Effects of Dichloromethane-Methanolic Leaf Extracts of Carissa edulis (Forsk) Vahl on Serum Lipid Profiles in Normal Rat Models

Obel H. Jorum1, Alex K. Machocho2 and Ngugi M. Piero1

1Department of Biochemistry and Biotechnology, Kenyatta University, P.O Box 43844-00100, Nairobi, Kenya
2Department of Chemistry, Kenyatta University, P.O Box 43844-00100, Nairobi, Kenya

Corresponding author: Obel H. Jorum, Department of Biochemistry and Biotechnology, Kenyatta University, P.O. Box 43844-00100, Nairobi, Kenya, Tel. : +254 728 671 219; E-mail: humphreyobel@gmail.com

Received date: January 01, 2016; Accepted date: March 19, 2016; Published date: March 29, 2016

Copyright: ©2016 Jorum OH, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

Abstract
Assessment of serum lipid profile is required for wellbeing of every individual since the blood can act as pathological reflector and also as an indicator of the physiological state of an animal. Disorders arising from lipid components imbalance are approaching epidemic levels while the methods used to conventionally manage them are not improving hence the need to continually seek alternative methods of management. Carissa edulis (Forsk.) Vahl belongs to the Apocynaceae family. This plant has been used extensively in traditional medicine to manage various diseases including those that are lipids related. This study, therefore, was designed to investigate the effects of Dichloromethane-Methanolic leaf extract of C. edulis on serum lipid profiles. The plant leaves were collected from Siakago-Mbeere North Sub-County, Embu County, Kenya. The samples were prepared and extraction of the active compounds carried out using organic solvents; dichloromethane and methanol in the ratio of 1:1. Experimental rats were divided into four groups each consisting of five normal rats. The groups received oral doses of 50, 75 and 100 mg/kgbw of the extract while one group was used as control and did not receive any dosage. Blood samples were drawn from the rats at intervals of seven days then the serum lipid profiles were analysed using an auto-analyzer machine. Screening for plants phytochemicals was conducted using the standard recommended procedures. The results of this study showed that DCM-MeOH leaf extract of C. edulis induced general changes in serum lipid profiles of normal rats. The triglycerides, total cholesterol and low density lipoprotein cholesterol levels decreased significantly at all the dose levels (p<0.05), while high density lipoprotein cholesterol levels increased significantly during this study (p<0.05). Qualitative phytochemical screening confirmed the presence of various phytochemicals which included alkaloid, flavonoids, tannins, phenols, terpenes and traces of steroids which have the ability to alter lipid metabolism. It was concluded that the plant extract may be useful in the management of lipid related disorders.

Keywords: Lipid profiles; Phytochemicals; Carissa edulis; Leaf extracts

Introduction
The term lipids describe an entire class of fats and fatlike substances in the blood. The most important lipids in the blood are fatty acids, cholesterol, cholesterol esters, TGLs, and phospholipids [1]. Assessment of plasma lipid profile is required for the state of wellbeing of every individual as cardiovascular diseases and coronary heart diseases are silent, serial killers of our age [2]. The assessment of serum lipid profile can be used to determine the effects and functions of compound in blood. A range of blood tests that are conducted to determine the level of lipid components in serum is known as the lipid profile test.

Triglycerides (TGs) are esters consisting of a glycerol molecule coupled to three fatty acid residues of varying carbon chain lengths and degrees of unsaturation [3]. They can be mobilized when required, for example, during starvation [4]. They are atherogenic because they are rich in Apo C-III, which delays the lipolysis of VLDL and inhibits its uptake and clearance from plasma [5]. Cholesterol is the major sterol in animal tissues. In addition to their roles as membrane constituents, the sterols serve as precursors for a variety of products with specific biological activities [6]. Steroid hormones, for example, are potent biological signals that regulate gene expression [7]. High Density Lipoproteins (HDL) picks up and transport excess cholesterol from the walls of arteries and brings it back to the liver for processing and removal. About 30% of blood cholesterol is carried in the form of HDL-C. Significant lowering of total cholesterol and rise in HDL-C is a very desirable biochemical state for prevention of atherosclerosis and ischemic conditions [8-11]. The roles of HDL includes promoting efflux and uptake of cholesterol from peripheral tissues, facilitates the conversion to cholesterol ester and subsequent delivery to the liver, either directly or via other lipoproteins [12]. Low Density Lipoproteins (LDL) is made by the liver to transport cholesterol to the body’s cells and tissues. They may form deposits on the walls of arteries and other blood vessels [4]. The main benefits of lowering LDL-C include, Decreases the chance of heart attack and/or stroke. Reduces the formation of new cholesterol plaques. Eliminates existing plaques. Prevents the rupture of existing plaques [13]. The LDL is even smaller than IDL. They contain predominantly cholesterol, protein and a very small amount of TG [14].

Very Low Density Lipoproteins (VLDL) is produced in the liver when there is an increased flux of FFAs or increased synthesis of fatty acids by the liver. They predominantly contain TG and smaller amounts of cholesterol and protein [15]. They transport endogenous triglycerides, phospholipids, cholesterol, and cholesteryl esters. They also functions as the body’s internal transport mechanism for lipids. In addition it serves for long-range transport of hydrophobic intercellular messengers [15]. Chylomicrons predominantly contain TG, very small
amounts of cholesterol and protein [16]. During chylomicron circulation, they rapidly deliver dietary TG to peripheral tissues. The Apo C-II stimulates lipoprotein lipase (LPL) which breaks down TG to glycerol and free fatty acids. The free fatty acids are released into the circulation to be extracted by organs such as muscle, adipose and mammary tissue. The metabolism of TG rapidly depletes the core of the chylomicron particle [16].

Hyperlipidemia refers to elevated levels of lipids and cholesterol in the blood, and is also identified as dyslipidemia, to describe the manifestations of different disorders of lipoprotein metabolism. Although elevated low density lipoprotein cholesterol (LDL) is thought to be the best indicator of atherosclerosis risk [17], dyslipidemia can also describe elevated total cholesterol (TC) or triglycerides (TG), or low levels of high density lipoprotein cholesterol (HDL).

Hyperlipidemia associated lipid disorders are considered to cause atherosclerotic cardiovascular disease. Among these hypercholesterolemia and hypertriglyceridemia are closely related to ischemic heart disease (IHD) [18]. The main aim of treatment in patients with hyperlipidemia is to reduce the risk of developing ischemic heart disease or the occurrence of further cardiovascular or cerebrovascular disease [19]. Hyperlipidemia can be either primary or secondary type, the primary disease may be treated by antilipidemic drugs, but the secondary type originating from diabetes, renal lipid nephrosis or hypothyroidism demands the treatment of the original disease rather than hyperlipidemia [20]. Medicinal plants play a major role in hypolipidemic activity and suggest that the lipid lowering action is mediated through inhibition of hepatic cholesterol biosynthesis and reduction of lipid absorption in the intestine [21].

Various conventional methods are currently being used to manage lipid related disorders. Primary treatment strategy is dietary management which entails decreased intake of saturated fat, decreased intake of trans-fatty acids which are thought to increase LDL-C levels and appear to lower HDL, encourage antioxidant food sources such as carotenoids, reduce serum homocysteine levels, adequate intakes of folate, vitamins B6 and B12 as well as total fat restriction may keep homocysteine levels low [22].

Pharmacological management of hyperlipidemia includes use of statins, a HMG-CoA (3-hydroxy-3-methylglutaryl-Coenzyme A) reductase inhibitor6. Fibrates are activators of lipoprotein lipase. It acts by; agonising peroxisome proliferator-activated receptor (PPAR); increasing hydrolysis of VLDL and chylomicrons; decreasing serum TGs; increasing clearance of LDL by liver and increase in HDL and expression of genes responsible for increased activity of plasma lipoprotein lipase enzyme [23]. Ezetimibe acts by inhibiting intestinal cholesterol, decrease in concentration of intrahepatic cholesterol; increase in uptake of circulating LDL; decrease in serum LDL cholesterol levels and compensatory increase in LDL receptors.

Nicotinic acid (Niacin) is an inhibitor of lipolysis, it acts as a potent inhibitor of lipolysis in adipose tissues, decreases mobilization of FFAs (major precursor of TGs) to the liver, increases HDL levels, decreases LDL and also decreases endothelial dysfunction and thrombosis [24].

Most conventional ways of managing lipid related disorders may be costly, have undesired side effects, painful to the patients or are not easily accessible. Use of Niacin can lead to dermatological conditions, hepatotoxicity and in extreme cases even birth defects [25]. Combination treatment of statins and fibrates causes a markedly increased risk of myopathy and rhabdomyolysis, so is only done under close supervision. In the view of these setbacks, there is therefore need to develop agents that are effective, cheaply available and with negligible side effects as the alternative medical intervention.

Many medicinal plants have been used traditionally to manage hematomal and serum lipid disorders. Unripe pods and leaves of Bauhinia purpurea, leaves of Cinnamomum tamala and the roots of Conomphora mukul herbs have been used by herbalists in India to manage lifestyle diseases such as obesity, heart conditions and hyperlipidemia. Like other terrestrial plants, Carissa edulis has ethnopharmacological relevance and has also been exploited by the local people in the search for remedies for various ailments to increase vitality and manage lipid related disorders [26]. Its effect in these activities has not been scientifically studied or validated. It was against this background that this study was undertaken to scientifically test the serum lipid profiles claimed effect of the plant extracts in normal rats.

Materials and Methods

Collection and preparation of plant material

Fresh leaves of Carissa edulis were collected from Siakago division, Mbeere North Sub-Country, Embu Country, Kenya. The fresh leaves were identified with the help of local herbalists. The information gathered included vernacular names, plant parts used and the ailment treated. The samples were collected with acceptable bio-conservative methods and were properly sorted out, cleaned, and transported in polythene bags to Kenyatta University, Biochemistry and Biotechnology laboratories for drying and crushing. The leaves of C. edulis were chopped into small pieces and air dried at room temperature for two weeks until properly dried. They were then ground into fine homogenous powder using an electric mill followed by sieving through mesh sieve and stored at room temperature awaiting extraction. The plant samples were provided to an acknowledged Taxonomist for botanical authentication and a voucher specimen deposited at the Kenyatta University Herbarium, Nairobi, Kenya for future reference.

Extraction

For each sample, 200 g of powder was soaked separately in a cold 1:1 mixture of DCM and MeOH and stirred for six hours to extract the active compounds. The successive extract was concentrate was put in an airtight container and stored at -4°C before use in bioassay studies.

Experimental animals

Male healthy winstar rats (20), aged between two to three months and weighing an average of 150 g were used in this study. They were bred in the animal house of the Department of Biochemistry and Biotechnology, Kenyatta University. The rats were housed in cages, maintained under standard laboratory conditions of 12 hour light and dark sequence, at ambient temperature of 25 ± 2°C and 35-60% humidity. The animals were fed with standard rat pellets obtained from Unga Feeds Limited, Kenya, and water ad libitum. Ethical guidelines and procedures for handling experimental animals were followed.

Experimental design

The rats were randomly assigned into four groups where each group was having five normal rats. The groups were named A-D and were
designed as follows: Group A was the control group and received normal saline (1 ml each) for 21 days. The other experimental groups were as follows; Group B animals were orally administered with the DCM-MeOH leaf extracts of *C. edulis* at a dose of 50 mg/kg bw for 21 days, Group C animals were orally administered with the DCM-MeOH leaf extracts of *C. edulis* at a dose of 75 mg/kg bw for 21 days and Group D animals were orally administered with the DCM-MeOH leaf extracts of *C. edulis* at a dose of 100 mg/kg bw for 21 days. The administration was done with the aid of a metal oropharyngeal cannula. Each rat was marked at the tail using a permanent marker pen to distinguish it from the lot. Daily cleaning of the cages was carried out.

Blood from rats in all groups was taken before the commencement of the first oral administration, then on the seventh, fourteenth and twenty-first days. During the entire period of study, rats were allowed free access to mice pellet and water ad libitum and observed for any signs of general illness, change in behaviour and/or mortality.

**Preparation of extracts doses for administration**

The dose level of 50 mg/kg bw was prepared by dissolving 0.038 g of the extract in 0.2 ml of 30% dimethylsulfoxide and topping up to 2.5 ml with distilled water, the dose level of 100 mg/kg bw was prepared by dissolving 0.076 g of 0.2 ml of 30% dimethylsulfoxide and topping up to 2.5 ml with distilled water.

**Collection of blood samples**

Blood samples were collected at the start of the experiment, then on the seventh day, the fourteenth day and finally on the twenty-first day from the tails of rats for the determination of hematological parameters. The tails were first sterilized by swabbing with 70% ethanol and then the tip of the tails pierced. Bleeding was enhanced by gently milking the tail from the body towards the tip. Blood of the tails of rats for the determination of hematological parameters using methods of analysis as described by [28,29]. Standard screening tests for detecting the presence of different chemical constituents were employed. Secondary metabolites tested for were flavonoids, phenolics, saponins, alkaloids, cardiac glycosides, sterols and terpenoids.

**Preparation of extracts doses for administration**

The dose level of 50 mg/kg bw was prepared by dissolving 0.038 g of the extract in 0.2 ml of 30% dimethylsulfoxide and topping up to 2.5 ml with distilled water, the dose level of 100 mg/kg bw was prepared by dissolving 0.076 g of 0.2 ml of 30% dimethylsulfoxide and topping up to 2.5 ml with distilled water.

**Preparation of extracts doses for administration**

The dose level of 50 mg/kg bw was prepared by dissolving 0.038 g of the extract in 0.2 ml of 30% dimethylsulfoxide and topping up to 2.5 ml with distilled water, the dose level of 100 mg/kg bw was prepared by dissolving 0.076 g of 0.2 ml of 30% dimethylsulfoxide and topping up to 2.5 ml with distilled water.

**Determination of serum lipid profiles**

Lipid profiles and indices were determined from blood samples using standard protocols described by Tietz et al. [27]. Serum lipid profiles were analysed by use of Roche modular (model P800, Mannheim, Germany) auto-analyzer machine. The assay kits for cholesterol, triacylglycerols, low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) were obtained from Roche Diagnostic GmbH, Mannheim, Germany. All other reagents used were of analytical grade and were supplied by Phillips Healthcare Technologies Ltd. Nairobi, Kenya.

**Qualitative phytochemical screening**

The extracts obtained were subjected to qualitative phytochemical screening to identify presence or absence of selected chemical constituents using methods of analysis as described by [28,29]. Standard screening tests for detecting the presence of different chemical constituents were employed. Secondary metabolites tested for were flavonoids, phenolics, saponins, alkaloids, cardiac glycosides, sterols and terpenoids.

**Data management and analysis**

Experimental data on different hematological parameters and serum lipid profiles were obtained from all the animals on the first day and compared with the seventh, fourteenth and twenty-first days for the three dose levels. It was recorded and tabulated on a broad spreadsheet sheet. Results were expressed as Mean ± Standard error of mean (SEM) for analysis. Statistical significance of difference among the groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey’s post hoc test to separate the means and obtain the specific significant differences among the different groups. The value of P ≤ 0.05 was considered significant. Analysis of the data was done using Minitab statistical software, Version 17.

**Results**

| PARAMETERS (mmol/l) | Control | 50mg/kgbw | 75mg/kgbw | 100mg/kgbw |
|---------------------|---------|-----------|-----------|------------|
| TG                  | Day 7   | Day 14    | Day 21    | Day 7      | Day 14    | Day 21    | Day 7      | Day 14    | Day 21    |
| 1.84 ± 0.05         | 1.72 ± 0.02 | 1.92 ± 0.10 | 1.30 ± 0.09 | 0.99 ± 0.06 | 0.82 ± 0.09 | 1.33 ± 0.09 | 0.95 ± 0.10 | 0.70 ± 0.08 | 1.33 ± 0.02 | 1.18 ± 0.05 | 0.80 ± 0.03 |
| TC                  | Day 7   | Day 14    | Day 21    | Day 7      | Day 14    | Day 21    | Day 7      | Day 14    | Day 21    |
| 1.81 ± 0.13         | 1.84 ± 0.09 | 2.00 ± 0.15 | 1.45 ± 0.07 | 1.00 ± 0.13 | 0.92 ± 0.10 | 1.27 ± 0.02 | 1.09 ± 0.22 | 0.95 ± 0.07 | 1.30 ± 0.03 | 1.22 ± 0.02 | 1.16 ± 0.19 |
| HDL-C               | Day 7   | Day 14    | Day 21    | Day 7      | Day 14    | Day 21    | Day 7      | Day 14    | Day 21    |
| 0.52 ± 0.06         | 0.57 ± 0.10 | 0.50 ± 0.14 | 1.13 ± 0.04 | 1.40 ± 0.04 | 1.52 ± 0.10 | 1.28 ± 0.05 | 1.40 ± 0.04 | 1.64 ± 0.02 | 1.20 ± 0.03 | 1.32 ± 0.02 | 1.36 ± 0.19 |
| LDL-C               | Day 7   | Day 14    | Day 21    | Day 7      | Day 14    | Day 21    | Day 7      | Day 14    | Day 21    |
| 1.42 ± 0.14         | 1.37 ± 0.08 | 1.46 ± 0.13 | 1.38 ± 0.09 | 1.33 ± 0.16 | 1.22 ± 0.07 | 1.20 ± 0.02 | 0.98 ± 0.13 | 0.97 ± 0.13 | 1.16 ± 0.07 | 0.94 ± 0.03 | 0.44 ± 0.05 |

Table 1: Effects of DCM-MeOH leaf extracts of *C. edulis* on serum lipid profiles in normal rats.

All values are expressed as mean ± SEM for five animals per group. Values followed by the same superscript are not significantly different (p<0.05 analysed by ANOVA and Tukey’s post hoc test). Means are compared among the days with the same extract dose level.
The DCM-MeOH leaf extracts of *C. edulis* at the dose level of 75 mg/kgbw caused significant decrease in the levels of TC after fourteen and twenty-one days of administration (p<0.05; Table 1). The levels of TC and LDL-C significantly decreased after twenty-one days of extract administration (p<0.05; Table 1). The HDL-C concentration, however, was not significantly affected by this dose level.

The DCM-MeOH leaf extracts of *C. edulis* at the dose level of 100 mg/kgbw caused significant decrease in the concentration of TG after seven, fourteen and twenty-one days of oral administration (p<0.05; Table 1). The levels of TC and LDL-C significantly decreased after twenty-one days of extract administration (p<0.05; Table 1). The HDL-C concentration, however, was not affected by this dose level.

When the levels of lipids and related parameter profiles in normal rats were compared on specific day across all the dose concentrations, it was evident that there were significant changes (Table 2). After seven days of DCM-MeOH leaf extracts of *C. edulis* administration, the levels of TG and TC decreased significantly at all the dose levels (p<0.05; Table 2). Concentration of HDL-C increased significantly at all the dose levels after seven days of administration of the extract (p<0.05; Table 2), while the levels of LDL-C decreased significantly at the dose levels of 75 mg/kgbw and 100 mg/kgbw (p<0.05; Table 2).

After fourteen days of DCM-MeOH leaf extracts of *C. edulis* administration, the levels of TG and TC decreased significantly at all the dose levels (p<0.05; Table 2). The concentration of HDL-C increased significantly at all the dose levels after fourteen days of administration (p<0.05; Table 2), while the levels of LDL-C decreased significantly at extract dose levels of 75 mg/kgbw and 100 mg/kgbw (p<0.05; Table 2).

After twenty-one days of DCM-MeOH leaf extracts of *C. edulis* administration, the levels of TG, TC and LDL-C decreased significantly at all the dose levels (p<0.05; Table 2). The concentrations of HDL-C, however, increased significantly at all the dose levels after twenty-one days of extract administration (p<0.05; Table 2).

**Phytochemical screening**

Qualitative phytochemical screening of the DCM-MeOH leaf extracts of *C. edulis* revealed the presence of alkaloids, flavonoids, phenolics, terpenoids and traces of steroids. However, saponins and cardiac glycosides were absent in the leaf extracts (Table 3).

| PARAMETER | Control | Dose 1 | Dose 2 | Dose 3 | Control | Dose 1 | Dose 2 | Dose 3 | Control | Dose 1 | Dose 2 | Dose 3 |
|-----------|---------|--------|--------|--------|---------|--------|--------|--------|---------|--------|--------|--------|
| Day 7     | TG      | 1.84 ± 0.05 | 1.30 ± 0.09 | 1.33 ± 0.08 | 1.33 ± 0.02 | 1.72 ± 0.02 | 0.99 ± 0.06 | 0.95 ± 0.10 | 1.18 ± 0.09 | 1.12 ± 0.10 | 0.82 ± 0.11 | 0.70 ± 0.18 | 0.80 ± 0.03 |
|           | TC      | 1.81 ± 0.13 | 1.45 ± 0.07 | 1.27 ± 0.02 | 1.30 ± 0.03 | 1.84 ± 0.09 | 1.00 ± 0.13 | 1.09 ± 0.22 | 1.22 ± 0.02 | 2.00 ± 0.15 | 0.92 ± 0.10 | 0.95 ± 0.07 | 1.16 ± 0.19 |
|           | HDL-C   | 0.52 ± 0.06 | 1.13 ± 0.04 | 1.28 ± 0.05 | 1.20 ± 0.03 | 0.57 ± 0.10 | 1.40 ± 0.04 | 1.40 ± 0.04 | 1.32 ± 0.02 | 0.50 ± 0.14 | 1.52 ± 0.09 | 1.64 ± 0.02 | 1.36 ± 0.19 |
|           | LDL-C   | 1.42 ± 0.14 | 1.38 ± 0.09 | 1.20 ± 0.02 | 1.16 ± 0.07 | 1.37 ± 0.08 | 1.33 ± 0.16 | 0.98 ± 0.13 | 0.96 ± 0.03 | 1.46 ± 0.13 | 1.22 ± 0.07 | 0.97 ± 0.13 | 0.44 ± 0.05 |

**Table 2**: Effects of DCM-MeOH leaf extracts of *C. edulis* on serum lipid profiles in normal rats.

All values are expressed as mean ± SEM for five animals per group. Values followed by the same superscript are not significantly different (p>0.05 analysed by ANOVA and Tukey’s post hoc test). Means are compared among the extract dose levels with the same day. Dose 1: 50 mg/kgbw; Dose 2: 75 mg/kgbw; Dose 3: 100 mg/kgbw.

Generally, during the twenty-one days of this experimental study, the levels of serum lipid profiles did not change significantly among the normal rat models in the controls of all groups (Tables 1 and 2).

## Effects of DCM-MeOH leaf extracts of *C. edulis* on serum lipid profiles in normal rats

The DCM-MeOH leaf extracts of *C. edulis* induced changes in lipids and related parameter profiles in normal rats (Table 1). After twenty-one days of administration of the extract at the dose level of 50 mg/kgbw, the TG and LDL-C levels significantly decreased (p<0.05; Table 1). After seven, fourteen and twenty-one days of extract administration at the same dose level, there was a significant decrease in TC levels (p<0.05; Table 1). The concentration of HDL-C, however significantly increased after fourteen and twenty one days of extract administration (p<0.05; Table 1).

The DCM-MeOH leaf extracts of *C. edulis* at the dose level of 75 mg/kgbw caused significant decrease in the levels of TG after fourteen and twenty-one days of administration (p<0.05; Table 1). The TC levels also decreased significantly after twenty-one days of administration at this dose level (p<0.05; Table 1). The levels of HDL-C, however, significantly increased after twenty-one days of administration of the extract at this dose level (p<0.05; Table 1), while the levels of LDL-C was not significantly affected by this extract dose level.

The DCM-MeOH leaf extracts of *C. edulis* at the dose level of 100 mg/kgbw caused significant decrease in the concentration of TG after seven, fourteen and twenty one days of oral administration (p<0.05; Table 1). The levels of TC and LDL-C significantly decreased after twenty-one days of extract administration (p<0.05; Table 1). The HDL-C concentration, however, was not affected by this dose level.

When the level of lipids and related parameter profiles in normal rats were compared on specific day across all the dose concentrations, it was evident that there were significant changes (Table 2). After seven days of DCM-MeOH leaf extracts of *C. edulis* administration, the levels of TG and TC decreased significantly at all the dose levels (p<0.05; Table 2). Concentration of HDL-C increased significantly at all the dose levels after seven days of administration of the extract (p<0.05; Table 2), while the levels of LDL-C decreased significantly at the dose levels of 75 mg/kgbw and 100 mg/kgbw (p<0.05; Table 2).

After fourteen days of DCM-MeOH leaf extracts of *C. edulis* administration, the levels of TG and TC decreased significantly at all the dose levels (p<0.05; Table 2). The concentration of HDL-C increased significantly at all the dose levels after fourteen days of administration (p<0.05; Table 2), while the levels of LDL-C decreased significantly at extract dose levels of 75 mg/kgbw and 100 mg/kgbw (p<0.05; Table 2).

After twenty-one days of DCM-MeOH leaf extracts of *C. edulis* administration, the levels of TG, TC and LDL-C decreased significantly at all the dose levels (p<0.05; Table 2). The concentrations of HDL-C, however, increased significantly at all the dose levels after twenty-one days of extract administration (p<0.05; Table 2).

## Phytochemical screening

Qualitative phytochemical screening of the DCM-MeOH leaf extracts of *C. edulis* revealed the presence of alkaloids, flavonoids, phenolics, terpenoids and traces of steroids. However, saponins and cardiac glycosides were absent in the leaf extracts (Table 3).

| Phytochemicals | Presence/Absence |
|----------------|------------------|
| Alkaloids      | ++               |
| Flavonoids     | ++               |
| Steroids       | + (trace)        |
| Saponins       | -                |
| Cardiac glycosides | -            |
| Phenolics      | ++               |
| Terpenoids     | ++               |

**Table 3**: Phytochemical composition of DCM-MeOH leaf extract of *Carissa edulis*.
Present phytochemicals are denoted by (+) sign, absent phytochemicals are denoted by (-) sign while (+) trace denote slightly present phytochemicals.

Discussion

Lipid profile is a panel of blood tests that serves as an initial broad medical screening tool for abnormalities in lipids such as high cholesterol and triglycerides levels [2]. Blood act as a pathological reflector of the status of exposed animals to toxicants and other conditions and/or agents [30]. In the present study, the DCM-MeOH leaf extracts of C. edulis demonstrated varying degrees of serum lipid profile changes in normal rats at the dose levels of 50, 75 and 100 mg/kg bw.

Alterations in the concentration of major lipids like cholesterol, high-density lipoprotein cholesterol, low density lipoprotein cholesterol and triglycerides could avail useful information on the lipid metabolism as well as predisposition of the heart to atherosclerosis and its associated coronary heart diseases [31]. Administration of DCM-MeOH leaf extracts of C. edulis caused significant reduction in the serum levels of total cholesterol, triglycerides and LDL-C and increased the level of HDL-C in normal rats. This suggests protective potential of the extract against hyperlipidemia, which may be beneficial in preventing diabetic complications and cardiovascular disorders.

High blood cholesterol concentration is one of the important risk factors for cardiovascular diseases [32]. Thus the reduction in serum total cholesterol concentration effected by the DCM-MeOH leaf extracts of C. edulis is beneficial and may reduce the risk of cardiovascular disease. Agents that have the ability to lower cholesterol concentration in the blood have been reported to reduce vascular resistance by improving endothelial function [32]. Similar alterations in lipid as well as haematological profiles were reported in various other plant extracts such as Bulbine natalensis, Bougainvillea spectabilis leaves [32], and Fadogia agrestis stem [33].

The extract may have achieved this by blocking cholesterol biosynthesis [34], probably through binding and reducing the production of bile acids or by increased generation of propionate, which has been shown to reduce cholesterol levels [35]. Further, the reduction of the lipid levels by DCM-MeOH leaf extracts of C. edulis may have been mediated by control of lipid metabolism [36]. Preliminary phytochemical screening of the plant revealed the presence of flavonoids, alkaloids and tannins. Many nutritional factors such as tannins have been reported to contribute to the ability of herbs to improve dyslipidemia [37]. The lipid lowering activity of DCM-MeOH leaf extracts of C. edulis may also be attributed to its phytochemical constituents. These findings suggest that C. edulis may be effective in ameliorating cardiac disease complications seen in hyperlipidemia situations. The prevention of oxidation of low density lipoprotein-cholesterol by the antioxidant compounds like polyphenolics and flavonoids is also important in the prevention of cardiovascular diseases. These phytochemicals are helpful in treating various other diseases [37].

The dose sensitive increase in the concentration of HDL-cholesterol and reduction of the VLDL-cholesterol in this study showed that C. edulis can be used to treat cardiovascular diseases and coronary heart diseases. This justifies its use in folk medicine for the treatment of cardiovascular diseases. Further to this is the presence of flavonoids and other poly-phenolic compounds in the DCM-MeOH leaf extracts of C. edulis, which have the ability to scavenge free radicals, therefore, acting as antioxidants [37]. It has been established that free radical scavengers help prevent cardiovascular diseases by interfering with the oxidation of the VLDLs and LDLs, which are key drivers of atherosclerosis. This is in agreement with a similar study carried out by [38], which showed that alcoholic leaf extracts of A. aspera had lipid lowering effects on triton induced hyperlipidemic rats.

Conclusion

In conclusion, the present study showed that oral administration of DCM-MeOH leaf extract of C. edulis in normal rats causes significant reduction in the levels of TG, TC and LDL-C as well as significant increase in the levels of HDL-C which shows that the extracts have potential to selectively regulate the components of serum lipid profiles. This is attributed to the presence of phytochemical compounds which have the ability to scavenge for free radicals, and also regulate the lipid metabolism processes. The present study, therefore, scientifically confirms and supports the traditional use of leaves of C. edulis in regulating serum lipid components and generally improving health.

Recommendations

1. A similar study should be undertaken to evaluate the effects of C. edulis on serum lipid profiles on rat models suffering from lipid related disorders.
2. Further studies should be conducted to verify mode of action of this plant.

Acknowledgement

The authors would wish to acknowledge support in form of the opportunity and facilities provided by Kenyatta University during this research study and the technical assistance provided by Mr. James Adino and Mr. Daniel Gitonga of Biochemistry and Biotechnology Department, Kenyatta University.

References

1. Vijaya K, Ananthan S, Nanithi R (1995) Antibacterial effect of the aalvin, polyphenon 60 Camella sinensis and E. hirta on Shigella spp, A culture study. Journal of Ethnopharmacology 49: 115-118.
2. Criqui MH, Heiss G, Cohn R, Cowan LD, Suchindran CM, et al. (1993) Plasma triglyceride level and mortality from coronary heart disease. National Journal of Medicine 328: 1220-1225.
3. Nelson DL, Cox MM (2000) Lehninger, Principles of Biochemistry. Worth Publishing: New York, 3: 153-156.
4. Aminoff MJ (2004) Nervous System In: Current medical diagnosis and Treatment. The McGraw- Hill Company 956-963.
5. Lampe MA, Burlingame AL, Whitney J, Williams ML, Brown BE, et al. (1983) Human stratum corneum lipids: characterization and regional variations. Journal of Lipid Research 24: 120–130.
6. Hanukoglu I (1992) Steroidogenic enzymes: structure, function, and role in regulation of steroid hormone biosynthesis. Journal of Steroidal Biochemistry and Molecular Biology 43: 779–804.
7. Frederickson DS, Lees RS (1965) A system for phenotyping hyperlipoproteinemia. Circulation Journal 31: 321–7.
8. Schwenke EC, Carew TE (1989) Initiation of atherosclerotic lesions in cholesterol fed rabbits. Atherosclerosis 9: 895-907.
9. Luc G, Fruchart JC (1991) Oxidation of lipoproteins and atherosclerosis. American Journal of Clinical Nutrition 53: 2065-2095.
