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(54) **Title:** SUCROSOMIAL® BERBERINE, ITS COMPOSITIONS AND THEIR USE

(57) **Abstract:** The present invention relates to a berberine formulation in solid form, called "sucrosomial® berberine", comprising or, alternatively, consisting of berberine, a lecithin and a first agent selected from a sucrester, a cyclodextrin, a fatty acid and a chitosan derivative, preferably a sucrester. Furthermore, the present invention relates to compositions comprising said sucrosomial® berberine and, optionally, red rice and to the use thereof in the treatment of a condition of dyslipidaemia or impairment of the lipid metabolism, in particular hypercholesterolemia, and/or impairment of the glucose metabolism.

"*Sucrosomial® berberine, its compositions and their use*"

The present invention relates to a berberine formulation in solid form, called "sucrosomial® berberine" (sucrosomial® is a registered trademark on behalf of Pharmanutra S.p.A and Alesco S.r.l.) or "Ultraberb", comprising or, alternatively, consisting of berberine, a lecithin and a first agent selected from a sucrester (alternatively called fatty acid carbohydrate ester), a cyclodextrin, a fatty acid, a chitosan derivative and a gum, preferably a sucrester or a gum.
Furthermore, the present invention relates to a composition, preferably in solid form, comprising said sucrosomial® berberine and, optionally, red fermented rice, and the use thereof in the treatment of a disease, symptom and/or disorder related with a dyslipidaemia or an impairment of the lipid metabolism, in particular hypercholesterolemia, and/or an impairment of the glucose metabolism.

Lastly, the present invention relates to a process for the preparation of said sucrosomial® berberine.

The present invention also relates to a bergamot formulation in solid form, called "Ultraberg", comprising or, alternatively, consisting of bergamot (or bergamot extract), a lecithin and a first agent selected from a sucrester (alternatively called fatty acid carbohydrate ester), a chitosan derivative and a gum, preferably a sucrester or a gum.
Furthermore, the present invention relates to a composition, preferably in solid form, comprising said sucrosomial® bergamot and, optionally, red fermented rice, and the use thereof in the treatment of a disease, symptom and/or disorder related with a dyslipidaemia or an impairment of the lipid metabolism, in particular hypercholesterolemia, and/or an impairment of the glucose metabolism.

Lastly, the present invention relates to a process for the preparation of said sucrosomial® bergamot.

Dyslipidaemias consist of a plasma increase in cholesterol, triglycerides, or both, or a low level of HDL cholesterol contributing to the development of atherosclerosis. The causes can be primitive (genetic) or secondary. The diagnosis is made by measuring the plasma levels of total cholesterol, triglycerides and individual lipoproteins. Treatment entails dietary changes, physical exercise and lipid-lowering drugs.

To date, various innovative nutritional strategies have been developed to control dyslipidaemias and ESC / EAS guidelines for the management of dyslipidaemias encourage the intake of so-called nutraceuticals as alternatives or supplementation to lipid-lowering drugs. Many nutraceuticals and the combinations thereof have demonstrated lipid-lowering properties, including bark extract of *Berberis aristata* (berberine).

Berberine is a quaternary ammonium salt (5,6-dihydro-9,10-dimethoxybenzo[g]benzo-1,3-dioxolo[5,6-a]quinolizinium, identified for example with CAS No. 2086-83-1, chemical formula $C_{20}H_{18}NO_4^+$) of the protoberberine group of benzylisoquinoline alkaloids present in plants of the Berberidaceae family of the genus Berberis (for example, plants of the species *Berberis vulgaris* (L.), common name barberry, or *Berberis aristata* (DC.), common name of tree turmeric) or in plants with genera other than *Berberis* (for example, plants of the species *Mahonia aquifolium* – Oregon grape, *Hydrastis canadensis* - goldenseal, *Xanthorhiza simplicodro* - yellowroot, *Phellodendron amurense* - *Amur cork tree*, *Coptis chinensis* – chinese Goldthread, *Tinospora cordifolia* , *Argemone mexicana* - mexican poppy, *Eschscholzia californica* - California poppy).
Berberine is usually found in roots, rhizomes, stems or trunks and in the bark.

Berberine has been shown to be effective in controlling LDL cholesterol, plasma triglyceride and HDL cholesterol levels, as well as blood sugar level.
Berberine has many mechanisms of action which are still subject of study. It seems that berberine can reduce the mRNA levels of PCSK9 (*Proprotein Convertase Subtilisin / Kexin* Type 9) and thus the plasma levels of this protein. On the other hand, berberine also exerts a direct effect on LDL receptors, stabilising the mRNA coding thereof. The combination of these two mechanisms (mRNA stabilisation and decrease in PCSK9 activity) leads to an increase in LDL receptors on the surface of hepatic cells and cellular LDL uptake, thus decreasing plasma LDL levels.
Berberine also decreases plasma triglyceride levels through opposite effects on Map kinase (which is inhibited) and on AMP kinase (enhanced).
Lastly, berberine has proven to be capable of increasing plasma HDL cholesterol levels by a few percentage points.

However, when berberine is administered through oral route (in short, per os) to a human subject, its low blood bioavailability (estimated in the literature about 2-3%) may lead to significant differences in the metabolic response of subjects, with cases of poor effectiveness.

CN 106511260 describes a xerogel comprising berberine, a cyclodextrin, and lecithin. This xerogel can then be processed to obtain a solid pellet. However, this document does not describe a solid formulation as claimed in the present context, it even less describes a similar formulation for use in the preventive and/or curative treatment of decrease or normalisation of the plasma levels of plasma levels of total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides.

Thus, there is a high need to provide a novel berberine formulation and compositions comprising said berberine formulation which are effective in the treatment of a disease, symptom and/or disorder related with a dyslipidaemia or an impairment of the lipid metabolism, in particular hypercholesterolaemia, and/or related with an impairment of the glucose metabolism, for all categories of subjects.

In particular, there arises the need for providing an effective solution for treating high blood levels of LDL cholesterol and/or glucose, for all categories of subjects.
Lastly, there arises the need for said novel berberine formulation and compositions comprising said berberine formulation to be stable over time, well tolerated and easy to prepare.

Following extensive research and development activity, the Applicant addresses and solves the aforementioned needs by providing a novel berberine formulation, called sucrosomial® berberine, and compositions comprising said sucrosomial® berberine.

An object of the present invention is to provide a novel berberine formulation, called sucrosomial® berberine, and compositions comprising said sucrosomial® berberine capable of increasing the effectiveness in the treatment of disorders related with dyslipidaemias or impairments of the lipid metabolism and/or impairments of the glucose metabolism.

An increase in effectiveness in the treatment of disorders related with dyslipidaemias or impairments of the lipid metabolism and/or impairments of the glucose metabolism of sucrosomial® berberine with respect to berberine as such, even by a slight degree, results in a decrease in the effective dose to be administered to a subject in need with respect to berberine as such, and, thus, it is also cost-effective.

Said increase in effectiveness in the aforementioned treatment with sucrosomial® berberine or the compositions thereof with respect to treatment with berberine as such, even by a slight degree, could be due to an increase in blood bioavailability of berberine, in turn due to an increase in intestinal or gastrointestinal absorption of sucrosomial® berberine with respect to berberine as such, when administered orally. However, said increase in effectiveness could be due to other mechanisms and reasons.

Said increase in effectiveness in the treatment of disorders related with dyslipidaemias or impairments of the lipid and/or glucose metabolism with sucrosomial® berberine or the compositions thereof, with respect to treatment with berberine as such, results in achieving a greater effect considering the same amount or concentration of berberine administered to a subject in need.

In addition, sucrosomial® berberine and the compositions thereof, subject of the present invention, are stable over time from the chemical-physical and organoleptic point of view.

Lastly, sucrosomial® berberine and the compositions thereof have no relevant side effects, they are well tolerated and, thus, they can be administered to all categories of subjects, including paediatric subjects, adolescents, pregnant women and the elderly.

Furthermore, the process for preparing said sucrosomial® berberine and the compositions thereof is easy to implement or prepare and cost-effective in proportion to the treatment potential.

For example, the berberine formulation of the invention (sucrosomial® berberine) is easily processable to provide the compositions of the present invention, preferably in solid form for oral use.

These and other objects, which will be clear from the detailed description that follows, are attained by the berberine formulation of the present invention (sucrosomial® berberine) and by the compositions thereof due to the technical characteristics claimed in the attached claims.

DESCRIPTION OF THE FIGURES

Figure 1: HPLC analysis on water-soluble extracts of berberine as such and sucrosomial® berberine powders.

Figure 2: cytotoxic effect of berberine as such and sucrosomial® berberine on the HepG2 cell line.

Figure 3: effect of berberine as such and sucrosomial® berberine on LDL cholesterol uptake in the Huh7 cell line.

Figure 4A and Figure 4B: effect of berberine as such and sucrosomial® berberine on levels of mRNA and LDL receptor cell proteins (LDLR) in the Huh7 cell line.

Figure 5: effect of berberine as such and sucrosomial® berberine on the expression of GK and AMPK in the Huh7 cell line.

Figure 6: effect of berberine as such and sucrosomial® berberine on the intracellular glycogen content in the Huh7 cell line.

Figures 7A, 7B, 7C, 7D: Cytotoxicity activity of Atomised berberine (BBR-A) and Ultra-berberine (U-BBR). Cells were seeded (8,000 cells/well of a 96-well tray) and cultured with MEM supplemented with 10% FCS. 24 hours later the medium was replaced with one containing 0.4% FCS and the nutraceuticals concentrations reported and incubation was continued for another 72 hours. At the end of this incubation period, cell viability was determined by means of SRB assay. Each bar represents the mean ± SD of a

representative of three independent experiments. Formulations vs control: * p <0.05; ** p <0.01; *** p <0.001.

Figure 8A, 8B, 8C: Effect of atomised berberine and ultra-berberine on the expression of LDLR and PCSK9 in Huh7 cells. Cells were cultured with MEM/0.4% FCS in the presence or absence of increasing concentrations of atomised berberine (BBR-A) and ultra-berberine (U BBR). After 24 hours, total protein extracts and protein expression evaluated by means of Western blot analysis were prepared. GAPDH was used as a load control. Densitometric readings were evaluated using ImageLab software. Formulations vs control: * p <0.05; ** p <0.01; *** p <0.001.

Figure 9. Effect of bergamot and ultra- bergamot on the expression of HMG-CoA reductase in Huh7 cells. Cells were cultured with MEM/0.4% FCS in the presence or absence of increasing concentrations of bergamot (BE) and ultra-bergamot (U-BE). After 24 hours, total protein extracts and protein expression evaluated by means of Western blot analysis were prepared. GAPDH was used as a load control. Densitometric readings were evaluated using ImageLab software. Formulations vs control: * p <0.05; ** p <0.01.

Figure 10: Effect of the U-BBR formulation on the LDL-DIO uptake by Huh7 cells. Cells were cultured with MEM/0.4% FCS in the presence or absence of increasing concentrations of ultra-berberine (U-BBR). After 24 hours, LDL-DIO was added to the growth medium and uptake was determined by means of cytofluorometry analysis. Formulations vs control: ** p <0.01; *** p <0.001.

DETAILED DESCRIPTION OF THE INVENTION

Forming an object of the present invention is a berberine formulation in solid form called sucrosomial® berberine (in short, berberine formulation of the invention) comprising or, alternatively, consisting of: (a) berberine, (b) a lecithin (c) at least one first agent selected from the group comprising or, alternatively, consisting of: (c-i) a fatty acid carbohydrate ester (alternatively called sucrester); (c-ii) at least one cyclodextrin, preferably an alpha-cyclodextrin; (c-iii) at least one fatty acid having a number of carbon atoms comprised in the range from C6 to C18, preferably from C12 to C18; (c-iv) at least one chitosan derivative, preferably carboxymethyl chitosan; (c-v) a gum selected from carrageenan, acacia gum and a mixture of carrageenan and acacia gum; and mixtures thereof.

Said sucrosomial® berberine may be an atomised “sucrosomial® berberine” or a non-atomised “sucrosomial® berberine”.

The term “atomised sucrosomial® berberine” is used to indicate a formulation comprising a berberine carried on a carrier and/or non-atomised), a lecithin and said first agent (such as (c-i)-(c-iv) or (c-v)). In “atomised sucrosomial® berberine” said berberine (a) is an atomised berberine.

The term “atomised berberine” is used to indicate a berberine carried on a carrier and obtained by means of an atomisation process (as described hereinafter): berberine is added to a carrier (or carrier material, for example a starch) in the presence of a solvent (for example water) to form a mixture (or intermediate product) and said mixture is subjected to an atomisation (or nebulisation) step to obtain said “atomised berberine”. Subsequently, the atomised “berberine” is processed with lecithin and said first agent (such as (c-i)-(c-iv) or (c-v)) to obtain said atomised “sucrosomial® berberine”.

The term “non-atomised sucrosomial® berberine” is used to indicate a formulation comprising a berberine as such (i.e. not carried on a carrier or non-atomised), a lecithin and said first agent (such as (c-i)-(c-iv) or (c-v)) and, optionally, a starch.

Both “atomised sucrosomial® berberine” and “non-atomised sucrosomial® berberine” are berberine formulations according to the invention, in short “sucrosomial® berberine”.

Unless otherwise indicated, in the present description the term “berberine” indicates both berberine as such and “atomised berberine”.

In a preferred embodiment of the present invention, a berberine formulation in solid form called sucrosomial® berberine (in short, berberine formulation of the invention) comprising or, alternatively, consisting of: (a) berberine (berberine as such or atomised berberine), (b) a lecithin and (c) at least one first agent comprising or, alternatively, consisting of (c-i) a fatty acid carbohydrate ester (alternatively called sucrester).

In the context of the present invention, the terms “fatty acid carbohydrate ester”, “sucrester” and “sucrose fatty acid ester”, whether in singular or plural form, are synonyms and they identify the same compound.

In an embodiment of the present invention, berberine (a), comprised in the berberine formulation of the invention (sucrosomial® berberine) together with (b), (c) such as (c-i) or (c-ii) or (c-iii) or (c-iv) or (c-v) and, optionally, (d), is obtained by extracting one or more parts (for example, roots, trunks, rhizomes, bark) of a plant of the genus *Berberis*, preferably of a plant of the species *Berberis aristata* (DC.) or *Berberis vulgaris* (L.), according to conventional extraction methods, preferably aqueous extraction.

Preferably, said berberine (a) is obtained by extracting the roots of the plant of the species *Berberis aristata* (DC.), preferably by means of aqueous extraction according to techniques known to the man skilled in the art.

In a more preferred embodiment, said berberine (a) is a berberine HCl, preferably berberine HCl granular or powder, obtained by the extraction of the species *Berberis aristata* (DC.), preferably by means of aqueous extraction according to techniques known to the man skilled in the art; for example, granular HCl granular obtained by extraction from *Berberis Aristata* DC. And identifiable withCAS No: 633-65-8 and

having the following technical characteristics: excipients: pre-gelatinised starch and microcrystalline cellulose, formula: $C_{20}H_{18}NO_4Cl$, MW: 371.81, loss on drying ≤13.0%, residue after ignition ≤0.2%, particle size ≥90% from 30 mesh to 120 mesh.

In an embodiment of the present invention, the (b) lecithin, comprised in the berberine formulation of the invention (sucrosomial® berberine) together with (a), (c) such as (c-i) o (c-ii) o (c-iii) o (c-iv)o (c-v) and, optionally, (d), is a lecithin in solid form, such as for example in the form of powder or granules, preferably solid lecithin for example E322, more preferably a solid lecithin for example E322 selected from sunflower lecithin, corn lecithin and soya lecithin; even more preferably solid sunflower lecithin E322, for example allergen free solid sunflower lecithin E322 (e.g. in form of powder or granules).

The term “allergen free” is used to indicate that it does not have any allergens as residues.

The term “E322” only defines that lecithin is a food additive according to the Directive 95/2/EC of 20.2.95 published in the Official Journal (in short, O.J.) of the European Union No. L61 of 18.3.95.
Directive 2008/84/EC of 27 August 2008 (published in the Official Journal of the European Union No L253) lays down the purity criteria which a lecithin must meet in order to be considered to meet food quality requirements (lecithin E322): insoluble in acetone (basically the active part of lecithin): 60 % min.; humidity: 2 % max.; acid number: 35 max.; peroxide number: 10 max.; insoluble in toluene (basically impurities): 0.3% max.

From a chemical point of view, the lecithin is a mixture of phosphoric acid, choline, fatty acids, glycerol, glycolipids, triglycerides and phospholipids. Phospholipids represent the main components thereof; the latter derive from the structure of the triglycerides, where a fatty acid is replaced by a phosphate group which confers a negative charge and thus polarity to the molecule; this molecule is known by the generic name phosphatide. A more complex organic molecule - generally serine, choline, ethanolamine, inositol or a single hydrogen atom - is bound to the phosphate group - through an ester bond - obtaining a phospholipid called, respectively, phosphatidylserine, phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol or phosphatidic acid. In its narrowest sense, lecithin is often represented by phosphatidylcholine. Phospholipids are characterised by a water-soluble polar head, which dissolves well in water, while the two saturated fatty acids represent the two non-polar tails, that are not water-soluble but lipophilic. Molecules of this type are called amphipathic and in the presence of water and fat they are arranged between the fat molecule and the water molecule emulsifying them. Due to its chemical-physical properties, lecithin is a natural emulsifier and it has a secondary antioxidant function being rich in natural antioxidant substances.

Should said (b) lecithin be a lecithin in powder or granular form, the lecithin may for example have a water content at a % by weight comprised in a range from 1.5% to 4.5% with respect to the lecithin, preferably from 2% to 4%, even more preferably from 2.5% to 3.5%.
Should said (b) lecithin be a sunflower lecithin E322, preferably in form of powder or granules, lecithin may for example have a glucose content at a % by weight comprised in the range from 20% to 60% with respect to the weight of lecithin, preferably from 30% to 50%, for example about 45%. A (b) sunflower lecithin E322, preferably in form of powder or granules, that can be used % in the context of the present invention may have the following composition at a % by weight (chemical/physical analysis): sunflower lecithin from 40% to 50%, carbohydrates from 40% to 50% (for example about 42%), proteins from 6% to 10%, ashes from 3% to 8%, moisture from 2% to 5% and another flowing agent from 0.5% to 1.5%.

In an embodiment of the present invention, said (c-i) sucrester, comprised in the berberine formulation of the invention (sucrosomial® berberine) together with (a), (b) and, optionally, (d), is a sucrester E473, preferably a sucrester E473, more preferably a sucrester E473 comprising at least 70% by weight, with respect to the total weight of the sucrester, of monoesters obtained through the esterification of sucrose with one or more fatty acids of plant origin, preferably stearic acid and/or palmitic acid.

The initials “E473 ” indicates that the sucresters are food grade additives allowed by the European law and regulated by the Italian Ministerial Decree No 1996.

In an embodiment of the present invention, said (c-i) sucrester, comprised in the berberine formulation of the invention (sucrosomial® berberine) together with (a), (b) and, optionally, (d), does not comprise or, alternatively, does not consist of a diglycerol fatty acid ester.

“Sucresters” are generally obtained from the esterification of the fatty acids or from the trans-esterification of fatty acids methyl esters with carbohydrates (also called saccharides). Sucrose (monosaccharide) and polysaccharides are generally the carbohydrates used. This is why sucresters are also referred to as “sucrose fatty acid esters”. The chemical-physical properties of these compounds depend on the number and the type of esterified fatty acids.
They are essentially emulsifiers and they are added to the compositions so as to determine a greater stabilisation of an aqueous phase with a fatty phase.
Should said (c-i) sucrester be a sucrester E473, said sucrester E473 can be a sucrester having a HLB (hydrophilic-lipophilic balance) value comprised in the range from 14 to 18, preferably a HLB value of about 15 or 16.

A (c-i) sucrester (E473) that can be used in the context of the present invention may have the following composition by weight: total ester content of at least 90%, of which at least 70% by weight, with respect to the total weight of the sucrester, of monoesters obtained through the esterification of sucrose with one or more fatty acids of plant origin, preferably stearic acid and/or palmitic acid; free fatty acid content (such as oleic acid) not exceeding 3 %; free sucrose content not exceeding 2 %; moisture not exceeding 4 %; acid value not exceeding 5. An example of (c-i) commercial sucrester that can be used in the context of the present invention is: sucrose esters SP70 produced by Chimab S.p.A –Italia.

In an embodiment of the present invention, the berberine formulation of the invention (sucrosomial® berberine) further comprises, together with (a), (b) and (c) such as (c-i) or (c-ii) or (c-iii) or (c-iv) or (c-v), a (d) starch of plant origin, preferably gelatinised or pre-gelatinised; preferably, said (d) starch of plant origin is selected from rice starch and/or corn starch; preferably, said (d) starch of plant origin is rice starch (*Oryza sativa*) or native rice starch; more preferably, said (d) starch of plant origin is pre-gelatinised rice starch.

A pre-gelatinised rice starch that can be used in the context of the present invention may for example have the following chemical/physical characteristics: moisture not exceeding 7%; protein content not exceeding 1%; ash content not exceeding 1%; pH (solution 10%) comprised from 5.5 to 7.5, density 0.40- 0.48 g/ cm3; minimum starch content at 97% and fats not exceeding 0.1%. An example of commercial pre-gelatinised rice starch is AX-FG-P produced by Reire Srl –Italia.

In a preferred embodiment of the present invention, the berberine formulation of the invention in solid form (sucrosomial® berberine) comprises or, alternatively, consists of: (a) berberine (berberine as such or atomised berberine), (b) a lecithin, (c-i) a sucrester and (d) a starch of plant origin.

In a more preferred embodiment of the present invention, the berberine formulation of the invention in solid form (sucrosomial® berberine) comprises or, alternatively, consists of: (a) berberine (berberine as such or atomised berberine), preferably berberine HCl granular or powder (b) a solid lecithin (E322), preferably solid sunflower lecithin (E322), more preferably allergen free solid sunflower lecithin (E322); (c-i) a sucrester (E473), preferably sucrester (E473) comprising at least 70% by weight, with respect to the total weight of the sucrester, of monoesters obtained through the esterification of sucrose with one or more fatty acids of plant origin, preferably stearic acid and/or palmitic acid; and (d) pre-gelatinised starch of plant origin, preferably pre-gelatinised rice starch.

In an even more preferred embodiment of the present invention, the berberine formulation of the invention in solid form (sucrosomial® berberine) comprises or, alternatively, consists of: (a) berberine (berberine as such or atomised berberine) or berberine HCl granular or powder; (b) an allergen free solid sunflower lecithin (E322); (c-i) a sucrester (E473) comprising at least 70% by weight, with respect to the total weight of the sucrester, of monoesters obtained through the esterification of sucrose with one or more fatty acids of plant origin, preferably stearic acid and/or palmitic acid; and (d) a pre-gelatinised rice starch.

In an alternative embodiment of the present invention, the berberine formulation of the invention (sucrosomial® berberine) comprises, together with (a), (b) and, optionally, (d) according to any one of the described embodiments, said at least one first agent (c) comprising or, alternatively, consisting of (c-ii) at least one cyclodextrin, preferably an alpha-cyclodextrin.

In an alternative embodiment of the present invention, the berberine formulation of the invention (sucrosomial® berberine) comprises, together with (a), (b) and, optionally, (d) according to any one of the described embodiments, said at least one first agent (c) comprising or, alternatively, consisting of (c-iii) at least one fatty acid having a number of carbon atoms comprised in the range from C6 to C18, preferably from C12 to C18.

In an alternative embodiment of the present invention, the berberine formulation of the invention (sucrosomial® berberine) comprises, together with (a), (b) and, optionally, (d) according to any one of the described embodiments, said at least one first agent (c) comprising or, alternatively, consisting of (c-iv) at least one chitosan derivative, preferably carboxymethyl chitosan. In other words, said (c-iv) replaces (c-i).

In a further alternative embodiment of the present invention, the berberine formulation of the invention (sucrosomial® berberine) further comprises, together with (a), (b), (c-i) and, optionally, (d), at least one or two or three of said first agents selected from the group comprising or, alternatively, consisting of: (c-ii) a cyclodextrin, (c-iii) a C6-C18 fatty acid isomer, (c-iv) a chitosan derivative, and mixtures thereof. In other words, one or more of said (c-ii), (c-iii) and/or (c-iv) are additional to (c-i)

The cyclodextrins (CyD or CD) are natural cyclic oligosaccharides consisting of 6, 7 or 8 monomers of D-(+)glucopyranose joined together by an α,1-4 glycosidic and closed-loop bond. Alpha-cyclodextrin is an oligosaccharide with 6 monomers of D-(+)glucopyranose. An example of a (c-ii) commercial cyclodextrin that can be used in the context of the present invention is the cyclodextrin Cavamax® W6 Food (CAS 10016-20-3) sold by Wecker Chemie AG.

Advantageously, said (c-iii) medium chain fatty acid with a number of carbon atoms C6-C18, preferably C12-C18, linear or branched, saturated or unsaturated, or mixtures of said fatty acids C6-C18 (in short, "C6-C18 fatty acid"), preferably it is lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0) and/or stearic acid (C18:0). Said first agent (c-iii) can be any isomer of a medium chain fatty acid C6-C18, for example any cis isomer or trans isomer of any fatty acid having from 6 to 18 carbon atoms.

Chitosan is a linear polysaccharide consisting of D-glucosamine and N-acetyl-D-glucosamine, linked by β(1- 4) bonds. The expression chitosan derivative is used to indicate, by way of non-limiting example in the context of the present invention, any compound that can be obtained using chitosan or a derivative thereof or a precursor thereof as the starting material and subjecting said starting material to chemical transformations known to the man skilled in the art, so that said chitosan derivative has characteristics such to confer to said (a) mineral (cation of the mineral) comprised in the preparation (or sucrosomial® mineral) an increased bioavailability and absorption capacity in the organism.

The following embodiments (FR) of the berberine formulation of the invention (sucrosomial® berberine), in solid form, are comprised in the present invention:

- FRa, comprising or, alternatively, consisting of (a), (b), (c-i) and (c-ii) and, optionally, (d);
- FRb, comprising or, alternatively, consisting of (a), (b), (c-i) and (c-iii) and, optionally, (d);
- FRc, comprising or, alternatively, consisting of (a), (b), (c-i) and (c-iv) and, optionally, (d);
- FRd, comprising or, alternatively, consisting of (a), (b), (c-i), (c-ii) and (c-iii) and, optionally, (d);
- FRe, comprising or, alternatively, consisting of (a), (b), (c-i), (c-ii) and (c-iv) and, optionally, (d);
- FRf, comprising or, alternatively, consisting of (a), (b), (c-i), (c-iii) and (c-iv) and, optionally, (d);
- FRg, comprising or, alternatively, consisting of (a), (b) and (c-i) and, optionally, (d);
- FRh, comprising or, alternatively, consisting of (a), (b) and (c-ii) and, optionally, (d);
- FRi, comprising or, alternatively, consisting of (a), (b) and (c-iii) and, optionally, (d);
- FRl, comprising or, alternatively, consisting of (a), (b) and (c-iv) and, optionally, (d);
- FRm, comprising or, alternatively, consisting of (a), (b), (c-ii) and (c-iii) and, optionally, (d);
- FRn, comprising or, alternatively, consisting of (a), (b), (c-ii) and (c-iv) and, optionally, (d);
- FRo, comprising or, alternatively, consisting of (a), (b), (c-iii) and (c-iv) and, optionally, (d);
- FRp, comprising or, alternatively, consisting of (a), (b), (c-ii), (c-iii) and (c-iv) and, optionally, (d);

wherein in all said embodiments comprised from FRa to FRp the components called (a), (b), (c-i), (c-ii), (c-iii) and (c-iv) are as defined in the present invention; and wherein it is not present in embodiments FRh to FRp (c-i).

In an embodiment of the berberine formulation of the invention (sucrosomial® berberine) in solid form, the components (a), (b), (c) such as (c-i) or (c-ii) or (c-iii) or (c-iv) and, optionally, (d) they are present at the following amounts at a % by weight with respect to the total weight of the berberine formulation (sucrosomial® berberine):

- said (a) berberine, such as berberine as such or atomised berberine, is comprised in a range from 10% to 90%, preferably from 30% to 80%, more preferably from 40% to 70%, for example about 51±1%;
- said (b) lecithin, preferably solid lecithin (E322), is comprised in a range from 0.05% to 20%, preferably from 0.1% to 10%, more preferably from 0.5% to 2%, for example about 1±0,5%;
- said (c) first agent such as (c-i) or (c-ii) or (c-iii) or (c-iv), preferably (c-i) sucrester (E473), is comprised in a range from 1% to 75% with respect to the weight of the preparation, preferably from 1% a 60%, more preferably from 5% to 40%, for example about 22±1%;
- if present, said (d) starch, preferably pre-gelatinised rice starch, is comprised in a range from 5% to 88%, preferably from 10% to 60%, more preferably from 15% to 40%, for example about 17±1% or 10±1%.

Forming an object of the present invention is a composition (in short, composition of the invention), preferably in solid form, comprising or, alternatively, consisting of: the berberine formulation of the invention (sucrosomial® berberine) comprising or, alternatively, consisting of (a), (b), (c) such as (c-i) or (c-ii) or (c-iii) or (c-iv) or (c-v) and, optionally, (d) according to any one of the embodiments described in the present invention and, optionally, at least one acceptable pharmaceutical or food grade additive and/or excipient.

Said at least one acceptable pharmaceutical or food grade additive and/or excipient can be selected from all substances known to the man skilled in the pharmaceutical art or skilled in preparing food products, such as preservatives, emulsifiers and/or thickeners such as for example hydroxymethyl cellulose, sweeteners, dyes such as for example E171 dye, natural and artificial flavours, antioxidants, stabilisers, fillers, anticaking agents such as for example vegetable magnesium stearate, fatty acid magnesium salts and silicon dioxide, bulking agents such as for example microcrystalline cellulose and mixtures thereof.

In a preferred embodiment, the composition of the invention comprises a berberine formulation of the invention (sucrosomial® berberine) comprising or, alternatively, consisting of (a), (b), (c-i) and, optionally, (d) according to any one of the described embodiments.

Advantageously, the composition of the invention, comprising a berberine formulation of the invention (sucrosomial® berberine) comprising or, alternatively, consisting of (a), (b), (c) such as (c-i) or (c-ii) or (c-iii) or (c-iv) or (c-v) and, optionally, (d) according to any one of the described embodiments, further comprises

at least one or more further active components selected from the group comprising or, alternatively, consisting of:

- (e) at least one vitamin selected from the group of vitamins comprising or, alternatively, consisting of: (e-i) a vitamin of group C, (e-ii) a vitamin of group E, (e-iii) a vitamin of group B, preferably vitamin B6 and/or B9 and/or B12, (e-iv) a vitamin of group D, preferably vitamin D3, and mixtures thereof; preferably (e-i) vitamin C (L-ascorbic acid and/or L-sodium ascorbate);
- (f) at least one organic salt or inorganic salt, preferably selected from the group comprising or, alternatively, consisting of: (f-i) magnesium glycinate, (f-ii) selenium methionine, (f-iii) zinc gluconate and mixtures thereof;
- (g) at least one antioxidant, preferably selected from the group comprising or, alternatively, consisting of: (g-i) N-acetylcysteine (NAC), (g-ii) Coenzyme Q10 (CoQ10), (g-iii) Acetyl-L-carnitine (ALC) and mixtures thereof;
- (h) at least one bacterial strain belonging to the genus *Lactobacillus* or *Bifidobatterium*, preferably of the species selected from the group comprising or, alternatively, consists of: *Lactobacillus casei, Lactobacillus acidophilus, Lactobacillus reuteri, Lactobacillus rhamnosus, Bifidobacterium lungum, Bifidobacterium lactis, Bifidobacterium infantis, Bifidobacterium bifidum* and mixtures thereof; and
- (i) folic acid;
- (l) red fermented rice (*Monascus purpureus*), preferably red fermented rice titrated in monacolin to a % by weight comprised in a range from 2% to 10%, preferably from 3% to 7%, more preferably to about 5%, with respect to the weight of red rice (in short, % w/w), wherein said monacolin is preferably monacolin K.

Red fermented rice is the product of the fermentation of the common rice (*Oryza sativa*) by the yeast *Monascus purpureus* (red yeast), according to the techniques known to the man skilled in the art. The production process of red fermented rice in Japan is known as Koji and is a very ancient technique. During fermentation, the common rice takes the red colouring typical of yeast and it is enriched with many active substances, including monacolin, including monacolin K.

In a preferred embodiment, the composition of the invention comprises a berberine formulation of the invention (sucrosomial® berberine) comprising or, alternatively, consisting of (a), (b), (c) such as (c-i) or (c-ii) or (c-iii) or (c-iv), or (c-v) and, optionally, (d) according to any one of the described embodiments and it further comprises (l) red fermented rice (*Monascus purpureus*), preferably red fermented rice titrated in monacolin to a % by weight comprised in a range from 2% to 10%, preferably from 3% to 7%, more preferably to about 5%, with respect to the weight of red rice, wherein said monacolin is preferably monacolin K.

In a more preferred embodiment, the composition of the invention comprises a berberine formulation of the invention (sucrosomial® berberine) comprising or, alternatively, consisting of (a), (b), (c-i) and, optionally, (d) according to any one of the described embodiments, and said composition further comprises (l) red fermented rice (*Monascus Purpureus*), preferably red fermented rice titrated in monacolin to a % by weight comprised in a range from 2% to 10%, preferably from 3% to 7%, more preferably to about 5% with respect to the weight of red rice, wherein said monacolin is preferably monacolin K.

Advantageously, said (l) red fermented rice (*Monascus purpureus*) titrated in monacolin or monacolin K to 2%-10% or 3%-7% or about 5% w/w, is present in the composition of the invention at a % by weight so that said monacolin or monacolin K is comprised in the composition in a range from 0.05% to 1.00% with respect to the total weight of the composition, preferably from 0.10% to 0.50%.

Furthermore, the following embodiments (FR) of the composition of the invention, preferably in solid form, are comprised in the present invention:

- FRa1, comprising or, alternatively, consisting of (l), (a), (b), (c-i) and (c-ii) and, optionally, (d);
- FRb1, comprising or, alternatively, consisting of (l), (a), (b), (c-i) and (c-iii) and, optionally, (d);
- FRc1, comprising or, alternatively, consisting of (l), (a), (b), (c-i) and (c-iv) and, optionally, (d);
- FRd1, comprising or, alternatively, consisting of (l), (a), (b), (c-i), (c-ii) and (c-iii) and, optionally, (d);
- FRe1, comprising or, alternatively, consisting of (l), (a), (b), (c-i), (c-ii) and (c-iv) and, optionally, (d);
- FRf1, comprising or, alternatively, consisting of (l), (a), (b), (c-i), (c-iii) and (c-iv) and, optionally, (d);
- FRg1, comprising or, alternatively, consisting of (l), (a), (b) and (c-i) and, optionally, (d);
- FRh1, comprising or, alternatively, consisting of (l), (a), (b) and (c-ii) and, optionally, (d);
- FRi1, comprising or, alternatively, consisting of (l), (a), (b) and (c-iii) and, optionally, (d);
- FRl1, comprising or, alternatively, consisting of (l), (a), (b) and (c-iv) and, optionally, (d);
- FRm1, comprising or, alternatively, consisting of (l), (a), (b), (c-ii) and (c-iii) and, optionally, (d);
- FRn1, comprising or, alternatively, consisting of (l), (a), (b), (c-ii) and (c-iv) and, optionally, (d);
- FRo1, comprising or, alternatively, consisting of (l), (a), (b), (c-iii) and (c-iv) and, optionally, (d);
- FRp1, comprising or, alternatively, consisting of (l), (a), (b), (c-ii), (c-iii) and (c-iv) and, optionally, (d);

wherein in all said embodiments comprised from FRa1 to FRp1, the components called (a), (b), (c-i), (c-ii), (c-iii) and (c-iv) are as defined in the present invention; and wherein it is not present in embodiments FRh1 to FRp1 (c-i).

Advantageously, if present, each single vitamin (e) and/or salt (f) is present in the composition of the invention at an amount equal to 100% RDA (recommended dietary allowance).

Advantageously, if present, each single antioxidant substance (g) is present in the composition of the invention in an amount equal to 100 mg/day.

In a preferred embodiment of the invention, the compositions of the invention are in solid form, for example in solid form as such or in mouth-soluble solid form (or mouth-dispersible form, or that dissolve in the oral cavity), preferably in solid form as such.

Alternatively, the compositions of the invention can be in liquid form, for example in the form of solution, dispersion or suspension of a solid in a liquid, or in semi-solid form, for example in form of creams or gels or soft-gels.

In a preferred embodiment of the invention, the compositions of the invention are formulated for oral administration (in short, per os).

Advantageously, the compositions of the invention are in solid form for oral administration, such as for example powder, granules, flakes, tablets or capsules.

If the compositions of the invention are in tablet form, said tablet may have a weight comprised in the range from 200 mg to 2000 mg, for example a hard tablet from 800 mg to 1000 mg.
Said tablets may be coated or filmed with one or more coating layers or films capable of going past the gastric barrier. Said coating may comprise bee wax or a sugar-based solution.

If the compositions of the invention are in the form of a capsule, said capsule may be of hard gelatine or soft gelatine or soft-gel; preferably a gelatine capsule may have a weight comprised in the range of from 100 mg to 1500 mg, more preferably about 800 mg.

The composition of the invention, comprising said berberine formulation of the invention (sucrosomial® berberine) according to any one of the embodiments described in the present invention, may be a pharmaceutical composition, a medical device composition, a dietary supplement, a food or novel food or nutraceutical composition.

In the context of the present invention, the expression “medical device” is used in the meaning according to the Italian Legislative Decree n° 46 dated 24 February 1997 or according to the new Medical Device Regulation (EU) 2017/745 (MDR).

Forming an object of the present invention is the composition of the invention and the berberine formulation of the invention (sucrosomial® berberine), according to any one of the described embodiments, for use as medicament.

Furthermore, forming an object of the present invention is the composition of the invention and the berberine formulation of the invention (sucrosomial® berberine), according to any one of the described embodiments, for use in a method for preventive and/or curative treatment of a disease, symptom and/or disorder related with a dyslipidaemia or an impairment of the lipid metabolism, in subjects in need.

Preferably, said diseases, symptoms and/or disorders related with a dyslipidaemia or impairment of the lipid metabolism are selected from:
(i) hypercholesterolemia,
(ii) abnormal levels of low-density lipoprotein (LDL or LDL cholesterol) in the blood or plasma (levels above the levels of a healthy subject),
(iii) abnormal levels of high-density lipoprotein (HDL or HDL cholesterol) in the blood or plasma (levels below the level of a healthy subject),
(iv) abnormal levels of blood or plasma triglyceride levels (levels above the levels of a healthy subject),
(v) atherosclerosis and relative coronary and cerebrovascular diseases, preferably stroke, and/or
(vi) metabolic syndrome,
and of symptoms and/or disorders related with said diseases or conditions (i)-(vi).

In the context of the present invention, the term “abnormal levels” is used to indicate higher or lower levels with respect to the ranges within which triglyceride or LDL or HDL levels fall in the blood or plasma of healthy subjects as determined by health care organisations (in short, normal or near optimal ranges). Said standard ranges may vary over time and from population to population.
For example, experts recommend keeping total cholesterol levels below 200 mg per decilitre (dl) of blood. LDL cholesterol should ideally not exceed 100 mg/dl, although levels below 160 mg/dl are considered normal. Lastly, HDL cholesterol should be equal to or above 50 mg/dl. Total cholesterol is considered “moderately high” when the blood concentrations thereof are between 200 and 239 mg/dl and “high” when equal to or above 240 mg/dl. LDL-cholesterol concentration is instead considered “almost optimal” for values between 100 and 129 mg/dl, “moderately high” for values between 130 and 159 mg/dl, “high” for values between 160 and 189 mg/dl, and “very high” for values exceeding 190 mg/dl. HDL cholesterol is considered to be “low” when the blood concentrations thereof fall below 40 mg/dl and “high” when they are equal to or above 60 mg/dl.

Preferably, the composition of the invention and the berberine formulation of the invention (sucrosomial® berberine) are for use in a method for the preventive and/or curative treatment of (i) hypercholesterolemia, more preferably the composition of the invention is for use in a method for preventive and/or curative treatment of (ii) modulation or decrease in plasma LDL cholesterol levels and/or of (iii) modulation or increase in plasma HDL cholesterol levels and/or (iv) modulation or decrease in plasma triglyceride levels.

Atherosclerosis is characterised by the presence of inhomogeneous intima plaques (atheromas) which invade the lumen of medium and large arteries; the plaques contain lipids, inflammatory cells, smooth muscle cells and connective tissue. Risk factors comprise dyslipidaemia, diabetes, cigarette smoking, family medical history, sedentary life, obesity and hypertension. Symptoms appear when plaque growth or rupture obstructs or decreases blood flow; symptoms vary depending on the artery involved.
Atherosclerosis is responsible for coronary and cerebrovascular diseases (for example, stroke).

Metabolic syndrome is characterised by a large waist circumference (due to excess abdominal fat), arterial hypertension, impaired fasting plasma glucose or insulin resistance and dyslipidaemia. The causes, complications, diagnosis and treatment are similar to those of obesity.

In the context of the present invention, the term “subject/s” is used to indicate human or animal subjects, preferably mammals (e.g. pets such as dogs, cats, horses, sheep or cattle). Preferably, the compositions of the invention are for use in treatment methods for human subjects.

Furthermore, forming an object of the present invention is the composition of the invention and the berberine formulation of the invention (sucrosomial® berberine), according to any one of the described embodiments, for use in a method for preventive and/or curative treatment of a disease, symptom and/or disorder related with an impairment of the glucose metabolism, in subjects in need.
Preferably, said disease, symptom and/or disorder related with an impairment of the glucose metabolism is selected from:

(I) diabetes, preferably type 2 diabetes mellitus,

(II) hyperglycaemia,

(III) insulin resistance,

(IV) high absorption of carbohydrates,

(V) deregulation of the blood or plasma glucose level, and

(VI) metabolic syndrome,

and symptoms and/or disorders related with said diseases or conditions (I)-(VI); preferably (I) diabetes, more preferably type 2 diabetes mellitus.

In the context of the present invention, the term “deregulation of blood glucose level” is used to indicate that the glucose level is lower or higher with respect to the range within which the glucose levels of healthy subjects fall as determined by health care organisations (in short, ranges or normal values). For example,” normal “blood glucose values are considered to be those comprised in the range from 60 to 110 mg/dL.

In order to treat a condition of dyslipidaemia or impairment of the lipid metabolism or impairment of the glucose metabolism, or symptoms and/or disorders related therewith, there is recommended the daily oral administration of an amount of the composition of the invention comprising or, alternatively, consisting of sucrosomial® berberine so as to provide the administration of an amount of berberine as such comprised in the range from 100 mg to 1,500 mg, preferably from 300 mg to 800 mg, more preferably from 400 mg to 600 mg, for example about 500 mg.

According to the embodiment in which said composition of the invention comprises the sucrosomial® berberine formulation and red fermented rice (*Monascus purpureus*), preferably red fermented rice titrated in monacolin or monacolin K to 2%-10% or 3%-6% or about 5% w/w, there is recommended the daily oral administration of an amount of the composition of the invention so as to provide the administration of an amount of berberine as such comprised in the range from 100 mg to 1,500 mg, preferably from 300 mg to 800 mg, more preferably from 400 mg to 600 mg, for example about 500 mg, and an amount of red fermented rice (*Monascus purpureus*), preferably red fermented rice titrated in monacolin or monacolin K to 2%-10% or 3%-6% or about 5% w/w, comprised in the range from 10 mg to 100 mg, preferably from 40 mg to 80 mg, more preferably from 50 mg to 70 mg, for example about 60 mg.

Preferably, in the embodiment in which the composition of the invention comprises the sucrosomial® berberine formulation and red fermented rice titrated in monacolin or monacolin K to about 5% w/w there is recommended the daily oral administration of an amount of the composition of the invention so as to provide the administration of an amount of berberine as such of about 500 mg and an amount of red fermented rice (*Monascus purpureus*) titrated in monacolin or monacolin K to about 5% w/w, of about 60 mg. For example, the composition of the invention is about 1000 mg and it comprises about 500 mg of berberine as such and about 60 mg of red fermented rice titrated in monacolin or monacolin K to about 5% w/w (equivalent to about 3 mg of monacolin or monacolin K).

The compositions of the invention or the berberine formulation of the invention (sucrosomial® berberine) can be administered for a period of time comprised in the range from 7 days to 120 days.

Forming an object of the present invention is to the use of the composition of the invention or of the berberine formulation of the invention (sucrosomial® berberine) in the therapeutic or non-therapeutic treatment of a disease, symptom and/or disorder related with a dyslipidaemia or impairment of the lipid metabolism, such as, for example, hypercholesterolemia, abnormal levels of blood triglycerides, abnormal levels of blood lipoproteins, atherosclerosis and related coronary and cerebrovascular diseases, preferably stroke and metabolic syndrome.

Furthermore, forming an object of the present invention is the use of the composition of the invention or the berberine formulation of the invention (sucrosomial® berberine) in the therapeutic or non-therapeutic treatment of a disease, symptom and/or disorder related with an impairment of the glucose metabolism, such as, for example, diabetes, preferably type 2 diabetes mellitus, hyperglycaemia, insulin resistance, high glucose uptake level, deregulation of the blood glucose level and metabolic syndrome conditions.

Forming an object of the present invention is a method for the preventive and/or curative treatment of a disease, symptom and/or disorder related with a dyslipidaemia or impairment of the lipid metabolism, such as, for example, hypercholesterolemia, abnormal levels of blood triglycerides, abnormal levels of blood lipoproteins, atherosclerosis and related coronary and cerebrovascular diseases, preferably stroke, and metabolic syndrome, wherein said method provides for the administration of the composition of the invention or of the berberine formulation of the invention (sucrosomial® berberine) to a subject in need.

Furthermore, forming an object of the present invention is a method for the preventive and/or curative treatment of a disorder related with an impairment of the glucose metabolism, such as, for example, diabetes, preferably type 2 diabetes mellitus, hyperglycaemia condition, insulin resistance conditions, high glucose uptake condition, deregulation of blood glucose level and metabolic syndrome conditions, and of symptoms and/or disorders related with said conditions, wherein said method provides for the administration of the composition of the invention or the berberine formulation of the invention (sucrosomial® berberine) to a subject in need.

Forming an object of the present invention is a process for the preparation of the berberine formulation of the invention (sucrosomial® berberine).

In a first embodiment of the invention, said process (in short, first process) is described in patent

document WO 2014/009806 A1 from page 7 line 1 to page 8 line 20, incorporated in the present application for reference.

In a second embodiment of the invention, said process (in short, second process) is described in patent document WO 2014/009806 A1 from page 8 line 22 to page 10 line 21, incorporated in the present application for reference.

Thus, in the context of the present invention, the term “sucrosomial® berberine” indicates a berberine formulation that can be obtained by processing berberine (a) together with a lecithin (b), a first agent (c) such as a sucrester (c-i) or a cyclodextrin (c-ii) or a fatty acid (c-iii) or a chitosan derivative (c-iv) and, optionally, a starch (d), according to said first process or said second process, described above.

The “atomised berberine” comprised in the formulation of the present invention (such as atomised sucrosomial berberine) can be obtained by means of the following process (berberine atomisation process), which forms an object of the present invention.
Said berberine atomisation process comprises the following steps:
(I) providing a first aqueous-based solvent, preferably water, in a container under stirring and heating and adding to said container a berberine, preferably a granular berberine, and plant starch, preferably rice starch, preferably at a by weight ratio comprised from 9:1 to 1:1, more preferably comprised from 4:1 to 2:1, understood as berberine/starch, to obtain an aqueous-based mixture of step (I);
(II) subjecting said mixture of step (I) to a nebulisation/atomisation step to obtain an atomised (or nebulised) berberine, and, optionally,
(III) subjecting said atomised (or nebulised) berberine to a sieving step to obtain a sieved atomised berberine (or atomised berberine).
The amount by weight of berberine (for example granular berberine) and starch, understood as the total solid fraction added to said aqueous-based solvent, preferably water, may be comprised from 20% to 60% by weight with respect to the total weight of said aqueous-based mixture of step (I), preferably comprised from 25% to 50%, even more preferably comprised from 20% to 45%. Preferably the berberine used in the berberine atomisation process is in granular form. The berberine in granular form has the advantage of being easy to handle and process.

The berberine used in the first step may be a commercially available food grade or pharmaceutically acceptable berberine as such, preferably granular berberine hydrochloride. For example, “Berberine Chloride Granular”, CAS No.633-65-8, produced and distributed by Shanghai Freemen LifeScience Co. Ltd. 2500 Xiupu Road, Building 19, Kangqiao, Pudong, Shanghai 201315, China, may be used. Berberine

used as starting product may also contain food or pharmaceutical grade excipients. For example, the berberine that can be used in the first step of the process may have the following composition:

name of the compound	CAS	% by weight
Hydrochloride berberine	633-65-8	88.1%
Pre-gelatinised starch	9005-25-8	8.8%
Microcrystalline cellulose	9004-34-6	3.1%

The berberine that can be used is free from allergens and harmful substances, such as heavy metals, pesticides or organic solvents. Lastly, the berberine that can be used in the present context may be derived from the “Berberis Aristata”.

Atomised/nebulised berberine, that can be obtained from the atomisation/nebulisation step, for example atomising for example by means of electrostatic and ultrasonic atomisers, is a berberine devoid of allergens and harmful substances, such as heavy metals, pesticides or organic solvents. It is also a pharmaceutical or food grade berberine.

For the sake of brevity from now on, the atomised/nebulised berberine, obtained from the atomisation/nebulisation step will be referred to as atomised berberine.

Nebulisation, also called atomisation, is the separation of a liquid into very fine parts (drops), which is obtained, for example, by striking the liquid with a jet of air at high speed or by forcing the liquid to pass through an orifice having a very narrow through-flow section. The narrower the through-flow section of the orifice, the greater the pressure the fluid is subjected to. There are electrostatic and ultrasonic atomisers, which can be used in this context.

The atomized berberine that can be obtained from the atomisation step of the process may have the following composition:

Hydrochloride berberine	90% by weight
Starch, for example a pre-gelatinised rice starch	10% by weight

Furthermore, atomised berberine may have a titre comprised from 70% to 90%, preferably comprised from 75% to 85%.

The plant starch that can be used in the process reported herein is as described in the present context, i.e. plant starch, preferably gelatinised or pre-gelatinised starch of plant origin.

For example, said starch of plant origin may be selected from the group comprising or, alternatively, consisting of rice starch and/or corn starch; preferably, it may be pre-gelatinised rice starch.

In an embodiment said process comprises the following steps:

(I) Loading an aqueous-based solvent, preferably water, at a temperature comprised from 30°C to 80°C (for example about 50°C or 60°C or 70°C), and subsequently loading a solid-form fraction comprising granular berberine, and rice starch, mix up to obtaining a homogeneous solution, called mixture of step (I);

(II) atomising said mixture obtained in step (I) obtaining an atomised berberine of step (II) (in powder form);

(III) Sieving said atomised berberine obtained in step (II) with a screen having a nominal sieve opening comprised from 20 µm to 500 µm, preferably comprised from 100 µm to 300 µm, for example an opening measuring 250 µm, to obtain said atomised berberine or sieved atomised berberine.

In an embodiment, said step (II) of atomising said mixture obtained in step (I) is carried out at a temperature comprised from 50°C to 250°C, preferably comprised from 150°C to 200°C, for a period of time comprised from 1 hour to 10 hours, preferably from 3 hours to 7 hours.

Temperatures are control parameters in the process; according to a preferred example:

- The input temperature is around 185°C and it is managed by the modulation of the burner flame;
- The output temperature is around 80°C and it is managed by sending more or less liquid into the drying chamber, trying to reach the required temperature. The more liquid flows into the chamber, the more the temperature tends to drop while the less liquid flows into the chamber the more the temperature tends to rise.

Furthermore, forming an object of the present invention is said atomised berberine that can be obtained from the berberine atomisation process reported above. Advantageously, not being a powder, said atomised berberine is extremely easy to handle and formulate.

Said atomised berberine may then be formulated by processing said berberine together with a lecithin (b), with a first agent (c) such as a sucrester (c-i) or a chitosan derivative (c-iv) or a gum (c-v) and, optionally, a starch (d), according to said first process or said second process, respectively described in WO 2014/009806 A1 from page 7 line 1 to page 8 line 20, and in WO 2014/009806 A1 from page 8 line 22 to

page 10 line 21.

Preferably, said atomised berberine may be formulated by processing such berberine together with a lecithin (b), a sucrester (c-i) and/or a gum (c-v), or mixtures thereof, according to said first process or said second process, respectively described in WO 2014/009806 A1 from page 7 line 1 to page 8 line 20, and in WO 2014/009806 A1 from page 8 line 22 to page 10 line 21.

Unless specified otherwise, the expression composition or other comprising a component at an amount "comprised in a range from x to y" is used to indicate that said component may be present in the composition or other at all the amounts present in said range, even though not specified, extremes of the range comprised.

Unless specified otherwise, the content of a component in a composition refers to the percentage by weight of that component with respect to the total weight of the composition.

Unless specified otherwise, the indication that a composition "comprises" one or more components means that other components - besides the one, or the ones, indicated specifically - can be present and the indication that a composition "consists" of determined components means that the presence of other components is excluded.

Preferred embodiments of the present invention are reported hereinafter. It is clear that what has been reported up to now in terms of definitions, purposes, uses, terms, processes and amounts also applies to these embodiments when appropriate.

The present invention also relates to a formulation in solid form, comprising or, alternatively, consisting of berberine (berberine as such or atomised berberine), and/or bergamot (or bergamot extract), a lecithin and a first agent selected from a sucrester (alternatively called fatty acid carbohydrate ester), and a gum selected from the group comprising or, alternatively, consisting of: carrageenan, acacia gum and mixtures thereof.

Furthermore, the present invention relates to a composition, preferably in solid form, comprising the solid formulation reported above and, optionally, red fermented rice, and the use thereof in the treatment of a disease, symptom and/or disorder related with a dyslipidaemia or an impairment of the lipid metabolism, in particular hypercholesterolemia.

An object of the present invention is to provide a novel berberine and/or bergamot formulation and compositions comprising said berberine and/or bergamot formulation capable of increasing the effectiveness in the treatment of disorders related with dyslipidaemias or impairments of the lipid metabolism.

An increase in effectiveness in the treatment of disorders related with dyslipidaemias or impairments of the lipid metabolism of berberine and/or of bergamot thus formulated with respect to berberine or bergamot as such, even by a slight degree, results in a decrease in the effective dose to be administered to a subject in need with respect to berberine and/or bergamot as such, and, thus, it reveals various advantages, including cost-effectiveness.

Said increase in effectiveness in the aforementioned treatment with the formulation according to the present invention or the compositions thereof with respect to treatment with berberine or bergamot as such, even by a slight degree, could be due to an increase in blood bioavailability of berberine or bergamot, in turn due to an increase in intestinal or gastrointestinal absorption of berberine or bergamot thus formulated with respect to berberine or bergamot as such, when administered orally. However, said increase in effectiveness could be due to other mechanisms and reasons.

Said increase in effectiveness in the treatment of disorders related with dyslipidaemias or impairments of the lipid metabolism with berberine or with bergamot formulated according to the present invention or the compositions thereof, with respect to treatment with berberine or with bergamot as such, results in achieving a greater effect considering the same amount or concentration of berberine administered to a subject in need.

In addition, berberine and/or bergamot and the compositions, subject of the present invention, are stable over time from the chemical-physical and organoleptic point of view.

Lastly, the berberine and/or the bergamot formulated according to the present invention and the compositions thereof have no relevant side effects, they are well tolerated and, thus, they can be administered to all categories of subjects, including paediatric subjects, adolescents, pregnant women and the elderly.

For example, the berberine and/or bergamot formulation of the invention is easily processable to provide the compositions of the present invention, preferably in solid form for oral use.

In the present context, said at least one agent (c-v) is a gum selected from the group comprising or, alternatively, consisting of carrageenan, acacia gum, and mixtures thereof.

The Carrageenan used in the present context is a food additive (thickener, stabiliser, gelling agent, emulsifier) allowed by European Union and Italian legislation and classified according to European Union legislation as a food additive “E407”.

Carrageenan (INCI Name: Carrageenan EU INCI Name: Chrondrus Chrispus Powder) is a product derived from carragheen, whose name derives from Carrageenan in Ireland. Carrageenans are a complex group of linear polysaccharides, often sulphates, extracted from red algae. They are compounds with high molecular weight, i.e. very large molecules, characterised by repeating units of galactose and 3,6-anhydrogalactose (3,6-AG), both sulphates and non-sulphates. The units alternate with alpha 1-3 and beta 1-4 glycosidic bonds. Due to their structure, carrageenans behave like highly flexible molecules which curl to form helical structures. The spatial arrangement of the molecule generally confers them the ability to form a wide variety of different gels at room temperature. There are mainly three commercial classes of carrageenan: lambda, kappa and iota.

Carrageenan essentially consists of calcium, potassium, sodium and magnesium salts, sulfuric esters of polysaccharides which, by hydrolysis, give galactose and 3,6-anhydrogalactose. Carrageenan must not be hydrolysed or otherwise degraded chemically. It is in the form of a powder of coarse to fine consistency, yellowish to colourless in colour and basically odourless.

Carrageenan is a gelatine widely used for food, medicine and industrial purposes (used to clarify honey, beer, for the manufacture of paper, starch and more), especially in Ireland and in Great Britain; it is obtained by boiling two red algae on the rocky coast of the North Atlantic (Chondrus crispus and Gigartina mamitiosa) known by the names Irish moss or carragheen.

An example of carrageenan that can be used in the present invention is a carrageenan type CSW-2 (Genuvisco®, registered trademark, CP Kelco) standardised with sucrose; CAS 9000-07-1, 57-50-1; according to European standard E407; pH (0.5% solution) 7.0-10.0; loss on drying <=12.0, instantaneous viscosity- high salts <=12; instantaneous viscosity- low salts >=50.

Said carrageenan may be obtained according to processes known to those skilled in the art, for example by means of extraction from seaweed with water and $Ca(OH)_2$ at high temperatures (e.g. slightly >1000C), neutralisation and precipitation with ethanol or isopropanol.

The acacia gum or gum arabic used in the present context is a food additive (thickener, stabiliser, emulsifier) allowed by European Union and Italian legislation and classified according to European Union legislation as a food additive “E414”.

Acacia gum is a natural gum of plant origin called acacia gum, since it is extracted from two species of sub-Saharan acacia: Acacia senegal (L) and Acacia seyal. In particular, acacia gum is a dry exudate obtained from trunks and branches of natural strains of acacias and the like.
Gum arabic is a complex mixture of high molecular weight polysaccharides (Mw 250,000-400,000), the calcium, magnesium and potassium salts thereof and glycoproteins which confer them one of its most important properties: the fact that it is fully edible (edibility). It consists of various components: arabinose, D-galactopyranose, rhamnopyranose, D-glucuronic acid, calcium, magnesium, potassium, sodium, mucilage.

Like almost all gums and resins of plant origin, it is produced by the plant following a natural process of "gummosis" that spontaneously activates to heal an insult (wound) to its surface integrity.

It is an excipient used primarily in the food industry as a "stabiliser", but it also has viscosity control functions in certain inks.

An example of acacia gum that can be used in the present invention is an acacia gum having CAS-No 232-519-5, EINECS 232-519-5, compliant with E414, min. purity 99.9%, max. loss on drying 10%, max. total ash 4%, viscosity (20.0C) min 60 cps., specific optical rotation 30-600 (commercial example 386° gum arabic produced by Chimab, code 306045).

Acacia gums are obtained according to methods known to the man skilled in the art comprising the centrifugation, filtering, heating, drying steps.

The bergamot that can be used in the present invention is a pharmaceutical or food grade bergamot extract. In the context of the present invention, the terms “bergamot” and “bergamot extract” are used interchangeably.

Bergamot is a plant similar to the orange of the family Rutacee (*Citrus bergamia*), with fragrant white flowers and fruits from whose skin an essential oil widely used in perfumery and liqueur industries is extracted. The extract can be obtained from fruits by grating, through a system of peeling machines that

«scratch» the external of the fruit (bark) in a stream of water obtaining an emulsion conveyed in centrifuges that separate the essence from the water for the specific weight. The bergamot extract according to the present invention is commercially available and is approved for pharmaceutical or food use. Preferably said bergamot extract derives from the plant Citrus Bergamia Risso et. poit of the family of Rutacee. The juice deriving from the fruit is normally used in this plant. Normally, the bergamot that can be used in the present context has an aromatic odour, a yellow colour and can be in powder form. For example, bergamot extract produced and sold by Herbal&antioxidant derivatives (H&AD), with the identification code D: BRGESTPG, can be used. The bergamot that can be used in the present context is devoid of allergens and other harmful substances.
An example of bergamot that can be used in the context of the present invention is the phytocompound described in patent document EP2364158 B1 from paragraph [0030] to [0033], incorporated in the present description for reference.

Thus, forming an object of the present invention is a solid formulation, comprising or, alternatively, consisting of:

(a) a berberine (as such or atomised) and/or bergamot (or a bergamot extract),

(b) a lecithin, and

(c) at least one first agent selected from the group comprising or, alternatively, consisting of:

(c-i) a sucrester or a fatty acid carbohydrate ester;

(c-v) at least one gum selected from the group comprising or, alternatively, consisting of: carrageenan, acacia gum and mixtures thereof;

and a mixture of (c-i) and (c-v).

A preferred embodiment of the present invention relates to a berberine formulation in solid form comprising or, alternatively, consisting of: (a) berberine (as such or atomised), (b) a lecithin (c) at least one first agent comprising or, alternatively, consisting of (c-i) a sucrester or a fatty acid carbohydrate ester.

A preferred embodiment of the present invention relates to a bergamot formulation in solid form comprising or, alternatively, consisting of: (a) bergamot, (b) a lecithin and (c) at least one first agent comprising or, alternatively, consisting of (c-i) a sucrester or a fatty acid carbohydrate ester.

A preferred embodiment of the present invention relates to a berberine formulation in solid form comprising or, alternatively, consisting of: (a) berberine (as such or atomised), (b) a lecithin and (c) at least one first agent comprising or, alternatively, consisting of (c-v) a gum selected from the group comprising or, alternatively, consisting of : carrageenan, acacia gum and mixtures thereof.

A preferred embodiment of the present invention relates to a bergamot formulation in solid form comprising or, alternatively, consisting of: (a) bergamot, (b) a lecithin and (c) at least one first agent comprising or, alternatively, consisting of (c-v) a gum selected from the group comprising or, alternatively, consisting of : carrageenan, acacia gum and mixtures thereof.

Optionally, the preferred formulations can comprise d) a starch, preferably of plant origin; even more preferably said starch of plant origin is gelatinised or pre-gelatinised; even more preferably said (d) starch of plant origin is selected from the group comprising or, alternatively, consisting of rice starch and/or corn starch; advantageously said (d) is rice starch, optionally pre-gelatinised.

Preferably, in the formulations of the invention in solid form, the components (a), (b), (c) such as (c-i) and/or (c-v) and, optionally, (d) they are each present independently at the following amounts at a % by weight with respect to the total weight of the formulation:
- said (a) berberine (as such or atomised), is comprised in a range from 10% to 90%, preferably from 30% to 80%, more preferably from 40% to 70%, for example about 51±1%;
- said (b) lecithin, preferably solid lecithin (E322), is comprised in a range from 0.05% to 20%, preferably from 0.1% to 10%, more preferably from 0.5% to 2%, for example about 1±0,5%;
- said (c) first agent such as (c-i) or (c-v), preferably (c-i) sucrester (E473), is comprised in a range from 1% to 75% with respect to the weight of the preparation, preferably from 1% to 60%, more preferably from 5% to 40%, for example about 22±1%;
- if present, said (d) starch, preferably pre-gelatinised rice starch, is comprised in a range from 1% to 88%, preferably from 1% to 50% or 60%, more preferably from 15% to 40%, or from 5% to 15% for example about 17% or 10%+1%.

Preferably, in the formulations of the invention in solid form, the components (a), (b), (c) such as (c-i) and/or (c-v) and, optionally, (d) they are each present independently at the following amounts at a % by weight with respect to the total weight of the formulation:
- said (a) bergamot, is comprised in a range from 10% to 90%, preferably from 30% to 80%, more preferably from 40% to 70%, for example about 51±1%;
- said (b) lecithin, preferably solid lecithin (E322), is comprised in a range from 0.05% to 20%, preferably from 0.1% to 10%, more preferably from 0.5% to 2%, for example about 1±0,5%;
- said (c) first agent such as (c-i) or (c-v), preferably (c-i) sucrester (E473), is comprised in a range from 1% to 75% with respect to the weight of the preparation, preferably from 1% to 60%, more preferably from 5% to 40%, for example about 22±1%;

- if present, said (d) starch, preferably pre-gelatinised rice starch, is comprised in a range from 1% to 70%, preferably from 1% to 50%, more preferably from 20% to 40%, for example about or 32+1%.

Preferably, in the formulations of the invention in solid form, the components (a), (b), (c) such as (c-i) and/or (c-v) and, optionally, (d) they are each present independently at the following amounts at a % by weight with respect to the total weight of the formulation:

- said (a) berberine (as such or atomised), is comprised in a range from 10% to 90%, preferably from 30% to 80%, more preferably from 40% to 70%, for example about 51±1%;
- said (b) lecithin, preferably solid lecithin (E322), is comprised in a range from 0.05% to 20%, preferably from 0.1% to 10%, more preferably from 0.5% to 2%, for example about 1±0,5%;

said (c) at least one first agent comprising or, alternatively, consisting of (c-v) at least one gum selected from the group comprising or, alternatively, consisting of carrageenan, acacia gum and mixtures thereof, at an amount by weight comprised from 1% to 75%, preferably from 10% to 60%, more preferably from 15% to 40% with respect to the total weight of the formulation, with respect to the total weight of the formulation; and

- if present, said (d) starch, preferably pre-gelatinised rice starch, is comprised in a range from 1% to 70%, preferably from 1% to 40%, more preferably from 5% to 20%, for example about or 10+1%.

Preferably, in the formulations of the invention in solid form, the components (a), (b), (c) such as (c-i) and/or (c-v) and, optionally, (d) they are each present independently at the following amounts at a % by weight with respect to the total weight of the formulation:

- said (a) bergamot, is comprised in a range from 10% to 90%, preferably from 30% to 80%, more preferably from 40% to 70%, for example about 51±1%;
- said (b) lecithin, preferably solid lecithin (E322), is comprised in a range from 0.05% to 20%, preferably from 0.1% to 10%, more preferably from 0.5% to 2%, for example about 1±0,5%;

said (c) at least one first agent comprising or, alternatively, consisting of (c-v) at least one gum selected from the group comprising or, alternatively, consisting of carrageenan, acacia gum and mixtures thereof, at an amount by weight comprised from 1% to 75%, preferably from 10% to 60%, more preferably from 15% to 40% with respect to the total weight of the formulation, with respect to the total weight of the formulation; and

- if present, said (d) starch, preferably pre-gelatinised rice starch, is comprised in a range from 1% to 70%, preferably from 10% to 60%, more preferably from 20% to 40%, for example about or 32+1%.

In the formulations described in the present context, advantageously when said first agent (c) comprises or alternatively consists of a mixture of (c-i) and (c-v), the latter are preferably at a weight ratio comprised

from 1:10 to 10:1, more preferably comprised from 1:5 to 5:1, even more preferably comprised from 1:3 to 3:1, for example 1:1.

According to an example of the formulation of the invention, both berberine and bergamot can be present. In the formulations described in the present context, advantageously when said (a) is a mixture of bergamot and berberine, the latter are preferably at a weight ratio comprised from 1:10 to 10:1, preferably comprised from 1:5 to 5:1, even more preferably comprised from 1:3 to 3:1, for example 1:1.

In an embodiment of the present invention, the berberine and/or bergamot formulation further comprises, together with (a), (b) and (c) such as (c-i) or (c-v) a (d) starch of plant origin, preferably gelatinised or pre-gelatinised; preferably, said (d) starch of plant origin is selected from rice starch and/or corn starch; preferably, said (d) starch of plant origin is rice starch (*Oryza sativa*) or native rice starch; more preferably, said (d) starch of plant origin is pre-gelatinised rice starch.

Furthermore, forming an object of the present invention is a composition, preferably in solid form, comprising or, alternatively, consisting of: the berberine and/or bergamot formulation comprising or, alternatively, consisting of (a), (b), (c) such as (c-i) or (c-v) and, optionally, (d) according to any one of the embodiments described in the present invention and, optionally, at least one acceptable pharmaceutical or food grade additive and/or excipient.

Said at least one acceptable pharmaceutical or food grade additive and/or excipient can be selected from all substances known to the man skilled in the pharmaceutical art or skilled in preparing food products, such as preservatives, emulsifiers and/or thickeners such as for example hydroxymethyl cellulose, sweeteners, dyes such as for example E171 dye, natural and artificial flavours, antioxidants, stabilisers, fillers, anticaking agents such as for example vegetable magnesium stearate, fatty acid magnesium salts and silicon dioxide, bulking agents such as for example microcrystalline cellulose and mixtures thereof.

In a preferred embodiment, the composition of the invention comprises a berberine and/or bergamot formulation of the invention comprising or, alternatively, consisting of (a), (b), (c-i) and, optionally, (d) according to any one of the described embodiments.

Advantageously, the composition of the invention, comprising a berberine and/or bergamot formulation comprising or, alternatively, consisting of (a), (b), (c) such as (c-i) or (c-v) and, optionally, (d) according to any one of the described embodiments, further comprises at least one or more further active components selected from the group comprising or, alternatively, consisting of:

- (e) at least one vitamin selected from the group of vitamins comprising or, alternatively, consisting of: (e-i) a vitamin of group C, (e-ii) a vitamin of group E, (e-iii) a vitamin of group B, preferably vitamin B6 and/or B9 and/or B12, (e-iv) a vitamin of group D, preferably vitamin D3, and mixtures thereof; preferably (e-i) vitamin C (L-ascorbic acid and/or L-sodium ascorbate);
- (f) at least one organic salt or inorganic salt, preferably selected from the group comprising or, alternatively, consisting of: (f-i) magnesium glycinate, (f-ii) selenium methionine, (f-iii) zinc gluconate and mixtures thereof;
- (g) at least one antioxidant, preferably selected from the group comprising or, alternatively, consisting of: (g-i) N-acetylcysteine (NAC), (g-ii) Coenzyme Q10 (CoQ10), (g-iii) Acetyl-L-carnitine (ALC) and mixtures thereof;
- (h) at least one bacterial strain belonging to the genus *Lactobacillus* or *Bifidobatterium*, preferably of the species selected from the group comprising or, alternatively, consists of: *Lactobacillus casei, Lactobacillus acidophilus, Lactobacillus reuteri, Lactobacillus rhamnosus, Bifidobacterium lungum, Bifidobacterium lactis, Bifidobacterium infantis, Bifidobacterium bifidum* and mixtures thereof; and
- (i) folic acid;
- (l) red fermented rice (*Monascus purpureus*), preferably red fermented rice titrated in monacolin to a % by weight comprised in a range from 2% to 10%, preferably from 3% to 7%, more preferably to about 5%, with respect to the weight of red rice (in short, % w/w), wherein said monacolin is preferably monacolin K.

Red fermented rice is the product of the fermentation of the common rice (*Oryza sativa*) by the yeast *Monascus purpureus* (red yeast), according to the techniques known to the man skilled in the art. The production process of red fermented rice in Japan is known as Koji and is a very ancient technique. During fermentation, the common rice takes the red colouring typical of yeast and it is enriched with many active substances, including monacolin, including monacolin K.

In a preferred embodiment, the composition of the invention comprises a berberine and/or bergamot formulation comprising or, alternatively, consisting of (a), (b), (c) such as (c-i) or (c-v) and, optionally, (d) according to any one of the described embodiments, and it further comprises (l) red fermented rice (*Monascus Purpureus*), preferably red fermented rice titrated in monacolin to a % by weight comprised in a range from 2% to 10%, preferably from 3% to 7%, more preferably to about 5% with respect to the weight of red rice, wherein said monacolin is preferably monacolin K.

In a more preferred embodiment, the composition of the invention comprises a berberine and/or bergamot formulation comprising or, alternatively, consisting of (a), (b), (c-i) and, optionally, (d) according to any one of the described embodiments, and said composition further comprises (l) red fermented rice (*Monascus*

Purpureus), preferably red fermented rice titrated in monacolin to a % by weight comprised in a range from 2% to 10%, preferably from 3% to 7%, more preferably to about 5% with respect to the weight of red rice, wherein said monacolin is preferably monacolin K.

In a more preferred embodiment, the composition of the invention comprises a berberine and/or bergamot formulation comprising or, alternatively, consisting of (a), (b), (c-v) and, optionally, (d) according to any one of the described embodiments, and said composition further comprises (l) red fermented rice (*Monascus Purpureus*), preferably red fermented rice titrated in monacolin to a % by weight comprised in a range from 2% to 10%, preferably from 3% to 7%, more preferably to about 5% with respect to the weight of red rice, wherein said monacolin is preferably monacolin K.

Advantageously, said (l) red fermented rice (*Monascus purpureus*) titrated in monacolin or monacolin K to 2%-10% or 3%-7% or about 5% w/w, is present in the composition of the invention at a % by weight so that said monacolin or monacolin K is comprised in the composition in a range from 0.05% to 1.00% with respect to the total weight of the composition, preferably from 0.10% to 0.50%.

Advantageously, if present, each single vitamin (e) and/or salt (f) is present in the composition of the invention at an amount equal to 100% RDA (recommended dietary allowance).

Advantageously, if present, each single antioxidant substance (g) is present in the composition of the invention in an amount equal to 100 mg/day.

In a preferred embodiment of the invention, the compositions of the invention are in solid form, for example in solid form as such or in mouth-soluble solid form (or mouth-dispersible form, or that dissolve in the oral cavity), preferably in solid form as such.

Alternatively, the compositions of the invention can be in liquid form, for example in the form of solution, dispersion or suspension of a solid in a liquid, or in semi-solid form, for example in form of creams or gels or soft-gels.

In a preferred embodiment of the invention, the compositions of the invention are formulated for oral administration (in short, per os).

Advantageously, the compositions of the invention are in solid form for oral administration, such as for example powder, granules, flakes, tablets or capsules.

If the compositions of the invention are in tablet form, said tablet may have a weight comprised in the range from 200 mg to 2000 mg, for example a hard tablet from 800 mg to 1000 mg.
Said tablets may be coated or filmed with one or more coating layers or films capable of going past the gastric barrier. Said coating may comprise bee wax or a sugar-based solution.

If the compositions of the invention are in the form of a capsule, said capsule may be of hard gelatine or soft gelatine or soft-gel; preferably a gelatine capsule may have a weight comprised in the range of from 100 mg to 1500 mg, more preferably about 800 mg.

The composition of the invention, comprising said berberine and/or bergamot formulation according to any one of the embodiments described in the present invention, may be a pharmaceutical composition, a medical device composition, a dietary supplement, a food or novel food or nutraceutical composition.

In the context of the present invention, the expression “medical device” is used in the meaning according to the Italian Legislative Decree n° 46 dated 24 February 1997 or according to the new Medical Device Regulation (EU) 2017/745 (MDR).

Forming an object of the present invention is the composition or berberine and/or bergamot formulation, according to any one of the described embodiments, for use as medicament.

Furthermore, forming an object of the present invention is the composition or the berberine and/or bergamot formulation, according to any one of the described embodiments, for use in a method for preventive and/or curative treatment of a disease, symptom and/or disorder related with a dyslipidaemia or impairment of the lipid metabolism, in subjects in need.

Preferably, said diseases, symptoms and/or disorders related with a dyslipidaemia or impairment of the lipid metabolism are selected from:
(i) hypercholesterolemia,
(ii) abnormal levels of low-density lipoprotein (LDL or LDL cholesterol) in the blood or plasma (levels above the levels of a healthy subject),
(iii) abnormal levels of high-density lipoprotein (HDL or HDL cholesterol) in the blood or plasma (levels below the level of a healthy subject),
(iv) abnormal levels of blood or plasma triglyceride levels (levels above the levels of a healthy subject),
(v) atherosclerosis and relative coronary and cerebrovascular diseases, preferably stroke, and/or

(vi) metabolic syndrome,
and of symptoms and/or disorders related with said diseases or conditions (i)-(vi).

In the context of the present invention, the term “abnormal levels” is used to indicate higher or lower levels with respect to the ranges within which triglyceride or LDL or HDL levels fall in the blood or plasma of healthy subjects as determined by health care organisations (in short, normal or near optimal ranges). Said standard ranges may vary over time and from population to population.
For example, experts recommend keeping total cholesterol levels below 200 mg per decilitre (dl) of blood. LDL cholesterol should ideally not exceed 100 mg/dl, although levels below 160 mg/dl are considered normal. Lastly, HDL cholesterol should be equal to or above 50 mg/dl. Total cholesterol is considered “moderately high” when the blood concentrations thereof are between 200 and 239 mg/dl and “high” when equal to or above 240 mg/dL. LDL-cholesterol concentration is instead considered “almost optimal” for values between 100 and 129 mg/dl, “moderately high” for values between 130 and 159 mg/dl, “high” for values between 160 and 189 mg/dl, and “very high” for values exceeding 190 mg/dl. HDL cholesterol is considered to be “low” when the blood concentrations thereof fall below 40 mg/dl and “high” when they are equal to or above 60 mg/dl.

Preferably, the composition of the invention and the berberine and/or bergamot formulation of the invention are for use in a method for the preventive and/or curative treatment of (i) hypercholesterolemia, more preferably the composition of the invention is for use in a method for preventive and/or curative treatment of (ii) modulation or decrease in plasma LDL cholesterol levels and/or of (iii) modulation or increase in plasma HDL cholesterol levels and/or (iv) modulation or decrease in plasma triglyceride levels.

Atherosclerosis is characterised by the presence of inhomogeneous intima plaques (atheromas) which invade the lumen of medium and large arteries; the plaques contain lipids, inflammatory cells, smooth muscle cells and connective tissue. Risk factors comprise dyslipidaemia, diabetes, cigarette smoking, family medical history, sedentary life, obesity and hypertension. Symptoms appear when plaque growth or rupture obstructs or decreases blood flow; symptoms vary depending on the artery involved.
Atherosclerosis is responsible for coronary and cerebrovascular diseases (for example, stroke).

Metabolic syndrome is characterised by a large waist circumference (due to excess abdominal fat), arterial hypertension, impaired fasting plasma glucose or insulin resistance and dyslipidaemia. The causes, complications, diagnosis and treatment are similar to those of obesity.

In order to treat a condition of dyslipidaemia or impairment of the lipid metabolism or symptoms and/or

disorders related therewith, there is recommended the daily oral administration of an amount of the composition of the invention comprising or, alternatively, consisting of the berberine and/or bergamot formulation according to the invention so as to provide the administration of an amount of berberine as such comprised in the range from 100 mg to 1,500 mg, preferably from 300 mg to 800 mg, more preferably from 400 mg to 600 mg, for example about 500 mg.

According to the embodiment in which said composition of the invention comprises the berberine and/or bergamot formulation and red fermented rice (*Monascus purpureus*), preferably red fermented rice titrated in monacolin or monacolin K to 2%-10% or 3%-6% or about 5% w/w, there is recommended the daily oral administration of an amount of the composition of the invention so as to provide the administration of an amount of berberine as such comprised in the range from 100 mg to 1,500 mg, preferably from 300 mg to 800 mg, more preferably from 400 mg to 600 mg, for example about 500 mg, and an amount of red fermented rice (*Monascus purpureus*), preferably red fermented rice titrated in monacolin or monacolin K to 2%-10% or 3%-6% or about 5% w/w, comprised in the range from 10 mg to 100 mg, preferably from 40 mg to 80 mg, more preferably from 50 mg to 70 mg, for example about 60 mg.

Preferably, in the embodiment in which the composition of the invention comprises the berberine and/or bergamot formulation and red fermented rice titrated in monacolin or monacolin K to about 5% w/w there is recommended the daily oral administration of an amount of the composition of the invention so as to provide the administration of an amount of berberine as such of about 500 mg and an amount of red fermented rice (*Monascus purpureus*) titrated in monacolin or monacolin K to about 5% w/w, of about 60 mg. For example, the composition of the invention is about 1000 mg and it comprises about 500 mg of berberine as such and about 60 mg of red fermented rice titrated in monacolin or monacolin K to about 5% w/w (equivalent to about 3 mg of monacolin or monacolin K).

The compositions of the invention or the berberine and/or bergamot formulation of the invention can be administered for a period of time comprised in the range from 7 days to 120 days.

Forming an object of the present invention is to the use of the composition of the invention or of the berberine formulation of the invention (sucrosomial® berberine) in the therapeutic or non-therapeutic treatment of a disease, symptom and/or disorder related with a dyslipidaemia or impairment of the lipid metabolism, such as, for example, hypercholesterolemia, abnormal levels of blood triglycerides, abnormal levels of blood lipoproteins, atherosclerosis and related coronary and cerebrovascular diseases, preferably stroke and metabolic syndrome.

Furthermore, forming an object of the present invention is the use of the composition of the invention or the berberine formulation of the invention (sucrosomial® berberine) in the therapeutic or non-therapeutic treatment of a disease, symptom and/or disorder related with an impairment of the glucose metabolism, such as, for example, diabetes, preferably type 2 diabetes mellitus, hyperglycaemia, insulin resistance, high glucose uptake level, deregulation of the blood glucose level and metabolic syndrome conditions.

Forming an object of the present invention is a method for the preventive and/or curative treatment of a disease, symptom and/or disorder related with a dyslipidaemia or impairment of the lipid metabolism, such as, for example, hypercholesterolemia, abnormal levels of blood triglycerides, abnormal levels of blood lipoproteins, atherosclerosis and related coronary and cerebrovascular diseases, preferably stroke, and metabolic syndrome, wherein said method provides for the administration of the composition of the invention or of the berberine formulation of the invention (sucrosomial® berberine) to a subject in need.

Furthermore, forming an object of the present invention is a method for the preventive and/or curative treatment of a disorder related with an impairment of the glucose metabolism, such as, for example, diabetes, preferably type 2 diabetes mellitus, hyperglycaemia condition, insulin resistance conditions, high glucose uptake condition, deregulation of blood glucose level and metabolic syndrome conditions, and of symptoms and/or disorders related with said conditions, wherein said method provides for the administration of the composition of the invention or the berberine formulation of the invention (sucrosomial® berberine) to a subject in need.

Forming an object of the present invention is a process for the preparation of the berberine formulation of the invention (sucrosomial® berberine).

In a first embodiment of the invention, said process (in short, first process) is described in patent document WO 2014/009806 A1 from page 7 line 1 to page 8 line 20, incorporated in the present application for reference.

In a second embodiment of the invention, said process (in short, second process) is described in patent document WO 2014/009806 A1 from page 8 line 22 to page 10 line 21, incorporated in the present application for reference.

Thus, in the context of the present invention, the term “sucrosomial® berberine” indicates a berberine formulation that can be obtained by processing berberine (a) together with a lecithin (b), a first agent (c) such as a sucrester (c-i) or a cyclodextrin (c-ii) or a fatty acid (c-iii) or a chitosan derivative (c-iv) and,

optionally, a starch (d), according to said first process or said second process, described above.

Furthermore, the following embodiments FRan are part of the present invention.

FRa1. A berberine formulation in solid form, called sucrosomial® berberine, comprising or, alternatively, consisting of:

(a) a berberine,

(b) a lecithin, and

(c) at least one first agent selected from the group comprising or, alternatively, consisting of:

(c-i) a sucrester or a fatty acid carbohydrate ester;

(c-ii) at least one cyclodextrin;

(c-iii) at least one fatty acid having a number of carbon atoms comprised in the range from C6 to C18;

(c-iv) at least one chitosan derivative;

and mixtures thereof.

FRa 2. The berberine formulation according embodiment 1, wherein said berberine formulation, called sucrosomial® berberine, comprises or, alternatively, consists of:

(a) a berberine,

(b) a lecithin, and

(c) at least one first agent comprising or, alternatively, consisting of (c-i) a sucrester or a fatty acid carbohydrate ester.

FRa 3. The berberine formulation according to embodiment 1 or 2, wherein said formulation further comprises (d) a gelatinised or pre-gelatinised starch of plant origin; preferably, wherein said (d) starch of plant origin is selected from rice starch and/or corn starch; preferably, wherein said (d) is pre-gelatinised rice starch.

FRa 4. The berberine formulation according to any one of embodiments 1- 3, wherein said (c) at least one first agent is selected from the group comprising or, alternatively, consisting of:

- said (c-i) sucrester, wherein said (c-i) is a sucrester E473; preferably a sucrester E473 comprising at least 70% by weight, with respect to the total weight of the sucrester, of monoesters obtained through the esterification of sucrose with one or more fatty acids of plant origin, preferably stearic acid and/or palmitic acid;

- said (c-ii) at least one cyclodextrin, wherein said (c-ii) is an alpha-cyclodextrin;

- said (c-iii) at least one fatty acid, wherein said (c-iii) is a fatty acid having a number of carbon atoms comprised in the range from C12 to C18;
- said (c-iv) at least one chitosan derivative, wherein said (c-iv) is a carboxymethyl chitosan;
and mixtures thereof.

FRa 5. A composition comprising or, alternatively, consisting of:
- the berberine formulation in solid form, called sucrosomial® berberine, according to any one of the embodiments 1- 4, and, optionally,
- at least one acceptable pharmaceutical or food grade additive and/or excipient.

FRa 6. The composition according to embodiment 5, wherein said composition further comprises red fermented rice; preferably red fermented rice titrated in monacolin at a by weight % comprised in a range from 2% to 10%, preferably from 3% to 7%, more preferably to about 5%, with respect to the weight of red rice.

FRa 7. The composition according to embodiment 5 or 6, wherein said composition is for use as medicament.

FRa 8. The composition for use according to embodiment 7, wherein said composition is for use in a method for the preventive and/or curative treatment of a disease, symptom and/or disorder related with a dyslipidaemia or an impairment of the lipid metabolism.

FRa 9. The composition for use according to embodiment 8, wherein said composition is for use in a method for the preventive and/or curative treatment of:
(i) hypercholesterolemia;
(ii) decrease in the blood or plasma LDL cholesterol levels,
(iii) increase in the blood or plasma HDL cholesterol levels,
(iv) decrease in the blood or plasma triglyceride levels,
(v) atherosclerosis and relative coronary and cerebrovascular diseases, preferably stroke, and/or
(vi) metabolic syndrome,
and symptoms and/or disorders related with said diseases (i)-(vi).

FRa 10. The composition for use according to embodiment 7, wherein said composition is for use in a method for the preventive and/or curative treatment of a disease, symptom and/or disorder related with an impairment of the glucose metabolism.

FRa 11. The composition for use according to embodiment 10, wherein said composition is for use in a method for the preventive and/or curative treatment of:

(I) diabetes, preferably type 2 diabetes mellitus,

(II) hyperglycaemia,

(III) insulin resistance,

(IV) high absorption of carbohydrates,

(V) deregulation of the blood or plasma glucose level, and/or

(VI) metabolic syndrome,

and symptoms and/or disorders related with said diseases (I)-(VI).

Furthermore, the following embodiments FRbn are part of the present invention.

FRb 1. A berberine formulation in solid form for use in a method for preventive and/or curative treatment of a disease, symptom and/or disorder related with a dyslipidemia or an impairment of the lipid metabolism, wherein said berberine formulation in solid form, called sucrosomial® berberine, comprising or, alternatively, consisting of:

(a) a berberine,

(b) a lecithin, and

(c) at least one first agent selected from the group comprising or, alternatively, consisting of:

(c-i) a sucrester or a fatty acid carbohydrate ester;

(c-ii) at least one cyclodextrin;

(c-iii) at least one fatty acid having a number of carbon atoms comprised in the range from C6 to C18;

(c-iv) at least one chitosan derivative;

and mixtures thereof.

FRb 2. The berberine formulation in solid form for use according to FRb 1, wherein said formulation is for use in a method for preventive and/or curative treatment of a hypercholesterolemia and of symptoms and/or disorders related therewith.

FRb 3. The berberine formulation in solid form for use according to FRb 1, wherein said formulation is for use in a method for preventive and/or curative treatment of decrease in the plasma LDL cholesterol levels.

FRb 4. The berberine formulation in solid form for use according to FRb 1, wherein said formulation is for

use in a method for preventive and/or curative treatment increase in the plasma HDL cholesterol levels.

FRb 5. The berberine formulation in solid form according to FRb 1, wherein said formulation is for use in a method for preventive and/or curative treatment of decrease in the blood or plasma triglyceride levels.

FRb 6. The berberine formulation in solid form for use according to FRb 1, wherein said formulation is for use in a method for preventive and/or curative treatment of an atherosclerosis or a relative coronary or cerebrovascular disease, preferably stroke, and of symptoms and/or disorders related therewith.

FRb 7. The berberine formulation in solid form for use according to FRb 1, wherein said formulation is for use in a method for preventive and/or curative treatment of a metabolic syndrome and of symptoms and/or disorders related therewith.

FRb 8. The berberine formulation in solid form for use according to any one of FRbs 1- 7, wherein said (c) at least one first agent comprises or, alternatively, consists of (c-i) a sucrester or a fatty acid carbohydrate ester.

FRb 9. The berberine formulation in solid form for use according to any one of FRbs 1-8, wherein said formulation further comprises (d) a gelatinised or pre-gelatinised starch of plant origin; preferably, wherein said (d) starch of plant origin is selected from rice starch and/or corn starch; preferably, wherein said (d) is pre-gelatinised rice starch.

FRb 10. The berberine formulation in solid form for use according to any one of FRbs 1- 9, wherein said (c) at least one first agent is selected from among the group comprising or, alternatively, consisting of:
- said (c-i) sucrester, wherein said (c-i) is a sucrester E473; preferably a sucrester E473 comprising at least 70% by weight, with respect to the total weight of the sucrester, of monoesters obtained through the esterification of sucrose with one or more fatty acids of plant origin, preferably stearic acid and/or palmitic acid;
- said (c-ii) at least one cyclodextrin, wherein said (c-ii) is an alpha-cyclodextrin;
- said (c-iii) at least one fatty acid, wherein said (c-iii) is a fatty acid having a number of carbon atoms comprised in the range from C12 to C18;
- said (c-iv) at least one chitosan derivative, wherein said (c-iv) is a carboxymethyl chitosan;
and mixtures thereof.
Furthermore, the following embodiments FRcn are object of the present invention.

FRc 1. A formulation in solid form comprising or, alternatively, consisting of:

(a) an extract of bergamot and/or berberine,

(b) a lecithin, and

(c) at least one first agent selected from the group comprising or, alternatively, consisting of:

(c-i) a sucrester or a fatty acid carbohydrate ester;

(c-v) at least one gum selected from the group comprising or, alternatively, consisting of: carrageenan, acacia gum,

and a mixture of (c-i) and (c-v).

FRc 2. A formulation in solid form according to FRc 1 comprising or, alternatively, consisting of:

(a) said bergamot extract,

(b) said lecithin, and

(c) said at least one first agent selected from the group comprising or, alternatively, consisting of:

(c-i) a sucrester or a fatty acid carbohydrate ester;

(c-v) at least one gum selected from the group comprising or, alternatively, consisting of: carrageenan, acacia gum

and a mixture of (c-i) and (c-v).

FRc 3. The formulation in solid form according to claim FRc 1, comprising or, alternatively, consisting of:

(a) said berberine,

(b) said lecithin, and

(c) said at least one first agent selected from the group comprising or, alternatively, consisting of:

(c-i) a sucrester or a fatty acid carbohydrate ester;

(c-v) at least one gum selected from the group comprising or, alternatively, consisting of: carrageenan, acacia gum, and

and a mixture of (c-i) and (c-v).

FRc 4. The formulation according to any one of the preceding FRcs, wherein said formulation further comprises (d) a starch; preferably, wherein said (d) starch is of plant origin; even more preferably said starch of plant origin is gelatinised or pre-gelatinised.

FRc 5. The formulation according to FRc 4, wherein said (d) starch of plant origin is selected from the group comprising or, alternatively, consisting of rice starch and/or corn starch; preferably, wherein said (d) is pre-gelatinised rice starch.

FRc 6. The formulation according to any one of the preceding FRcs, wherein said formulation comprises, or alternatively, consists of:

(a) said berberine and/or said bergamot extract;

(b) a lecithin,

(c) at least one first agent comprising or, alternatively, consisting of (c-i) a sucrester or a fatty acid carbohydrate ester; and

(d) a starch.

FRc 7. The formulation according to any one of the preceding FRcs, wherein said formulation comprises or, alternatively, consists of:

(a) a berberine and/or bergamot;

(b) a lecithin, and;

(c) at least one first agent comprising or, alternatively, consisting of (c-v) at least one gum selected from the group comprising or, alternatively, consisting of carrageenan, acacia gum and mixtures thereof; and

(d) a starch.

FRc 8. The formulation according to any one of the preceding FRcs, wherein said formulation comprises or, alternatively, consists of:

(a) a berberine and/or bergamot;

(b) a lecithin;

(c) at least one mixture of said first agent comprising or, alternatively, consisting of (c-i) a sucrester or a fatty acid carbohydrate ester and (c-v) at least one gum selected from the group comprising or, alternatively, consisting of carrageenan, acacia gum and mixtures thereof; and

(d) a starch.

FRc 9. The formulation according to FRc 8, wherein said mixture (c-i) and (c-v) are at a weight ratio comprised from 1:10 to 10:1, preferably comprised from 1:5 to 5:1, even more preferably comprised from 1:3 to 3:1, for example 1:1.

FRc 10. A composition comprising or, alternatively, consisting of:

- the formulation in solid form according to any one of claims 1-9, and optionally,
- at least one acceptable pharmaceutical or food grade additive and/or excipient.

FRc 11. The composition according to FRc 10, wherein said composition further comprises red fermented rice; preferably fermented red rice titrated in monacolin to a weight % comprised from 2% to 10%, preferably from 3% to 7%, more preferably to about 5%, with respect to the weight of red rice.

FRc 12. A formulation according to any one of FRcs 1-9 or a composition according to claim 10 or 11, wherein said composition is for use as medicament.

FRc 13. The formulation or the composition for use according to claim 11, wherein said formulation or composition is for use in a method for the preventive and/or curative treatment of a disease related with dyslipidaemia and/or hypercholesterolaemia.

FRc 14. The formulation or the composition for use according to FRc 13, wherein said formulation or composition is for use in a method for the preventive and/or curative treatment of decrease or normalisation of the plasma levels of total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides.

FRc 15. The formulation or the composition for use according to FRc 13, wherein said formulation or composition is for use in a method for the preventive and/or curative treatment of decrease in the plasma LDL cholesterol levels.

FRc 16. The formulation or the composition for use according to FRc 13, wherein said formulation or composition is for use in a method for the preventive and/or curative treatment of decrease in the blood or plasma triglyceride levels.

FRc 17. The formulation or the composition for use according to FRc 13, wherein said formulation or composition is for use in a method for the preventive and/or curative treatment of a disease related with atherosclerosis or a coronary or cerebrovascular disease, preferably stroke.

FRc 18. The formulation or the composition for use according to FRc 13, wherein said formulation or composition is for use in a method for the preventive and/or curative treatment of a metabolic syndrome and symptoms and/or disorders related therewith.

FRc 19. A process for the preparation of an atomised berberine carried on a carrier, wherein said process comprises the following steps:

(I) providing an aqueous-based solvent, preferably water, in a container under stirring and heating and adding to said container a berberine, preferably a granular berberine, and plant starch, preferably rice

starch, to obtain a mixture of step (I);

(II) subjecting said mixture of step (I) to a nebulisation or atomisation step to obtain an atomised or nebulised berberine, and, optionally,

(III) subjecting said atomised/nebulised berberine to a sieving step to obtain a sieved atomised berberine.

FRc 20. An atomised berberine carried on a carrier obtainable according to the process according to FRc 19.

EXPERIMENTAL PART

1. Study design

The purpose of the present trial is to predict the cholesterol lowering properties of the berberine formulation according to the present invention (sucrosomial® berberine) using in vitro experimental models in LDL cholesterol metabolism. To this end, human hepatocarcinoma cell lines (HepG2 and Huh7) were used to test the berberine formulations according to the present invention (sucrosomial® berberine) on their ability to modulate the expression of genes involved in cholesterol and lipid metabolisms, such as LDL receptor, PCSK9, HMG-CoA reductase, fatty acid synthase (FAS) and effect on LDL cholesterol uptake.

2. Materials used.

- berberine as such (solid) in the form of berberine HCl, formulation not according to the invention.
- sucrosomial® berberine (solid), berberine formulation according to the invention, as reported in Table 1.

Ingredients list	Amt per 100g	Amt per unit
Berberine HCl	60.0 g (51.0 g berberine as such)	600.000 mg
Saccharide fatty acid esters (sucrester)	21.700 g	217.000 mg
Pre-gelatinised rice starch (*Oryza sativa* L.)	17.300 g	173.000 mg
Sunflower lecithin (E-322)	1.000 g	10.000 mg
Total	100.000 g	1000.000 mg

Table 1

3. In *vitro* evaluation of sucrosomial® berberine on LDL cholesterol uptake.

3.1. Berberine concentration after extraction with water.

In order to compare the differential effect of berberine as such and sucrosomial® berberine (formulation according to the invention), the concentration of berberine was first measured after extraction with water of 100 mg berberine as such and sucrosomial® berberine. HPLC analysis showed that the amounts of berberine after extraction - with water at room temperature - of 100 mg berberine as such (powder) and 100 mg sucrosomial® berberine (powder) with 1 ml of water were similar to each other and were about 3.1 mg/ml each (Figure 1 and Table 2). With berberine having an MW of 336.36 g/mol, the molar concentration of these basic preparations is equal to 9.2 mM.

Sample	Berberine concentration (mg/ml)
Berberine	3.21
Sucrosomial® berberine	3.12

Table 2

3.2. Cytotoxic effect.

The potential cytotoxic effect of sucrosomial® berberine was compared with berberine as such in HepG2 cells after 24 hours of incubation. The range of concentrations tested was comprised in the range from 2.5 µM to 40 µM. Cell viability was determined by means of SRB assay (sulforhodamine B assay)

No significant cytotoxic effects were observed from both samples tested (Figure 2).

3.3. Determination of LDL cholesterol uptake.

Regardless of the mechanism of action of berberine, the final cellular outcome for the effects of cholesterol is determined by the ability of hepatocytes to uptake LDL cholesterol. Thus, several experiments were conducted with the aim of determining the effect of both preparations of berberine, berberine as such and sucrosomial® berberine, on hepatocyte cell lines.

HuH7 cells were incubated with berberine as such or sucrosomial® berberine at 10 µM and 20 µM for 24 hours. Subsequently, fluorescent-labelled LDL-cholesterol (DiO-LDL) was added to the growth medium (10 µg / ml of DiO-LDL) for 3 hours and cell fluorescence was determined by means of flow cytometry analysis (percentage of positive fluorescent cells).

As shown in Figure 3, both berberine as such and sucrosomial® berberine significantly induced LDL cholesterol uptake with respect to untreated control cells (in short, Crt). It should be noted that sucrosomial® berberine is more efficient than berberine as such to induce the absorption of LDL cholesterol, as observed at the low concentration tested, i.e. 10 µM.

Caption in Figure 3: ***p<0.001 vs Ctr; **p<0.01 vs Ctr; #p<0.05 vs Berberine 10µM.

3.4. Effect on mRNA expression of genes involved in lipid metabolism and cholesterol metabolism.

A better effectiveness of sucrosomial® berberine with respect to berberine as such on LDL cholesterol uptake suggests a potential positive effect on expression of LDL receptor (in short, LDLR). It is known in the literature that berberine as such increases the stability of the mRNA of the LDL receptor (LDLR) and, thus, the expression of LDLR. Thus, the effect of berberine as such and sucrosomial® berberine on LDLR mRNA levels and LDLR cell protein levels in the Huh7 cell line was evaluated by means of real time quantitative PCR (simultaneous DNA amplification and quantification method) and western blot analysis, respectively.

The expression of LDLR mRNA was significantly increased after only 24 hours of incubation with 20 µM of sucrosomial® berberine (Figure 4A). This induction is significantly higher than the LDLR mRNA levels in Huh7 cells incubated with berberine as such at 20µM (Figure 4A).

Even more importantly, 10 µM and 20 µM sucrosomial® berberine significantly induced the LDL receptor (LDLR) (quantification of LDLR cell proteins) more efficiently with respect to berberine as such considering the same concentrations (Figure 4B).

3.5. Conclusion on the results of points 3.3 and 3.4.

As a whole, these results indicate that sucrosomial® berberine more efficiently induces LDL cholesterol uptake (in short, LDL-C) and the expression of LDL receptor (LDLR) with respect to berberine as such.

4. In vitro evaluation of sucrosomial® berberine on proteins involved in glucose metabolism.

In the literature it is known that, besides a hypocholesterolemic action, berberine shows a significant glucose lowering effect and an enhancement in the related metabolic disorders both in clinical and animal trials. AMPK and glucokinase (in short, GK) is the main intracellular target of the berberine mediating this action. Thus, the effect of sucrosomial® berberine on these two pathways was studied using Huh7 cells exposed to high glucose levels (25mM).

As shown in figure 5, both berberine as such and sucrosomial® berberine induced the expression of GK and the phosphorylation state of AMPK. This effect is slightly more pronounced with sucrosomial ® berberine with respect to berberine as such.

In detail, Huh7 cells were incubated in DMEM (Dulbecco's Modified Eagle's Medium) containing 25mM of glucose with indicated concentrations of berberine as such and sucrosomial® berberine for 24 hours. After this period , the total cellular proteins were extracted for analysis.

GK catalyses the conversion of glucose to glucose-6-phosphate and it is considered the guardian of

glucose metabolism in hepatocytes. As a matter of fact, glucose-6-phosphate can be further transformed into glucose-1-phosphate (G-1-P) and synthesised into glycogen for storage or used by the cell through the tricarboxylic acid cycle and the pentose phosphate pathway.

Biochemical quantification of the intracellular amount of glycogen showed that berberine as such and sucrosomial® berberine both drastically reduced the levels thereof after 24 hours of treatment (Figure 6).

In detail, Huh7 cells were incubated in DMEM containing 25mM of glucose with indicated concentrations of berberine as such and sucrosomial® berberine for 24 hours. After this period, the total glycogen content was determined by means of ELISA test.

5. Conclusions.

The limited bioavailability of berberine as such may limit its favourable action on lipid and glucose metabolisms. For this reason, a novel berberine formulation according to the present invention based on sucrosomial technology (sucrosomial® berberine), which can enhance its cellular uptake and its therapeutic action, has been analysed in the present trial.

Sucrosomial® berberine was more effective with respect to berberine as such, evidenced by a significant improvement of sucrosomial® berberine with respect to berberine as such on the *in vitro* determination of the uptake of LDL cholesterol (LDL-C), on the expression of LDL receptor (both mRNA and proteins), GK and the phosphorylation status of AMPK.

6. Experimental details.

6.1. Reagents.

Eagle's minimum essential medium (MEM) was bought from Sigma, trypsin-EDTA, penicillin, streptomycin, sodium pyruvate, non-essential amino acid solution, foetal calf serum (FCS), petri dishes and capsules were bought from EuroClone. DiO was bought from Sigma. Berberine as such and sucrosomial® berberine were prepared by dissolving 10 mg of each compound in 1 ml of water.

6.2. *Culture cellular*.

HepG2, Huh7 and Caco2 cells were cultured in MEM supplemented with 10% FCS, L-glutamine, sodium pyruvate and non-essential amino acids, penicillin / streptomycin at 37°C in a humidified atmosphere at 5% CO_2 and 95% air.

6.3. Cell viability test.

Cell viability was determined by means of sulforhodamine B (SRB) assay, as described above. The cells

were seeded in a 96-well plate (8 x 10^3 cells/well) and the SRB test was conducted after 48 hours of incubation with increasing concentrations of nutraceutical compounds.

6.4. LDL isolation and labelling.

Total LDL (d >1.019 <1.063 g / mL) was isolated from human plasma by means of ultracentrifugation at 4°C. To remove the excess of EDTA, LDL samples were transferred in dialysis tubes and dialysed in physiological solution (0.9% NaCl in deionised water) at 4 °C, changing the solution three times (4 hours, 48 hours). Purified LDLs were sterilised using a 0.22 µm filter and stored at 4°C. The protein content was evaluated by the BCA assay, using BSA as a standard. For labelling, LDLs were incubated with the DiO fluorescent dye (250 µg of DiO / mg of LDL protein) for 18 hours at 4°C. LDL-DiO was purified on a Sephadex G25 (PD10) column with 0.01% PBS-EDTA (pH 7.4), to remove unbound DiO.

6.5. Assay based on uptake of LDL fluorescent cells.

Cells were seeded in a 96-well plate (25 x 10^3 cells / well in a complete medium) and after 24 hours they were treated in 0.4% FCS medium. 24 hours after treatment, the cells were incubated with 100 µg/mL LDL-DiO (3,3'-dioctadecyloxacarbocyanine). After 3 hours incubation at 37°C, the cells were analysed by means of flow cytometry analysis.

6.6. Reverse transcription and quantitative PCR (RT-qPCR).

"RNA Preparation" e "Quantitative Real Time PCR-Total RNA" were extracted using the reagents for the preparation of cDNA synthesis (Bio-Rad), iScript™ RT-qPCR Sample Preparation Buffer (BIO-RAD), according to the manufacturer's instructions. Reverse synthesis of transcription-polymerase first strand cDNA was performed using the Maxima first Strand cDNA synthesis kit (Thermo Scientific). qPCR was then performed using *PowerUp™ SYBR™ Green Master Mix (Thermo Scientific) and primers* specific to selected genes. The analyses were performed with Mx3000P qPCR System (Agilent), under the following cycle conditions: 95°C, 2 min; 95°C, 15 seconds and 60°C, 1 minute for 40 cycles. The data were expressed as Ct values and used for the relative quantification of objectives with ΔΔthe Ct calculation. ΔΔThe Cts were corrected by multiplying the value of the ratio between the efficiency of the specific primer and the "*house keeping* 18S".

6.7. Western blot analysis.

The cells were washed twice with PBS and lysed with a 50 mM solution of Tris pH 7.5, 150 mM NaCl, 0.5% Nonidet-P40, containing a cocktail of protease and phosphatase inhibitors (SIGMA, Milan, Italy) for 30 minutes, on ice. Twenty µg of proteins and a molecular mass marker (Thermo Scientific) were

separated on SDS-PAGE 4-12% (BIO-RAD) under denaturation and reduction conditions. The proteins were then transferred onto a nitrocellulose membrane using the Trans-Blot® Turbo™ (BIO-RAD) transfer system. The membranes were washed with Tris-Tween 20 (TBS-T) buffered saline solution and non-specific binding sites were blocked in TBS-T containing 5% fat-free dried milk for 60 minutes at room temperature. The blots were incubated overnight at 4°C with a diluted solution (5% fat-free powdered milk) of the following primary human antibodies: anti-GK (Biovision, rabbit polyclonal; dilution 1: 1,000), anti LDLR (mouse monoclonal antibody, Millipore clone 2H7.1; dilution 1: 1,000), anti-AMPK and phospho-AMPK (cell signalling, polyclonal antibodies) and anti-α-tubulin (mouse monoclonal antibody, Sigma clone DM1A; dilution 1: 2,000). The membranes were washed with TBS-T and then exposed for 90 minutes at room temperature to a diluted solution (5% fat-free powdered milk) of secondary antibodies (goat anti-rabbit peroxidase-conjugated and anti-mouse, Jackson Immunoresearch). Immunoreactive bands were detected by exposing the membranes to the Western ClarityTM ECL (Bio-Rad) chemiluminescent substrates for 5 minutes and images were acquired using a VersaDoc 4000 Imaging System (Bio-Rad). Densitometric readings were evaluated using the ImageLab™ software as described above.

6.8. Glycogen analysis protocol.

Cells were homogenised with dH_2OR on ice (10^6 cells with 200 µl of dH_2O). The homogenates were boiled for 10 minutes to inactivate the enzymes and centrifuge at 18,000 x g for 10 minutes to remove insoluble material. The supernatant was then analysed using the Biovision test kit (catalogue number K646-100).

6.9. Statistical analysis.

Statistical analysis was performed using the Prism statistical analysis package version 5.01 (GraphPad Software, San Diego, CA). When possible, the values of p (*p-values*) were determined by the Student's t-test. Otherwise, differences between treatment groups were evaluated by one-way ANOVA test. A probability value of $p < 0.05$ was considered statistically significant. The experiments were performed in triplicate at least three times.

Experimental part II

The cholesterol-lowering properties of formulations 1 to 4 (see materials) were tested using in vitro experimental models of LDL-cholesterol metabolism. To this end, Huh7 (human hepatocarcinoma) are used to test the ability to modulate the expression of genes involved in cholesterol and lipid metabolisms, such as LDLR, PCSK9, HMG-CoA reductase, fatty acid synthase (FAS), stearoyl-CoA desaturase (SCD1) and evaluation of the effect on LDL uptake.

Materials

1) Atomised berberine (BBR-A): Understood as a formulation comprising berberine carried on starch

2) Ultra-berberine (U-BBR) (atomised sucrosomial berberine): Understood as a formulation comprising berberine carried on starch, lecithin, sucrester

3) Bergamot: 100% bergamot extract

4) Ultra-bergamot (U-BE): Understood as a formulation comprising bergamot, lecithin, sucrester and starch

Atomised berberine (BBR-A) can be obtained through the atomisation/nebulisation process described in the present invention.

Determination of cytotoxicity.

The potential cytotoxic effect of the tested formulations was initially determined in HuH7 cells after incubation for 72 hours with increasing concentrations in 0.4% medium / FCS. None of the materials showed a relevant cytotoxic effect (Figure 7).

Activity 2. Effect on the expression of the major actors of the lipid and cholesterol metabolisms

Huh7s were incubated for 24 hours with increasing concentrations of formulations 1-4. At the end of the incubation the total proteins were extracted and the expression of LDLR, HMG-CoA and PCSK9 was determined by means of western blot analysis. As expected, berberine significantly increases expression of LDLR and reduces levels of PCSK9 (Figure 8). The effect of atomised berberine and ultra berberine on the epsression of LDLR and PCSK9 in Huh7 cells was evaluated. Cells were cultured with MEM/0.4% FCS in the presence or absence of increasing concentrations of atomised berberine (BBR-A) and ultra-berine (U BBR). After 24 hours, total protein extracts and protein expression evaluated by means of Western blot analysis were prepared. GAPDH was used as a load control. Densitometric readings were evaluated using ImageLab software. Formulations vs control: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (Figure 8).

The effect of bergamot and ultra-bergamot on the expression of HMG-CoA reductase in Huh7 cells was also tested. Cells were cultured with MEM/0.4% FCS in the presence or absence of increasing

concentrations of bergamot (BE) and ultra-bergamot (U-BE). After 24 hours, total protein extracts and protein expression evaluated by means of Western blot analysis were prepared. GAPDH was used as a load control. Densitometric readings were evaluated using ImageLab software. Formulations vs control: * $p < 0.05$; ** $p < 0.01$ (Figure 9).

Activity 3. Determination of LDL cholesterol uptake.

Huh7 was treated with the U-BBR formulation for 24 hours and then incubated with fluorescence-labelled LDL (LDL-DiO) for 3 hours. After incubation, LDL-DiO uptake was evaluated by means of cytofluorometry analysis. As shown in Figure 10, simvastatin and berberine increased LDL-DiO uptake by 2.6 and 3.4 folds, respectively. Ultra-berberine (U-BBR) (atomised sucrosomial berberine) induced high LDL uptake by more than 5 folds. No differences between atomised berberine and ultra-berberine were observed (Figure 10).

The effect of Ultra-berberine (U-BBR) (atomised sucrosomial berberine) on LDL-DiO by Huh7 cells was tested. Cells were cultured with MEM / 0,4% FCS in the presence or absence of increasing concentrations of atomised berberine (BBR-A) and ultra-berberine (U-BBR). After 24 hours, LDL-DiO was added to the growth medium and uptake was determined by means of cytofluorometry analysis. Formulations vs control: ** $p < 0.01$; *** $p < 0.001$.

Conclusions

In the light of the above, it can be deduced that the formulations according to the present invention are effective in the treatment of hypercholesterolaemia and of the diseases related therewith.

Experimental plan

Reagents

Eagle minimal essential Medium (MEM) was bought from Sigma, trypsin-EDTA, penicillin, streptomycin, sodium pyruvate, non-essential amino acid solution, foetal calf serum (FCS), Petri dishes and capsules were bought from EuroClone. DiO will be bought from Sigma. The natural products were supplied by PharmaNutra S.p.A (Pisa, Italy). Atomised berberine and ultra-berberine were dissolved in DMSO. Bergamot will be dissolved in saline solution.

Cell cultures

Huh7s were cultured in MEM supplemented with 10% FCS, L-glutamine, sodium pyruvate and non-essential amino acids, penicillin/streptomycin at 37°C in a humidified atmosphere with 5% CO_2 and 95%

air.

Cell viability test

Cell viability was determined by means of the sulforhodamine B (SRB) assay, as described above 38. The cells were seeded in a 96-well tray (8 x 103 cells / well) and the SRB assay was conducted after 72 hours of incubation with increasing concentrations of nutraceutical compounds.

Analysis based on LDL uptake fluorescent cells

Huh7 cells were seeded in a 96-well tray (25 x 103 cells / well in a complete medium) and they were treated with nutraceuticals in a 0.4% FCS medium after 24 hours. 24 hours after treatment, the cells were incubated with 10 µg / mL di LDL-DiO (CABRU s.a.s.). After 3 hours of incubation at 37°C, the cells were detached using trypsin and fluorescence intensity was determined by means of cytofluorometry analysis.

Reverse transcription and quantitative PCR (RT-qPCR)

RNA preparation and Quantitative real-time PCR Total RNA were extracted using the iScript™ RT-qPCR sample preparation reagent (Bio-Rad) according to the manufacturer's instructions. One-step qPCR was performed using TranScriba 1step PCR Mix SYBR (CABRU s.a.s) and primers specific to selected genes. Analyses were performed using the CFX96 Touch Real-Time PCR Detection System (Bio-Rad). The data was expressed as Ct values and used for the relative quantification of the targets using the ΔΔCt. calculation. The II ΔΔCt was corrected by multiplying the value of the ratio between the efficiency of the specific primer and the housekeeping 18S.

Western blotting

The samples were processed as described above. SDS-PAGEs were performed under denaturing conditions using Mini-PROTEAN® Tetra cell System (Bio-Rad) and proteins were transferred into nitrocellulose membranes using Trans-Blot TURBO® Transfer System (Bio-Rad). The membranes were incubated with appropriate primary and secondary antibodies conjugated with HRP and then analysed at c400 Azure Molecular Imager (Aurogene).

Statistical analysis

Statistical analysis was performed using the Prism statistical analysis package version 5.01 (GraphPad Software, San Diego, CA). The p-values were determined by the Student's t-test. A probability value of $p < 0.05$ was considered statistically significant. The experiments were carried out in triplicate at least three times.

CLAIMS

1. A formulation in solid form comprising or, alternatively, consisting of:

(a) an extract of bergamot and/or berberine,

(b) a lecithin, and

(c) at least one first agent selected from the group comprising or, alternatively, consisting of:

(c-i) a sucrester or a fatty acid carbohydrate ester;

(c-v) at least one gum selected from the group comprising or, alternatively, consisting of: carrageenan, acacia gum,

and a mixture of (c-i) and (c-v).

2. A formulation in solid form according to claim 1 comprising or, alternatively, consisting of:

(a) said bergamot extract,

(b) said lecithin, and

(c) said at least one first agent selected from the group comprising or, alternatively, consisting of:

(c-i) a sucrester or a fatty acid carbohydrate ester;

(c-v) at least one gum selected from the group comprising or, alternatively, consisting of: carrageenan, acacia gum

and a mixture of (c-i) and (c-v).

3. A formulation in solid form according to claim 1, comprising or, alternatively, consisting of:

(a) said berberine,

(b) said lecithin, and

(c) said at least one first agent selected from the group comprising or, alternatively, consisting of:

(c-i) a sucrester or a fatty acid carbohydrate ester;

(c-v) at least one gum selected from the group comprising or, alternatively, consisting of: carrageenan, acacia gum, and

and a mixture of (c-i) and (c-v).

4. The formulation according to any one of the preceding claims, wherein said formulation further comprises (d) a starch; preferably, wherein said (d) starch is of plant origin; even more preferably said starch of plant origin is gelatinised or pre-gelatinised.

5. The formulation according to claim 4, wherein said (d) starch of plant origin is selected from the group comprising or, alternatively, consisting of rice starch and/or corn starch; preferably, wherein said (d) is pre-gelatinised rice starch.

6. The formulation according to any one of the preceding claims, wherein said formulation comprises, or alternatively, consists of:

(a) said berberine and/or said bergamot extract;

(b) a lecithin,

(c) at least one first agent comprising or, alternatively, consisting of (c-i) a sucrester or a fatty acid carbohydrate ester; and

(d) a starch.

7. The formulation according to any one of the preceding claims, wherein said formulation comprises or, alternatively, consists of:

(a) a berberine and/or bergamot;

(b) a lecithin, and;

(c) at least one first agent comprising or, alternatively, consisting of (c-v) at least one gum selected from the group comprising or, alternatively, consisting of carrageenan, acacia gum and mixtures thereof; and

(d) a starch.

8. The formulation according to any one of the preceding claims, wherein said formulation comprises or, alternatively, consists of:

(a) a berberine and/or bergamot;

(b) a lecithin;

(c) at least one mixture of said first agent comprising or, alternatively, consisting of (c-i) a sucrester or a fatty acid carbohydrate ester and (c-v) at least one gum selected from the group comprising or, alternatively, consisting of carrageenan, acacia gum and mixtures thereof; and

(d) a starch.

9. The formulation according to claim 8, wherein in said mixture (c-i) and (c-v) are at a weight ratio comprised from 1:10 to 10:1, preferably comprised from 1:5 to 5:1, even more preferably comprised from 1:3 to 3:1, for example 1:1.

10. A composition comprising or, alternatively, consisting of:

- the formulation in solid form according to any one of claims 1-9, and optionally,

- at least one acceptable pharmaceutical or food grade additive and/or excipient.

11. The composition according to claim 10, wherein said composition further comprises red fermented rice; preferably fermented red rice titrated in monacolin to a weight % comprised from 2% to 10%, preferably from 3% to 7%, more preferably to about 5%, with respect to the weight of red rice.

12. A formulation according to any one of claims 1-9 or a composition according to claim 10 or 11, wherein said composition is for use as medicament.

13. The formulation or the composition for use according to claim 11, wherein said formulation or composition is for use in a method for the preventive and/or curative treatment of a disease related with dyslipidaemia and/or hypercholesterolaemia.

14. The formulation or the composition for use according to claim 13, wherein said formulation or composition is for use in a method for the preventive and/or curative treatment of decrease or normalisation of the plasma levels of total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides.

15. The formulation or the composition for use according to claim 13, wherein said formulation or composition is for use in a method for the preventive and/or curative treatment of decrease of plasma LDL cholesterol levels.

16. The formulation or the composition for use according to claim 13, wherein said formulation or composition is for use in a method for the preventive and/or curative treatment of decrease in the blood or plasma triglyceride levels.

17. The formulation or the composition for use according to claim 13, wherein said formulation or composition is for use in a method for the preventive and/or curative treatment of a disease related with atherosclerosis or a coronary or cerebrovascular disease, preferably stroke.

18. The formulation or the composition for use according to claim 13, wherein said formulation or composition is for use in a method for the preventive and/or curative treatment of a metabolic syndrome and symptoms and/or disorders related therewith.

19. A process for the preparation of an atomised berberine carried on a carrier, wherein said process comprises the following steps:

(I) providing an aqueous-based solvent, preferably water, in a container under stirring and heating and adding to said container a berberine, preferably a granular berberine, and plant starch, preferably rice starch, to obtain a mixture of step (I);

(II) subjecting said mixture of step (I) to a nebulisation or atomisation step to obtain an atomised or nebulised berberine, and, optionally,

(III) subjecting said atomised/nebulised berberine to a sieving step to obtain a sieved atomised berberine.

20. An atomised berberine carried on a carrier obtainable according to the process according to claim 19.

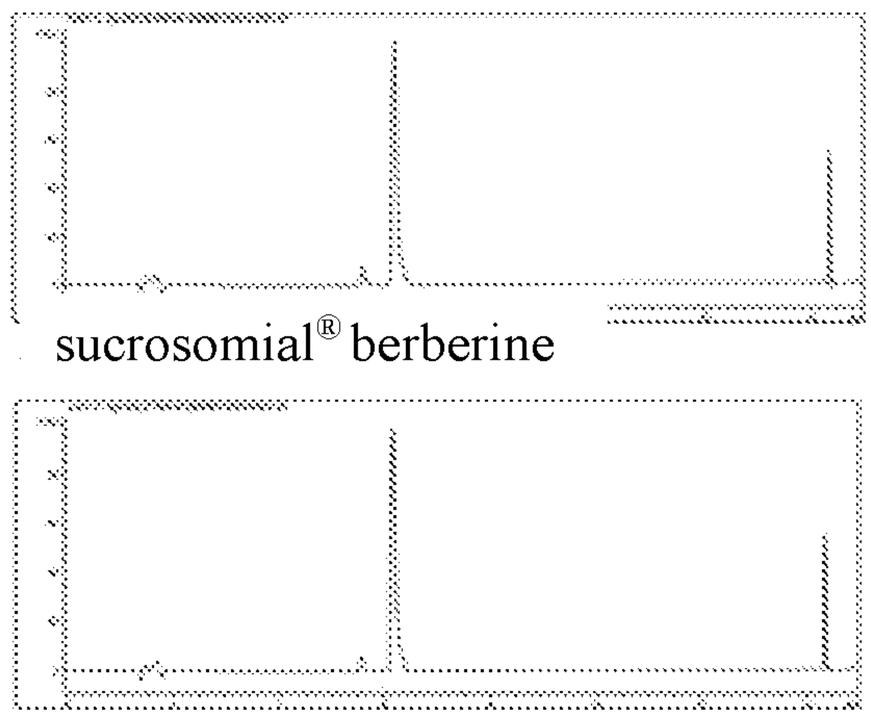


Figure 1

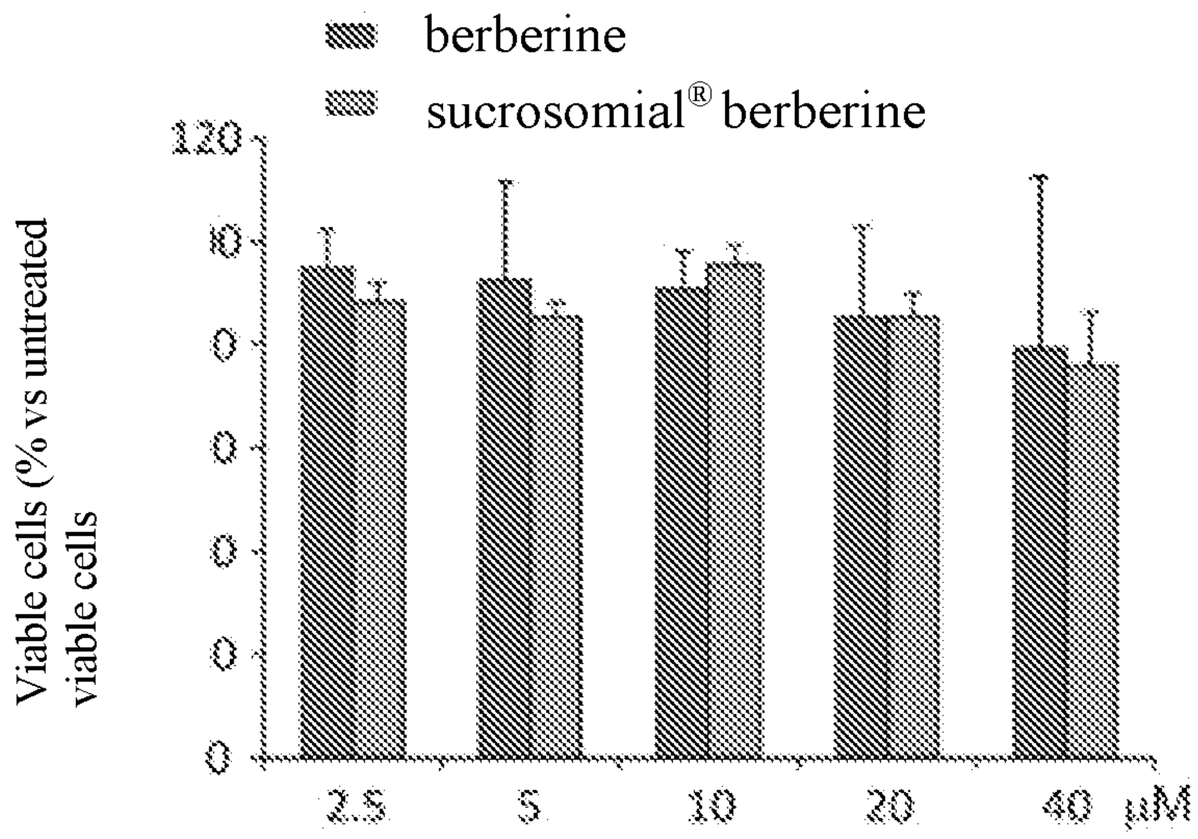


Figure 2

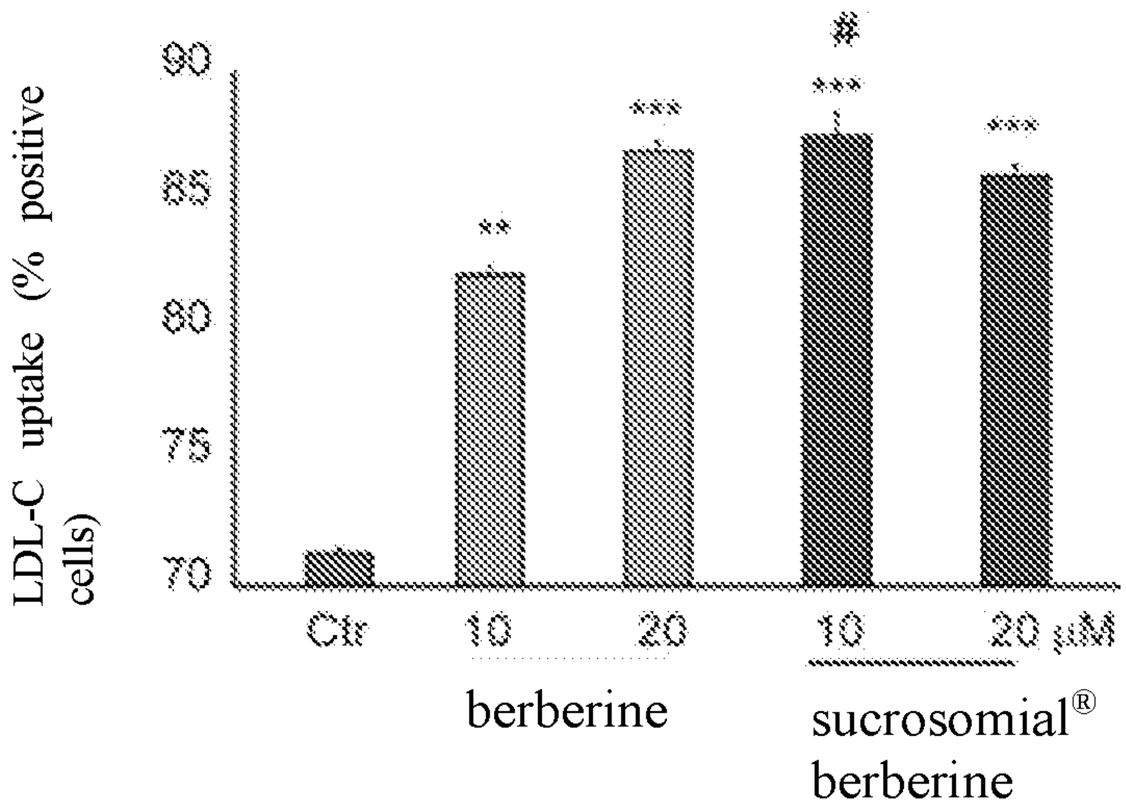


Figure 3

A

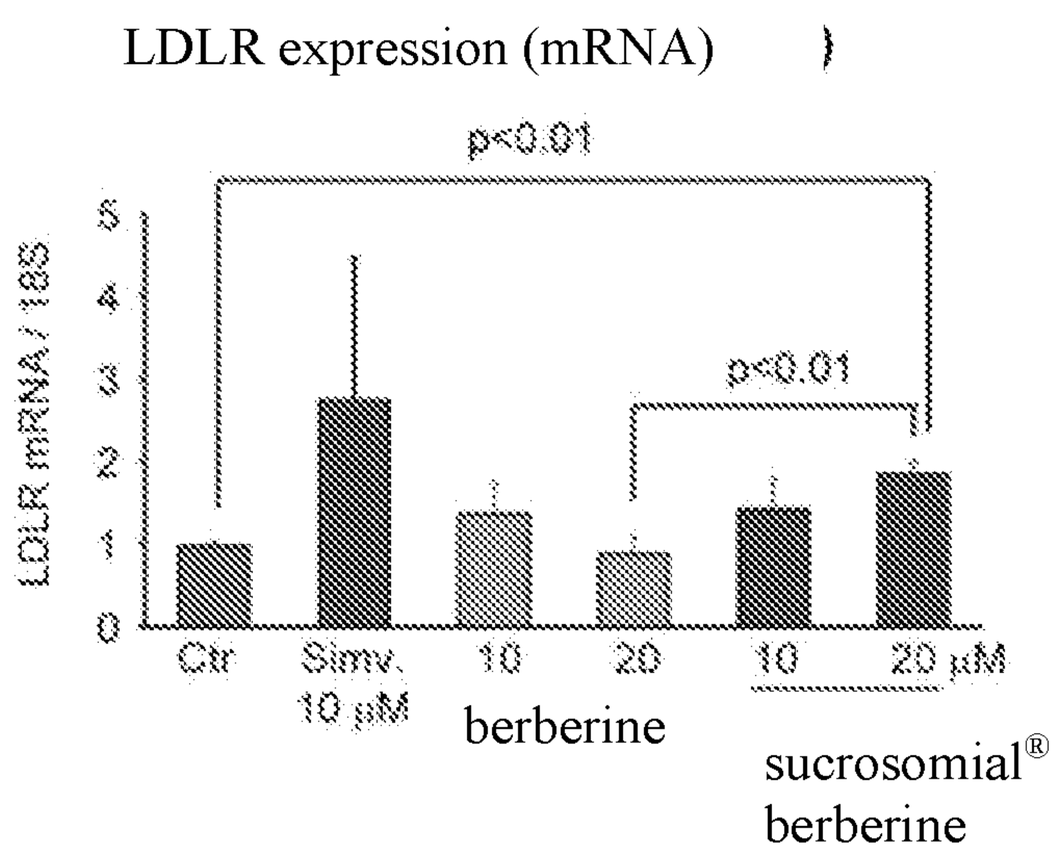


Figure 4A

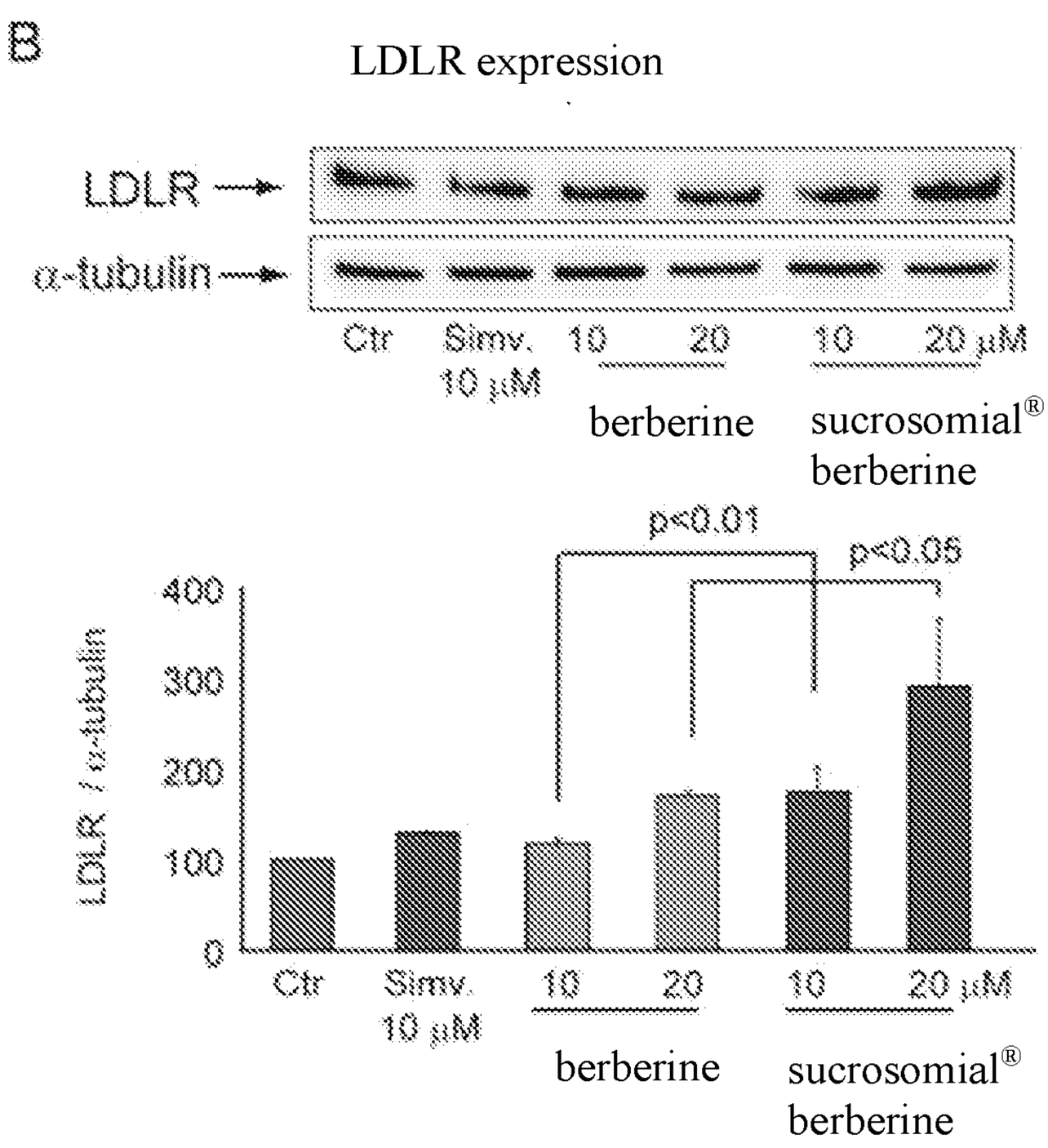

B
LDLR expression
LDLR
α-tubulin
Ctr
Simv. 10 μM
10
20
10
20 μM
berberine
sucrosomial® berberine
p<0.01
p<0.05
LDLR / α-tubulin
400
300
200
100
0
Ctr
Simv. 10 μM
10
20
10
20 μM
berberine
sucrosomial® berberine

Figure 4B

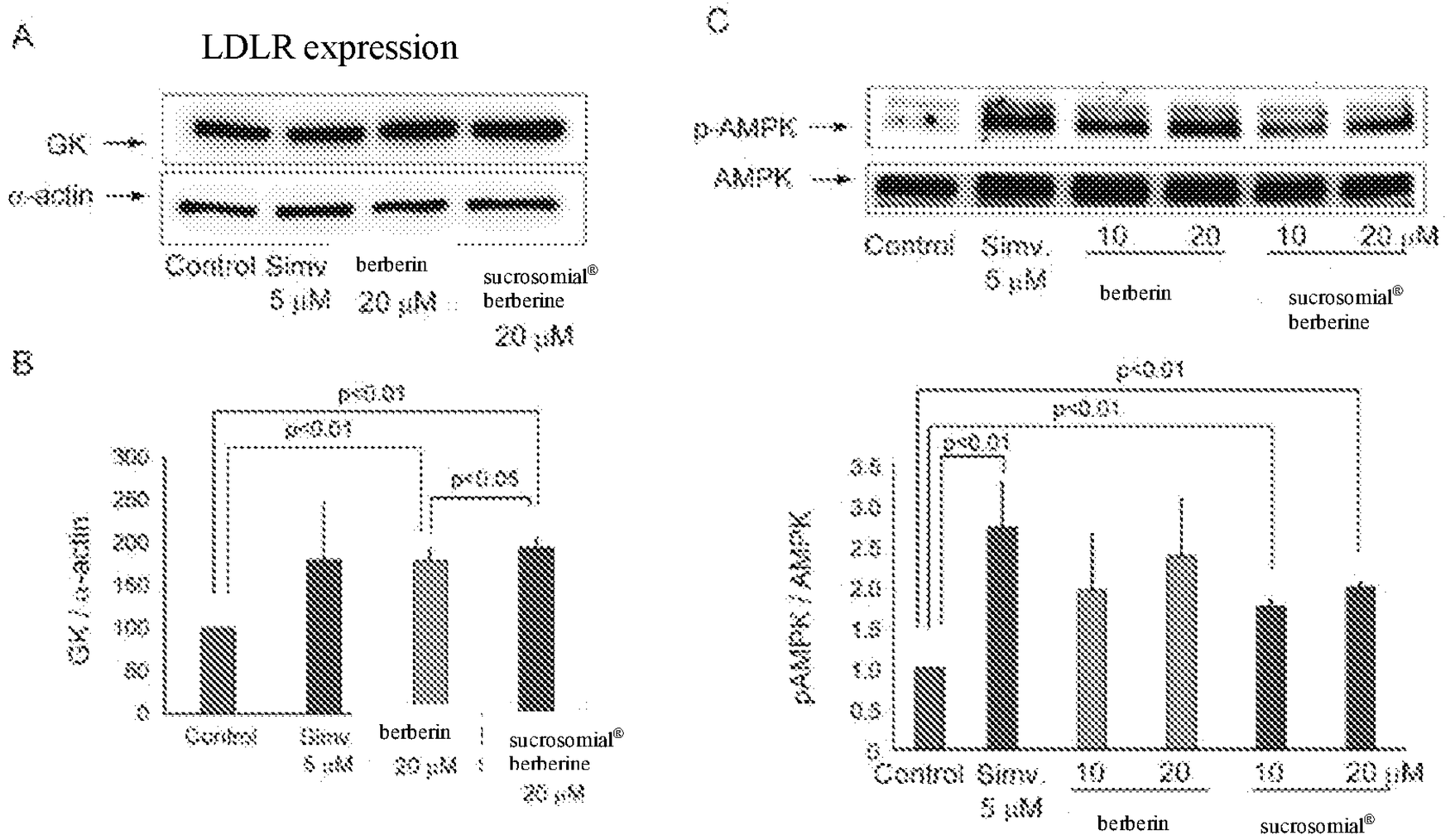


Figure 5

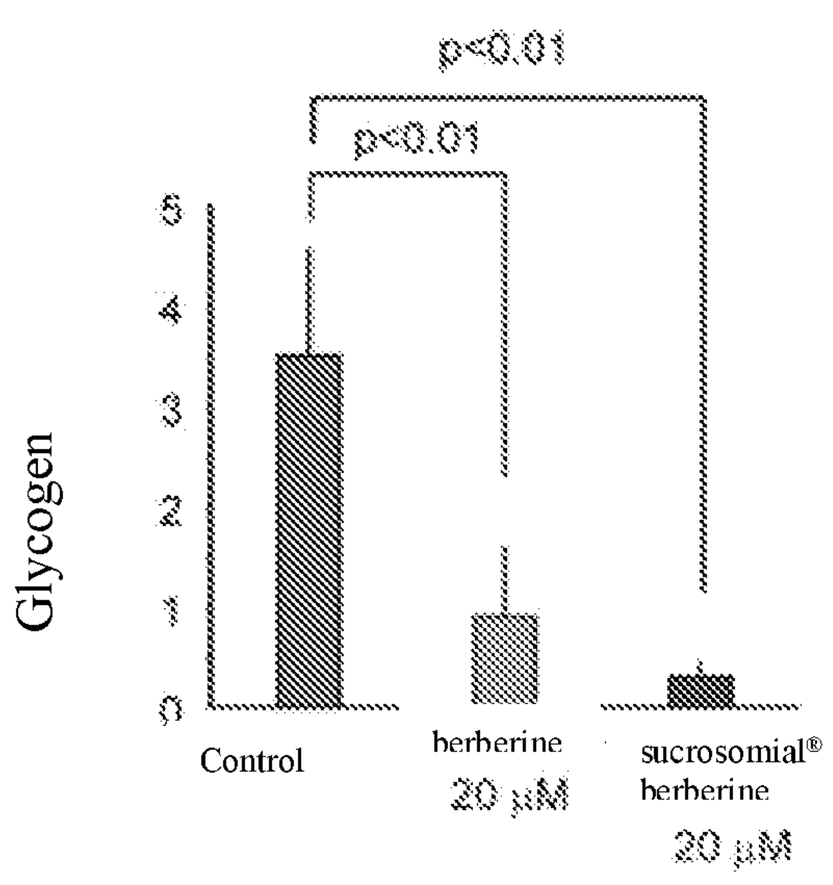


Figure 6

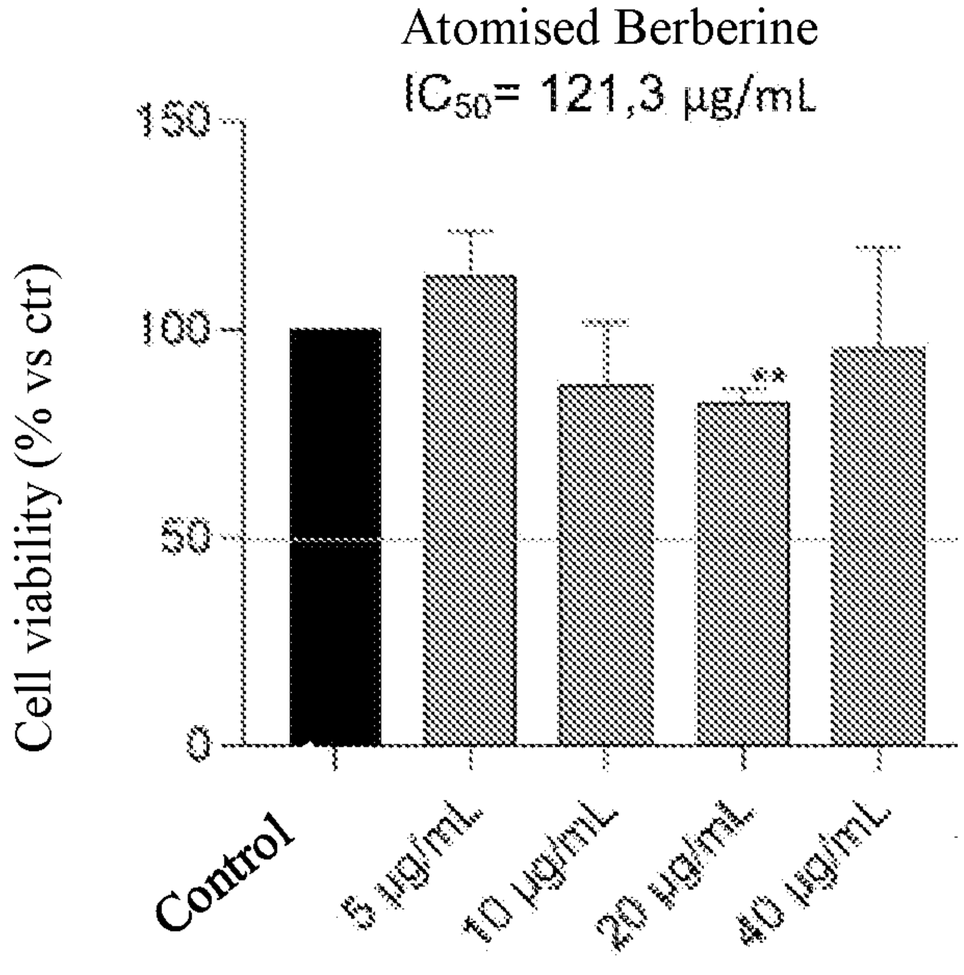


Figure 7A

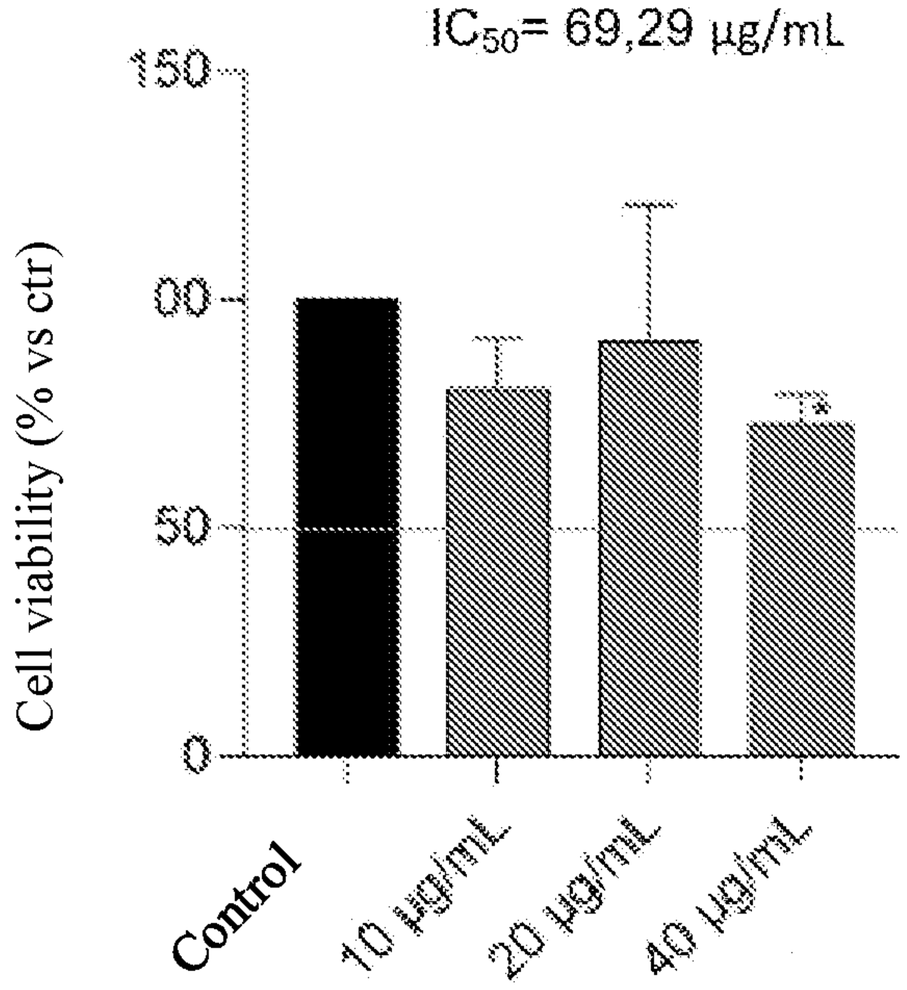


Figure 7B

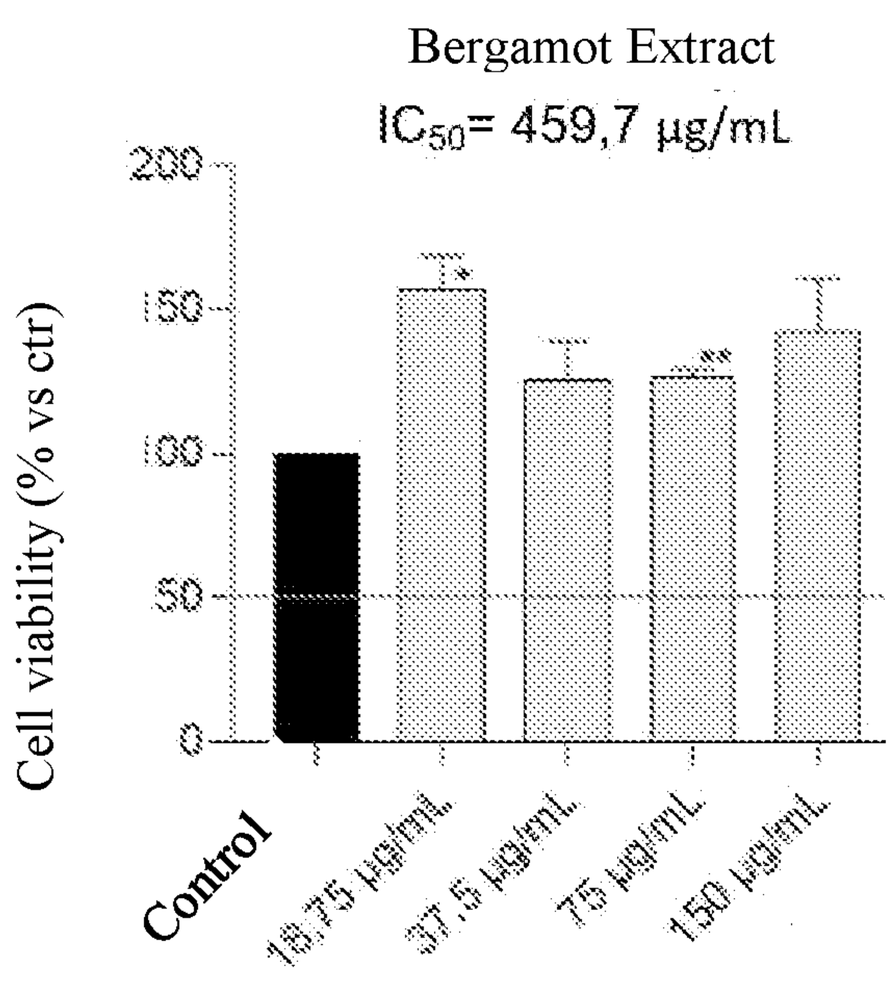


Figure 7C

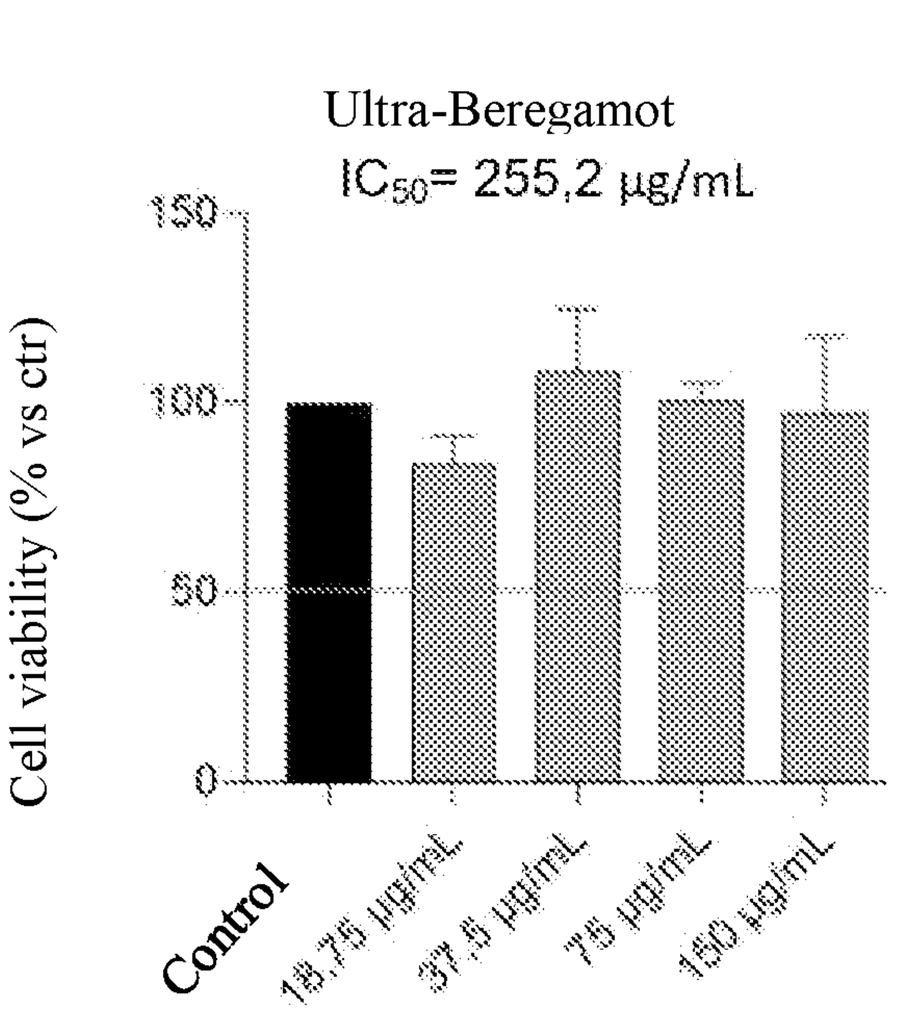


Figure 7D

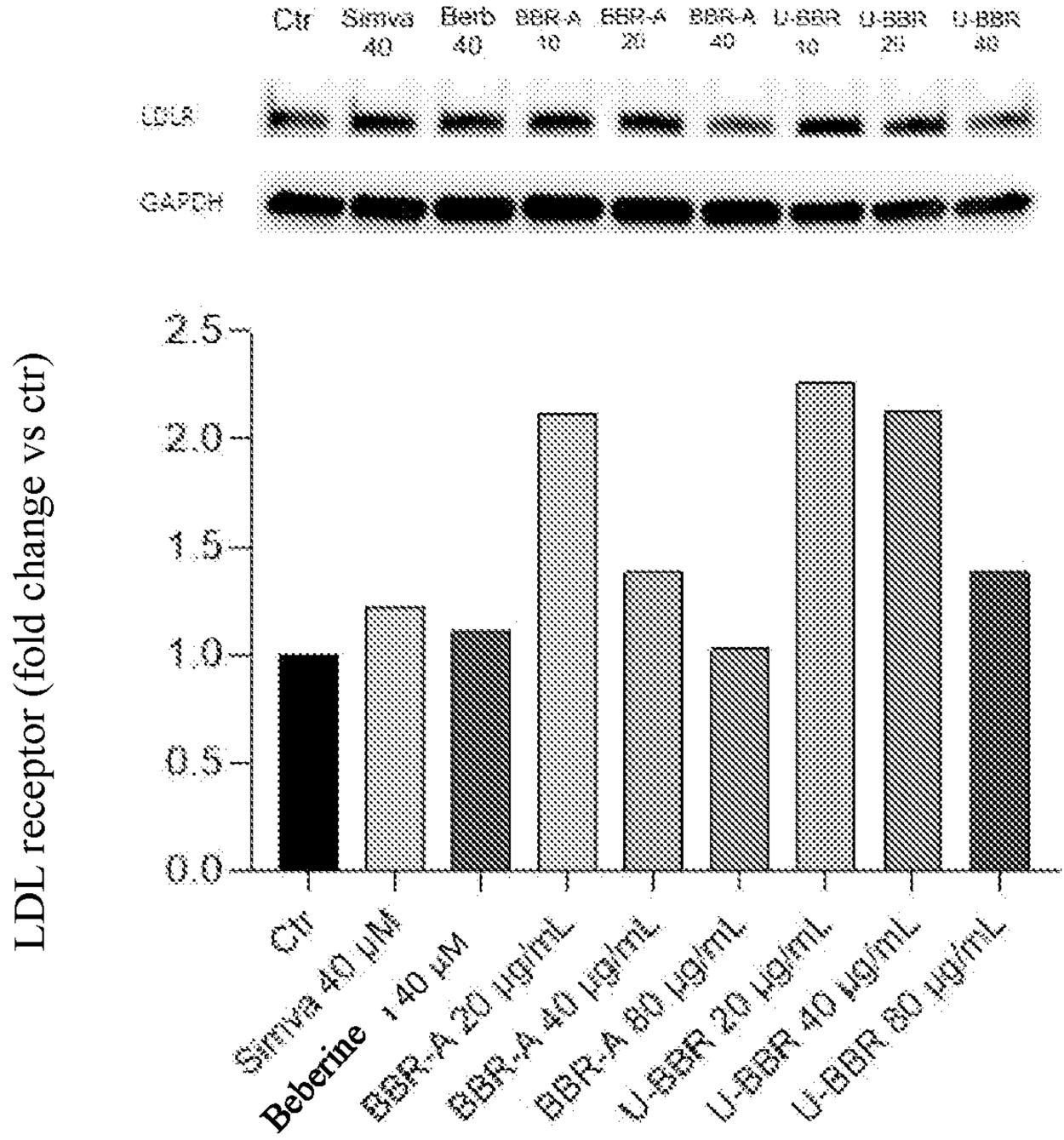

Ctr
Simva 40
Berb 40
BBR-A 10
BBR-A 20
BBR-A 40
U-BBR 10
U-BBR 20
U-BBR 40
LDLR
GAPDH
LDL receptor (fold change vs ctr)
0.0
0.5
1.0
1.5
2.0
2.5
Ctr
Simva 40 µM
Beberine 40 µM
BBR-A 20 µg/mL
BBR-A 40 µg/mL
BBR-A 80 µg/mL
U-BBR 20 µg/mL
U-BBR 40 µg/mL
U-BBR 80 µg/mL

Figure 8A

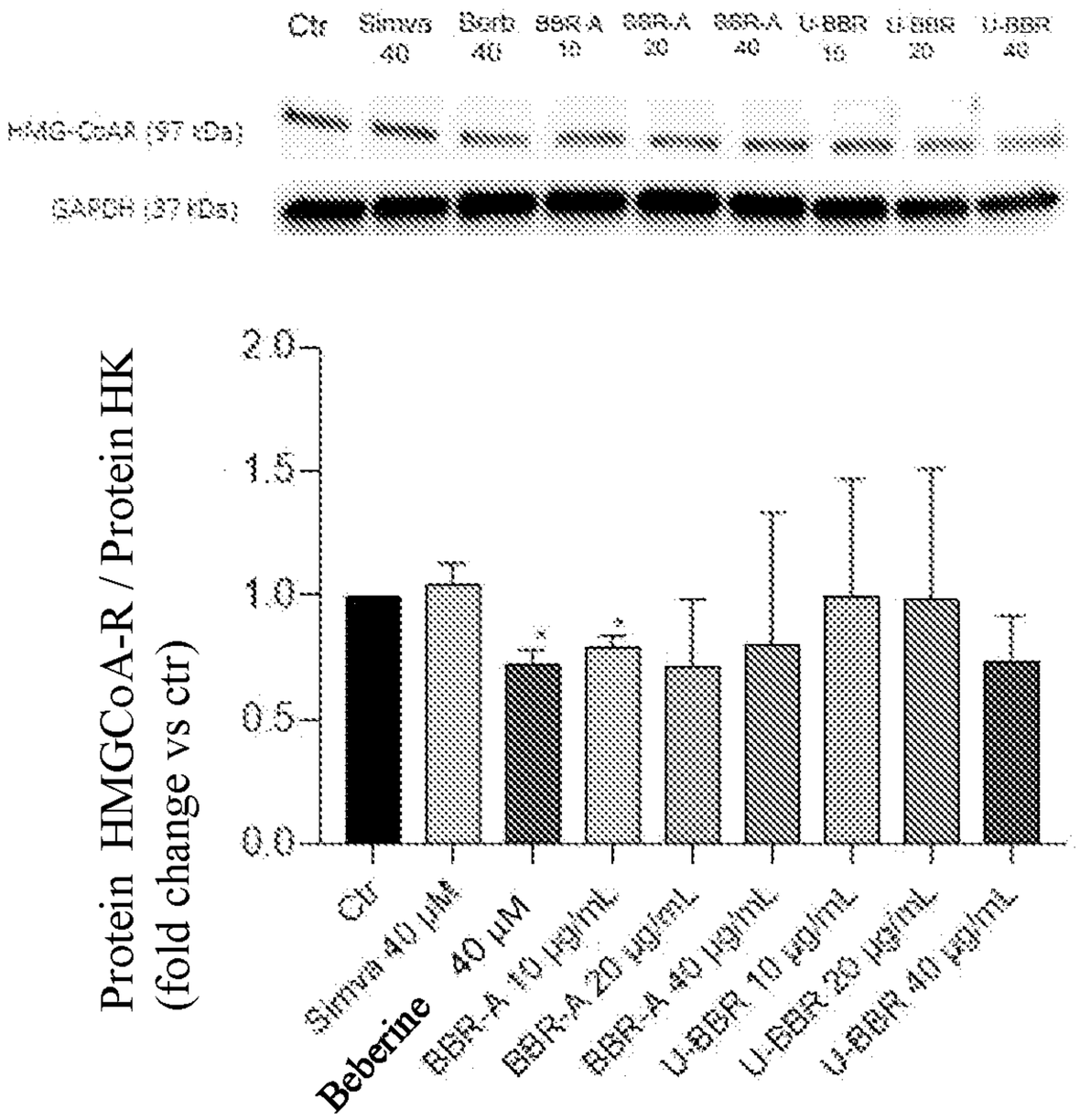
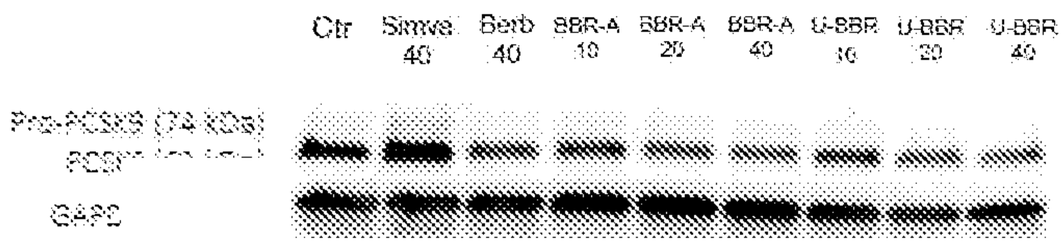

Ctr
Simva 40 μM
Beb 40 μM
BBR-A 10 μg/mL
BBR-A 20 μg/mL
BBR-A 40 μg/mL
U-BBR 10 μg/mL
U-BBR 20 μg/mL
U-BBR 40 μg/mL
HMG-CoAR (97 kDa)
GAPDH (37 kDa)
Protein HMGCoA-R / Protein HK
(fold change vs ctr)
2.0
1.5
1.0
0.5
0.0

Figure 8B



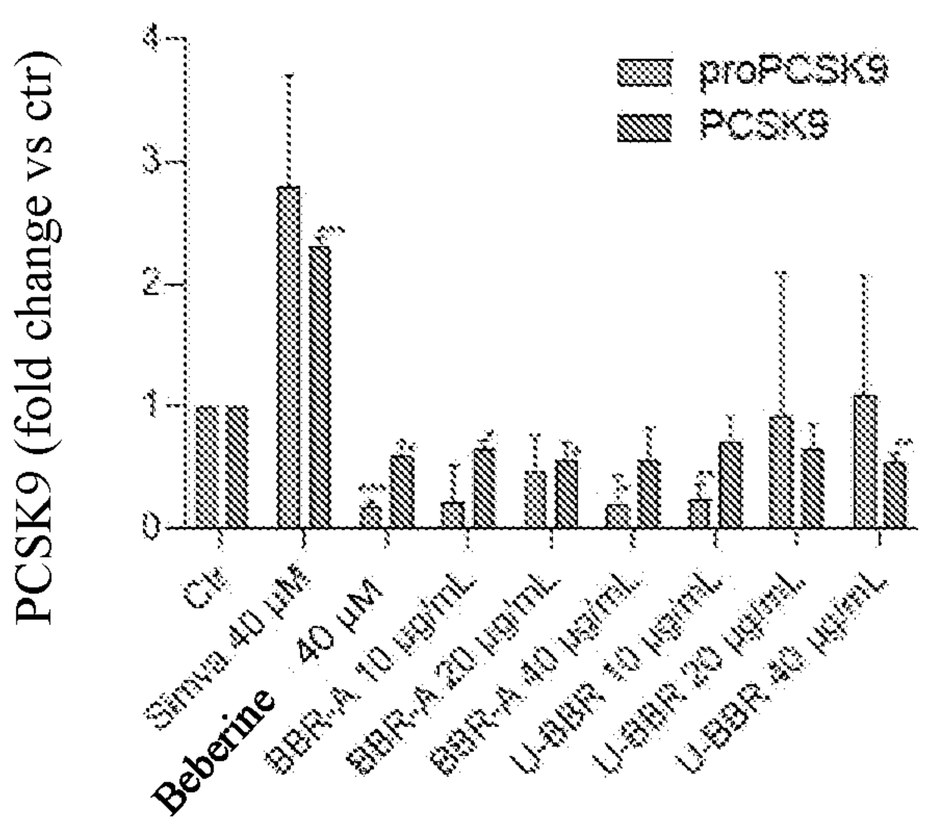


Figure 8C

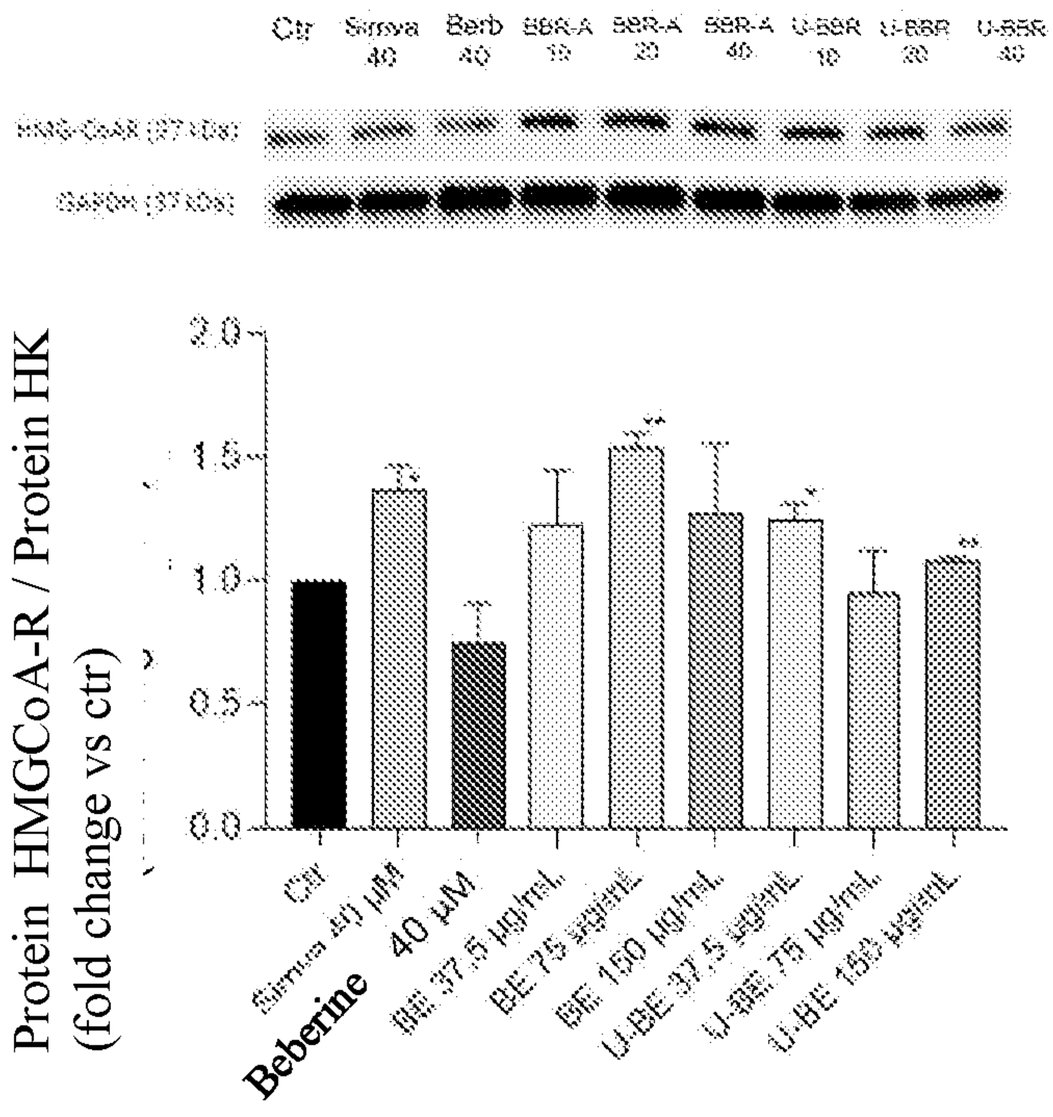


Figure 9

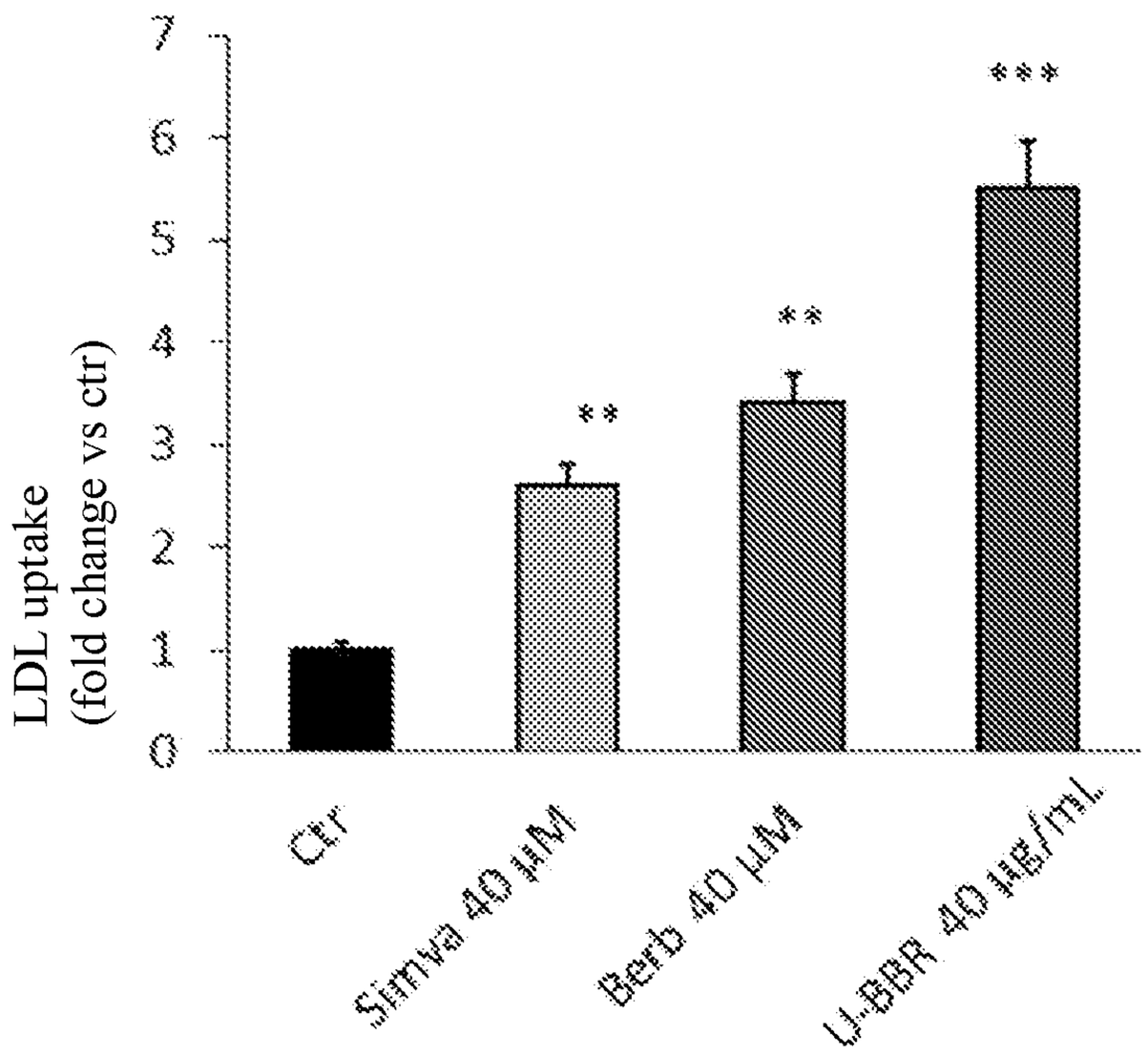

LDL uptake
(fold change vs ctr)
0
1
2
3
4
5
6
**
**
***
Ctr
Simva 40 µM
Berb 40 µM
U-BBR 40 µg/mL

Figure 10