



(51) International Patent Classification:

A61K 38/16 (2006.01) A61K 31/404 (2006.01)
A61K 31/03 (2006.01) A61K 31/409 (2006.01)
A61K 31/12 (2006.01) A61K 31/4418 (2006.01)
A61K 31/137 (2006.01) A61K 31/47 (2006.01)
A61K 31/202 (2006.01) A61K 31/505 (2006.01)
A61K 31/22 (2006.01) A61K 35/74 (2006.01)
A61K 31/366 (2006.01) A61P 9/00 (2006.01)
A61K 31/40 (2006.01)

(21) International Application Number:

PCT/IB2013/051987

(22) International Filing Date:

13 March 2013 (13.03.2013)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

2595/MUM/2011 13 March 2012 (13.03.2012) IN

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— with international search report (Art. 21(3))

— with amended claims and statement (Art. 19(1))

(54) Title: PHARMACEUTICAL COMPOSITIONS FOR TREATMENT OF CARDIOVASCULAR DISEASES

(57) Abstract: The present invention relates to LDL-lowering and HDL-increasing pharmaceutical compositions comprising HMG CoA reductase inhibitor; NADPH oxidase inhibitor, and optionally oil containing long-chain polyunsaturated fatty acids for the treatment of cardiovascular diseases. The present invention also relates to method of preparation of the said pharmaceutical compositions.



**PHARMACEUTICAL COMPOSITIONS FOR TREATMENT OF
CARDIOVASCULAR DISEASES**

FIELD OF THE INVENTION

5 The present invention relates to a synergistic LDL-lowering and HDL-increasing pharmaceutical composition comprising a) HMG CoA reductase inhibitor, b) NADPH oxidase inhibitor, and optionally c) oil containing long-chain polyunsaturated fatty acids, for the treatment of cardiovascular diseases. The invention also discloses a method of preparation for the said composition.

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BACKGROUND OF THE INVENTION

Cardiovascular disease (CVD) is a complex and multifactorial disease and characterized by multiple factors. Epidemiologic studies have identified these as elevated serum lipids (cholesterol and triglycerides), increased plasma fibrinogen and coagulation factors,
15 increased platelet activation, alterations in glucose metabolism, and smoking. The oxidative modification of low density lipoprotein (LDL) by reactive oxygen species (ROS) is also now considered an important mechanism in the development of atherosclerosis, as it is the pathogenesis of hypertension. There is also considerable evidence supporting the involvement of platelets in the development of atherosclerosis.
20 Increased high density lipoprotein (HDL) levels are negatively correlated with cardiovascular disease. Normalization of abnormal lipids and lipoproteins, inhibition of platelet aggregation, and an increase in antioxidant status are believed to improve cardiovascular disease.

25 Hypercholesterolemia is the presence of high levels of cholesterol in the blood. It is not a disease but a metabolic derangement that can be caused by many diseases, notably cardiovascular disease. It is closely related to the terms "hyperlipidemia" (elevated levels of lipids in the blood) and "hyperlipoproteinemia" (elevated levels of lipoproteins in the blood).

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Elevated cholesterol in the blood is due to abnormalities in the levels of lipoproteins, the particles that carry cholesterol in the bloodstream. This may be related to diet, genetic

factors (such as LDL receptor mutations in familial hypercholesterolemia) and the presence of other diseases such as diabetes and an underactive thyroid. The type of hypercholesterolemia depends on which type of particle (such as low-density lipoprotein) is present in excess.

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Although hypercholesterolemia itself is asymptomatic, longstanding elevation of serum cholesterol can lead to atherosclerosis. Over a period of decades, chronically elevated serum cholesterol contributes to formation of atheromatous plaques in the arteries. This leads to progressive narrowing or even complete blockage of the involved arteries. Blood supply to the tissues and organs served by these occluded arteries gradually diminishes until organ function becomes impaired.

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The inadequate blood flow to the heart muscles is caused by the narrowed coronary arteries, which are the vessels that supply blood to the heart, lead to myocardial ischemia or angina, which may ultimately result in myocardial infarction, heart attack or failure.

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Therapeutic agents used to prevent myocardial ischemia or infarction generally include β -blockers, statins and aspirin. β -Blockers exert their beneficial effects predominantly through heart rate control, whereas statins reduce blood cholesterol levels and prevent development and rupture of atherosclerotic plaque and aspirin reduces platelet activation and vasoconstriction, thereby limiting ischemic events.

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HMG-CoA reductase inhibitors, often called "statins"; these are drugs that block an enzyme called "HMG-CoA reductase." These are the most effective cholesterol lowering agents available and in recent years have received increased attention for their benefits beyond helping patients with high cholesterol.

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Other agents, such as omega-3 fatty acids, have been used to treat post-myocardial infarction (MI) and adult endogenous hyperlipidemias of hypercholesterolemias and of hypertriglyceridemias by lowering serum triglycerides, increasing serum HDL-cholesterol, lowering systolic and diastolic blood pressure and the pulse rate, and lowering the activity of the blood coagulation factor VII-phospholipid complex.

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US 20100172971 disclose phycobilins as inhibitors of NADPH oxidase activity and are useful in the prophylaxis and/or treatment of medical conditions associated with or linked to an NADPH oxidase activity. NADPH oxidase overactivity can stimulate pro-inflammatory mechanisms, promote tissue fibrosis and bone resorption, and, in the
5 vascular system, antagonize the crucial protective activity of nitric oxide.

US 5763496 disclose a method for the prevention and treatment of atherosclerosis and its related diseases in which an NADPH oxidase inhibitor, preferably apocyanin is administered. The NADPH oxidase inhibitor prevents the production of reactive oxygen
10 species upon exposure of endothelial cells to atherogenic LDL levels, resulting in decreased endocytosis and vascular hyperpermeability.

WO 2006096806 discloses a composition of statin and omega-3 fatty acids and method of preparation for the said composition
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Although individual therapeutic agents such as HMG CoA reductase inhibitor, aspirin, β -Blockers, omega-3 fatty acids, NADPH oxidase inhibitor and combination of statin and omega-3 fatty acids are available, there exists a need of pharmaceutical compositions that can provide a synergistic effect for the treatment of cardiovascular diseases in a unit
20 dosage form.

The inventors of the present invention have surprisingly found that the combination of HMG CoA reductase inhibitor, NADPH oxidase inhibitor and optionally a polyunsaturated fatty acids synergistically lowers the low density lipoprotein (LDL) and
25 increase the high density lipoprotein (HDL) in patients with hyperlipidemia.

OBJECT OF INVENTION

An object of the present invention is to provide a synergistic LDL-lowering and HDL-increasing pharmaceutical composition comprising:

- 30 a) HMG CoA reductase inhibitor,
 b) NADPH oxidase inhibitor, and optionally
 c) polyunsaturated fatty acids.

Another object of the present invention is to provide a synergistic LDL-lowering and HDL-increasing pharmaceutical composition comprising:

- a) HMG CoA reductase inhibitor,
- 5 b) NADPH oxidase inhibitor, and optionally
- c) omega-3 fatty acid.

Yet another object of the present invention is to provide a process for preparing a synergistic LDL-lowering and HDL-increasing pharmaceutical composition comprising:

- 10 a) HMG CoA reductase inhibitor,
- b) NADPH oxidase inhibitor, and optionally
- c) polyunsaturated fatty acids.

SUMMARY OF THE INVENTION

- 15 According to an aspect of the present invention there is provided a synergistic LDL-lowering and HDL-increasing pharmaceutical composition comprising
- a) HMG CoA reductase inhibitor in the range from 5 to 80 mg;
- b) NADPH oxidase inhibitor in the range from 5 to 300 mg, and optionally
- c) omega-3 fatty acid in the range from 100 to 1000 mg.

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DESCRIPTION OF THE INVENTION

- The present invention relates to a synergistic LDL-lowering and HDL-increasing pharmaceutical composition comprising a) HMG CoA reductase inhibitor, b) NADPH oxidase inhibitor, and optionally c) long-chain polyunsaturated fatty acids, for the
- 25 treatment of cardiovascular diseases, specifically hyperlipidemia.

In first embodiment, the present invention provides a synergistic LDL lowering and HDL increasing pharmaceutical composition comprising:

- a) HMG CoA reductase inhibitor,
- 30 b) NADPH oxidase inhibitor, and optionally
- c) polyunsaturated fatty acids.

In another embodiment, the present invention provides a synergistic LDL lowering and HDL increasing pharmaceutical composition comprising:

- a) HMG CoA reductase inhibitor,
- b) NADPH oxidase inhibitor.

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The use of the terms "a" and "an" and "the" and similar referents in the context of describing the invention are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context.

- 10 The term "synergistic" as used herein means the combined activity of the active agents is more when used in combination as compare to the activity of the individual active agent, when used alone.

- 15 The term "pharmaceutical composition" or "dosage form" as used herein means any physical form of the formulation that contains an amount of active substance(s) with pharmaceutically acceptable excipient (s) sufficient to produce a therapeutic effect on administration.

- 20 The term "pharmaceutically acceptable" in context with excipient(s) means approved by a regulatory agency of the federal or a state government or listed in recognized pharmacopoeia for use in humans.

- 25 HMG-CoA reductase (or 3-hydroxy-3-methyl-glutaryl-CoA reductase) is the rate-limiting step in cholesterol synthesis and represents the sole major drug target for contemporary cholesterol-lowering drugs. The medical significance of HMG-CoA reductase has continued to expand beyond its direct role in cholesterol synthesis following the discovery that it can offer cardiovascular health benefits independent of cholesterol reduction. HMG-CoA reductase inhibitors inhibit the HMG-CoA reductase enzyme and lower cholesterol level. HMG CoA reductase inhibitor as used in the present invention is selected from the group of statins comprising of atorvastatin, simvastatin, pitavastatin, lovastatin, fluvastatin, pravastatin, cerivastatin, rosuvastatin or a combination thereof. Statins as used herein includes free base, or pharmaceutically acceptable salts
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thereof or mixtures thereof. It also includes anhydrous form, hydrous form, different crystalline forms, amorphous form, prodrugs, metabolites, enantiomers or mixtures thereof.

5 The NADPH oxidase (Nicotinamide adenine dinucleotide phosphate-oxidase) is a membrane-bound enzyme complex. It can be found in the plasma membrane as well as in the membrane of phagosome. It generates superoxide by transferring electrons from NADPH inside the cell across the membrane and coupling these to molecular oxygen to produce the superoxide, which is a reactive free-radical.

10 Superoxide, in turn, can spontaneously form hydrogen peroxide that undergoes further reactions to generate reactive oxygen species (ROS). NADPH oxidase is a major cause of atherosclerosis. Atherosclerosis is caused by the accumulation of macrophages containing cholesterol (foam cells) in artery walls. NADPH oxidase produces ROSs. These ROSs activate an enzyme that makes the macrophages adhere to the artery wall. NADPH

15 oxidase inhibitor as used in the present invention is selected from the group comprising of phycocyanin, C-phycocyanin, phycocyanobilin, Spirulina extract, aminoethyl-benzenesulfonylfluoride, apocynin, diphenylene iodonium or a combination thereof. In a preferred aspect of the present invention, NADPH oxidase inhibitor is phycocyanin or C-phycocyanin.

20 Polyunsaturated fatty acid containing oil or omega-3 fatty acid source, as used in the present invention includes, but not limited to Krill oil, Fish Oil, Flax-seed oil, Chia-seed oil, or any other oil containing omega-3 fatty acid or oleoresin or extract derived from any terrestrial or aquatic plants or algae, or any marine animal species including fish and

25 crustaceans or a combination thereof. In a preferred aspect, polyunsaturated fatty acid is oil containing omega-3 fatty acid. The said oil containing omega-3 fatty acid has a double bond ($C=C$) starting after the third carbon atom from the end of the carbon chain. The said Omega-3 fatty acid is chemically synthesized or produced through enzymatic or microbial transformation of the precursor materials. Omega-3 fatty acid is extracted from

30 the raw materials using an appropriate extraction method, preferably an organic solvent or CO_2 extraction method. Polyunsaturated fatty acid is selected from the group comprising

of cis 5,8,11,14,17-eicosapentanoic acid (EPA) and cis 4,7,10,13,16,19-docosahexanoic acid (DHA), one of their esters or pharmaceutically acceptable salts.

Phospholipid as used in the present invention includes, but not limited to naturally occurring lecithins, phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol or a combination thereof. In a preferred aspect of the present invention, phospholipid is lecithin.

In one embodiment, the present invention provides a synergistic LDL-lowering and HDL-increasing pharmaceutical composition comprising:

- a) HMG CoA reductase inhibitor,
- b) NADPH oxidase inhibitor,
- c) Oil containing polyunsaturated fatty acids.

In further embodiment, the present invention provides a synergistic LDL-lowering and HDL-increasing pharmaceutical composition comprising:

- a) HMG CoA reductase inhibitor,
- b) NADPH oxidase inhibitor, and
- c) Oil containing omega-3 fatty acid.

In further embodiment, the present invention provides a synergistic LDL-lowering and HDL-increasing pharmaceutical composition comprising:

- a) Statin,
- b) Phycocyanin, and
- c) Oil containing omega-3 fatty acid.

Yet another embodiment, the present invention provides a process for preparing a synergistic LDL-lowering and HDL-increasing pharmaceutical composition comprising:

- a) HMG CoA reductase inhibitor,
- b) NADPH oxidase inhibitor, and optionally
- c) Oil containing polyunsaturated fatty acids.

Yet another embodiment, the present invention provides a process for the preparation of synergistic LDL-lowering and HDL-increasing pharmaceutical composition, comprising the steps of:

- 1) Dissolving statin and NADPH oxidase inhibitor optionally with an equi-molar concentration of PC (80 % purity grade of soya-phospholipids), in a suitable liquid media and mixing subsequently.
- 2) Evaporating the liquid media under vacuum at desired temperature in a rotary vacuum evaporator to obtain dry residue.
- 3) Placing the dry residue in vacuum desiccators overnight and then mixing it with oil containing omega-3 fatty acids.
- 4) Placing the mixture from the above step, optionally in the presence of surfactant/co-surfactant, to a micro-fluidizer or a high-pressure homogenizer to create a homogeneous suspension.

Yet another embodiment, the present invention provides a process for the preparation of synergistic LDL-lowering and HDL-increasing pharmaceutical composition, comprising the steps of:

- 1) Premixing the required amounts of statins and NADPH oxidase inhibitor, by first dissolving them in their respective liquid media and subsequently mixing the two in desired ratio.
- 2) Evaporating the liquid media under vacuum at desired temperature in a rotary vacuum evaporator to obtain dry residue.
- 3) Placing the dry residue in vacuum desiccators overnight and then mixing it with oil containing omega-3 fatty acids.
- 4) Optionally adding the required amount of surfactants and/or co-surfactants to the premix and subjecting the mixture to a micro-fluidizer or high-pressure homogenizer to create a population of particles having diameters ranging between 10-200 nm.

Yet another embodiment, the present invention provides a process for the preparation of synergistic LDL-lowering and HDL-increasing pharmaceutical composition, comprising the steps of:

- 1) Dissolving statin and NADPH oxidase inhibitor optionally with an equimolar concentration of PC (80 % purity grade of soya-phospholipids), in a liquid media and mixing subsequently.
- 2) Evaporating the liquid media under vacuum at desired temperature in a rotary vacuum evaporator to obtain dry residue.
- 3) Formulating the dry residue in a pharmaceutical formulation with the aid of suitable pharmaceutical acceptable excipient in a desired range.

“Liquid media” used in the process for the preparation according to the present invention include, but not limited to water, saline solution, ringers solution, dextrose, short chain alcohols, suitable organic solvent or a mixture thereof.

“Surfactant and/or co-surfactant” used in the process for the preparation according to the present invention include, but not limited to Capryol, Gelucire, Cremophor, Imwitor, Labrafil, Lauroglycol 90, Tween20, Tween80, PEG400, glycerin, ethylene glycol, propylene glycol, or a mixture thereof.

Other agents may also used for the process for the preparation according to the present invention include, but not limited to emulsifying agent, preservatives, stabilizers, wetting agent, secondary emulsifier, buffer.

Yet another aspect of the present invention, the amount present in the synergistic pharmaceutical formulation of HMG CoA reductase inhibitor is in a range from 5 to 80 mg, NADPH oxidase inhibitor is in a range from 4 to 300 mg, and omega-3 fatty acid is in a range from 100 to 2000 mg.

The compositions of the present invention may possibly act in reducing dose of individual active ingredients when administered together either in one fixed dose composition or as a kit separately as distinct units.

In another embodiment, the present invention provides a method of use of the synergistic lipid lowering pharmaceutical composition comprising:

- a) HMG CoA reductase inhibitor
- b) NADPH oxidase inhibitor, and optionally
- c) Omega-3 fatty acid,

for the treatment of cardiovascular diseases.

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In further embodiment, the present invention provides a method of use of said synergistic lipid lowering pharmaceutical composition in maintaining healthy lipid profile, reducing LDL oxidation, inhibiting platelet aggregation and vascular inflammation.

10 In a preferred embodiment the present invention provides a synergistic lipid lowering pharmaceutical composition in the treatment of atherosclerosis, myocardial infarction, myocardial ischemia, angina pectoris, stroke and any other cardiovascular events which involve deposition of lipids in blood vessels.

15 Yet another embodiment, the present invention provides additional advantage of the synergistic lipid lowering pharmaceutical composition for clinical management of cardiovascular diseases, whereby composition may help in reducing some of the side effects associated with statins.

20 Yet another embodiment, the present invention provides a pharmaceutical kit comprising the said synergistic lipid lowering pharmaceutical composition in a single or multiple units in a single or multiple pack/container which comprising:

- a) HMG CoA reductase inhibitor,
- b) NADPH oxidase inhibitor, and optionally
- 25 c) Oil containing polyunsaturated fatty acids/natural phospholipid.

Suitable dosage forms according to the present invention include, without limitation, solid dosage forms (eg. powders and granules, capsules, and/or tablets); liquid dosage forms (eg. solutions and disperse systems); and/or sterile dosage forms and delivery systems (eg
30 parenterals, and/or biologics).

Methods of administering the synergistic pharmaceutical composition according to the present invention by any suitable route include, but are not limited to, intramuscular, intrathecal, intradermal, intraperitoneal (ip), intravenous (iv), subcutaneous (sc), intranasal, epidural, intradural, intracranial, intraventricular, and oral routes. Convenient routes for administration include, for example, infusion or bolus injection, topical, absorption through epithelial or mucocutaneous linings, ophthalmic, nasal, and transdermal. Administration can be systemic or local.

The invention is further exemplified with following examples and is not necessarily limited to the present formulations. It is obvious to those skilled in the art to find out the composition for other dosage forms and substitute the equivalent excipients as described in this specification or with the one known to the art.

Example 1

Ingredients	mg/capsule
Omega3 Fish oil	500
Lovastatin	20
C-Phycocyanin	100
Soy Lecithin	100
Cremophor	10
propylene glycol	10
Polysorbate-80	10

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Example 2

Ingredients	mg/capsule
Flax seed oil	500
Simvastatin	20
C-Phycocyanin	100
Sunflower Lecithin	100
Cremophor	10
Polysorbate-80	10

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Example 3

Ingredients	mg/capsule
Krill oil	500
Fluvastatin	20
Apocyanin	100
Soy Lecithin	100
Cremophor	10
Polyethylene glycol	10
Polysorbate-80	10

Example 4

Ingredients	mg/capsule
Krill oil	500
Lovastatin	20
C-Phycocyanin	100
Soy Lecithin	100
Cremophor	10
Tocotrienols	100
Polysorbate-80	10

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Examples 1-4 can be formulated using any of the manufacturing processes described above or by any other process known to the person skilled in the art.

Example 5: For the purpose of examples the data generated with Atorvastatin represents the class of HMG CoA reductase inhibitors (statins)

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Repeat dose pharmacology Study of male Rats was conducted to evaluate and compare the efficacy of Atorvastatin and its combinations in male adult Wistar rats with hyperlipidemia. Atorvastatin and its combinations were evaluated and compared the efficacy of in terms of changes in High density lipoprotein (HDL) and Low density lipoprotein (LDL) levels in blood. Duration of the study was 42 days and the period of treatment was 28 days. Hyperlipidemia was induced experimentally in wistar rats by feeding the rats with high fat synthetic diet for 2 weeks. For the next four weeks the rats

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were on the same high fat synthetic diet along with the simultaneous administration of the study drug. Two hours prior to dosing the diet were withheld from the cages.

Atorvastatin, Phycocyanin, Omega 3 fatty acid oil compositions were evaluated

5 accordingly six study groups were designed as below.

T1= Atorvastatin (1.45mg/kg).

T2= Atorvastatin (1.45mg/kg) + Omega3 fatty acid oil 300mg + Phycocyanin
50mg.

10 **T3**= Atorvastatin (1.45mg/kg) + Phycocyanin 50mg.

T4= Atorvastatin (1.45mg/kg) + Omega3 fatty acid oil 300mg.

C1= Omega3 fatty acid oil 300mg

Methodology:

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Rats were weighed and marked. Before initiation of the study on day 1 the weight, HDL, LDL, TC and TG of the individual animal was estimated. Then the rats were fed on high fat diet for 14 days of the study for the induction of hyperlipidemia. On day 14, HDL and LDL and body weight was assessed to confirm the occurrence of hyperlipidemia and to
20 rule out any kind of hepatotoxicity. From Day 14 the study drug as per the groups assigned were administered to the rats for 4 weeks. After 2 weeks of study treatment on day 28 HDL and LDL body weight was assessed. Final evaluation was done on day 42 including the LDL and HDL levels assessment. Blood was withdrawn from the tail vein to analyze lipid profiles.

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Route of administration: Oral route was used in this study as it is preferred route for humans. Rats were administered test items orally every day for 28 days, after physical restriction of the animal.

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**Table 1 : HDL levels measured in various treatment groups from day 0 to day 28
(Treatment period)**

Table 1: Evaluation of HDL(mg/dl) (Day 0 to Day 28 of treatment)											
GROUP	DAY 0 of treatment			Day 14 of treatment			P Value	Day 28 of treatment			P Value
	MEAN ±S.D.	Range		MEAN ±S.D.	Range			MEAN ±S.D.	Range		
		Min	Max		Min	Max			Min	Max	
C1	16.78 ± 2.486	13	20.7	22.61± 2.514	19.4	25.7	0.0078	26.85± 1.586	24.3	29.1	0.0078
T1	20.88 ± 4.761	14.9	28.9	28.41± 4.334	22.3	34.7	0.0078	36.36± 3.392	29.7	39.7	0.0098
T2	19.53 ± 2.908	15.9	24.2	29.04± 7.674	12.8	39.7	0.0156	44.02± 2.673	41.7	48.3	0.0013
T3	15.51 ± 2.060	13.8	19.7	26.52± 2.659	22.9	29.8	0.0313	40.88± 2.051	38.3	43.8	0.0625
T4	15.38 ± 1.567	12.9	17.8	23.26± 3.353	19.8	28.3	0.0078	36.90± 4.748	30.6	45.3	0.0054

**5 Table 2 : LDL levels measured in various treatment groups from day 0 to day 28
(Treatment period)**

Table 2: Evaluation of LDL(mg/dl) (Day 0 to Day 28 of treatment)											
GROUP	DAY 0 of treatment			Day 14 of treatment			P Value	Day 28 of treatment			P Value
	MEAN	Range		MEAN	Range			MEAN	Range		
		±S.D.	Min		Max	±S.D.			Min	Max	
C1	108.3± 7.627	99.4	123.4	96.94± 3.926	91.3	102.4	0.0078	82.35± 5.064	75.4	89.4	0.0068
T1	109.5± 7.330	99.7	121.9	89.38± 9.350	74.3	105.8	0.0068	69.44± 6.070	60.2	79.4	0.0078
T2	105.8± 12.32	89.9	124.3	81.20± 12.46	68.9	105.7	0.0059	55.38± 5.705	48.9	61.2	0.0013
T3	126.9± 46.33	87.8	198.2	107.2± 47.65	71.2	182.3	0.0156	65.14± 4.392	61.3	72.4	0.0625
T4	102.6± 13.25	84.2	123.4	90.38± 11.62	74.5	102.4	0.0048	75.55± 10.76	60.33	90.2	0.0051

Table 3: Percentage changes in HDL in various treatment groups measured as mg/dl

Group	0 day treatment	28 day treatment	% change wrt 0 day
C1 : Omega-3 fatty acid	16.78	26.85	60
T1 : Atorvastatin	20.88	36.36	74
T2: Atorvastatin+Omega-3 fatty acid+ Phycocyanin	19.53	44.02	125
T3 : Atorvastatin + Phycocyanin	15.51	40.88	163
T4 : Atorvastatin + Omega-3 fatty acid	15.38	36.90	139

Table 4 : Percentage changes in LDL in various treatment groups measured as mg/dl

Group	0 day treatment	28 day treatment	% Reduction wrt 0 day
C1 : Omega-3 fatty acid	108.3	82.35	23.96
T1 : Atorvastatin	109.5	69.44	36.58
T2: Atorvastatin +Omega-3 fatty acid + Phycocyanin	105.8	55.38	47.65
T3 : Atorvastatin + Phycocyanin	126.9	65.14	48.66
T4 : Atorvastatin + Omega-3 fatty acid	102.6	75.55	26.36

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Data in table 3 indicates a marked increase in the HDL levels i.e. 163% in the group treated with combination of Atorvastatin and Phycocyanin at the end of 28 day treatment. The group treated with Atorvastatin 1.45mg/kg + Omega-3 300mg + Phycocyanin 50mg showed an increase of 125% when compared to the group treated with Atorvastatin alone i.e. 74%.

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Data in table 4 indicates that there was a significant reduction in Low density lipoprotein levels in the group treated with Atorvastatin 1.45mg/kg + Phycocyanin 50mg i.e 48.66% and group treated with (Atorvastatin 1.45mg/kg + Omega 3 300mg + Phycocyanin 50mg) showed a decrease of 47.65% when compared to the group treated with Atorvastatin alone, which showed only 36.58% reduction in low density lipoproteins.

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The compositions of the present invention depict a synergistic effect as compared to the individual components as demonstrated by the results in table 1-4. The compositions of the present invention can reduce intake of the high dose of HMG CoA reductase inhibitor by administrating them along with NADPH oxidase inhibitor optionally with an omega-3
5 fatty acid source, exhibiting both increase in high density lipoproteins and decrease in low density lipoproteins for treatment of cardiovascular diseases in humans. The pharmaceutical compositions of the present invention are suitable for veterinary use.

The combination of atorvastatin and phycocyanin and combination of atorvastatin,
10 phycocyanin and omega-3 fatty acid source exhibits superior efficacy in reduction of LDL and boosting HDL levels as compared to statin administered alone.

It will be understood that various modifications may be made to the embodiments disclosed herein. Therefore the above description should not be construed as limiting, but
15 merely as exemplifications of preferred embodiments. For example, the functions described above and implemented as the best mode for operating the present invention are for illustration purposes only. Other arrangements and methods may be implemented by those skilled in the art without departing from the scope and spirit of this invention.

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CLAIMS

1. A LDL-lowering and HDL-increasing oral pharmaceutical composition comprising:
 - a) HMG CoA reductase inhibitor in the range from 5 to 80 mg;
 - 5 b) NADPH oxidase inhibitor in the range from 5 to 300 mg, and optionally,
 - c) omega-3 fatty acid in the range from 5 to 300 mg.
2. The LDL-lowering and HDL-increasing oral pharmaceutical composition according to claim 1, wherein the HMG CoA reductase inhibitor is selected from
10 atorvastatin, simvastatin, pitavastatin, lovastatin, fluvastatin, pravastatin, cerivastatin, or rosuvastatin.
3. The LDL-lowering and HDL-increasing oral pharmaceutical composition according to claim 1 wherein, the NADPH oxidase inhibitor is selected from
15 phycocyanin, C-phycocyanin, phycocynobilin, Spirulina extract, aminoethyl-benzenesulfonylfluoride, apocynin, or diphenylene iodonium.
4. The LDL-lowering and HDL-increasing oral pharmaceutical composition according to claim 3 wherein the most preferred NADPH oxidase inhibitor is
20 phycocyanin or c-phycocyanin.
5. The LDL-lowering and HDL-increasing pharmaceutical composition according to claim 1, wherein omega-3 fatty acid is obtained from krill oil, fish oil, flax-seed oil, chia-seed oil, oleoresin, extract derived from terrestrial or aquatic plants or
25 algae, or marine animal species including fish and crustaceans.
6. A method of lowering LDL and increasing HDL for the treatment of cardiovascular diseases, wherein the said method comprises administration to a subject in need thereof, an oral pharmaceutical composition comprising:
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 - a) HMG CoA reductase inhibitor in the range from 5 to 80 mg;
 - b) NADPH oxidase inhibitor in the range from 5 to 300 mg, and optionally,
 - c) omega-3 fatty acid in the range from 5 to 300 mg.

7. The method according to claim 6 wherein the oral pharmaceutical composition comprises:
- a) HMG CoA reductase inhibitor selected from atorvastatin, simvastatin, pitavastatin, lovastatin, fluvastatin, pravastatin, cerivastatin, or rosuvastatin;
 - b) NADPH oxidase inhibitor selected from phycocyanin, C-phycocyanin, phycocynobilin, Spirulina extract, aminoethyl-benzenesulfonylfluoride, apocynin, or diphenylene iodonium, and optionally,
 - c) omega-3 fatty acid obtained from krill oil, fish oil, flax-seed oil, chia-seed oil, oleoresin, extract derived from terrestrial or aquatic plants or algae, or marine animal species including fish and crustaceans.
8. The use of a LDL-lowering and HDL-increasing oral pharmaceutical composition for the treatment of cardiovascular diseases comprising:
- a) HMG CoA reductase inhibitor in the range from 5 to 80 mg;
 - b) NADPH oxidase inhibitor in the range from 5 to 300 mg.
9. The use of LDL-lowering and HDL-increasing oral pharmaceutical composition according to claim 8 wherein the HMG CoA reductase inhibitor is selected from atorvastatin, simvastatin, pitavastatin, lovastatin, fluvastatin, pravastatin, cerivastatin, or rosuvastatin.; and wherein the NADPH oxidase inhibitor is selected from phycocyanin, C-phycocyanin, phycocynobilin, Spirulina extract, aminoethyl-benzenesulfonylfluoride, apocynin, or diphenylene iodonium.

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AMENDED CLAIMS

received by the International Bureau on 25 July 2013 (25.07.13)

1. A pharmaceutical composition in the treatment of cardiovascular diseases comprising an effective amount of phycocyanin in combination with an HMG CoA reductase inhibitor in a pharmaceutically acceptable carrier for lowering low density lipoproteins and increasing high density lipoproteins.
2. The pharmaceutical composition according to claim 2 wherein, in the HMG CoA reductase inhibitor is selected from the group consisting of lovastatin, pravastatin, fluvastatin, simvastatin, pitavastatin, rosuvastatin and atorvastatin.
3. The pharmaceutical composition according to claim 2 wherein, in the HMG CoA reductase inhibitor is atorvastatin.
4. A method of lowering low density lipoproteins and increasing high density lipoproteins for the treatment of cardiovascular diseases comprising administering to a mammal in need thereof an effective amount of phycocyanin in combination with an effective amount of HMG CoA reductase inhibitors.
5. The method according to claim 4 wherein the HMG CoA reductase inhibitor is selected from the group consisting of lovastatin, pravastatin, fluvastatin, simvastatin, pitavastatin, rosuvastatin and atorvastatin.
6. The method according to claim 5 wherein the HMG CoA reductase inhibitor is atorvastatin.
7. Use of phycocyanin and HMG CoA reductase inhibitor for the manufacture of a medicament for lowering low density lipoproteins and increasing high density lipoproteins for the treatment of cardiovascular diseases.

8. The use of phycocyanin and HMG CoA reductase inhibitor according to claim 7 wherein the HMG CoA reductase inhibitor is selected from the group consisting of lovastatin, pravastatin, fluvastatin, simvastatin, pitavasatin, rosuvastatin and atorvastatin.

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9. The use phycocyanin and HMG CoA reductase inhibitor according to claim 8 wherein the HMG CoA reductase inhibitor is atorvastatin.

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STATEMENT UNDER ARTICLE 19 (1)

Please refer to the Written Opinion date of mailing May 28, 2013 issued by the ISA, EPO on the above application. The written opinion has mentioned that the present application lacks novelty and inventive step and the applicants respectfully disagree with the finding of the written opinion.

With respect to objections raised under Item V, our humble submission is as follows:

D1: Agata et al "Targeting NADPH oxidases in vascular pharmacology": The amended claim 1, 4 and 7 refer to phycocyanin in combination with HMG CoA reductase inhibitor. It is respectfully submitted that in view of the presently amended claim the present invention is novel and inventive over teachings of D1.

D2: US2010/172971: In view of the amended claims 1, 4 and 7, it is respectfully submitted that the objection may be waived.

D3: WO2006/096806: D3 relates to pharmaceutical composition comprising statin and omega-3 fatty acid. Amended claims of the present invention are directed to pharmaceutical composition comprising effective amount of phycocyanin in combination with HMG Co-A reductase inhibitor. In view of the amendment, the pharmaceutical composition of D3 is thus different from the present invention.

D4. WO2004062729: D4 primarily teaches pharmaceutical composition comprising telmisartan and simvastatin and/or diuretic. There is no teaching in D4 on phycocyanin. The approach in the present invention is thus different from D4.

D5. US2011/213004: D5 claims pharmaceutical formulation comprising angiotensin II receptor blocker and statin for use in treatment of cardiovascular diseases. There is no teaching on addition of phycocyanin to the pharmaceutical formulation of D5.

D6: Chang-Jiang Ge et Al: D6 disclose pharmaceutical formulation comprising calcium channel blocker amlodipine and statin atorvastatin for use in the treatment of cardiovascular diseases. The pharmaceutical composition of the present invention comprises phycocyanin in combination with HMG CoA reductase inhibitor. In view of the amended claims, the subject matter of the claims is novel and inventive over D6.

D7: US2002086889: D7 essentially relates to combination of amlodipine and atorvastatin. The present invention does not involve amlodipine. It is respectfully submitted that synergistic effect of amlodipine and atorvastatin may not be extrapolated to combination of phycocyanin and atorvastatin. In view of the amendment, the present application is novel and inventive over D7.

D8: Vascular NADPH oxidases as drug targets for novel antioxidant strategies by Guzik et al.: D8 teaches that NADPH oxidases referred as 'Nox enzyme' play central role in preventing formation of Reactive oxygen species (ROS). However, there is no motivation in the said prior art to arrive at a pharmaceutical composition of phycocyanin and atorvastatin.

In view of the aforesaid the applicant believes that the present application is novel and inventive over the prior art. The said amendments may be regarded as amendments with Statement Under Article 19.

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2013/051987

A. CLASSIFICATION OF SUBJECT MATTER				
INV.	A61K38/16	A61K31/03	A61K31/12	A61K31/137
	A61K31/22	A61K31/366	A61K31/40	A61K31/404
	A61K31/4418	A61K31/47	A61K31/505	A61K35/74
				A61P9/00
According to International Patent Classification (IPC) or to both national classification and IPC				
B. FIELDS SEARCHED				
Minimum documentation searched (classification system followed by classification symbols) A61K				
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched				
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) EPO-Internal, BIOSIS, CHEM ABS Data, EMBASE, SCISEARCH, WPI Data				
C. DOCUMENTS CONSIDERED TO BE RELEVANT				
Category*	Citation of document, with indication, where appropriate, of the relevant passages			Relevant to claim No.
X,P	AGATA SCHRAMM ET AL: "Targeting NADPH oxidases in vascular pharmacology", VASCULAR PHARMACOLOGY, vol. 56, no. 5-6, 1 May 2012 (2012-05-01), pages 216-231, XP055063203, ISSN: 1537-1891, DOI: 10.1016/j.vph.2012.02.012 figure 3 page 223, left-hand column, paragraph 1 - page 227, left-hand column, line 3 abstract ----- -/--			1-3,5-9
☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.				
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family				
Date of the actual completion of the international search 21 May 2013			Date of mailing of the international search report 28/05/2013	
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016			Authorized officer Herdemann, Matthias	

INTERNATIONAL SEARCH REPORT

International application No
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y	paragraph [0045] paragraphs [0082] - [0107] claims 1, 13	1-9
X	WO 2004/062729 A1 (BOEHRINGER INGELHEIM INT [DE]; BOEHRINGER INGELHEIM PHARMA [DE]; RIEDE) 29 July 2004 (2004-07-29) claims 13, 14	1,2,5,6, 8
Y		1-9
X	US 2011/213004 A1 (KIM SUNG WUK [KR] ET AL) 1 September 2011 (2011-09-01) claims 1-4	1,2,5,6, 8
Y	paragraphs [0216] - [0217]; tables 9-10 paragraphs [0222] - [0223]	1-9
X	CHANG-JIANG GE ET AL: "Synergistic effect of amlodipine and atorvastatin on blood pressure, left ventricular remodeling, and C-reactive protein in hypertensive patients with primary hypercholesterolemia", HEART AND VESSELS, SPRINGER-VERLAG, TO, vol. 23, no. 2, 4 April 2008 (2008-04-04), pages 91-95, XP019627848, ISSN: 1615-2573	1,2,5,6, 8
Y	abstract page 92, left-hand column, paragraph 1	1-9
X	US 2002/086889 A1 (MASON R PRESTON [US]) 4 July 2002 (2002-07-04) claims 1, 4, 5	1,5,6,8
Y	paragraph [0016]	1-9
X	GUZIK T J ET AL: "Vascular NADPH oxidases as drug targets for novel antioxidant strategies", DRUG DISCOVERY TODAY, ELSEVIER, RAHWAY, NJ, US, vol. 11, no. 11-12, 1 June 2006 (2006-06-01), pages 524-533, XP027889298, ISSN: 1359-6446 [retrieved on 2006-06-01] abstract page 529, right-hand column, paragraph 3 - page 530, right-hand column, paragraph 6	1-9
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International application No
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	----- HEUMÜLLER SABINE ET AL: "Apocynin is not an inhibitor of vascular NADPH oxidases but an antioxidant", HYPERTENSION, LIPPINCOTT WILLIAMS & WILKINS, US, vol. 51, no. 2, 1 February 2008 (2008-02-01), pages 211-217, XP009134766, ISSN: 0194-911X, DOI: 10.1161/HYPERTENSIONAHA.107.100214 [retrieved on 2007-12-17] abstract -----	1-9

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