcu, Meltem Oznur, Erdogan Selcuk Seber, Asuman Gedikbasi and Tarkan Yetisyigit, (2016). Protective effects of onion (*Allium cepa*) extract against doxorubicin-induced hepatotoxicity in rats. *Toxicology and Industrial Health*, 32(3), 551–557.

Nair, D. R., Sharifi, M., & Al-Rasadi, K. (2014). Familial hypercholesterolaemia. *Current opinion in cardiology*, *29*(4), 381-388.

Nelson, D. L., & Cox, M. M. (2013). Course Smart International E-Book for Principles of Biochemistry. Palgrave Macmillan.

Nidhi Shauma, Preeti Shauma, Nakuleshawar, Dut Jasuja and Sureh Chand Joshi, (2013): Hypocholesterolemia and Antioxidant Potentials of some plants and Herds. *Journal of Zoological Sciences*, 1(2), 2347-2294.

Okoro N, Nelson CA, Reginald AO, (2007). Antibacterial activity of *Allium cepa* (onion) and Zingiber officinale (ginger) on Staphylococcus aureus and Pseudomonas aeruginosa isolated from high vaginal swab. *International Journal of Tropical Medicine*, 3, 1–5.

Oladapo O.O., Solako L, Sodiq O., Shoyinka K., Adedapo K., Falase AO, (2010): A prevalence of cardiometabolic risk factors among a rural Yoruba South-Western Nigerian population: a population-based survey. *Cardiovasc. J Afr, 21*, 26 – 31.

Olfa Belguith-Hadriche, Sonda Ammar, Maria del Mar Contreras, Mouna Turki, Antonio Segura-Carretero, Abdelfattah El Feki, Fatma Makni-Ayedi and Mohamed Bouaziz , (2016). Anti-hyperlipidemic and Antioxidant Activities of Edible Tunisian Ficuscarica L. Fruits in High Fat Diet-Induced Hyperlipidemic Rats. *Plant Foods Hum Nutr*, 71, 183–189.

Peer Abdul Hannan, Jamshaid Ali Khan, Irfan Ullah and Safi Ullah, (2016): Synergistic Combinatorial anti-hyperlipidemic study of selected natural antioxidants; modulatory effects on lipid profile and endogenous antioxidants. *Lipids in Health and Disease*,15,151.

Perere O.S, Liyange L, Weththasinghe P, Jayawardana B.C, Vidanarachchi J.K, Fernando P.and Sivakanesan R., (2016). Modulating effects of Cowpea incorporated diets on serum lipds and serum antioxidant activity in Wistar rats. *J. Natn. Sci. Foundation Sri Lanka , 44 (1), 69-76.*

Phung OJ, Baker WL, Matthews LJ, Lanosa M, Thorne A, Coleman CI, (2010). Effect of green tea catechins with or without caffeine on anthropometric measures: A systematic review and meta-analysis. *Am J ClinNutr*,91, 73-81.

Prisk Victor, (2014). Green Tea and Liver Toxicity. Available at: http://www.advanced molecularlabs.com/fat-loss/green-tea-and-your-liver/. Accessed. Saturday, 11 January, 2014.

Shashank Kumar and Abhay K. Pandey (2013). Chemistry and Biological activities of flavonoids. *The Scientific World Journal*, 2013, 16.

Shogo Matsunaga, Kazuo Azuma,Mayumi Watanabe, TekeshiTsuka, Tomohiro Osuki, and Yoshiharu, (2013). Onion peel tea ameliorates obesity and affects blood parameters in a mouse model of high-fat-diet-induced obesity. *Experimental and Therapeutical Medicine*, 7, 379-382.

Shrank William, Jane F. Barlow, Troyen A. Brennan, (2015). New Therapies in the Treatment of High Cholesterol. An Argument to Return to Goal-Based Lipid Guidelines. *JAMA*, 314(14), 1443-1444.

Steyn K, Sliwa K, Hawken S, Commerford P, Onen C, Damasceno A, (2005). Risk factors associated with myocardial infarction in Africa: the INTERHEART Africa study. Circulation 112: 3554–3561.PMID: 16330696. *Therapeutic Advances in Chronic Disease*, 3(2), 87–106.

Visavadiya, N. P., & Narasimhacharya, A. V. R. L. (2011). Ameliorative effects of herbal combinations in hyperlipidemia. *Oxidative medicine and cellular longevity*, *2011*.

Wedro Benjamin, Bhupinder S. Anand, Melissa Conrad Stöppler, (2015). Anatomy and Function of the Liver. Available at: htt://www.medicinenet.com/. (Accessed Thursday 18 June 2015).

World Health Organization, (2014a). Non communicable diseases (NCDs), *Country Profiles, 2014*. *www.who.int/nmh/countries/uga* en. Accessed; 27th Jun 2014.

World Health Organization, (2014b). The top 10 causes of death. In: Media centre fact sheets. 2014. *http://www.who.int/ media centre/factsheets/fs310/en*. Accessed; 20th Nov 2014.

Young, D. S., & Friedman, R. B. (2001). Effects of disease on clinical laboratory tests. (Vol.1). *Amer Assn for Clinical Chemistry*.