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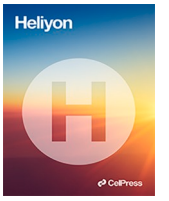

Research article

In vivo toxicity, anti-hyperlipidaemic, antioxidant and anti-atherogenic activities of 'LIPO A' A traditional herbal product in rodents

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ABSTRACT

Hyperlipidemia accounts for about 17 million deaths worldwide each year. High cost and side effects have limited the use of conventional anti-lipidaemic agents in some cases, majority of whom resort to traditional medicine. The current research focused on validating the safety and efficacy of a herbal product, 'LIPO A' used in the management of hyperlipidaemia. Induction of hyperlipidaemia was achieved by oral administration of 3 mL of cholesterol in coconut oil for 4 weeks in male Sprague Dawley rats with water available as 40 % sucrose. Subsequently, the animals were treated with 100, 200 and 400 mg/kg of the product 'LIPO A' for 4 additional weeks with atorvastatin as reference drug (at 2 mg/kg body weight). Blood samples were taken for serum biochemistry and atherogenic ratios were then calculated. 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) scavenging assay, total antioxidant capacity, physicochemical and phytochemical analysis were also carried out using standard methods. Treatment resulted in a dose-dependent reduction in total cholesterol with maximum reduction of 46.01 % at 400 mg/kg compared to atorvastatin with 49.30 %. There were significant changes in the low-density lipoprotein cholesterol and high-density lipoprotein cholesterol (LDL-c/HDL-c) and Total Cholesterol (TC/HDL-c) ratios which measures the atherogenic and coronary risk indices respectively. Acute and subacute toxicity studies did not reveal any signs of toxicity. High Performance Liquid Chromatography (HPLC) fingerprint revealed six well resolved peaks with two prominent compounds with retention times 24.88 and 23.95 min, which could serve as quality control markers for the product. The herbal product showed considerable antihyperlipidemic and antioxidant actions in rodent models and lend credence to its use in traditional medicine for hyperlipidaemia.

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1. Introduction

Hyperlipidaemia refers to the persistent elevated levels of lipids in the bloodstream. Such lipids include triglycerides, phospholipids, very low-density proteins (VLDL), low density lipoproteins (LDL) and high-density lipoproteins (HDL). It is a major risk factor for cardiovascular diseases (CVDs), cerebrovascular accidents, diabetes and several other diseases [1]. CVDs are responsible for over 17.3 million mortalities yearly, with a projection to greater than 23.6 million by the year 2030 [2].

Persistent elevated lipids in the blood could lead to atherosclerotic plaques forming within the vascular walls of veins, arteries and capillaries. A dislocation of such plaques could result in the formation of a thrombus or an embolus which could lead to cardiovascular diseases such as deep vein thrombosis and pulmonary embolism [3]. It could also result in ischaemic stroke [4]. The mainstay conventional therapy for hyperlipidaemia involves the use of drugs such as the statins, fibric acid derivatives, bile acid sequestrants, nicotinic acid derivatives and ezetimibes. It is recommended that for the treatment of hyperlipidaemia, statins should be the first drug of choice to achieve a maximal effective response before the addition and/or substitution with any other anti-hyperlipidaemic drug [5]. Hence, the statins are the widely used drugs worldwide and are even assumed to be the most effective anti-hyperlipidaemic drug with its additional advantage of preventing cardiovascular complications [6]. Over the years, they have shown major successes in the reduction of blood cholesterols [7]. Currently, statins still remain the first-choice drug in the pharmacological approach to the reduction of blood lipid levels [8].

The adverse effects of the first line treatment drugs (statins) which includes hepatotoxicity, muscle disease, acute renal failure, cataracts, and an increased risk of diabetes mellitus are now of great concern [9]. Aside the adverse effects, the high cost of these conventional drugs affects compliance [10,11]. In low- and middle-income countries, patients find it difficult to afford such drugs over a long period of time, thereby leading to poor compliance, and thus, ineffective therapy. Sometimes the availability of the drugs poses a challenge especially, to people in the rural communities. Therefore, majority resort to alternatives which are easily available and affordable with minimal adverse effects. There are several reports of plants and their products being used to manage hyperlipidaemia. Examples include *Trigonella adscendens* [12], *Monascus purpureus* [13] and *Allium sativum* [14,15] which act by lowering blood cholesterol, triglycerides and/or low-density lipoprotein cholesterol (LDL-c) levels. Other medicinal plants are also known to increase the levels of high-density lipoprotein cholesterol (HDL-c) [16,17].

In Ghana, it is reported that over 60 % of the people use herbal medicines because of its cultural acceptability, acclaimed safety and efficacy, affordability and availability [18]. Herbal medicines are used in several hospitals for the management of hyperlipidaemia in Ghana as the country has integrated traditional medicine to mainstream orthodox care. However, some of these herbal remedies have not been scientifically validated. This work therefore aimed at validating the antihyperlipidaemic potential of one of such indigenous products coded 'LIPO A'. It is an aqueous decoction prepared from *Citrus limon* and *Allium sativum* and used for the management of obesity and hypercholesterolemia. The aim of the study was to establish whether 'LIPO A'could be utilized as an alternative to conventional antilipidaemic agents in clinical use.

2. Materials and methods

2.1. Chemicals, reagents, media and equipment

Analytical grade solvents chloroform and methanol were obtained from VWR chemicals BDH (France). HPLC grade methanol and trifluoroacetic acid were purchased from Sigma-Aldrich (Steinheim, Germany). Similarly, analytical grade reagents and chemicals such as ammonium molybdate, sulphuric acid, Di-Sodium Hydrogen Phosphate Dodecahydrate (Na_2HPO4), Ascorbic acid, DPPH, Nitric Acid ($HNO_{3)}$ and Hydrochloric Acid (HCl) were obtained from Sigma-Aldrich (Steinheim, Germany). Atorvastatin (Lipitor) was obtained from Pfizer, USA. All media for the microbiological analysis; MacConkey agar, Dextrose agar, cetrimide nutrient agar, Bismuth sulphite agar and nutrient agar were obtained from Oxoid limited, UK. Equipment used included Mettler Toledo electronic balance (ME204 ME, Vietnam), Gallenkamp incubator and oven (United Kingdom), autoclave (model YX-12L, China) and Stuart colony counter (SC6, England). EDTA and gel separator tubes were obtained from BD Biosciences, San Jose, Canada.

2.2. Product (test sample) information

The herbal product used for the study, coded 'LIPO A', was obtained from a traditional medicine practitioner in Kumasi, Solak Herbal Industry, Ghana. According to the practitioner it was prepared by boiling a mixture of *Cymbopogon citratus* Stapf, *leaves* (1 kg), and *Allium sativum* L (1 kg) with 10 L of water for 1 h. The resultant decoction is allowed to cool and bottled. Twenty-four samples of the product (in 750 mL ambered coloured plastic bottles) were provided by the practitioner for this research. It is brown in colour with a bitter taste and characteristic odour of garlic. It was manufactured on the August 6, 2021 with a shelf life of two years. According to the manufacturer information, 45 mL is to be taken by an adult twice daily for 21 days for hyperlipidaemia.

2.3. Animal husbandry and groupings

Ten female and eighty normolipidaemic (NC) male Sprague Dawley rats, weighing between 160 and 180 g were purchased from the Centre for Plant Medicine Research (CPMR), Mampong, Akwapim, in the Eastern region of Ghana and sent to the animal house of the Department of Pharmacology, KNUST, where they were acclimatized for two weeks. Animals were housed in groups of 10 at room temperature and had access to food and water *ad libitum*.

2.4. Acute toxicity studies

The test was done using the Organisation for Economic Co-operation and Development (OECD) guidelines [19]. Ten female Sprague-Dawley (SD) rats were acclimatized to laboratory conditions for 7 days prior to the initiation of dosing. The animals were fasted overnight prior to testing. The rats were given the product 'LIPO A' by gavage using a ball-tipped intubation needle fitted on a syringe at a dose of 3000 mg/kg. Administration of the product to the rats was a single event. Observations of pharmacotoxic signs, if any, were made at 10, 30, 60, and 120 min and at 4 and 6 h after dosing during the first day and daily thereafter for 14 days. The time of onset, intensity, and duration of any symptoms, if any, was recorded.

2.5. Antihyperlipidaemic assay

Animal experimental procedures was done after ethical approval for the study was obtained from the Committee on Animal Research, Publication, and Ethics, Department of Pharmacology, Kwame Nkrumah University of Science and Technology with approval number (KNUST/Cology/012). Also, guidelines for the Helsinki declaration for the care of laboratory animals were strictly followed [20]. The animals were randomly assigned to six groups: normolipidaemic/normal control (NC), hyperlipidaemic group/-negative control (HL), atorvastatin group (2 mg/kg body weight) and three treatment groups for LIPO A (100, 200 and 400 mg/kg body weight). NC and HL groups had 10 animals whereas the other groups had five animals each.

Induction of hyperlipidaemia was done using the protocol outlined by Kim et al. [21], with some modifications. Instead of a cholesterol rich diet, coconut oil was used to prepare 2%w/v cholesterol which was administered 3 mL daily for 28 days (4 weeks). Additionally, the animals had free access to water containing 40 % sucrose and standard rat pellets diet. The NC group, however, did not receive the 2 % cholesterol in coconut oil and had access to clean tap water instead of 40%w/v sucrose. The animals were observed daily and their body weight checked weekly. Five animals each from the NC and HL (negative control) groups were euthanized after 4 weeks and blood drawn through cardiac puncture into Ethylenediaminetetraacetic Acid (EDTA) tubes for haematological analysis and gel separator tubes for serum biochemistry. The sera were separated through centrifugation at 3000 rev/min for 10 min and stored at −20 °C for the biochemical analysis. The parameters analysed included the total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-c), very low-density lipoprotein cholesterol (VLDL-c), Low density lipoprotein cholesterol (LDL-c), liver enzymes and creatinine as a measure of renal function. This was to help ascertain the establishment of hyperlipidaemia in the HL and other groups fed with 2 % cholesterol in coconut oil and to obtain baseline data for the establishment of the toxicity or otherwise of the traditional medicinal product 'LIPO A'. Successful induction of hyperlipidaemia was done by comparing the lipid levels of NC and HL groups. Subsequently the animals were treated with 100, 200 and 400 mg/kg body weight doses of 'LIPO A' and atorvastatin (2 mg/kg) for four additional weeks. The HL group did not receive any treatment but had access to standard rat pellet and water. At the end of the period, the animals were sacrificed humanely under diethyl ether euthanasia by introducing the animals into a saturated chamber containing 500 mL of diethyl ether mixed with wood shavings. They were subsequently sacrificed, blood samples drawn, and sera separated as described previously. The haematological and biochemical analysis followed the same protocol. The ratios of atherogenicity and coronary risk, TC/HDL and HDL/LDL, were then calculated [22].

2.6. Antioxidant assays

The DPPH radical scavenging activity and total antioxidant capacity of the herbal product was assessed as a measure of the protective effect of the herbal product 'LIPO A' against oxidative damage which characterises hyperlipidaemia.

In the DPPH radical scavenging assay, 'LIPO A' and the standard antioxidant ascorbic acid were tested at concentrations of 15–500 μg/mL, prepared by two-fold dilution. The test was carried out as reported by Govindappa et al. (2011) [21]. Briefly 1 mL of the different concentrations of the product was added to 3 mL of DPPH (0.002 % in methanol) and incubated in the dark for half an hour after which absorbance was measured at 517 nm using a spectrophotometer (Cecil CE 7200 spectrophotometer, England). DPPH in methanol (0.002 %) was used at the blank. The same procedure was followed for ascorbic acid. All experiments were done in triplicates. The percentage free radical scavenging activity was calculated using the formula:

$$\%\ DPPH\ inhibition = \left\| 1 - \left[\frac{A_{agent}}{A_{control}} \right] x\ 100 \right\|$$

With A (agent) representing the absorbance of the extract and A (control) as the absorbance of the control.

In the total antioxidant capacity assay, the method outlined by Prieto et al. [23], was followed. An aliquot of 1 mL of the product 'LIPO A' (500–15 μg/mL) was mixed with 3 mL of a reagent consisting of 6 mM sulphuric acid, 28 mM disodium hydrogen phosphate and 4 mM of ammonium molybdate. The mixture was incubated at 95 °C for 1 h 30 min and 200 μL of the cooled mixture transferred into a micro-titre plate and the absorbances read at 695 nm. The reference antioxidant ascorbic acid at concentrations of 100–3.13 μg/mL was taken through the same procedure and used to generate a standard calibration curve. The total antioxidant capacity was then calculated from the calibration curve as ascorbic acid equivalent (AAE) in mg per g of the product.

2.7. Physicochemical assessment

The physicochemical properties of the product such as the pH, weight per mL and content of toxic metals were all assessed.

2.7.1. pH and weight per unit volume

The pH of the product was determined using Mettler-Toledo pH meter FE20/FG2 (Fisher Scientific UK Ltd). About 250 mL of the herbal product was transferred into 500 mL beaker. The probe of the pH meter was then inserted into the solution until a steady pH was obtained. This was repeated two more times using other samples. In the determination of the weight per mL, three batches, 250 mL each, of the product was evaporated to dryness in an oven at 70 °C for 48 h until constant weight was achieved. The average weight per mL was then determined from the dried extract [24,25].

2.7.2. Heavy metal content of LIPO A

The heavy metal content of the herbal product LIPO A was estimated using an auto sampler atomic absorption Spectrophotometer (AAS) (Model ICE3000; Thermo Scientific, USA). Iron, cadmium, lead, zinc, copper and arsenic content were determined for the product using the method reported by Turkson et al. [25],. One mL of the product was digested with a 3:1:1 mixture of HNO_3, concentrated HCl and water in a 250 mL beaker. The mixture was heated at 150 °C until the solution became very clear. After the digestion period, the residual solution was made up to the 50 mL mark with deionized water and analysed using AAS. Samples were analysed in duplicates.

2.8. Microbial quality analysis of LIPO A

In the microbial load evaluation of the product LIPO A, the method outlined in the British Pharmacopoeia [26] was followed. MacConkey agar was used for the total viable bacteria count whereas potato dextrose agar served as the media for moulds and yeast. Cetrimide Nutrient agar and Bismuth sulphite were used for *Pseudomonas* and *Salmonella* spp. respectively. All media were prepared in accordance with the manufacturer's specifications.

In the test, the product was shaken vigorously to uniformly distribute the microbial content, if any. One mL was then suspended in 9 mL of sterile distilled aseptically to form the stock solution. The stock solution (1 mL) was then diluted serially by tenfold to afford concentrations up to 10^{-6}. In the estimation of the total viable bacteria count, 1 mL of the dilute solutions were seeded with 20 mL of McConkey agar and mixed thoroughly. For the total fungal count, 1 mL of the dilute solutions was streaked on dried potato dextrose agar. Incubation for bacteria was done at 37 °C for 48 h and 25 °C for five days for yeast and moulds. Specific selective media were used to detect the presence or absence of *E. coli*, *Pseudomonas* and *Salmonella* species. All experiments were done in duplicates. The Stuart colony counter (model SC6) was used for counting and the and the microbial load estimated as the colony forming units per mL (CFU/g). The product of the average number of colonies and the dilution factor, gives the CFU/mL. Values were compared to reference values [26].

2.9. Phytochemical screening and HPLC fingerprint of herbal product

The presence of plant metabolites such as tannins, alkaloids, flavonoids, coumarins, triterpenoids, glycosides and phytosterols were determined as described by standard methods [27,28].

In the chromatographic analysis, a PerkinElmer Flexar HPLC system consisting of a binary LC pump, LC autosampler, PDA plus detector, in-line degasser equipped with Chromera software was used to develop an HPLC fingerprint of the herbal product and one of its plant components, *Cymbopogon citratus*. Ten (10) mL of the product was measured and filtered through a membrane filter (0.45 μm). It was then sonicated for about 10 min. The samples were then filtered using 0.45 μm membrane filter (Thermo Fischer Scientific, USA) into 2.5 mL vials and set in the HPLC autosampler for injection. A decoction of lemon grass, prepared by boiling 500 g of the material with 5L of distilled water for 2 h, was also prepared for HPLC analysis as described above. Injections (injection volume 20 μL) were done in triplicates. The components were eluted on a 3.9 × 300 mm Phenomenex C18, 5 μm column at a flow rate of 1 mL/min using 0.05 % TFA (A) and methanol (B) as mobile phase in a linear gradient from 95 % TFA to 95 % methanol in 40 min. The wavelength for detection was 225 nm.

2.10. Statistical analysis

Data were computed as means ± standard deviation (SD). Graph Pad Prism 8 software (San Diego, CA, USA) was used for all statistical and graphical representation of data. For the animal studies, comparisons of means of treated groups with negative control and normal control groups were done using one-way ANOVA followed by Dunnett's post-hoc multiple comparison test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Acute toxicity studies

'LIPO A' had no-adverse-effect following oral administration at a dose of 3000 mg/kg per body weight. All the SD rats survived and physical observation did not reveal any signs of toxic effect such as changes on skin, eyes and mucus, behaviour patterns, trembling, diarrhoea, falling of the fur, sleep or coma. No observable changes were seen in their body weights after 14 days.

3.2. Antihyperlipidaemic activity of LIPO A

3.2.1. Changes in body weight

Oral administration of 2 % cholesterol in coconut oil with 40 % sucrose water, available *ad libitum,* for 4 weeks (induction period) resulted in considerable increase in body weight of all the animal groups except the normal control group which did not receive the cholesterolaemic agents and were also not given the 40 % sucrose supplementation. Slight increase in body weight was seen after the first week which increased steadily up to the 4th week (Fig. 1). The normolipidaemic group (NC) recorded a mean percentage increment of 2.29 ± 0.397 by week 4 of induction compared to 6.81 ± 0.864 of the hyperlipidaemic negative control (HL). The highest increase in body weight, for the experimental group, was seen in those designated to receive 400 mg of the herbal product (Fig. 1).

The increase in weight of the normal control (NC) group was compared to the other animal groupings using one way analysis of variance (ANOVA) with Dunnett's *post hoc* test. There was significant ($P < 0.05$) increase in weight of all groups compared to the NC group. The percentage increase in weight of negative control (HL) compared to the other treatment groups was, not significant ($P > 0.05$).

3.2.2. Anti-hyperlipidaemic activity

Treatment of hyperlipidaemic rats for 4 weeks using the traditional herbal product 'LIPO A' resulted in a dose-dependent inhibition of any further increase in body weight due to consumption lipogenic agent. There was also considerable reduction in the weight of animals treated with atorvastatin and 200, 400 mg/kg body weight of 'LIPO A' (Fig. 2). Atorvastatin dosed orally at 2 mg/kg, resulted in a reduction in body weight by 23.15 % after 4 weeks of treatment whereas 'LIPO A' dosed at 200 and 400 mg/kg resulted in respective body weight reductions of 9.19 and 18.82 % in the rodents. At the 100 mg/kg dose, treatment with the herbal product resulted in a slight increase in body weight by 5.44 %. The negative control (HL) group recorded a 36.54 % increase in weight after the treatment phase whereas the normal control group, fed standard rat pellet diet within the same period registered a marginal increase in body weight (Fig. 2). A two tailed unpaired *t*-test, with Welch's correction of each group after induction of hyperlipidaemia and after treatment, showed no significant difference for the normal control and the groups treated with the 100 and 200 mg/kg doses of the herbal product. The changes in body weight after induction and treatment was, however, significant ($P < 0.05$) for the 400 mg/kg of 'LIPO A', atorvastatin and the negative control groups (Fig. 2).

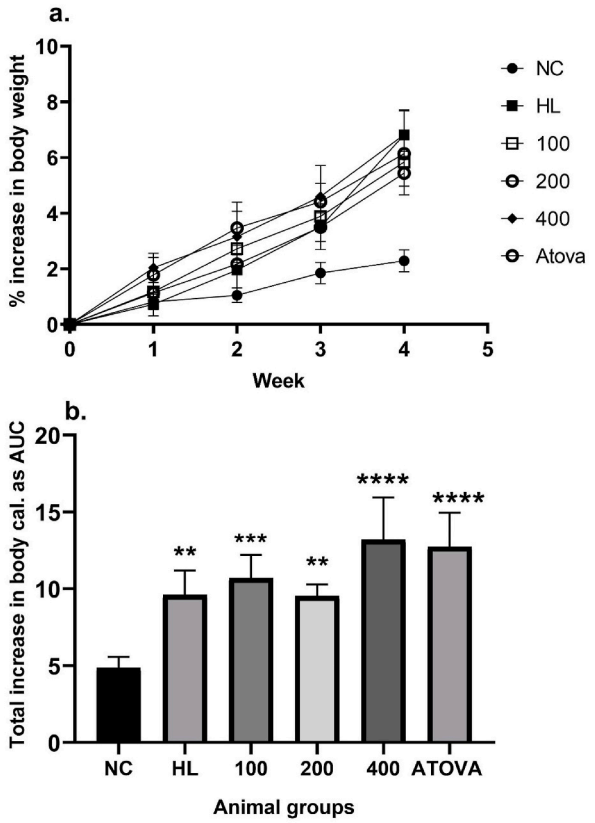

Fig. 1. Changes in body weight of animal groups over 4 weeks induction with lipogenic agent. **a.** time course curve and **b**. represented as area under time course curve. Values are mean ± SD (N = 5). NC: normal control, HL: negative control. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.001 compared to the normal control (One-way ANOVA followed by Dunnett's *post hoc* test).

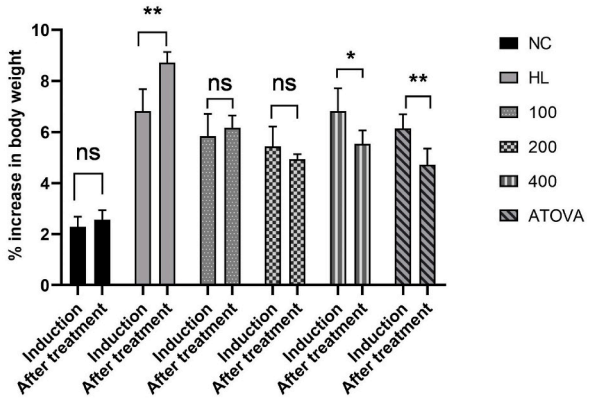

Fig. 2. Changes in body weight of animal groups after 4 weeks induction of hyperlipidaemia and 4 weeks of treatment. Values are mean ± SD (N = 5). NC: normal control, HL: negative control. Two-tailed unpaired *t*-test with Welch's correction used to compare each group before and after treatment. *P < 0.05, **P < 0.01.

The NC group (normal control) recorded significantly (P < 0.05) lower lipid levels compared to the negative control (HL) group indicating that 4 weeks administration of 2 % cholesterol in coconut oil and sucrose water was enough to induce a state of hyperlipidaemia in rodents (Fig. 3). Similar elevated lipid levels were also observed in the other treatment groups after the induction period. However, the differences in lipid levels among the treatment groups and the negative control was not statistically significant (P > 0.05).

Daily supplementation of normal rat pellets with 2 % cholesterol in coconut oil for 4 weeks and 40%w/v sucrose resulted in significant increase in total cholesterol levels (TC) by 47.89 %, triglyceride levels (TG) by 47.89 %, low density lipoprotein cholesterol (LDL-c) by 201.75 and very low density lipo-protein cholesterol (VLDL-c) by 96.29 % in the negative control group (HL) relative to the normal control (Table 1). These changes were attenuated by treatment with LIPO A and atorvastatin. The herbal product showed significant (P < 0.05) dose dependent decrease in serum cholesterol with maximum reduction of 46.01 % at 400 mg/kg body weight compared to atorvastatin with 49.30 % at 2 mg/kg. In addition, administration of LIPO A for 4 weeks resulted in a significant (P < 0.05) reduction of 64 and 63 % in the levels of LDL-c and VLDL-c respectively at the maximum dose (400 mg/kg). This was comparable to atorvastatin which registered reductions of 68 and 67 % respectively for LDL-c and VLDL-c cholesterol (Table 1). The product and atorvastatin improved the levels of HDL cholesterol, but this was not significant (P > 0.05) compared to the negative control group (Table 1). Significant increase in serum triglycerides were also recorded in the HL group (negative control) but again these elevations were inhibited by treatment with LIPO A and atorvastatin. The product was not active at the lowest dose used (100 mg/kg).

3.3. Ratios of atherogenicity

Atherogenic ratios, LDL-c/HDL-c and TC/HDL-c, which are strong markers for predicting the risk of atherosclerosis and coronary heart disease [22] were determined for all treatment groups (Table 2).

There were significant changes in the LDL-c/HDL-c and TC/HDL-c ratios which measures the atherogenic and coronary risk indices respectively. Atherogenic index decreased from 2.53 in hyperlipidaemic rats to 0.85 in the group treated with LIPO A 400 mg/kg (representing 67.19 %). Similarly, that of atorvastatin decreased to 0.7 (a 72.33 % reduction). In the coronary risk index assessment,

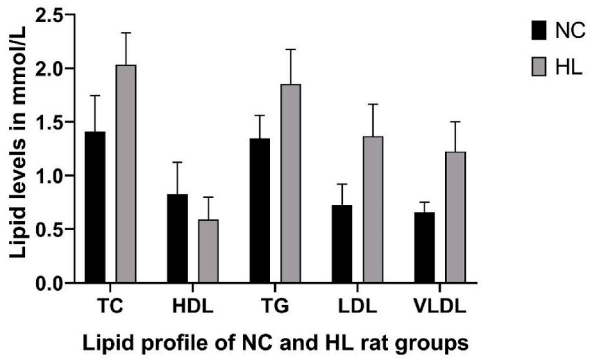

Fig. 3. Lipid profile of normal control and hyperlipidaemic groups after 4 weeks of induction with 2 % cholesterol in coconut oil. Values are mean ± SD (n = 5). Two-tailed unpaired *t*-test with Welch's correction used to compare lipid levels of NC and HL groups after induction. NC: Normal control, HL: negative control.

Table 1
Effect of LIPO A and atorvastatin on serum lipid levels after 4 weeks of treatment.

Treatment Groups	TC	HDL-c	TG	LDL-c	VLDL-c
NC rats + Water	1.28 ± 0.55	0.78 ± 0.57	1.19 ± 0.58	0.57 ± 0.19	0.54 ± 0.62
HL rats + water	2.13 ± 0.15	0.68 ± 0.06	1.76 ± 0.35	1.72 ± 0.12	1.46 ± 1.88
HL + LIPO A 100 mg/kg	1.35 ± 0.34	0.66 ± 0.05	1.48 ± 0.15	1.58 ± 0.16	1.30 ± 1.67
HL + LIPO A 200 mg/kg	1.52 ± 0.09	0.68 ± 0.17	1.34 ± 0.05	*0.85 ± 0.12	*0.67 ± 0.05
HL + LIPO A 400 mg/kg	*1.15 ± 0.11	0.73 ± 0.34	*1.28 ± 0.38	*0.62 ± 0.15	*0.54 ± 0.17
HL + Atorvastatin 2 mg/kg	*1.08 ± 0.11	0.79 ± 0.10	*1.03 ± 0.10	*0.55 ± 0.12	*0.470 ± 0.04

Values are mean ± standard deviation (n = 5), One Way ANOVA followed by Dunnett's post hoc test: Values were compared to the negative control (HL) group. *P < 0.05. TC: total cholesterol, TG: triacyl glyceride, HDL-c: high density lipoprotein cholesterol, LDL-c, low density lipoprotein cholesterol, VLDL-c: very low-density lipoprotein cholesterol, NC: normolipidaemic/normal control, HL: hyperlipidaemic/negative control group, LIPO A: traditional herbal product.

Table 2
Atherogenic index and coronary risk index of treated and untreated groups.

Treatment Groups	AI LDL-c/HDL-c	CRI TC/HDL-c
NC rats + Water	0.73	2.28
HL rats + water	2.53	3.13
HL rats + LIPO A 100 mg/kg	2.39	2.76
HL rats + LIPO A 200 mg/kg	1.25	2.72
HL rats + LIPO A 400 mg/kg	*0.85	*2.40
HL rats + Atorvastatin 2 mg/kg	*0.70	*2.13

AI: Atherogenic index, CRI: coronary risk index, HDL-c: high density lipoprotein cholesterol, LDL-c, low density lipoprotein cholesterol, NC: normal/naive control, HL: hyperlipidaemic control group, LIPO A: is the traditional herbal product. Values are mean (n = 5), *P < 0.05.

treatment with 'LIPO A' and atorvastatin resulted in significant (P < 0.05) reduction in the TC/HDL-c ratios (Table 2).

3.4. Effect of treatment on vital organs

Four weeks of induction of hyperlipidaemia and 4 weeks of treatment with the herbal product LIPO A and atorvastatin as reference agent, did not result in significant changes in the organ weights, including the heart, liver, kidney, and spleen of the various groups of experimental animals. However, the spleen of the hyperlipidaemic (untreated) group was significantly (P < 0.001) larger than the normolipidaemic control (Table 3). Examination of the liver of NC and HL groups revealed fatty deposition, evidenced by the pale colour of the organ.

3.5. Subacute toxicity

Daily administration of the animals with LIPO A and atorvastatin for 4 weeks did not result in significant (P > 0.05) changes in haematological parameters (Table 4) of the treated and untreated groups (NC and HL). Similarly, there were no significant changes in the levels of the liver enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP). Similarly, serum globulins, albumins and total proteins were not significantly (P > 0.05) affected by treatment when compared to the normolipidaemic group (Table 5). Blood creatinine levels did not show significant differences (P > 0.05) between control and treatment groups. Overall, toxicologically significant changes were not observed in the parameters in relation to treated and the control groups (Tables 4 and 5).

Table 3
Organ weights of animals after treatment with LIPO A.

Treatment Groups	**Organ weights of treated and untreated animals (g)** Kidney	Liver	Heart	Spleen
NC rats + Water	0.77 ± 0.21	7.85 ± 1.96	0.91 ± 0.14	0.63 ± 0.16
HL rats + water	0.73 ± 0.02	7.78 ± 1.96	0.77 ± 0.083	*1.53 ± 0.11
HL rats + LIPO A 103.5 mg/kg	0.71 ± 0.07	7.82 ± 0.20	0.99 ± 0.07	0.62 ± 0.13
HL rats + LIPO A 207 mg/kg	0.75 ± 0.03	7.98 ± 0.45	0.93 ± 0.17	0.73 ± 0.16
HL rats + LIPO A 414 mg/kg	0.78 ± 0.05	8.50 ± 0.39	0.92 ± 0.20	0.63 ± 0.10
HL rats + Atorvastatin 2 mg/kg	0.76 ± 0.12	7.44 ± 0.59	0.89 ± 0.23	0.73 ± 0.34

Values are mean ± standard deviation (n = 5), One Way ANOVA followed by Dunnett's post hoc test: Values were compared to the normolipidaemic control. *P < 0.0001. NC normolipidaemic group, HL hyperlipidaemic group.

Table 4
Effect of treatment with the herbal product LIPO A and atorvastatin on haematological parameters.

Haematological parameters	NC	HL (control)	LIPO A 100 mg/kg	LIPO A **200** mg/kg	LIPO A **400** mg/kg	ATORVASTATIN 2 mg/kg
WBC (10^9/L)	12.9 ± 1.31	12.24 ± 1.84	10.77 ± 1.53	12.7 ± 2.45	10.77 ± 0.52	16.63 ± 4.85
HGB (g/dl)	15.1 ± 0.20	11.72 ± 2.71	14.57 ± 0.91	14.4 ± 1.28	15.3 ± 1.02	12.27 ± 3.99
RBC (10^{12}/L)	7.88 ± 0.20	6.45 ± 1.44	7.81 ± 0.29	7.76 ± 0.47	7.98 ± 0.75	6.51 ± 1.91
HCT (%)	38.57 ± 1.54	30.52 ± 7.58	38.53 ± 2.53	38.13 ± 2.76	39.87 ± 2.25	32.43 ± 9.79
MCV (fL)	49.07 ± 2.77	47.16 ± 3.26	49.37 ± 1.40	49.13 ± 0.61	50.13 ± 1.90	49.87 ± 1.42
MCH (pg)	19.13 ± 0.55	18.08 ± 1.03	18.6 ± 0.46	18.47 ± 0.55	19.13 ± 0.57	18.63 ± 0.61
MCHC (%)	39.13 ± 1.10	38.56 ± 1.25	37.73 ± 0.15	37.67 ± 0.61	38.3 ± 0.56	37.53 ± 1.27
PLT (10^9/L)	575.30 ± 79.0	495.2 ± 160.7	636 ± 178.6	660.7 ± 148.4	590.3 ± 24.01	606.70 ± 22
MPV (fL)	7.53 ± 0.40	8.7 ± 3.19	9.43 ± 3.70	7.2 ± 0.2	7.27 ± 0.23	7.47 ± 0.23
PCT (%)	0.42 ± 0.06	0.43 ± 0.15	0.62 ± 0.10	0.47 ± 0.09	0.43 ± 0.02	0.45 ± 0.17

NC: Normal control, HL: Negative control.

Table 5
Effect of treatment with the herbal product 'LIPO A' and atorvastatin on liver parameters NC: Normal control, HL: Negative control. Values are mean ± SD (n = 5). Values were compared to NC and HL groups by one way ANOVA followed by Dunnett's multiple comparison test. $P > 0.05$. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), serum globulins (GLO), albumins (ALB) and total proteins (TP), TB: Total bilirubin, DB: Direct bilirubiun, IND: Indirect bilirubin, serum creatinine (CRK).

Biochemical parameters	NC	HL	LIPO A 100 mg/kg	LIPO A 200 mg/kg	LIPO A 400 mg/kg	ATORVASTATIN 2 mg/kg
AST **(IU/L)**	178.70 ± 12.580	180.40 ± 16.990	171.40 ± 24.060	189.10 ± 11.930	173.30 ± 19.190	190.4 ± 8.633
ALT **(IU/L)**	92.02 ± 6.252	108.1 ± 7.610	82.03 ± 11.470	97.32 ± 10.620	115.80 ± 40.530	90.01 ± 7.239
GGT **(IU/L)**	1.82 ± 0.153	1.80 ± 0.316	1.82 ± 0.198	1.747 ± 0.064	1.85 ± 0.051	1.78 ± 0.065
ALP **(IU/L)**	12.92 ± 6.871	9.63 ± 2.573	12.21 ± 5.579	10.82 ± 5.757	6.65 ± 0.848	5.86 ± 2.289
GLO **(g/L)**	36.55 ± 0.7485	35.48 ± 2.165	36.72 ± 1.998	40.7 ± 0.876	38.01 ± 2.730	37.64 ± 3.299
ALB **(g/L)**	29.05 ± 0.998	37.51 ± 2.996	35.88 ± 2.681	36.23 ± 1.492	37.19 ± 2.309	35.44 ± 2.106
TP **(g/L)**	65.60 ± 1.362	72.99 ± 1.658	72.60 ± 1.503	76.93 ± 1.244	75.2 ± 0.636	72.84 ± 0.869
TB (μmol/L)	13.19 ± 1.30	0.59 ± 0.10	11.11 ± 0.59	10.78 ± 2.31	10.38 ± 0.97	10.11 ± 0.64
DB (μmol/L)	2.26 ± 0.28	1.03 ± 0.33	1.86 ± 0.19	2.42 ± 0.52	2.41 ± 0.07	2.53 ± 1.34
IND (μmol/L)	10.93 ± 1.35	10.93 ± 0.32	9.25 ± 0.65	8.36 ± 2.60	7.97 ± 1.02	7.58 ± 1.95
CRK **(mmol/L)**	3.195 ± 0.195	2.79 ± 0.081	2.09 ± 0.599	2.79 ± 0.178	2.55 ± 0.117	1.91 ± 0.658

3.6. Antioxidant activity of LIPO A

In the DPPH radical scavenging assay, the product and the reference compound ascorbic acid showed concentration dependent (Fig. 4) activity. The IC_{50} of the product was 80.59 μg/mL whereas that of ascorbic acid was 19.28 μg/mL; thus, about four times more potent than LIPO A. In the total antioxidant capacity assay, 1 g of the herbal product recorded antioxidant effect that was equivalent to the activity of 5.96 mg of ascorbic acid, estimated from the calibration curve.

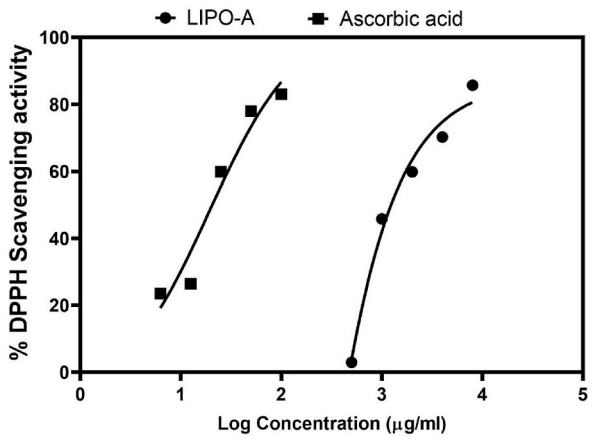

Fig. 4. DPPH scavenging activity of product 'LIPO A' and ascorbic acid.

3.7. Physicochemical parameters of LIPO A

The concentration of the heavy metals Pb, Zn, As, Cd, Cu and Fe in each batch of the product were recorded (Table 6). The levels of the heavy metals in the two batches of the product were not significantly different ($P > 0.05$) from each other. This was seen in a two-tailed unpaired *t*-test with Welch's correction. Cadmium, lead, zinc and arsenic were within permissible levels set by the world health organization [29]. The maximum permissible level of copper and iron have not been set by WHO, but several countries have their own limits.

3.8. Microbial quality analysis

Evaluation of LIPO A for the load of microorganisms revealed pathogenic microbes such as *S. typhi, E. coli* and *P. aeruginosa* to be absent from both batches of the product. Similarly, the total aerobic counts, yeast and moulds were within permissible levels (Table 7). The two batches did not differ significantly ($P > 0.05$) from each other in microbial composition.

3.9. Phytochemical constituents of LIPO A

Phytochemical analysis of the secondary metabolites in the herbal product revealed the presence of alkaloids, triterpenoids, condensed tannins, coumarins and glycosides (Table 8).

3.10. HPLC fingerprint of herbal product

The HPLC chromatogram of the herbal product LIPO A, revealed six conspicuous peaks (Fig. 5) which eluted between 2.57 and 24.87 min. Two prominent peaks eluting at 24.88 and 23.95 min could be used as marker compounds for the quality analysis of the herbal product LIPO A. HPLC fingerprint of one of the plant components listed on the label, lemon grass, revealed three well resolved spots (Fig. 6) which was consistent with some of the compounds in the herbal product 'LIPO A'. For instance, two major peaks eluting at 24.79 and 23.95, had the same retention time and intensity as those in the product. Similarly, a low intense peak eluting at 2.575 min was the same as that in the product. The solvent or blank chromatogram did not reveal any recognizable peak and thus did not contribute to the peaks in the products.

4. Discussion

In this study, daily supplementation of SD rats with 3 mL of 2 % cholesterol in coconut oil and sucrose water (40%w/v) available *ad libitum* for 4 weeks resulted in significant weight gain by the experimental animals compared to the normal control group fed on standard rat pellet diet. Thus, the use of cholesterol in coconut oil and sucrose water in a hyperlipidemic model compares with other well-known procedures such as the high fructose diet, high cholesterol diet, hydrocortisone and Triton induced hyperlipidaemic methods which have yielded excellent results [30].

Daily treatment of the animals for 4 weeks mitigated any increase in the weight of the animals in a dose-dependent fashion. Significant reduction in body weight was seen in the highest dose (400 mg/kg) of LIPO A which was comparable to atorvastatin (2 mg/kg) used as reference drug. Several studies have shown that medicinal plants could be used to control body weight and they do so through stimulation of thermogenesis, inhibition of pancreatic lipase activity, control of appetite, prevention of adipogenesis and promotion of lipolysis [31]. *Allium sativum* (garlic), one of the component herbs of this product, is a known anti-obesity traditional medicine. It is reported that consumption of garlic mitigates weight gain through a thermogenic effect that increases energy expenditure [32,33]. Elsewhere, lemon grass (*Cymbopogon citratus*), also a component of LIPO A, reportedly lowered cholesterol levels in mice after repeated 21-day oral administration [34]. Thus, considerable weight reduction elicited by the product could probably, in part, be due to thermogenesis attributable to its garlic component.

The significant reduction in TC, TG, LDL-c and VLDL-c by the 400 mg/kg dose of the herbal product lends support to the traditional claims of the product as an anti-hyperlipidaemic agent. The reduction in triglycerides levels by plant extracts is ascribed to increased lipoprotein lipase activity which hydrolyses triglycerides to fatty acid or by inhibition of the synthesis of TG's from fatty acids [35]. Other reports suggest that anti-cholesterolaemic medicinal plants exert their actions by inhibiting the absorption of cholesterol and accelerating the catabolism of LDL-c to free up cholesterol for steroid synthesis [36] or transform them into bile acids by the liver. Decreasing the levels of LDL-c in the liver and in serum is the target for several antihyperlipidaemic drugs used in conventional medicine [22]. The reduction in levels of TC, TG, LDL-c and VLDL-c by 'LIPO A' was associated with corresponding elevations in the levels of HDL-c (good cholesterol) at all doses, but these were not significant ($P > 0.05$) when compared to the negative control. Several studies have found, lemon grass and garlic (the main components of the product Lipo A) to exert potent lipid lowering effect [34, 37–39]. In a randomized control clinical trial, 112 individuals who were given a mixture of garlic and lemon grass recorded significant lowering of TC and LDL-c by 40.8 ± 6.1 and 29.8 ± 2.6 % respectively [40]. This corroborates with our findings where 46 % and 63 % reductions in TC and LDL-c respectively were recorded after treatment of hyperlipidaemic rats with LIPO A (garlic and lemon mixture) for 4 weeks.

Phytochemical analysis of the herbal product revealed the presence of alkaloids, tannins, triterpenoids, glycosides and coumarins. Several of these phytochemicals have shown lipid lowering activities in preclinical and clinal studies. The triterpenoid curcumin has been shown to inhibit the intestinal absorption of cholesterol [36]. Other triterpenoids such as ursolic and oleanolic acids have shown

Table 6
Heavy metal content of the herbal product LIPO A.

Element	Concentration in LIPO-A (mg/kg) B1 B2		*Reference (mg/kg)
Iron	84.69 ± 1.12	82.64 ± 2.70	NS
Copper	36.49 ± 0.90	37.71 ± 0.35	NS
Zinc	15.05 ± 0.61	15.11 ± 0.63	50
Cadmium	0.05 ± 0.07	0.05 ± 0.18	0.3
Lead	0.01 ± 0.00	0.01 ± 0.000	10
Arsenic	0.28 ± 0.01	0.03 ± 0.02	5

B1 and B2 are two batches of the same product, *(Dghaim et al., 2015; World Health Organization, 2007), NS: Not set, values are means ± sd (N = 2).

Table 7
Microbial content of herbal product 'LIPO A'.

Microbes	Load (CFU) B1 B2		*BP 2018
Total aerobic counts	1×10^3	1.1×10^3	$\leq 1.0 \times 10^4$
Total yeast and moulds count	1×10^2	0.9×10^2	$\leq 1.0 \times 10^2$
S. aureus	Not detected	Not detected	Absent in 1 mL
P. aeruginosa	Not detected	Not detected	Absent in 1 mL
E. coli	Not detected	Not detected	Absent in 1 mL
S. typhi	Not detected	Not detected	Absent in 1 mL

B1 and B2 are two batches of the same product.

Table 8
Phytochemical constituents of herbal product 'LIPO A'.

Secondary metabolite	Inference
Alkaloids	a
Tannins	a
Glycosides (general)	a
Triterpenoids	a
Coumarins	a
Phytosterols	b
Flavonoids	b

[a] detected.
[b] not detected.

cholesterol lowering effect by enhancing it esterification [41,42]. The phenolic catechin and alkaloid palmatine displayed hypocholesterolaemic effect by promoting the utilization and excretion of cholesterol [43,44]. The polyherbal product LIPO A, containing several secondary metabolites, may exert its anti-hyperlipidaemic activity by one or more of these mechanisms. The product could be explored for the treatment of diabetes, obesity, and cerebrovascular diseases, which are all associated with hyperlipidaemia.

Several evidence indicates that LDL-c, a major contributor of total cholesterol, is a significant risk factor to cardiovascular diseases [45]. As the most atherogenic lipoprotein, circulating LDL-c leaks into arterial endothelial walls where they become oxidized and form atherosclerotic plaques [36]. The ratios TC/HDL-c and HDL-c/LDL-c, which are indexes of atherogenicity and coronary risk [22] were assessed in the present study. The significant reductions in the levels of LDL-c and TC as well as the marginal increments in HDL-c by the herbal product were associated with significant reductions in the atherogenic and coronary risk indexes at the 200 and 400 mg/kg doses of the herbal product compared to the hyperlipidaemic control (Table 2). Similar results were obtained for the reference agent atorvastatin. By lowering the atherogenic index, the herbal product protects the coronary artery, heart, kidney and liver from oxidative damage as well as foam cells or plaque deposition in these organs [46].

The hyperlipidaemic state predisposes organisms to oxidative stress. Thus, further protection of various organs of the body of experimental animals in hyperlipidaemia is afforded by antioxidants. The herbal product scavenged the DPPH radical with IC_{50} that was four times less potent than ascorbic acid. It also showed considerable total antioxidant capacity measured as the ascorbic acid equivalent. Despite promising results seen in animal studies on the role of antioxidants in the prevention of atherosclerosis and cardiovascular diseases in hyperlipidaemic groups, that of human studies have not yielded the desired results. Large-scale population-based studies did not confirm the association between decreased plasma antioxidant or supplementation with antioxidants and decreased cardiovascular risk [47]. It is argued that wrong choice or dose of antioxidants and patient selection were limiting factors in these trials and thus should be given careful consideration in future clinical trials [47].

Induction of hyperlipidaemia and subsequent treatment with the herbal product and atorvastatin did not result in significant changes in organ weight of the animals except the spleen of the negative control group (Table 3) that showed marked changes

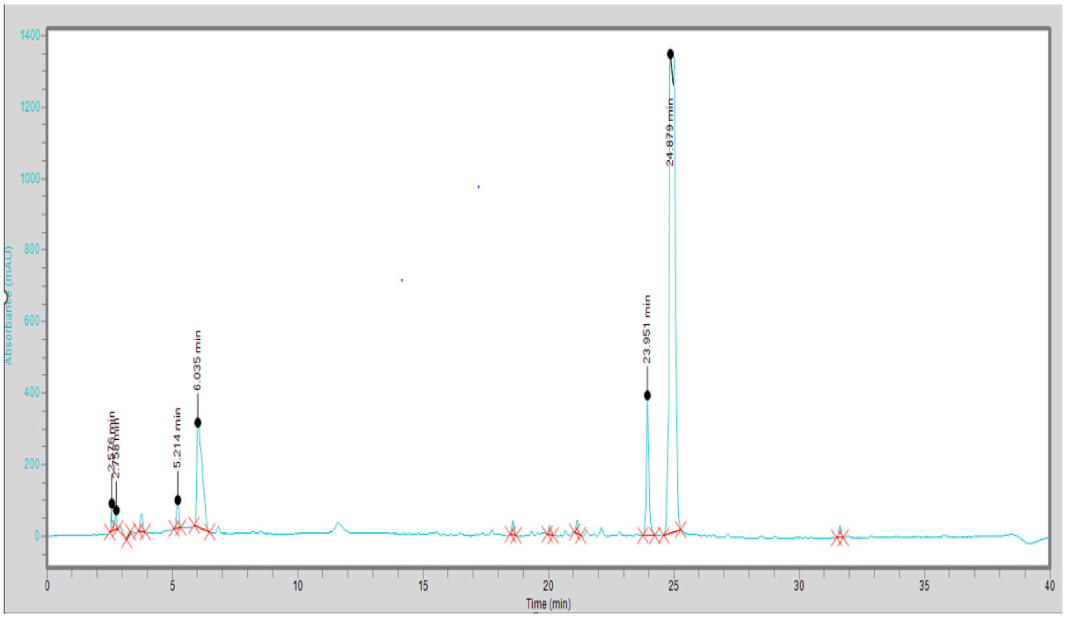

Fig. 5. HPLC fingerprint of aqueous herbal product LIPO A.

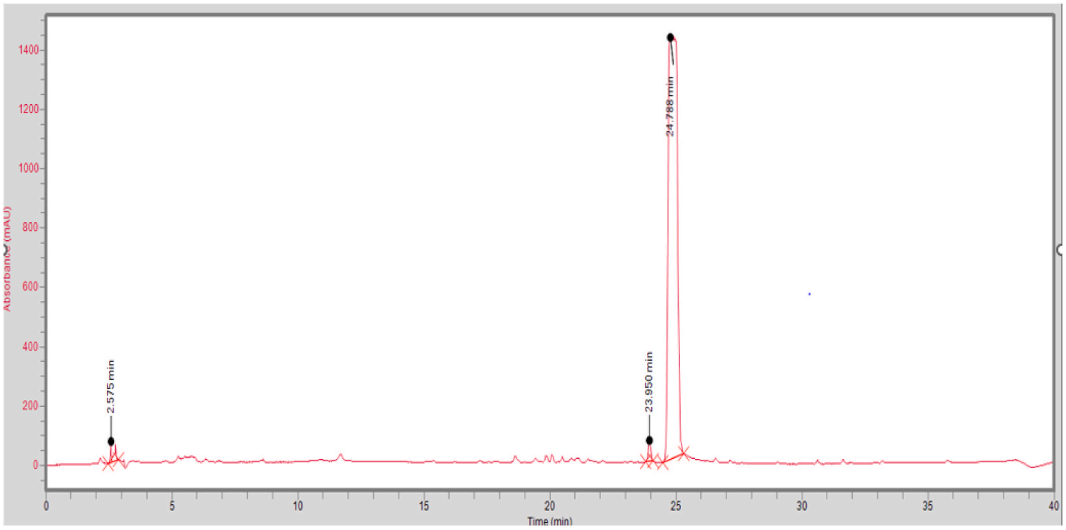

Fig. 6. HPLC fingerprint of decoction of *Cymbopogon citratus*.

compared to the normal control group. The LD_{50} of the herbal product was estimated to be more than 3000 mg/kg body weight. Treatment of the animals for four weeks did not result in significant changes in the haematological parameters (Table 4). Also, no changes in parameters related to the liver toxicity was observed in the serum biochemical analysis. No toxicologically significant changes were observed in the serum biochemical parameters of the treated and control groups in relation to renal and hepatic function (Table 5). Thus, LIPO A is safe to use for the duration of treatment used in the study. The components of LIPO A, *Allium sativum* (garlic) and *Cymbopogon citratus* (lemon grass), are age-old medicinal plants with several medical and culinary applications. Toxicity of these plants are not widespread and thus contributed to the non-toxic nature of the product.

The product also contained levels of toxic metals (As, Cd and Pb) which were within specifications [48]. Thus, the plant ingredients were possibly harvested from geographical locations which are not heavily contaminated with toxic metals. The maximum level of copper has not been set by WHO but countries such as Singapore and China have set maximum permissible levels at 150 and 20 mg kg^{-1}, respectively. Similarly, that of iron has not been set but the daily intake of iron is limited to 18 mg/day [49]. From the weight per mL of the product (1.125) at a dose of 90 mL per day, daily administration of the product will afford 8.37 mg of iron, which is far below the maximum daily limit. Thus, consumption of the product would not lead to toxicity from heavy metal contamination.

The product also complied with microbial quality specified in the British Pharmacopoeia [26]. 'LIPO A' did not contain any pathogenic microorganism. Contamination of plant ingredients used in herbal preparation can affect the final product if good manufacturing practices are not followed. Sometimes microbial contamination of herbal products occurs during harvesting, handling, drying, packaging, and transportation [50]. Microbial load above the recommended limits have been associated with product spoilage and toxic adverse effects in patients [51]. The herbal product satisfied the requirement for microbial load. However, the level of yeast and moulds were just at the upper limit of specification and so steps should be taken by practitioner to control them.

Physicochemical and chromatographic parameters were developed for the herbal product as quality control tools. The average pH of the herbal product was within the acceptable range (4–8) for oral dosage forms [52]. The pH of finished herbal products affects their stability since it is an important factor in the selection and actions of preservatives [53]. It also encourages or discourages the growth of microbes. The excellent pH of the product could be inferred to discourage the growth of microorganisms and thus, in part, accounted for the microbial load which was consistent with regulatory requirements.

Herbal products are made up of a vast array of secondary metabolites, often with unknown active ingredients. Therefore, a quality control measure widely accepted by the WHO, medicines agency of Europe and the Food and Drug Administration of the USA, is fingerprinting which provides a comprehensive chemical pattern reflecting the complex chemical composition of herbal products [54]. In this study, HPLC fingerprinting was used as a quality control tool for the herbal product. The chromatogram revealed six well resolved peaks, all eluting within 25 min (Fig. 5). The highly intense peak with retention time 24.88 min could be used as a marker compound for the quality control of this product. Different batches of the product could be assessed for consistency using this marker compound. HPLC fingerprint of an aqueous extract of *Cymbopogon citratus*, (lemon) one of the listed plant ingredients of the products, revealed three signals which were consistent with that of the product (Fig. 6). The intense peak at retention time of 24.88 min was found in the chromatogram of the plant. This confirms that, contrary to some views that herbal manufacturers under declare the medicinal plant ingredients of their products [55], LIPO A contains lemon grass as listed by the manufacturer and the presence of garlic in the product is also not in doubt.

5. Conclusion

Treatment of hyperlipidaemic rats for 4 weeks with the traditional herbal product 'LIPO A' resulted in a dose-dependent anti-hyperlipidaemic effect with the activity peaking at the highest dose used (400 mg/kg body weight). The product also showed significant reduction in total cholesterol, triglycerides, LDL-c and VLDL-c and slight increase in HDL-c at the maximum dose administered. The ability of the product to prevent oxidative stress and its associated pathologies in hyperlipidemic individuals was demonstrated by its antioxidant actions. The product was also found to be safe based on the acute and sub-acute toxicity studies. This was further corroborated by the heavy metal content and microbial load, all of which were within specifications. The study therefore supports the use of LIPO A, a bi-herbal product for the management of hyperlipidemia and could therefore be considered as viable alternative to conventional antilipidaemic agents in clinical use.

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CRediT authorship contribution statement

Solomon Appiah Kubi: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft. **Isaac Kingsley Amponsah:** Conceptualization, Methodology, Formal analysis, Investigation, Supervision. **Bernard Kofi Turkson:** Methodology, Resources. **Evelyn Asante-Kwatia:** Formal analysis, Investigation, Resources. **Desmond Nkrumah:** Methodology, Writing – review & editing. **Rita Akosua Dickson:** Writing – review & editing, Resources.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] R.H. Nelson, Hyperlipidemia as a risk factor for cardiovascular disease, in: Primary Care - Clinics in Office Practice, Elsevier, 2013, pp. 195–211, https://doi.org/10.1016/j.pop.2012.11.003, 40, Issue 1.

[2] N. Verma, Introduction to hyperlipidemia and its treatment: a review, Int. J. Curr. Pharmaceut. Res. 9 (1) (2016) 6, https://doi.org/10.22159/ijcpr.2017v9i1.16616.

[3] J. Stewart, T. McCallin, J. Martinez, S. Chacko, S. Yusuf, Hyperlipidemia. Pediatrics in Review 41 (8) (2020) 393–402, https://doi.org/10.1542/pir.2019-0053.

[4] D.C. Brooks, J.L. Schindler, Management of hyperlipidemia after stroke, Curr. Treat. Options Cardiovasc. Med. (2019), https://doi.org/10.1007/s11936-019-0774-8.

[5] S.M. Grundy, N.J. Stone, A.L. Bailey, C. Beam, K.K. Birtcher, R.S. Blumenthal, L.T. Braun, S. De Ferranti, J. Faiella-Tommasino, D.E. Forman, R. Goldberg, P. A. Heidenreich, M.A. Hlatky, D.W. Jones, D. Lloyd-Jones, N. Lopez-Pajares, C.E. Ndumele, C.E. Orringer, C.A. Peralta, J. Yeboah, Guideline on the management of blood cholesterol: a report of the American College of Cardiology/American heart association Task Force on clinical practice guidelines, Jl of the American College of Cardiology 73 (24) (2019) 285–e350, https://doi.org/10.1016/j.jacc.2018.11.003.

[6] F. Mach, C. Baigent, A.L. Catapano, K.C. Koskina, M. Casula, L. Badimon, M.J. Chapman, G.G. De Backer, V. Delgado, B.A. Ference, I.M. Graham, A. Halliday, U. Landmesser, B. Mihaylova, T.R. Pedersen, G. Riccardi, D.J. Richter, M.S. Sabatine, M.R. Taskinen, R.S. Patel, ESC/EAS guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk, Atherosclerosis 290 (1) (2019) 140–205, https://doi.org/10.1016/J.ATHEROSCLEROSIS.2019.08.014, 2019.

[7] S.J. Nicholls, E.M. Tuzcu, I. Sipahi, A.W. Grasso, P. Schoenhagen, T. Hu, K. Wolski, T. Crowe, M.Y. Desai, S.L. Hazen, S.R. Kapadia, S.E. Nissen, Statins, high-density lipoprotein cholesterol, and regression of coronary atherosclerosis, JAMA, J. Am. Med. Assoc. 297 (5) (2007) 499–508, https://doi.org/10.1001/jama.297.5.499.

[8] L. Tokgözoglu, J.L. Zamorano, Current perspectives on the use of statins in the treatment of dyslipidaemic patients: focus on pitavastatin, Drugs Context (US) 9 (2020), https://doi.org/10.7573/DIC.2020-4-4.

[9] B.A. Golomb, M.A. Evans, Statin adverse effects: a review of the literature and evidence for a mitochondrial mechanism, Am. J. Cardiovasc. Drugs: Drugs, Devices, and Other Interventions 8 (6) (2008) 373–418, https://doi.org/10.2165/0129784-200808060-00004.

[10] A. Grover, H.S. Rehan, L.K. Gupta, M. Yadav, Correlation of compliance to statin therapy with lipid profile and serum HMGCoA reductase levels in dyslipidemic patients, Indian Heart J. 69 (1) (2017) 6–10, https://doi.org/10.1016/j.ihj.2016.07.007.

[11] Y.C. Li, W.L. Huang, Effects of Adherence to statin therapy on health care Outcomes and utilizations in Taiwan: a population-based study, BioMed Res. Int. (2015), https://doi.org/10.1155/2015/149573.

[12] M. Prasanna, Hypolipidemic effect of fenugreek: a clinical study, Indian J. Pharmacol. 32 (1) (2000) 34–36.

[13] O. Gheith, H. Sheashaa, M. Abdelsalam, Z. Shoeir, M. Sobh, Efficacy and safety of Monascus purpureus Went rice in children and young adults with secondary hyperlipidemia: a preliminary report, Eur. J. Intern. Med. 20 (3) (2009), https://doi.org/10.1016/J.EJIM.2008.08.012.

[14] J. Kojuri, A.R. Vosoughi, M. Akrami, Effects of anethum graveolens and garlic on lipid profile in hyperlipidemic patients, Lipids Health Dis. 6 (1) (2007) 1–5, https://doi.org/10.1186/1476-511X-6-5, 2007.

[15] Y.E. Sun, W. Wang, J. Qin, Anti-hyperlipidemia of garlic by reducing the level of total cholesterol and low-density lipoprotein: a meta-analysis, Méd. 97 (18) (2018), https://doi.org/10.1097/MD.0000000000010255.

[16] M. Bahmani, M. Mirhoseini, H. Shirzad, M. Sedighi, N. Shahinfard, M. Rafieian-Kopaei, A review on promising natural agents effective on hyperlipidemia, J Evid-Based Complementary and Alternative Med 20 (3) (2015) 228–238, https://doi.org/10.1177/2156587214568457.

[17] M. Kafeshani, M. Mirhosseini, A. Momeni, R. Rabiei, M. Rafieian-Kopaei, Impact of Kelussia odoratissima Mozaffarian lipid profile and fasting blood sugar in hyperlipidemia patients, J Nephropharmacol 6 (1) (2017). pmc/articles/PMC5295657/.

[18] T. Agbovie, K. Amponsah, O.R. Crentsil, F. Dennis, G.T. Odamtten, W. Ofosuhene-Gyan, Conservation and Sustainable Use of Medicinal Plants in Ghana, Prep Phytopharm for the Management of Disorders, 2002, pp. 409–427.

[19] M. Jonsson, M. Jestoi, A.V. Nathanail, U.M. Kokkonen, M. Anttila, P. Koivisto, P. Karhunen, K. Peltonen, Application of OECD Guideline 423 in assessing the acute oral toxicity of moniliformin, Food Chem. Toxicol. 53 (2013) 27–32, https://doi.org/10.1016/j.fct.2012.11.023.

[20] Y. Touitou, F. Portaluppi, M.H. Smolensky, L. Rensing, Ethical principles and standards for the conduct of human and animal biological rhythm research, Chronobiol. Int. 21 (1) (2004) 161–170, https://doi.org/10.1081/CBI-120030045.

[21] J.H. Kim, O.K. Kim, H.G. Yoon, J. Park, Y. You, K. Kim, Y.H. Lee, K.C. Choi, J. Lee, W. Jun, Anti-obesity effect of extract from fermented Curcuma longa L. through regulation of adipogenesis and lipolysis pathway in high-fat diet-induced obese rats, Food Nutr. Res. 60 (2016), https://doi.org/10.3402/fnr.v60.30428.

[22] N. Fidèle, B. Joseph, T. Emmanuel, D. Théophile, Hypolipidemic, antioxidant and anti-atherosclerogenic effect of aqueous extract leaves of *Cassia occidentalis* Linn (Caesalpiniaceae) in diet-induced hypercholesterolemic rats, BMC Compl. Alternative Med. 17 (1) (2017), https://doi.org/10.1186/s12906-017-1566-x.

[23] M. Govindappa, S. Naga Sravya, M.N. Poojashri, T.S. Sadananda, C.P. Chandrappa, G. Santoyo, P. Sharanappa, N.V. Anil Kumar, Antimicrobial, antioxidant and in vitro anti-inflammatory activity and phytochemical screening of water extract of *Wedelia trilobata* (L.) hitchc, J. Med. Plants Res. 5 (24) (2011) 5718–5729.

[24] P. Prieto, M. Pineda, M. Aguilar, Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E, Anal. Biochem. 269 (2) (1999) 337–341, https://doi.org/10.1006/abio.1999.4019.

[25] J. Ma, S. Wu, N.V.R. Shekhar, S. Biswas, A.K. Sahu, Determination of physicochemical parameters and levels of heavy metals in food waste water with environmental effects, Bioinorgan. Chem. Appl. (2020), https://doi.org/10.1155/2020/8886093.

[26] B.K. Turkson, M.L.K. Mensah, G.H. Sam, A.Y. Mensah, I.K. Amponsah, E. Ekuadzi, G. Komlaga, E. Achaab, Evaluation of the microbial load and heavy metal content of two polyherbal antimalarial products on the Ghanaian market, Evid-Based Complementary and Alternative Med (2020), https://doi.org/10.1155/2020/1014273.

[27] British Pharmacopoeia. British Pharmacopoeia, Her Majesty's Stationary Office, 2018.

[28] N. Fischer, M. Isman, H. Stafford, Modern Phytochemical Methods, Modern Phytochemi Methods, 2012, https://doi.org/10.1007/978-1-4684-9060-2.

[29] J.B. Harborne, P.M. Richardson, 42(2, in: second ed.Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, Brittonia, 1998 https://doi.org/10.2307/2807624, 115.

[30] World Health Organization, WHO Guidelines for Assessing Quality of Herbal Medicines with Reference to Contaminants and Residues, 2007.

[31] R. Dghaim, S. Al Khatib, H. Rasool, M.A. Khan, Determination of Heavy Metals Concentration in Traditional Herbs Commonly Consumed in the United Arab Emirates, J Environ Pub Health, 2015, https://doi.org/10.1155/2015/973878.

[32] R. Asija, C. Singh, Comprehensive review on antihyperlipidemic activity of various medicinal plants, Int J Current Pharm Rev and Res 7 (6) (2016) 407–415.

[33] M. Kazemipoor, G.A. Cordell, M.M.R. Sarker, C.W.J.B.W.M. Radzi, M. Hajifaraji, En kiat alternative treatments for weight loss: safety/risks and effectiveness of anti-obesity medicinal plants, Int. J. Food Prop. 18 (9) (2015) 1942–1963, https://doi.org/10.1080/10942912.2014.933350.

[34] M.S. Lee, J.Y. Park, H. Freake, I.S. Kwun, Y. Kim, Green tea catechin enhances cholesterol 7α-hydroxylase gene expression in HepG2 cells, Br. J. Nutr. 99 (6) (2008) 1182–1185, https://doi.org/10.1017/S0007114507864816.

[35] Y. Oi, M. Okamoto, M. Nitta, Y. Kominato, S. Nishimura, T. Ariga, K. Iwai, Alliin and volatile sulfur-containing compounds in garlic enhance the thermogenesis by increasing norepinephrine secretion in rats, J. Nutr. Biochem. 9 (2) (1998) 60–66, https://doi.org/10.1016/S0955-2863(97)00167-8.

[36] C.A.R.A. Costa, L.T. Bidinotto, R.K. Takahira, D.M.F. Salvadori, L.F. Barbisan, M. Costa, Cholesterol reduction and lack of genotoxic or toxic effects in mice after repeated 21-day oral intake of lemongrass (*Cymbopogon citratus*) essential oil, Food Chem. Toxicol. 49 (9) (2011) 2268–2272, https://doi.org/10.1016/j.fct.2011.06.025.

[37] K.R. Feingold, C. Grunfeld, Introduction to Lipids and Lipoproteins, 2015. https://www.ncbi.nlm.nih.gov/books/NBK305896/.

[38] S.U. Islam, M.B. Ahmed, H. Ahsan, Y.S. Lee, Recent molecular mechanisms and beneficial effects of phytochemicals and plant-based whole foods in reducing ldl-c and preventing cardiovascular disease, Antioxidants 10 (Issue 5) (2021), https://doi.org/10.3390/antiox10050784.

[39] N. Abbas, M. Hamed Al-Sueaadi, A. Rasheed, E. Sayed Ahmed, Study of antidiabetic effect of lemongrass (Cymbopogon citratus) aqueous roots and flower extracts on albino mice, Int. J. Pharma Sci. Res. 9 (8) (2018) 3552, https://doi.org/10.13040/IJPSR.0975-8232.10(6).2785-89.

[40] Y.E. Sun, W. Wang, J. Qin, Anti-hyperlipidemia of garlic by reducing the level of total cholesterol and low-density lipoprotein, Med (United States). Lippincott Williams and Wilkins 97 (Issue 18) (2018), https://doi.org/10.1097/MD.0000000000010255.

[41] Y.Y. Yeh, L. Liu, Cholesterol-lowering effect of garlic extracts and organosulfur compounds: human and animal studies, J. Nutr. 131 (3 SUPPL) (2001) 989S–993S, https://doi.org/10.1093/jn/131.3.989s.

[42] N. Aslani, M.H. Entezari, G. Askari, Z. Maghsoudi, M.R. Maracy, Effect of garlic and lemon juice mixture on lipid profile and some cardiovascular risk factors in people 30-60 years old with moderate hyperlipidaemia: a randomized clinical trial, Int. J. Prev. Med. (1) (2016) 95, https://doi.org/10.4103/2008-7802.187248.
[43] Y. Lin, M.A. Vermeer, E.A. Trautwein, Triterpenic acids present in hawthorn lower plasma cholesterol by inhibiting intestinal ACAT activity in hamsters, Evid-Based Complementary and Alternative Med (2011), https://doi.org/10.1093/ecam/nep007.
[44] Y. Wang, X. Yi, K. Ghanam, S. Zhang, T. Zhao, X. Zhu, Berberine decreases cholesterol levels in rats through multiple mechanisms, including inhibition of cholesterol absorption, Metab., Clin. Exp. 63 (9) (2014) 1167–1177, https://doi.org/10.1016/j.metabol.2014.05.013.
[45] M.S. Lee, I.H. Kim, C.T. Kim, Y. Kim, Reduction of body weight by dietary garlic is associated with an increase in uncoupling protein mRNA expression and activation of AMP-activated protein kinase in diet-induced obese mice, J. Nutr. 141 (11) (2011) 1947–1953, https://doi.org/10.3945/jn.111.146050.
[46] N. Ning, K. He, Y. Wang, Z. Zou, H. Wu, X. Li, X. Ye, Hypolipidemic effect and mechanism of palmatine from *Coptis chinensis* in hamsters fed high-fat diet, Phytother Res. 29 (5) (2015) 668–673, https://doi.org/10.1002/ptr.5295.
[47] M. Hedayatnia, Z. Asadi, R. Zare-Feyzabadi, M. Yaghooti-Khorasani, H. Ghazizadeh, R. Ghaffarian-Zirak, A. Nosrati-Tirkani, M. Mohammadi-Bajgiran, M. Rohban, F. Sadabadi, H.R. Rahimi, M. Ghalandari, M.S. Ghaffari, A. Yousefi, E. Pouresmaeili, M.R. Besharatlou, M. Moohebati, G.A. Ferns, H. Esmaily, M. Ghayour-Mobarhan, Dyslipidemia and cardiovascular disease risk among the MASHAD study population, Lipids Health Dis. 19 (1) (2020), https://doi.org/10.1186/s12944-020-01204-y.
[48] D. Dhingra, D. Lamba, R. Kumar, P. Nath, S. Gauttam, Antihyperlipidemic activity of aloe succotrina in rats: possibly mediated by inhibition of hmg-coa reductase, ISRN Pharmacol (2014) 1–9, https://doi.org/10.1155/2014/243575.
[49] J.A. Leopold, Antioxidants and coronary artery disease: from pathophysiology to preventive therapy, Coron. Artery Dis. 26 (2) (2015) 176–183, https://doi.org/10.1097/MCA.0000000000000187.
[50] World Health Organization, Quality Control Methods for Herbal Materials, World Health Organization, 2011.
[51] C.M. De Sousa Lima, M.A.T. Fujishima, B. De Paula Lima, P.C. Mastroianni, F.F.O. De Sousa, J.O. Da Silva, Microbial contamination in herbal medicines: a serious health hazard to elderly consumers, BMC Complementary Med and Therapies 20 (1) (2020) 1–9, https://doi.org/10.1186/s12906-019-2723-1.
[52] D. Abba, H.I. Inabo, S.E. Yakubu, O.S. Olonitola, Contamination of herbal medicinal products marketed in Kaduna metropolis with selected pathogenic bacteria, Afr. J. Tradit., Complementary Altern. Med. 6 (1) (2009) 70–77, https://doi.org/10.4314/ajtcam.v6i1.57076.
[53] M.E. Aulton, Taylor Kmg Aulton's, Pharmaceutics- the Design and Manufacture of Medicines, 5th-Edition, 2018. London, United Kingdom.
[54] I.C. Oladipo, D.T. Adeleke, A.O. Adebiyi, The effect of pH and chemical preservatives on the growth of bacterial isolates from some Nigerian packaged fruit juices, Pakistan J. Nutr. 8 (11) (2010) 1796–1801, https://doi.org/10.3923/pjn.2009.1796.1801.
[55] A. Bārzdiņa, A. Paulausks, D. Bandere, A. Brangule, The potential use of herbal fingerprints by means of HPLC and TLC for characterization and identification of herbal extracts and the distinction of Latvian native medicinal plants, Mol 27 (8) (2022), https://doi.org/10.3390/molecules27082555.