(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
5 November 2009 (05.11.2009)

PCT

(10) International Publication Number
WO 2009/133458 A1

(51) International Patent Classification:
A61K 36/718 (2006.01) *A61K 36/734* (2006.01)
A61K 31/473 (2006.01) *A61P 3/06* (2006.01)
A61K 36/638 (2006.01) *A61P 9/00* (2006.01)

(21) International Application Number:
PCT/IB2009/005461

(22) International Filing Date:
1 May 2009 (01.05.2009)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/049,588 1 May 2008 (01.05.2008) US

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

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

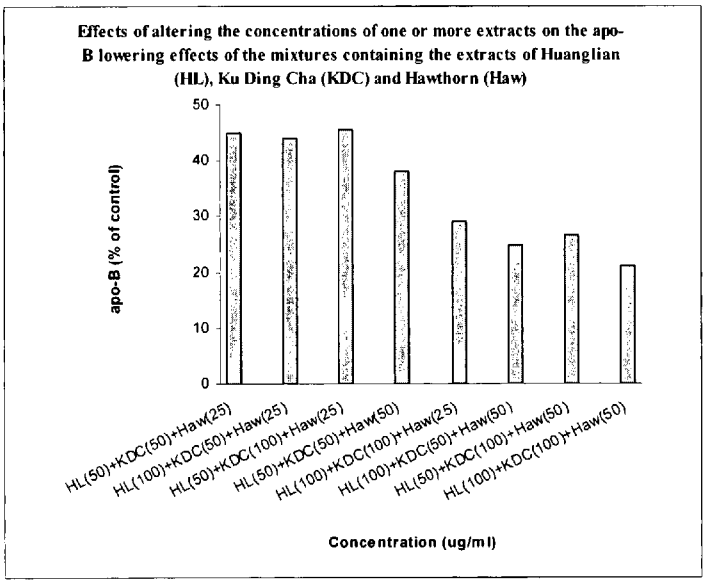
Published:

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

[Continued on next page]

(54) Title: PHARMACEUTICAL COMPOSITION COMPRISING EXTRACTS OF HUANGLIAN AND KU DING CHA EFFECTIVE FOR LOWERING BLOOD LIPID LEVELS

FIG. 7



(57) Abstract: A medicinal combination of herbs, herbal extracts, and their chemical constituents that is synergistically effective for promoting human and veterinary health. The combination is a blood lipid level lowering synergistic composition comprising Ku ding cha, berberine or a berberine-containing plant extract, and other optional active ingredients. Methods of extraction and combination in synergistic amounts are also disclosed. The compositions according to the present invention may be used to treat or reduce the chance of contracting or the progression of a number of diseases or conditions in a subject, particularly cardiovascular diseases.

WO 2009/133458 A1

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

Pharmaceutical composition comprising extracts of huanglian and Ku ding cha effective for lowering blood lipid levels

FIELD OF THE INVENTION

This invention relates to medicinal compositions, particularly to combinations of herbs, herbal extracts and their chemical constituents for promoting human and veterinary health, particularly cardiovascular health.

BACKGROUND OF THE INVENTION

Cardiovascular disease (CVD) is the leading cause of death in most industrialized nations of the world. In the United States alone, 61 million people comprising approximately 25% of the total population have been reported to suffer from some type of CVD. Reducing the levels of blood cholesterol, in particular the low-density lipoprotein cholesterol, is known to be one of the effective strategies for the prevention of CVD [1].

A class of drugs, called statins, has been found to be highly effective in the reduction of blood cholesterol levels. Statins work by suppressing the activity of HMG-CoA reductase, the rate limiting enzyme in the synthesis of cholesterol in cells. The resultant decrease in intracellular concentrations results in a compensatory up-regulation of surface low-density lipoprotein (LDL) receptors, which in turn mediates clearance of LDL-cholesterol from plasma. Clinically, HMG-CoA reductase inhibitors are effective in lowering blood LDL-cholesterol levels by 20-40% and have also been found to be effective in reducing the levels of triglyceride and inducing modest increases in the levels of HDL-cholesterol in blood [2,3]. Despite their success in the management of blood

lipid concentrations, statin therapy however cannot be prescribed to all patients with hyperlipidemia. In particular, these drugs are contraindicated in patients with cholestasis or impaired hepatic functions. In some patients, statins have also been reported to cause elevations in various hepatocellular enzymes such as creatine kinase. Statins have also been found to have potential adverse effects on muscle, kidney and the nervous system of some patients. There have also been reports of myopathy during the treatment with statins. Although myopathy occurs in only 0.1 to 0.2% of patients receiving statins, its risk increases with the increase in dose. In addition, if left untreated, myopathy could lead to a more serious and life-threatening condition called rhabdomylosis. Consequently, following the development of myopathy immediate cessation of statin therapy is required [4,5].

Treatment of hypercholesterolemic patients with berberine (BBR) at a dose of 1 g/day for 3 months was found to reduce serum cholesterol concentrations by 29%, triglycerides by 35% and LDL-cholesterol by 25% [6]. Experimental studies also support the cholesterol-lowering efficacy of BBR. Oral administration of BBR to hyperlipidemic hamsters at a dose of 100 mg/day for 2 weeks was found to reduce serum total- and LDL-cholesterol levels by up to 30% [6-8]. A number of *in vitro* studies utilizing human hepatoma cell lines such as Hep G2 cells have also been carried out to determine the underlying mechanisms of cholesterol-lowering effects of BBR. Results from these studies indicate that BBR increases the expression of LDL receptors in the cells through a post-transcriptional mechanism that increases the stabilization of *m*RNA for these receptors. BBR was also found to inhibit the synthesis of cholesterol and triglyceride in the cells

through activation of an AMP-activated protein kinase [6-8]. In a more recent study, BBR was found to inhibit differentiation of 3T3-L1 adipocytes and to inhibit lipogenesis in the cells through PPAR pathways [9].

Although evidence from a number of studies indicates that BBR has cholesterol-lowering properties, a dose of approximately 1g/day appears to be required to achieve clinical benefits. Safety of chronic administration of this dosing remains a concern. In an experimental study, intra-peritoneal administration of BBR at doses exceeding 15 mg/Kg for 10 days to mice was found to be lethal to the animals [10]. Higher doses of BBR were also found to be toxic to cells in culture. For example, treatment of L929 cells with BBR in doses greater than 40 μg/ml was found to induce cytotoxicity. More recently, incubation of human liver microsomes with BBR at concentrations of 20 μM was found to decrease the activities of various CYP 450 enzymes including CYP2D6, CYP3A4 and CYP2E1 [11].

An herbal tea, Ku Ding Cha (also referred to as Kudingcha, Kuding tea, bitter tea, ku cha), is also known to have cardiovascular benefits. It is widely consumed in China as a normal tea or functional drink. The original plant of Ku Ding Cha consists of about 10 most commonly known species in the genus Ligustrum, Oleaceae, and Ilex, Aquifoliaceae including *Ligustrum pedunclare, Ligustrum purpurascens, Ligustrum japonicum, Ligustrum robustrum, Ilex cornuta, Ilex kudincha C. J. Tseng (Aquifoliaceae), Ilex latifolia, Cratoxylum prunifolium, Ehretia thyrsiflora, Photinia serruiata* [12] and *Ilex paraguariensis.* The extract from these ground plants in hot water is consumed as a

tea. In animal studies extracts of some of these species have been found to promote circulation of blood, lower blood pressure, have anti-oxidative effects and reduce levels of lipids in plasma [13-15]. For example, in an experimental study daily gavaging of water extracts of *Ilex paraguariensis* to hypercholesterolemic rats at a dose of 500 mg/day for 2 weeks was found to significantly reduce the plasma concentrations of cholesterol and triglycerides [16]. In another study, daily administration of extracts of *Ligustrum japonicum* for 1-3 months reduced the levels of blood total cholesterol in hypercholesterolemic rabbits [17]. The clinical benefits of the tea in the management of human blood cholesterol concentrations however have not been determined.

The biologically active components of Ku Ding Cha have yet to be determined. Aqueous and alcoholic extracts of leaves of various Ilex species contain numerous terpenoids, flavonoids [18-23], triterpenes, phenylethanoid glycosides, caffeoyl quinic acid and derivatives thereof. Some of these compounds are implicated in the cholesterol-lowering effects of Ku Ding Cha; recent studies have found that a number of mono-and tri-terpenes isolated from the leaves and twigs of two species of Ku Ding Cha including *Ilex kudingcha* and *Ilex macropoda* were found to inhibit the activity of acyl-CoA: cholesterol acyltransferase (ACAT) *in vitro* [24-27]. ACAT activity is important for regulation of cholesterol absorption from the gut and esterification of cholesterol with fatty acids in mammals. Inhibition of ACAT in hepatocytes has been shown to decrease the secretion of apolipoprotein-B containing lipoproteins, such as very low density lipoprotein (VLDL) particles from the liver [28].

Many of the triterpenoid saponins have been isolated from *Ilex kudingcha*: ilekudinosides A-S, ilexoside XL VIII, cynarasaponin C, latifolosides A, C, G and H, kudinoside G. Some of them exhibited significant ACAT inhibitory activity [26].

Some monoterpenoid and phenylethanoid glycosides have also been isolated from Ku Ding Cha (*Ligustrum pendunculare*) leaves: lipedosides A-I and A-II as phenylthanoid glycosides, and lipedosides B-I, B-II, B-III, B-IV, B-V, and B-VI as monoterpenoid glycosides. Lipedoside B-III has been identified as inhibitor of ACAT with IC_{50} of 269 μM [23]

Ku Ding Cha is also known to contain ursolic acid, betulin, lupeol, and chlorogenic acid.

Ursolic acid (3β-Hydroxy-12-ursen-28-ic acid), has been identified as an inhibitor of several enzymes, including adenosine deaminase, arachidonate lipoxygenase, aromatase, cyclooxygenase, DNA ligase I, elastase, protein kinases A and C, and RNA-directed DNA polymerase [29]. Recently, ursolic acid has also been identified as an inhibitor of nucleoside triphosphate hydrolase (NTPase, IC_{50} 0.6 μM) and acyl-CoA: cholesterol acyltransferase (ACAT, IC_{50}, 58.8 μM) [30-31]. Ursolic acid is also a strong antitumorigenic and chemopreventive agent [32].

Betulin (lup-20[29]-ene-3β, 28-diol) and betulinic acid (3-hydroxy-20[29]-lupen-28-oic acid) are anti-inflammatory and cytotoxic against a variety of tumor cell lines [33]. U.S. Patent No. 5,679,828 has found that betulinic acid and its derivatives have strong anti-HIV infection activity. Betulinic acid has also been identified as an inhibitor of acyl

CoA: diacylglycerol acyltransferase (DGAT, IC_{50}, 9.6 μM) [34]. Furthermore, betulin and betulinic acid are good ACAT inhibitors with IC_{50} of 83 and 16.2 μM, respectively [24].

Lupeol (3β-Hydroxy-20(29)-lupene, or 20(29)-Lupen-3β-ol) has been found to have anti-cancer activity via down-regulation of NF-kB and has been identified as an inhibitor of acyl-CoA: cholesterol acyltransferase (ACAT IC50, 48 μM) [24,35].

Chlorogenic acid, a chemical component of Ku Ding Cha, has been identified as squalene synthase inhibitor, with an IC_{50} of 0.1 μM [36].

Hawthorn, a plant cultivated widely in Europe and China (called shanzha in China), has been found to have various health-promoting properties. In Europe, the species of hawthorn which are commonly accepted in herbal medicine include *Crataegus oxyacantha, Crataegus pentagyna* Waldst, *Crataegus nigra* Waldst, *Crataegus azarolus* L. and *Crataegus monogyna*. In China, two other varieties of hawthorn including *Crataegus pinnatifida* and *Crataegus pinnatifida* Bge var. *major* NE Br., have been used in traditional Chinese medicine. Studies indicate that both European as well as Chinese varieties have various health benefits including anti-oxidant, anti-inflammatory and hypolipidemic effects. These varieties have also been found to have protective effects on the brain and vascular endothelium and endothelium-dependent relaxation. In addition, some clinical studies also indicate that hawthorn has blood pressure lowering properties and is effective in the treatment of mild forms of arrhythmia. Flavonoids, in particular

proanthocyanidines are believed to be the active components of hawthorn. Studies utilizing various flavonoids indicate that these compounds possess cholesterol-lowering properties and more specifically they have been found to increase the hepatic uptake of cholesterol [37].

SUMMARY OF THE INVENTION

The present inventors have found that certain compositions comprising huanglian and Ku Ding Cha, as herbs, extracts or chemical combinations thereof, can synergistically promote cardiovascular health. The synergistic combination can be obtained either through extracting one or more herbs together or extracting each herb separately and then combining the extracts; the latter method is preferred. The present inventors have also discovered that berberine and berberine related alkaloids, e.g. palmitine chloride, that are found within the huanglian extract, interact synergistically with each other to promote cardiovascular health. Naturally- or synthetically-derived chemical constituents may be used to make the synergistic combinations effective for promoting cardiovascular health.

Ku Ding Cha and its extracts can be obtained from any part of the fresh or dried plant, whole leaves or milled powder by using liquid extraction, or supercritical fluid extraction, or high-pressure extraction using conventional liquid solvents and combined with small amounts of supercritical fluids, although liquid extraction from finely ground powder of dried KDC leaves is preferred. The most preferred species of Ku Ding Cha is derived from *Ilex kudingcha,* although the species *Ilex aquifoliacese, Ilex latifolia* Thunb,

Ligustrum robustum, Ehretia thyrsiflora, Clerodendrum fortunatum L. and *Ilex cornuta* Lindl. ex paxt. also show the same synergistic effect. Suitable solvents for liquid extraction include water, acidic water, water-alcohol mixtures, water-lower alkyl (C_{1-6}) alcohol mixtures, alcohols, especially lower alkyl (C_{1-6}) alcohols, esters, especially C_{2-10} esters, ethers, especially C_{4-12} ethers, hydrocarbon chlorides or other organic solvents or mixtures thereof. Water, alcohol, ethyl acetate and ethyl acetate-based solvent mixtures are preferred, although an alcoholic extraction is most preferred. Extraction can be carried out by using liquid extraction, including pressurized liquid extraction (PLE, also called accelerated solvent extract, ASE), maceration, sonication, microwave assist extraction or supercritical fluid extraction, soaking, agitation, or supersonic aid extraction in a proportion e.g. 1 ml–1 Litre, preferably 2-500 ml, more preferably 2-200ml, more preferably 2.5-50 ml, most preferably 10 ml of solvent per gram of finely ground powder of Ku Ding Cha, most preferably obtained from the leaf, for a time between e.g. one minute to 100 hours, preferably 2 minutes to fifty hours, more preferably 5 minutes to twenty-four hours, even more preferably for 20 minutes to 5 hours, most preferably 2 hours, depending on the chosen extraction procedure. The extractions were carried out at a temperature of about 4-120° C, or preferably 15-100° C, or more preferably 30-90° C, or more preferably 50-80° C, or even more preferably 60-80° C, most preferably 70-78° C, or even most preferably at 78° C, in the high-pressure container and/or under reflux conditions, followed by cooling to room temperature and reducing the pressure to atmospheric state, then separating of the Ku Ding Cha extract solution from the solid phase by filtration, centrifugation, or subside and decant or other separation techniques. Filtration is the preferred method of separation. The resulting solutions can be

concentrated to liquid or solid products through evaporation at a temperature of 20 to 80°C, preferably 20 to 50°C, more preferably 30 to 40° C, and most preferably 30° C, by freeze-drying preferably, although concentration may also occur by using an evaporator or spray-drying techniques.

Ku Ding Cha includes any part of a Ku Ding Cha plant, its extracts, chemically synthesized compounds that are found in Ku Ding Cha, or pure chemical species identified from any part of the fresh or dried plant, and will hereinafter, including in the claims, be referred to as KDC. KDC has been found to contain multiple active ingredients in apoB secretion lowering tests, and also has a synergistic effect in combination with huanglian and its extracts. A few of the active ingredients from KDC were identified as tri-terpenes: lupeol, ursolic acid, betulin and betulinic acid and related derivatives; a group of tri-terpenoid saponins; a group of mono-terpenoid glycosides, a group of phenylethanoid glycosides, caffeoyl quinic acids and their derivatives. These compounds may also be isolated from a variety of other plants. Plant species with the highest content of ursolic acid include, but are not limited to, Greek Sage (*Salvia triloba L.*), Oleander (*Nerium oleander L.*) and Rosemary (*Rosmarius officinalis L.*). Plant species with the highest content of betulin and betulinic acid include, but are not limited to, bark of *Betula alba, B. pendula, B. platyphylla* and seed of *Ziziphus vulgaris* var. *spinosus*, bark of birch. Plant species with the highest content of lupeol include, but are not limited to, *Alnus glutinosa*, *Aloe vera*, *Apocynum cannabinum* and *Arbutus unedo*. Chlorogenic acid can also be isolated from coffee, black tea or Eucommia bark (Du zhong, *Eucommia ulmoides Oliv.*).

Huanglian and its extracts, chemically synthesized compounds that are found in Huanglian, berberine and berberine containing extracts, berberine-derivatives, analogs, and berberine related alkaloids will hereinafter, including in the claims, be referred to as HL. Huanglian, berberine and berberine-related alkoloids such as palmitine chloride are most preferably obtained from *Coptis chinensis Franch* by using liquid extraction from the dried and powdered herb. *Coptis chinensis* is also sometimes known as *Coptis* root, Golden Thread, Goldthread, Huang Lian, Huanglian, *Rhizoma Coptidis,* or *Coptis chinensis* rhizomes. HL may also be preferably obtained from *Coptis deltoidea CY.Cheng et Hsiao* and *Coptis teeta Wall.* Furthermore, berberine, berberine-derivatives, analogs, and berberine related alkaloids may also be found in a variety of plants, including goldenseal (*Hydrastis canadensis*), San-Ke-Zhen (*Berberis spp.*) *Berberis pratti Schneid, Berberis sargentiana Schneid.,Berberis virgetorum Schneid., Berberis vernae Schneid., Berberis soufleana. Schneid., Berberis pruinosa Franch., Berberis poiretii Schneid., Berberis wilsonae Heml., Berberis dictyophylla Fr., Berberis julianae Schneid., Berberis jamesiana Forrest et W. W. Sm., Berbeis heteropoda Schrenk., Berberis brachypoda Maxi, Berberis sargentiana, Berberis thunbergii, Berberis vulgaris* (barberry), and Oregon grape (*Berberis aquifolium*) as well as *Corydalis Tuber (Yan hu suo), Coptis japonica, Coptis Trifolia, Coptis teeta, Coptis quiquefolia, Coptis deltodea, Phellodendron amurence* (Amur cork tree or *Huang bai*), *Argemone mexicaca L.* (prickly poppy), *Andira inermis* (cabbage bark), *Corydalis Spp* (Fumewort), *Mahonia aquifolium* (blue barberry), *Chelidinium majus* L. (Celandine), *Menispermum canadense* L. (moonseed), *Tinospora Cordifolia* (Guduchi), and *Eschscholzia californica* (California

poppy). Suitable solvents include water, acidic water, water-alcohol mixtures, acid water-alcohol mixtures, water-lower alkyl (C_{1-6}) alcohol mixture, acid water-lower alkyl (C_{1-6}) alcohol mixture, alcohol, especially lower alkyl (C_{1-6}) alcohol, esters, especially C_{2-10} ester, ethers, especially C_{4-12} ether, hydrocarbon chlorides or other organic solvents or mixture thereof. Water, acid water, aqueous alcohol, acidic aqueous alcohol, ethyl acetate and ethyl acetate-based solvent mixture are preferred, although an aqueous alcohol extraction is most preferred. Extraction can be carried out by using liquid extraction, including pressurized liquid extraction (PLE, also called accelerated solvent extraction, ASE), maceration, sonication, microwave assist extraction or supercritical fluid extraction, soaking, agitation, supersonic aid extraction, in a proportion e.g. 1 ml –1 Litre, preferably 2-500 ml, more preferably 2 ml-200ml, more preferably 2.5-50 ml, most preferably 10ml of solvent per gram of *Coptis chinensis* milled powder for a time between e.g. one minute to 100 hours, preferably 2 minutes to fifty hours, more preferably 5 minutes to twenty-four hours, even more preferably for 20 minutes to 5 hours, most preferably 2 hours, at a temperature e.g. 4-120° C, preferably 15-100° C, more preferably 30-90° C, more preferably 50-80° C, even more preferably 60-80° C, most preferably 70-78° C, most preferably at 78° C, in a high-pressure container and/or under reflux conditions; followed by cooling and reducing pressure to room temperature and atmospheric state, then separating of the *Coptis chinensis* solution from the solid phase by filtration, centrifugation, subside and decant, or other separation techniques. Filtration is the most preferred separation technique. The resulting solutions can be concentrated to liquid or solid extract products most preferably with freeze-drying, although evaporator or spray-drying techniques are also preferred.

Berberine and berberine related alkaloids found within the huanglian extract include, but are not limited to, berberine, and berberine-related or analog compounds, e.g. berberine sulfate, berberine chloride, palmatine chloride, coptisine chloride, worenine chloride, jatrorrhizine chloride, colchicines, magnoflorine, oxyberberine, dihydroberberine, (-)-canadine, β-hydrastine, hydrastinie, and salts thereof. Combinations of these alkaloids were found to have synergistic effects in lowering apoB secretion.

The invention further includes the use of compositions comprising KDC in combination with HL, further comprising at least one additional active ingredient selected from the group consisting of Gymnemic acid and Gymnemic acid containing extract from Gymnema (*Gymnema Sylvestre*), [Wu Xue Teng]; Gypenosides and Gypenosides containing extract from Gynostemma pentaphyllum (*Gynostemma pentaphylla (thumb.) makino*), [Jiao Gu Lan]; Isoflavones and Isoflavones containing extract from Puerariae (*Pueraria lobata O.*), [Ge Gan]; Flavones and Flavones containing extract from Hawthorn (*Crataegus pinnatifida,* and *pinnatifida* Bge. Var. major NE Br.), [Shan zha]; Diosgenine and Diosgenine containing extract from Wild Chinese Yam (*Dioscorea Opposita Thunb*), [Shan Yao]; Andrographolides and Andrographolides containing extract from Andrographis (*Andrographis paniculatae*), [Chuan Xin Lian]; orange peel and orange peel extracts; tangerine and tangerine peel extracts; hawthorn and/or hawthorn extracts; *Semen Cassiae (*Jue ming zi) and Semen extracts; Rhizoma Alismatis (ze xie) and Rhizoma Alismatis exracts; Fructus Lycii (Gou Qi Zi) and Fructus Lycii extracts; Radix Glycyrrhizae (gang cao) and Radix Glycyrrhizae extracts; Radix Bupleuri

(cai hu) and Radix Bupleuri extracts; Radix et Rhizoma Rhei and Radix et Rhizoma Rhei extracts; Fleeceflower Root (shou wu) and Fleeceflower root extracts; Semen Ziziphi Spinosae (suan zao ren) and Semen Ziziphi Spinosae extracts; Rhizoma Polygonati Odorati (yu zhu) and Rhizoma Polygonati Odorati extracts; Epimedium Herb (yin yang huo) and Epimedium Herb extracts; Radix Scrophulariae (xuan shen) and Radix Scrophulariae extracts; Radix Angelicae Sinensis (dang gui) and Radix Angelicae Sinensis extracts; Ginseng and Ginseng extracts; Rhizoma Curcumae Longae (Jiang Huang) and Rhizoma Curcumae Longae extracts; Ganoderma Lucidumseu Sinensis (lingzhi); and Ganoderma Lucidumseu Sinensis extracts; Cortex Eucommiae (du zhong) and Cortex Eucommiae extracts; and Fourstamen Stephania Root and Fourstamen Stephania Root extract.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1. shows the dose-related effects of a water extract of HL on the secretion of apolipoprotein-B containing lipoproteins (apo-B) from Hep-G2 cells.

FIG. 2. shows the dose-related effects of a water extract of KDC on the secretion of apolipoprotein-B containing lipoproteins (apo-B) from Hep-G2 cells.

FIG. 3 shows the dose-related effects of an ethanol extract of HL and an ethyl acetate extract of KDC on the secretion of apolipoprotein-B containing lipoproteins (apo-B) from Hep-G2 cells.

FIG. 4 shows the dose-related effects of a hawthorn extract on the secretion of apolipoprotein-B containing lipoproteins (apo-B) from Hep-G2 cells.

FIG. 5 shows the dose-related effects of addition of a hawthorn extract to a methanol extract of HL on the secretion of apolipoprotein-B containing lipoproteins (apo-B) from Hep-G2 cells.

FIG. 6 shows the dose-related effects of addition of a hawthorn extract to an ether extract of Ku Ding cha (KDC) on the secretion of apolipoprotein-B containing lipoproteins (apo-B) from Hep-G2 cells.

FIG. 7 shows the dose-related effects of mixtures of HL, KDC and hawthorn (Haw) extracts on the secretion of apolipoprotein-B containing lipoproteins (apo-B) from Hep-G2 cells.

FIG. 8 shows the dose-related effects of an ethanol extract of HL and an ethyl acetate extract of KDC, both separately and in a 1:1 ratio combination with each other, on the secretion of apolipoprotein-B containing lipoproteins (apo-B) from Hep-G2 cells.

FIG. 9 is a non-linear blending curve plotting how combining an ethanol extract of HL and an ethyl acetate extract of KDC in various proportions for a combined total dose of 150 ug/ml affects the secretion of apolipoprotein-B containing lipoproteins (apo-B) from HepG2 cells.

FIG. 10 is a non-linear blending curve plotting how combining an ethanol extract of HL and an ethyl acetate extract of KDC in various proportions for a combined total dose of 200 ug/ml affects the secretion of apolipoprotein-B containing lipoproteins (apo-B) from HepG2 cells.

FIG. 11 is a non-linear blending curve plotting how combining an ethanol extract of HL and an ethyl acetate extract of KDC in various proportions for a combined total dose of 250 ug/ml affects the secretion of apolipoprotein-B containing lipoproteins (apo-B) from HepG2 cells.

FIG. 12 is an isobologram plotting how combining the ethanol extract of HL and the ethyl acetate extract of Ku Ding Cha (KDC) in various proportions affects the IC_{50} values of the mixture.

FIG. 13 is a flow diagram illustrating an extraction process for Ku Ding Cha (KDC) leaves.

FIG. 14 is a non-linear blending curve plotting how combining an ethanol extract of HL and an ethanol extract of KDC in various proportions for a combined total dose of 150 ug/ml affects the secretion of apolipoprotein-B containing lipoproteins (apo-B) from HepG2 cells.

FIG. 15 is a non-linear blending curve plotting how combining an ethanol extract of HL and an ethanol extract of KDC in various proportions for a combined total dose of 200 ug/ml affects the secretion of apolipoprotein-B containing lipoproteins (apo-B) from HepG2 cells.

FIG. 16 is a non-linear blending curve plotting how combining an ethanol extract of HL and an ethanol extract of KDC in various proportions for a combined total dose of 250 ug/ml affects the secretion of apolipoprotein-B containing lipoproteins (apo-B) from HepG2 cells.

FIG. 17 is a non-linear blending curve plotting how combining an ethanol extract of HL and the organic phase extract of KDC obtained following ethanol and ethyl acetate extractions (5-74-A) in various proportions for a combined total dose of 150 ug/ml affects the secretion of apolipoprotein-B containing lipoproteins (apo-B) from hepG2 cells.

FIG. 18 is a non-linear blending curve plotting how combining an ethanol extract of HL and the organic phase extract of KDC obtained following ethanol and ethyl acetate extractions (5-74-A) in various proportions for a combined total dose of 200 ug/ml affects the secretion of apolipoprotein-B containing lipoproteins (apo-B) from hepG2 cells.

FIG. 19 is a non-linear blending curve plotting how combining an ethanol extract of HL and the water phase extract of KDC obtained following ethanol and ethyl acetate extractions (5-74-B) in various proportions for a combined total dose of 150 ug/ml affects the secretion of apolipoprotein-B containing lipoproteins (apo-B) from hepG2 cells.

FIG. 20 is a non-linear blending curve plotting how combining an ethanol extract of HL and the water phase extract of KDC obtained following ethanol and ethyl acetate extractions (5-74-B) in various proportions for a combined total dose of 200 ug/ml affects the secretion of apolipoprotein-B containing lipoproteins (apo-B) from hepG2 cells.

FIG. 21 is a non-linear blending curve plotting how combining an ethanol extract of HL and the water phase extract of KDC obtained following ethanol and ethyl acetate extractions (5-74-B) in various proportions for a combined total dose of 250 ug/ml affects the secretion of apolipoprotein-B containing lipoproteins (apo-B) from hepG2 cells.

FIG. 22 is a non-linear blending curve plotting how combining each of 3 different extracts of Ku Ding Cha (KDC) with ethanol extracts of HL in various proportions for a combined total dose of 150 ug/ml affects the secretion of apolipoprotein-B containing lipoproteins (apo-B) from hepG2 cells. 5-21-1 is the ethanol extract of KDC; 5-74-A is the organic phase extract obtained following ethanol and ethyl acetate extraction of KDC; and 5-74-B is the water phase extract obtained following ethanol and ethyl acetate extraction of KDC.

FIG. 23 is a non-linear blending curve plotting how combining each of 3 different extracts of Ku Ding Cha (KDC) with ethanol extracts of HL in various proportions for a combined total dose of 200 ug/ml affects the secretion of apolipoprotein-B containing lipoproteins (apo-B) from hepG2 cells. 5-21-1 is the ethanol extract of KDC; 5-74-A is the organic phase extract obtained following ethanol and ethyl acetate extraction of KDC; and 5-74-B is the water phase extract obtained following ethanol and ethyl acetate extraction of KDC.

FIG. 24 is a non-linear blending curve plotting how combining an ethanol extract of HL with ursolic acid in various proportions for a combined total dose of 50 ug/ml affects the secretion of apolipoprotein-B containing lipoproteins (apo-B) from hepG2 cells.

FIG. 25 is a non-linear blending curve plotting how combining an ethanol extract of HL with ursolic acid in various proportions for a combined total dose of 30 ug/ml affects the secretion of apolipoprotein-B containing lipoproteins (apo-B) from hepG2 cells.

FIG. 26 is a non-linear blending curve plotting how combining an ethanol extract of HL with betulin in various proportions for a combined total dose of 50 ug/ml affects the secretion of apolipoprotein-B containing lipoproteins (apo-B) from hepG2 cells.

FIG. 27 is a non-linear blending curve plotting how combining an ethanol extract of HL with betulin in various proportions for a combined total dose of 75 ug/ml affects the secretion of apolipoprotein-B containing lipoproteins (apo-B) from hepG2 cells.

FIG. 28 is a non-linear blending curve plotting how combining an ethanol extract of HL with lupeol in various proportions for a combined total dose of 50 ug/ml affects the secretion of apolipoprotein-B containing lipoproteins (apo-B) from hepG2 cell.

FIG. 29 is a non-linear blending curve plotting how combining an ethanol extract of HL with lupeol in various proportions for a combined total dose of 30 ug/ml affects the secretion of apolipoprotein-B containing lipoproteins (apo-B) from hepG2 cells.

FIG. 30 is a non-linear blending curve plotting how combining an ethanol extract of HL and a sub-fraction of a water phase extract of KDC (94-1), in various proportions for a combined total dose of 250 ug/ml affects the secretion of apolipoprotein-B containing lipoproteins (apo-B) from hepG2 cells.

FIG. 31 is a non-linear blending curve plotting how combining the ethanol extract of HL and a sub-fraction of water phase extract of KDC (94-2), in various proportions for a combined total dose of 250 ug/ml, affects the secretion of apolipoprotein-B containing lipoproteins (apo-B) from hepG2 cells.

FIG. 32 is a non-linear blending curve plotting how combining the ethanol extract of HL and a subfraction of water phase extract of KDC (94-3), in various proportions for a combined total dose of 250 ug/ml, affects the secretion of apolipoprotein-B containing lipoproteins (apo-B) from hepG2 cells.

FIG. 33 is a non-linear blending curve plotting how combining each of 3 different sub-fractions of water phase extracts of KDC with an ethanol extract of HL in various proportions for a combined total dose of 250 ug/ml affects the secretion of apolipoprotein-B containing lipoproteins (apo-B) from hepG2 cells. 94-1 is the first sub-fraction of 5-74-B; 94-2 is the second sub-fraction of 5-74-B: 94-3 is the third sub-fraction of 5-74-B.

FIG. 34 is a non-linear blending curve plotting how combining an ethanol extract of HL with chlorogenic acid in various proportions for a combined total dose of 50 ug/ml affects the secretion of apolipoprotein-B containing lipoproteins (apo-B) from hepG2 cells.

FIG. 35 is a non-linear blending curve plotting how combining an ethanol extract of HL with chlorogenic acid in various proportions for a combined total dose of 75 ug/ml affects the secretion of apolipoprotein-B containing lipoproteins (apo-B) from hepG2 cells.

FIG. 36 is a non-linear blending curve plotting how combining berberine (BBR) with lupeol in various proportions for a combined total dose of 50 ug/ml affects the secretion of apolipoprotein-B containing lipoproteins (apo-B) from hepG2 cells.

FIG. 37 is a non-linear blending curve plotting how combining berberine (BBR) with betulin in various proportions for a combined total dose of 50 ug/ml affects the secretion of apolipoprotein-B containing lipoproteins (apo-B) from hepG2 cells.

FIG. 38 is a non-linear blending curve plotting how combining berberine (BBR) with ursolic acid in various proportions for a combined total dose of 50 ug/ml affects the secretion of apolipoprotein-B containing lipoproteins (apo-B) from hepG2 cells.

FIG. 39 is a non-linear blending curve plotting how combining berberine (BBR) with chlorogenic acid in various proportions for a combined total dose of 50 ug/ml affects the secretion of apolipoprotein-B containing lipoproteins (apo-B) from hepG2 cells.

FIG. 40 is a non-linear blending curve plotting how combining berberine (BBR) with palmitine chloride in various proportions for a combined total dose of 50 ug/ml affects the secretion of apolipoprotein-B containing lipoproteins (apo-B) from hepG2 cells.

DETAILED DESCRIPTION OF THE INVENTION

Evidence from numerous studies indicates that berberine (BBR), an alkaloid isolated primarily from the herbs huanglian (*Coptis chinensis*) and goldenseal (*Hydrastis canadensis*), possesses cholesterol-lowering properties. In particular, berberine has been found to enhance expression of LDL receptors in liver cells through a post-transcriptional mechanism that apparently stabilizes the mRNA for the receptors. This increased expression of LDL receptors enhances the hepatic uptake of cholesterol, consequently resulting in lowering the levels of LDL-cholesterol in plasma.

The cholesterol-lowering properties of KDC extracts appear to be related to their content of terpenes, terpenoids, flavonoids, phenylethanoid glycosides, caffeoyl quinic acid and its derivatives. Recent studies have shown that these terpenoids inhibit a hepatic microsomal enzyme, ACAT, which is required for the esterification and secretion of cholesterol from liver cells.

Although berberine and KDC have been reported to possess cholesterol-lowering properties, they appear to act through different mechanisms. Without being held to any particular mechanism of action, berberine has been found to enhance the hepatic uptake of cholesterol whereas KDC has been reported to reduce the hepatic secretion of cholesterol. Cholesterol is transported to the tissues by LDL and apolipoprotein B (apo-B) is the primary apolipoprotein of LDL. High levels of apo-B can lead to atherosclerosis. The present invention relates to compositions comprising HL and KDC that have been found to act synergistically to lower apo-B secretion. The compositions are useful in their lipid lowering effects to promote human and veterinary health, particularly cardiovascular health and to prevent or treat cardiomyopathy.

The compositions according to the present invention may be used to treat or reduce the chance of contracting or the progression of a number of diseases or conditions in a subject. Diseases or conditions include, for example, cardiomyopathy, cardiovascular disease, hyperlipidemia, hyperlipoproteinemia, high levels of lipoprotein (a), ischemia, coronary heart disease, atherosclerosis, angina, cerebrovascular disease, stroke, overweight or obesity, metabolic syndrome, polycystic ovary syndrome, myocardial infarction, diabetes, insulin resistance, sclerotic disorder, hyperglycemia, hypertension,

arrhythmia, diseases of the central nervous system, diseases of the brain, diseases of the peripheral nervous system and inflammation.

The invention also includes pharmaceutical compositions, comprising HL and KDC in combination with a pharmacologically acceptable carrier. Those of skill in the art are familiar with any pharmaceutically acceptable carrier that would be useful in this regard, and therefore the procedure for making pharmaceutical compositions in accordance with the invention will not be discussed in detail. Suitably, the pharmaceutical compositions may be in the form of tablets, capsules, liquids, lozenges, pastes, salves, powders, lotions, aerosol, solutions suitable for injection or suppositories.

The oral compositions can include an inert diluent or an edible carrier. For the purpose of oral therapeutic administration, compositions comprising HL and KDC can be incorporated with excipients and used in the form of tablets, troches, powders, or capsules, e.g., gelatin capsules. Oral compositions can also be prepared using a fluid carrier. Pharmaceutically compatible binding agents or other carrier materials can be included as part of the composition. Such binding agents and carriers can be a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, or corn starch; a lubricant such as magnesium stearate; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent. HL and KDC compositions may also be admixed with a food or beverage and taken orally. Fortified foods and beverages may be made by adding HL and KDC during the manufacturing of the food or beverage. Alternatively, the consumer may add HL and KDC to the food or beverage near the time

of consumption. Each ingredient may be added to the food or beverage together with the other ingredients or separately from the other ingredients. Examples of foods and beverages are, but not limited to, cereals, snack bars, dairy products, fruit juices, powdered food and dry powder beverage mixes.

The lipid level lowering composition according to the present invention can be administered by any suitable route including oral, aerosol, or other device for delivery to the lungs, nasal spray, intravenous, intramuscular, intraperitoneal, vaginal, rectal, and topical, transmucosal or transdermal. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art. Transmucosal administration can be accomplished through the use of nasal sprays, suppositories or retention enemas for rectal delivery. The suppositories can include conventional suppository bases such as cocoa butter and other glycerides. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

HL and KDC may be administered simultaneously or sequentially over a period of time. The effective time range for administering HL and KDC sequentially is up to and including 60 minutes. Preferably, HL and KDC are co-administered. Compositions comprising HL and KDC may be used alone or in combination with another medicament.

Dosages of HL and KDC in accordance with the invention depend upon the particular condition to be treated, as well as the age, sex and general health condition of the patient. Effective dosages are administered in a synergistically effective amount of HL and KDC

in amounts sufficient to produce a synergistic effect. However, suitable dosages may be found in the range between 0.1-500 mg/kg body weight per day, in between 1 and 10 daily doses. Preferably, effective dosages would range between 1-400 mg/kg body weight per day, administered in between 2 to 6 times a day in divided doses. More preferably, the effective dosage may be 2-100 mg/kg body weight per day, administered in between 2 to 4 times a day in divided doses. Most preferably, the effective dosage may be 10-60 mg/kg body weight per day, administered in 3 times per day in divided doses.

A HL:KDC weight ratio of about 1% to 99% is synergistic; however, 25% to 99% is preferable, 20% to 80% is more preferable, 25% to 75% is even more preferable; 33% to 75% is more preferable; and 40% to 67% is more preferable, and an approximately 1:1 ratio is most preferred.

A HL ethanol extract:KDC ethanol extract weight ratio of about 1% to 99% is synergistic and preferable; however, 25 to 75% more preferable, 30% to 67% is even more preferable; and an approximately 1:2 ratio is most preferred.

A HL ethanol extract:KDC ethyl acetate extract weight ratio of about 1% to 99% is synergistic and preferable; however, 25% to 75% is more preferable, 33% to 67% is even more preferable, and an approximately 2:1 ratio is most preferred.

A HL ethanol extract:KDC water extract weight ratio of about 1% to 99% is synergistic; however, 5% to 85% is preferable, 25% to 75% is more preferable, and an approximately 3:1 ratio is most preferred.

A HL ethanol extract:chlorogenic acid weight ratio of about 1% to 99% is synergistic; however, 25% to 75% is preferable, and an approximately 1:3 ratio is most preferred.

A berberine:lupeol weight ratio of about 1% to 65% is synergistic and preferable; however, 5 to 60% is more preferable, 30% to 55% is even more preferable, and an approximately 1:1 ratio is most preferred.

A berberine:betulin weight ratio of about 1% to 99% is synergistic and preferable; however, 30 to 70% is more preferable, 30% to 60% is even more preferable; and an approximately 1:1 ratio is most preferred.

A berberine:ursolic acid weight ratio of about 1% to 50% is synergistic and preferable; however, 1 to 49% is more preferable, 30 to 40% is yet more preferable; and an approximately 1:2 ratio is most preferred.

A berberine:chlorogenic acid weight ratio of about 1% to 75% is synergistic and preferable; however, 5 to 74% is more preferable, 20% to 50% is yet more preferable; and an approximately 1:3 ratio is most preferred.

A berberine:palmitine chloride weight ratio of about 1% to 99% is synergistic and preferable; however, 25% to 75% is more preferable; and an approximately 2:1 ratio is most preferred.

The terms "effective amount" and/or "therapeutic amount" means a dosage sufficient to provide prevention and/or treatment for the disease state being treated. This will vary depending on the patient, the condition disease and the treatment being effected. The patient may be human or animal, including birds and mammals. Thus, the composition has both medical and veterinarian uses.

In the Examples to follow, Apo-B secretion experiments were carried out on HepG2 cells. These cells were obtained from the American Type Culture Collection (cat #: ATCC HB-8065). HepG2, a human hepatoblastoma-derived cell line, is one of the most commonly used models to study lipoprotein uptake and metabolism in intact human liver. These cells secrete most of the plasma proteins secreted from human liver cells including apolipoprotein-B [38]. In addition, most of the enzymes involved in intra- and extracellular cholesterol metabolism including LCAT, ACAT, HMG-CoA reductase and Cholesterol 7-α-hydroxylase are expressed in these cells [39]. They also exhibit both saturable high-affinity and nonsaturable low-affinity LDL binding sites [40].

The invention will now be further elucidated by the following Examples.

Example 1

Determining the dose related effects of HL and KDC on the secretion of Apo-B from HepG2 cells

The effects of HL, KDC and hawthorn extracts, when used in isolation as well as in combination, on the secretion of apo-B from human hepatoma cells, Hep-G2 was determined. Water extracts of *Coptis chinesis* (Huanglian) and Ku Ding Cha (*Ilex kudingcha)* were developed and tested; results are indicated in Figures 1 and 2, respectively. Ethanolic extracts of *Coptis chinesis* (Huanglian) and ethyl acetate extracts of Ku Ding Cha (*Ilex kudingcha)* were also developed and hawthorn extract was used for these experiments shown in Figures 3-12. Isolated huanglian or Ku Ding Cha extracts reduce the secretion of apo-B from Hep-G2 cells in a dose-related manner (Fig. 3) and the hawthorn extract also has an inhibitory effect on the secretion of apo-B from the cells (Fig. 4). HL had greater efficacy than KDC. The IC_{50} values for HL and KDC extracts were found to be 146 μg/ml and 238.6 μg/ml, respectively. The addition of hawthorn extract to the HL (Fig. 5), as well as to KDC (Fig. 6), was found to increase the efficacy of both extracts in a dose-dependent manner.

Further, the effect of combining HL and KDC extracts in the weight ratio of 1:1 on the secretion of apo-B (Fig. 8) indicates synergism. As is apparent, the combination was found to have greater efficacy than isolated extracts of either HL or KDC, thus indicating synergism between the extracts.

Example 2

Determining the dose related effects of HL, KDC and hawthorn on the secretion of Apo-B from HepG2 cells

The effects of mixtures containing HL, KDC, and hawthorn extracts, at two different concentrations, on the secretion of apo-B are shown (Fig. 7). In the presence of hawthorn, increasing the concentrations of either HL or KDC alone had little additional effect on the inhibitory effects of the mixture on apo-B secretion, whereas increasing the concentration of hawthorn was found to increase the overall efficacy of the combined extract. However, when the concentrations of any two of these three extracts were increased simultaneously, the overall efficacy of the mixture was found to be increased. Increasing the concentration of all three extracts was found to have the greatest efficacy. Factorial analysis of the data revealed that among the three extracts, hawthorn extract contributed the most to the overall efficacy of the mixture, followed by HL and KDC had a minimal effect. The nature of interaction between HL and KDC was found to be synergistic.

Example 3

Determining the IC_{50} values for combinations of HL and KDC extracts

The IC_{50} values for HL and KDC were found to be 146 ug/ml and 239.1 ug/ml, respectively. When the concentration of HL was kept constant at 50 ug/ml and KDC varied, the IC_{50} value was found to be 163.5 ug/ml; this result indicates that 113.5 ug/ml of KDC extract should be added to 50 ug/ml HL for 50% efficacy. Furthermore, with HL concentration constant at 100 ug/ml and KDC varied, the IC_{50} was found to be 123.3 ug/ml. Thus, 100 ug/ml of HL should be added to 23.3 ug/ml of KDC extract for 50%

efficacy. The lower IC_{50} values of the combined extracts suggest that the two extracts acted synergistically in lowering the secretion of apo-B from hepG2 cells.

Additional experiments were subsequently carried out to confirm the synergism between the HL and KDC extracts. Three commonly used approaches including non-linear blending, isobologram and combination index were utilized to determine this interaction.

Non-linear blending curves for a mixture of HL and KDC for total doses of 150 µg/ml, 200 µg/ml and 250 µg/ml are illustrated in Figures 9, 10 and 11, respectively. At all these concentrations, increasing the proportion of HL in the mixture was found to increase the efficacy of the extracts, which was reflected in the curving of the line inwards, thereby indicating that the nature of interaction between the two extracts was synergistic.

Further, the isobologram based on IC_{50} values (Table 1) plotted for various combinations of HL and KDC extracts was found to bend inwards (Fig. 12), further indicating that the nature of interaction between the two extracts was synergistic. Figure 12 is based on the IC_{50} values of the two extracts (indicating 50% efficacy). It indicates that at HL concentration of 50 ug/ml, 113.2 ug/ml of KDC will be required to achieve 50% inhibition in the secretion of apo-B from HepG2 cells. Whereas when the concentration of HL is increased to 100 ug/ml, then only 23.3 ug of KDC will be required to achieve this effect.

Table 1: IC_{50} values of HL and KDC extracts and for various combinations

Extracts	IC_{50}
KDC	239.1 ug/ml
HL	146.0 ug/ml
KDC mixed with 50 ug/ml HL	163.2 ug/ml
KDC mixed with 100 ug/ml HL	123.3 ug/ml

The combination index

Determination of a drugs combination index (CI) provides quantitative information of the nature of drug interaction. It is calculated through the following equation:

$$CI = (C_{A,X} / IC_{X,A}) + (C_{B,X} / IC_{X,B})$$

$C_{A,X}$ and $C_{B,X}$, are the concentrations of drug A and drug B used in the combination to achieve x% drug effect. $IC_{X,A}$ and $IC_{X,B}$ are the concentrations for single agents to achieve the same effect. A CI of less than, equal to, and more than 1 indicates synergy, additivity, and antagonism, respectively.

For HL 50 µg/ml: **0.81**
For HL 100 µg/ml: **0.78**

The Combination indexes computed for both the HL concentrations were found to be less than 1. This further indicates that HL and KDC interacted synergistically in reducing the secretion of apo-B from hep-G2 cells.

The combination index was computed from the IC_{50} values and because the IC_{50} value of HL alone was found to be less than 150 ug/ml, combination indexes (CI) for the mixtures containing the amount of HL greater than 150 ug/ml cannot be calculated.

Example 4

Determining if the method of extraction affects observed biological activity of KDC

To determine if the method of extraction affects the biological activity of KDC, 3 different extracts of KDC were prepared. Figure 13 indicates the flow diagram revealing the processing techniques utilized in the preparation of these extracts. Raw KDC leaves were initially extracted with ethanol and the extract obtained (5-21-1) was freeze dried and subsequently utilized for the preparation of other extracts and assessment of biological activity. The dried extract (5-21-1) was dissolved in water and further extracted with ethyl acetate. Both organic (5-74-A) and aqueous (5-74-B) phase extracts obtained following ethyl acetate extraction were collected. All extracts obtained from raw leaves during KDC processing were tested for biological activity, both alone as well as in combination, with the ethanol extract of HL.

Interaction between ethanol extracts of KDC and HL: Figures 14-16 show the non-linear blending curves between ethanol extract of HL and ethanol extract of KDC (5-21-1) for the combined total doses of 150 ug/ml, 200 ug/ml and 250 ug/ml. Mixtures of these extracts in different proportions were found to have efficacy consistently higher than that of the either of these extracts individually. The results, therefore, indicate synergism between ethanol extracts of HL and KDC in reducing the secretion of apo-B related lipoproteins from hepG2 cells.

Interaction between the ethyl acetate organic phase extract of KDC (5-74-A) and HL: Figures 17 and 18 illustrate the interaction between the ethanol extract of HL (HL)

and the organic phase extract of KDC obtained following both ethanol and then ethyl acetate extractions (5-74-A) for the combined total doses of 150 and 200 ug/ml, respectively. Thus, 5-74-A was found to interact synergistically with the HL extract for both the doses tested in these experiments.

Interaction between the water phase extract of KDC (5-74-B) and HL: Figures 19, 20, and 21 show the non-linear blending curves for the mixtures containing the ethanol extract of HL and the water phase extract of KDC obtained following both ethanol and then ethyl acetate extractions (5-74-B). Figures 19-21 demonstrate that HL interacts synergistically with the 5-74-B extract of KDC.

As demonstrated in Figures 14 to 21, the method of extraction for KDC does not affect its synergistic interaction with HL. Among the three extracts, 5-74-A was found to have the greatest synergism with HL (Figures 22 and 23).

Example 5

Interaction between triterpenoids, ursolic acid, betulin, and lupeol, and HL

Chemical analysis of 5-74-A revealed that it contained various triterpenoids and, in particular, significant amounts of lupeol, betulin and ursolic acid were found in this extract. The next sets of experiments, therefore, were designed to determine the nature of interaction between each of these triterpenoids and the ethanol extract of HL.

The experiments described in Figures 24- 29 were based on the prior laboratory analysis indicating that HL contains about 20% BBR. Therefore, a value of 20% of BBR in HL was combined with either ursolic acid or lupeol to prepare a mixture of combined total doses of 30 or 50 ug/ml. For example in Figure 24, for a total dose of 50 ug/ml with 40% BBR content, 20 ug/ml of BBR is required; thus, 100 ug/ml of HL was added to 30 ug/ml of ursolic acid (for 60% ursolic acid content). In a similar fashion, 20% of BBR in HL was combined with betulin to prepare a mixture of combined total doses of 50 and 75 ug/ml. The non-linear blending curves of each of these terpenoids with HL are shown in Figures 24-29. It appears, therefore, that triperpenoids, in particular ursolic acid, betulin and lupeol, are likely a few of the constituents of KDC interacting with HL.

Example 6

Interactions between KDC aqueous sub-fractions, 94-1, 94-2, 94-3, and huanglian

Subsequent to completion of above described experiments, 5-74-B, the water phase extract that was obtained following the ethanol and ethyl acetate extraction of KDC, was further fractionated by water elution through a Sephadex-15 column and 3 fractions were successively collected and named fractions 94-1, 94-2 and 94-3.

Interaction between 94-1 and ethanol extract of HL: Figure 30 depicts the non-linear blending curve for various mixtures of 94-1 and the HL extract. As is evident from Figure 30, 94-1 interacted synergistically with HL.

Interaction between 94-2 and ethanol extract of HL: Figure 31 demonstrates that 94-2 is also synergistic when combined with HL.

Interaction between 94-3 and the ethanol extract of HL: Figure 32 demonstrates that 94-3 is also synergistic when combined with HL. The effects are most apparent in a 94-3 to HL weight of 3:1.

As demonstrated in figures 30 to 32, each sub-fraction of 5-74-B are synergistic with HL. Among the 3 sub-fractions of the water phase extract 5-74-B, 94-3 has the greatest synergy with HL (see Figure 33).

Example 7

Chemical analysis of 94-3 revealed that the one of the major constituents of this fraction was a caffeoyl quinic acid called chlorogenic acid. Subsequent experiments, therefore, were designed to determine the nature of interaction between chlorogenic acid and the ethanol extract of HL.

Interaction between chlorogenic acid and the ethanol extract of HL: Chlorogenic acid and the ethanol extract of HL were mixed together in various proportions to study its interactions. The effects of these combinations are illustrated at the combined total dose of 50 and 75 ug/ml in Figures 34 and 35. Chlorogenic acid also expressed synergism with the HL extract, indicating that the chlorogenic acid content of the KDC extracts is likely contributing to the demonstrated synergism between HL and KDC.

Example 8

Experiments were devised to consider the synergism between BBR and various potential active components of KDC (pure compounds including lupeol, betulin, ursolic acid, and chlorogenic acid). The following graphs illustrate the synergism between BBR and these pure compounds in Figures 36 – 39.

Example 9

An experiment was also carried out to determine the nature of interaction between different components of HL. Therefore, the nature of interaction between berberine and palmitine chloride was determined and is presented in Figure 40. Palmitine chloride was also found to interact synergistically with berberine.

FULL CITATIONS FOR REFERENCES REFERRED TO IN THE SPECIFICATION

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CLAIMS

1. A synergistic blood lipid lowering composition comprising synergistically effective amounts of HL and KDC.
2. The composition of claim 1, further comprising an effective amount of a hawthorn extract.
3. The composition of claim 1, wherein the weight ratio of HL to KDC is between about 15 and 85%.
4. The composition of claim 3, wherein the weight ratio of HL to KDC is between 25 and 75%.
5. The composition of claim 4, wherein the weight ratio of HL to KDC is between 33 and 67%.
6. The composition of claim 5, wherein the weight ratio of HL to KDC is between 40 and 60%.
7. The composition of claim 6, wherein the weight ratio of HL to KDC is about 1:1
8. A method of lowering the blood lipid levels in a patient in need of such lowering, comprising administering to the patient synergistically effective amounts of HL and KDC.
9. The method of claim 8, wherein at least one of the HL or KDC is orally administered to the patient in a food or beverage.
10. The composition of claim 1, wherein the composition further comprises a carrier which is a food or beverage.

11. The pharmaceutical composition of claim 1 in combination with another medicament or with one or more pharmaceutical acceptable carriers including food items for the lowering of blood lipid levels
12. Use of the composition of claim 1 for the preparation of a pharmaceutical composition, a food or a beverage for the lowering of blood lipid levels.
13. A blood lipid level lowering synergistic composition comprising a berberine containing plant extract and Ku Ding Cha extract.
14. The composition of claim 13, further comprising an effective amount of a hawthorn extract.
15. The composition of claim 13, wherein the berberine containing plant extract is from *Coptis chinensis Franch, Coptis deltoidea C. Y. Cheng et Hsiao, Coptis teeta Wall or Hydrastis Canadensis.*
16. The composition of claim 13 , wherein the Ku Ding Cha extract is from *Ilex kudincha C. J. Tseng (Aquifoliaceae), Ilex aquifoliacese, Ilex latifolia* Thunb, *Ligustrum robustum, Ehretia thyrsiflora, Clerodendrum fortunatum L.* or *Ilex cornuta* Lindl. ex paxt.
17. The composition of claim 13, wherein the weight ratio of the berberine containing plant extract to Ku Ding Cha extract is between about 10% to 90%.
18. The composition of claim 17, wherein the weight ratio of the berberine containing plant extract to Ku Ding Cha extract is between 25% to 75%.
19. The composition of claim 18, wherein the weight ratio of the berberine containing plant extract to Ku Ding Cha extract is between 33% to 67%.

20. The composition of claim 19, wherein the weight ratio of the berberine containing plant extract to Ku Ding Cha extract is between 40% to 60%.

21. The composition of claim 20, wherein the weight ratio of the berberine containing plant extract to Ku Ding Cha extract is about 1:1.

22. The pharmaceutical composition of claim 13 in combination with another medicament or with one or more pharmaceutical acceptable carriers including food items for the lowering of blood lipid levels

23. Use of the composition of claim 13 for the preparation of a pharmaceutical composition, a food or a beverage for the lowering of blood lipid levels.

24. A process for producing a blood lipid level lowering composition comprising obtaining a Ku ding cha extract using liquid extraction in 1 to 1000 mL of solvent per gram of Ku ding cha in a solvent, recovering the Ku ding cha product, obtaining a berberine product from a berberine containing plant using liquid extraction in 5 to 20 mL of solvent per gram of said plant in a solvent, recovering the berberine product and further comprising mixing the Ku ding cha and berberine products.

25. The process of claim 24, wherein the berberine containing plant is *Coptis chinensis Franch, Coptis deltoidea C. Y. Cheng et Hsiao, Coptis teeta Wall* or *Hydrastis Canadensis.*

26. The process of claim 24, wherein the Ku Ding Cha extract is from *Ilex kudincha C. J. Tseng (Aquifoliaceae), Ilex aquifoliacese, Ilex latifolia* Thunb, *Ligustrum robustum, Ehretia thyrsiflora, Clerodendrum fortunatum L.* or *Ilex cornuta* Lindl. ex paxt.

27. The process of claim 24, wherein the solvent is water, acid water, aqueous alcohol, acidic aqueous alcohol, ethyl acetate and ethyl acetate-based solvent mixture.
28. The process of claim 24, wherein the solvent is an aqueous alcohol extraction.
29. The process of claim 24, wherein the solvent is ethyl acetate or an ethyl acetate-based solvent mixture.
30. The process of claim 24, wherein the weight ratio of berberine solution to Ku ding cha solution is between about 10% to 90%
31. The process of claim 30, wherein the weight ratio of berberine solution to Ku ding cha solution is between 25% to 75%.
32. The process of claim 31, wherein the weight ratio of berberine solution to Ku ding cha solution is between 33% to 67%
33. The process of claim 32, wherein the weight ratio of berberine solution to Ku ding cha solution is between 40% to 60%.
34. The process of claim 33, wherein the weight ratio of berberine solution to Ku ding cha solution is about 1:1.
35. A method of lowering the blood lipid level in a patient in need of such lowering comprising administering to the patient effective amounts of a berberine containing plant extract and a Ku ding cha extract.
36. The method of claim 35, wherein the berberine containing plant extract and Ku ding cha extract are administered simultaneously.
37. The method of claim 35, wherein the berberine containing plant extract and Ku ding cha extract are administered sequentially.

38. The method of claim 36, wherein the berberine containing plant extract and Ku ding cha extract are administered over a time period of up to and including 60 minutes.

39. The method of claim 35, wherein the berberine containing plant extract is from *Coptis chinensis Franch, Coptis deltoidea C. Y. Cheng et Hsiao, Coptis teeta Wall* or *Hydrastis Canadensis.*

40. The method of claim 35, wherein the Ku Ding Cha extract is from *Ilex kudincha C. J. Tseng (Aquifoliaceae), Ilex aquifoliacese, Ilex latifolia* Thunb, *Ligustrum robustum, Ehretia thyrsiflora, Clerodendrum fortunatum L.* or *Ilex cornuta* Lindl. ex paxt.

41. The method of claim 35, wherein the weight ratio of berberine containing plant extract to Ku Ding Cha extract is between about 10% and 90%.

42. The method of claim 41, wherein the weight ratio of berberine containing plant extract to Ku Ding Cha extract is between 25% and 75%.

43. The method of claim 42, wherein the weight ratio of berberine containing plant extract to Ku Ding Cha extract is between 33% and 66%.

44. The method of claim 43, wherein the weight ratio of berberine containing plant extract to Ku Ding Cha extract is between 40% and 60%.

45. The method of claim 44, wherein the weight ratio of berberine containing plant extract to Ku Ding Cha extract is about 1:1.

46. The method of claim 35, wherein at least one of the extracts of the composition is administered orally.

47. The method of claim 35, wherein the blood lipid levels that are lowered comprise triglyceride levels.

48. The method of claim 35, wherein the blood lipid levels that are lowered comprises low-density-lipoprotein cholesterol levels.

49. The method of claim 35, wherein the blood lipid levels that are lowered comprises total cholesterol levels.

50. The method of claim 35 for treating or preventing cardiovascular disease, hyperlipidemia, cardiomyopathy, hyperlipoproteinemia, high levels of lipoprotein (a), ischemia, coronary heart disease, atherosclerosis, angina, cerebrovascular disease, stroke, overweight or obesity, myocardial infarction, diabetes, insulin resistance, sclerotic disorder, hyperglycemia, hypertension, arrhythmia, diseases of the central nervous system, diseases of the brain, diseases of the peripheral nervous system or inflammation in a mammal.

51. The method of claim 35, wherein the mammal is a human.

52. A kit containing a preparation for lowering blood lipid levels in a mammal in need of said lowering, comprising in separate containers a berberine containing plant extract and a Ku ding cha extract.

53. The kit of claim 52, wherein the berberine containing plant extract is *Coptis chinensis Franch, Coptis deltoidea C. Y. Cheng et Hsiao, Coptis teeta Wall* or *Hydrastis Canadensis*.

54. The kit of claim 52, wherein the Ku Ding Cha extract is from *Ilex kudincha C. J. Tseng (Aquifoliaceae), Ilex aquifoliacese, Ilex latifolia* Thunb, *Ligustrum robustum, Ehretia thyrsiflora, Clerodendrum fortunatum L.* or *Ilex cornuta* Lindl. ex paxt.

55. The composition of claim 1, further comprising at least one herbal extract selected from the group consisting of Gymnemic acid and/or Gymnemic acid containing extract from Gymnema (*Gymnema sylvestre*), Gypenosides and/or

Gypenosides containing extract from Gynostemma pentaphyllum (*Gynostemma pentaphylla (thumb.) makino*), Isoflavones and/or Isoflavones containing extract from Puerariae (*Pueraria lobata O.*), Flavones and/or Flavones containing extract from Hawthorn (*Crataegus pinnatifida,* and *pinnatifida* Bge. Var. major NE Br.), Emodin and/or Emodin containing extract from Rhubarb (*Rheum officinale baill.*), Diosgenine and/or Diosgenine containing extract from Wild Chinese Yam (*Dioscorea Opposita Thunb*), and Andrographolides and/or Andrographolides containing extract from Andrographis (*Andrographis paniculatae*).

56. The method of claim 35, further comprising administering at least one herbal extract selected from the group consisting of Gymnemic acid and/or Gymnemic acid containing extract from Gymnema (*Gymnema sylvestre*), Gypenosides and/or Gypenosides containing extract from Gynostemma pentaphyllum (*Gynostemma pentaphylla (thumb.) makino*), Isoflavones and/or Isoflavones containing extract from Puerariae (*Pueraria lobata O.*), Flavones and/or Flavones containing extract from Hawthorn (*Crataegus pinnatifida,* and *pinnatifida* Bge. Var. major NE Br.), Emodin and/or Emodin containing extract from Rhubarb (*Rheum officinale baill.*), Diosgenine and/or Diosgenine containing extract from Wild Chinese Yam (*Dioscorea Opposita Thunb*), and Andrographolides and/or Andrographolides containing extract from Andrographis (*Andrographis paniculatae*).

57. The composition of claim 13, further comprising an effective amount of a flavone from hawthorn.

Figures

FIG. 1

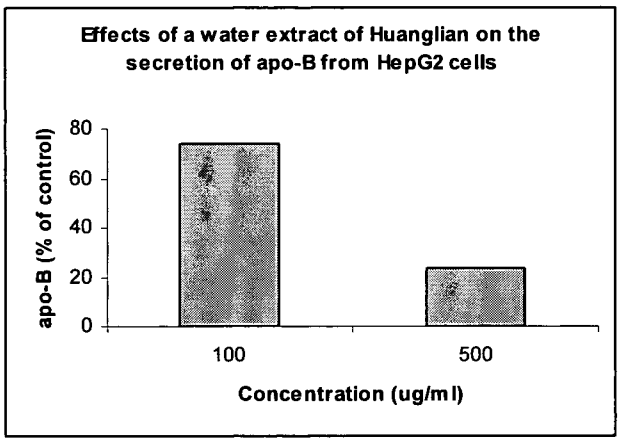


FIG. 2

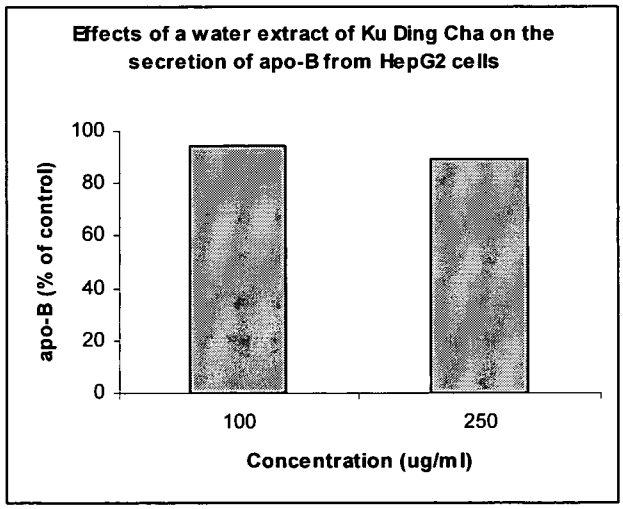


FIG. 3

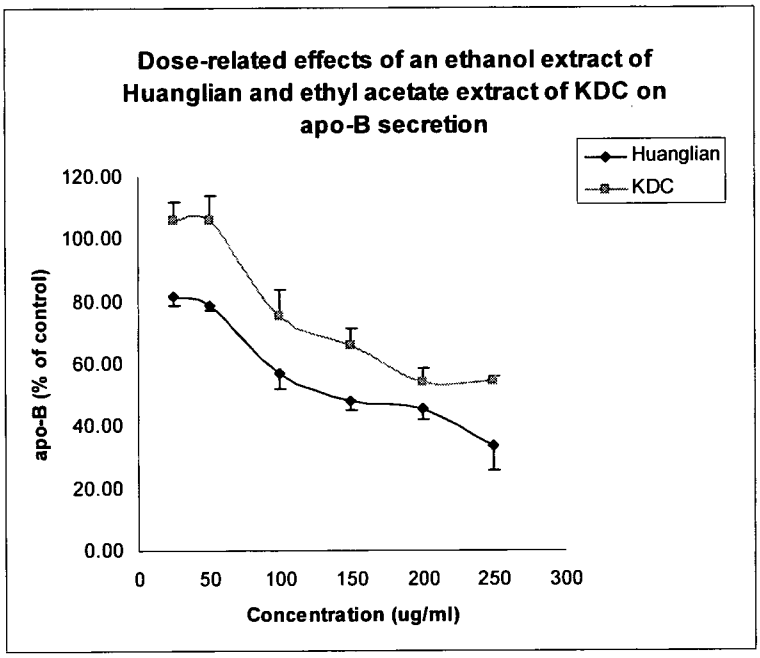

Dose-related effects of an ethanol extract of Huanglian and ethyl acetate extract of KDC on apo-B secretion
Huanglian
KDC
120.00
100.00
80.00
60.00
40.00
20.00
0.00
apo-B (% of control)
0
50
100
150
200
250
300
Concentration (ug/ml)

FIG. 4

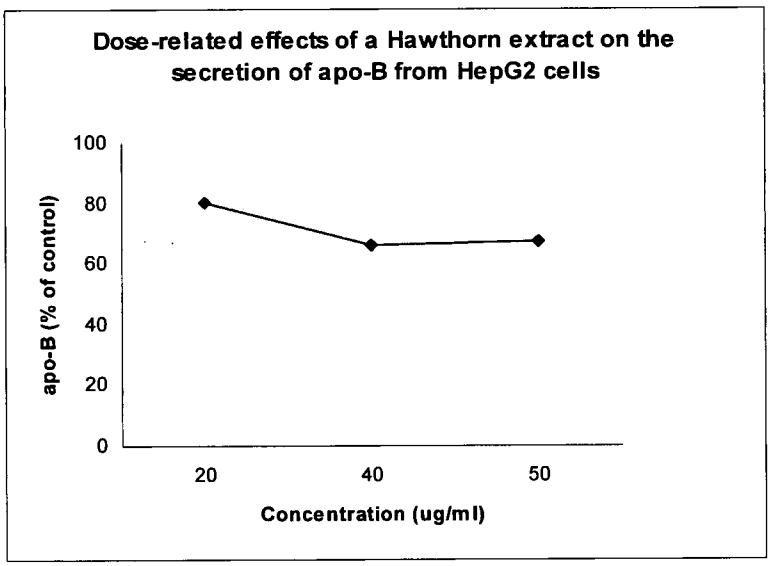

Dose-related effects of a Hawthorn extract on the secretion of apo-B from HepG2 cells
100
80
60
40
20
0
apo-B (% of control)
20
40
50
Concentration (ug/ml)

FIG. 5

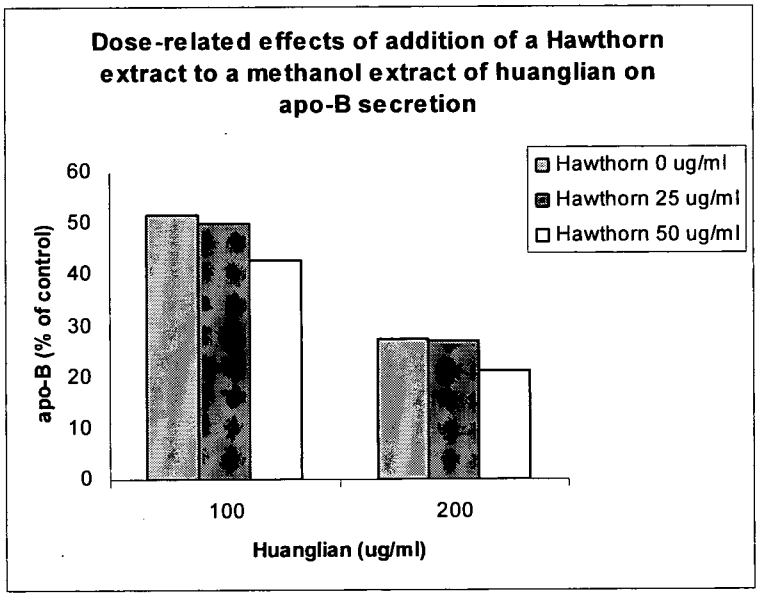

Dose-related effects of addition of a Hawthorn extract to a methanol extract of huanglian on apo-B secretion
Hawthorn 0 ug/ml
Hawthorn 25 ug/ml
Hawthorn 50 ug/ml
apo-B (% of control)
60
50
40
30
20
10
0
100
200
Huanglian (ug/ml)

FIG. 6

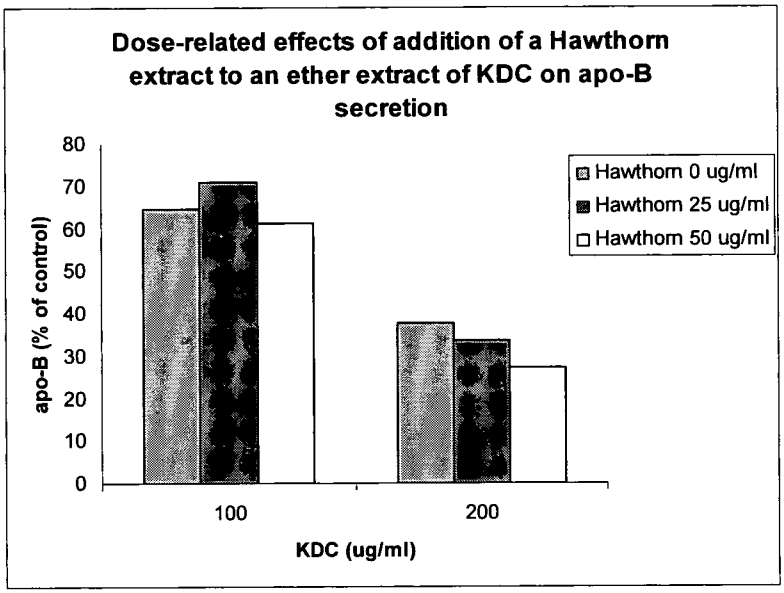

Dose-related effects of addition of a Hawthorn extract to an ether extract of KDC on apo-B secretion
Hawthorn 0 ug/ml
Hawthorn 25 ug/ml
Hawthorn 50 ug/ml
apo-B (% of control)
80
70
60
50
40
30
20
10
0
100
200
KDC (ug/ml)

FIG. 7

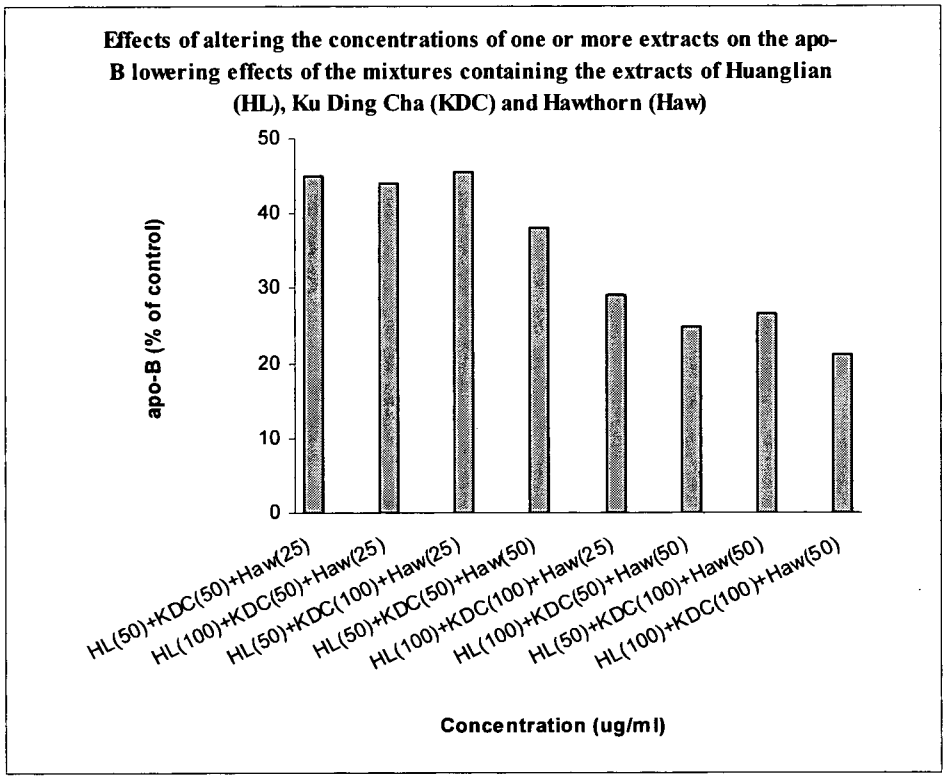

Effects of altering the concentrations of one or more extracts on the apo-B lowering effects of the mixtures containing the extracts of Huanglian (HL), Ku Ding Cha (KDC) and Hawthorn (Haw)
apo-B (% of control)
50
40
30
20
10
0
HL(50)+KDC(50)+Haw(25)
HL(100)+KDC(50)+Haw(25)
HL(50)+KDC(100)+Haw(25)
HL(50)+KDC(50)+Haw(50)
HL(100)+KDC(100)+Haw(25)
HL(100)+KDC(50)+Haw(50)
HL(50)+KDC(100)+Haw(50)
HL(100)+KDC(100)+Haw(50)
Concentration (ug/ml)

FIG. *8*.

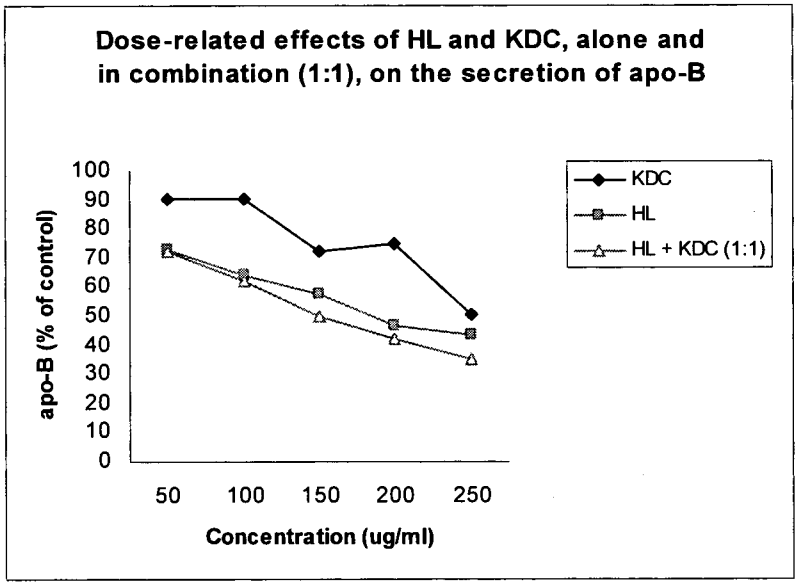

Dose-related effects of HL and KDC, alone and in combination (1:1), on the secretion of apo-B
apo-B (% of control)
100
90
80
70
60
50
40
30
20
10
0
50
100
150
200
250
Concentration (ug/ml)
KDC
HL
HL + KDC (1:1)

FIG. 9

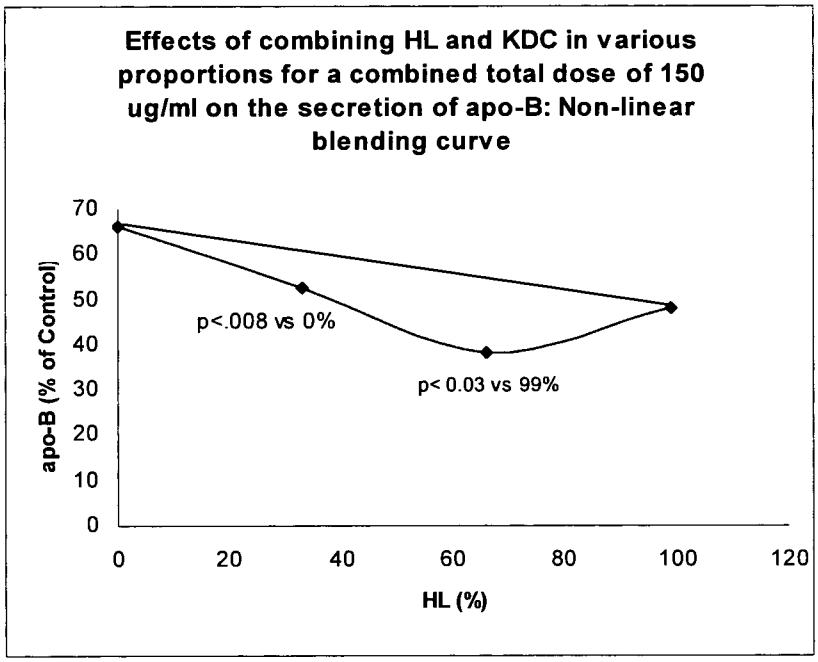

Effects of combining HL and KDC in various proportions for a combined total dose of 150 ug/ml on the secretion of apo-B: Non-linear blending curve
apo-B (% of Control)
70
60
50
40
30
20
10
0
p<.008 vs 0%
p< 0.03 vs 99%
0
20
40
60
80
100
120
HL (%)

FIG. 10

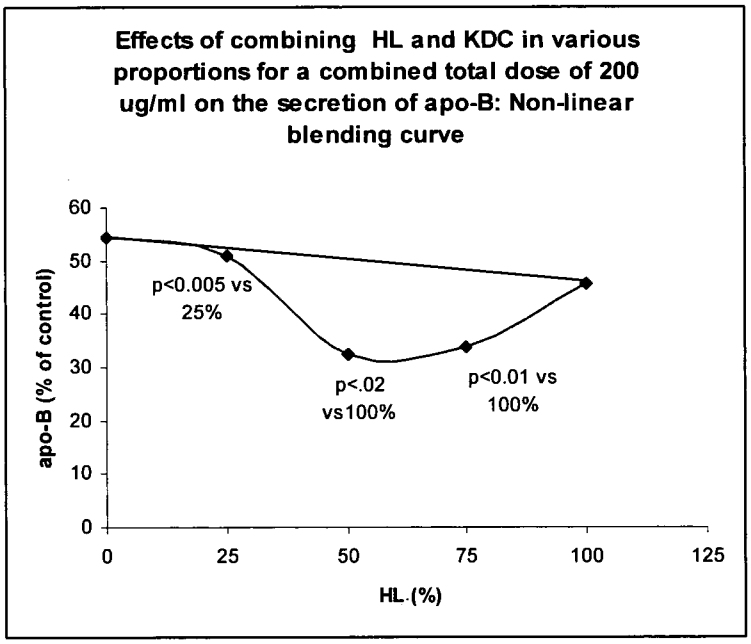

Effects of combining HL and KDC in various proportions for a combined total dose of 200 ug/ml on the secretion of apo-B: Non-linear blending curve
apo-B (% of control)
60
50
40
30
20
10
0
p<0.005 vs 25%
p<.02 vs100%
p<0.01 vs 100%
0
25
50
75
100
125
HL (%)

FIG. 11

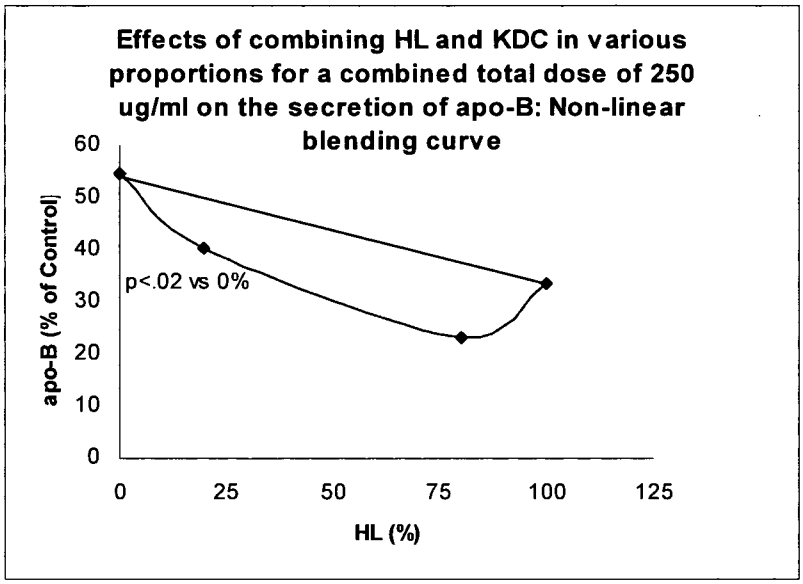

Effects of combining HL and KDC in various proportions for a combined total dose of 250 ug/ml on the secretion of apo-B: Non-linear blending curve
apo-B (% of Control)
60
50
40
30
20
10
0
p<.02 vs 0%
0
25
50
75
100
125
HL (%)

FIG. 12

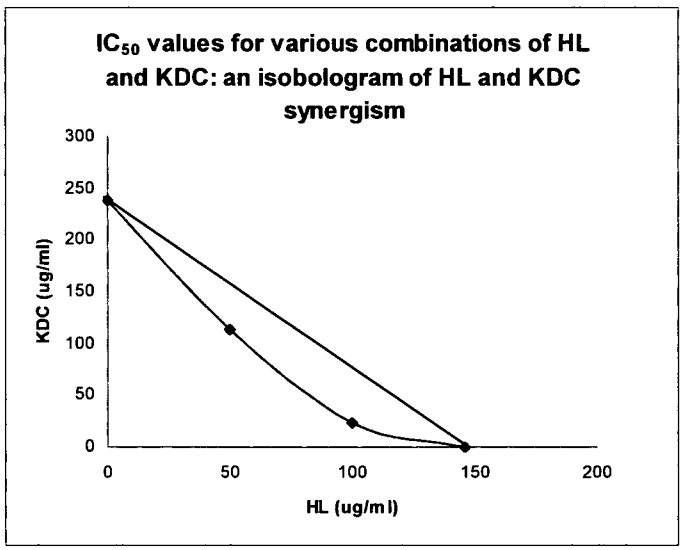

IC50 values for various combinations of HL and KDC: an isobologram of HL and KDC synergism
KDC (ug/ml)
300
250
200
150
100
50
0
0
50
100
150
200
HL (ug/ml)

FIG. 13

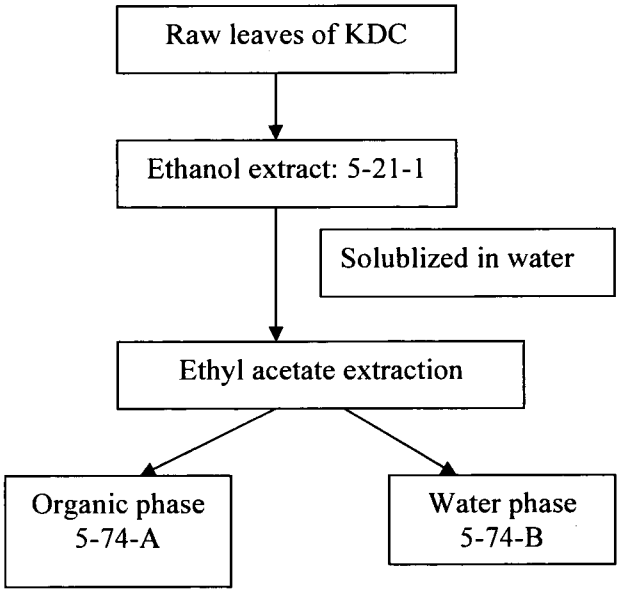

Raw leaves of KDC
Ethanol extract: 5-21-1
Solublized in water
Ethyl acetate extraction
Organic phase
5-74-A
Water phase
5-74-B

FIG. 14

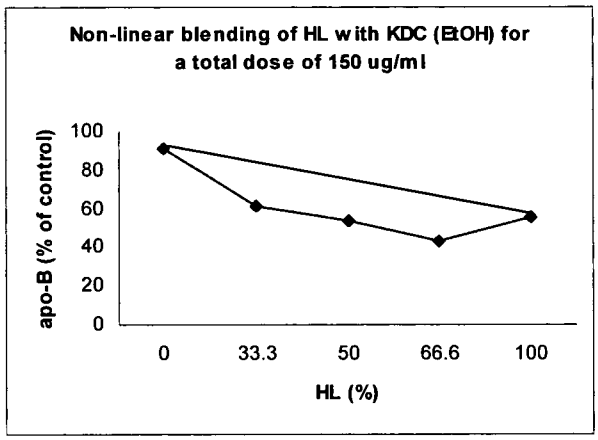

Non-linear blending of HL with KDC (EtOH) for a total dose of 150 ug/ml
apo-B (% of control)
100
80
60
40
20
0
0
33.3
50
66.6
100
HL (%)

FIG. 15

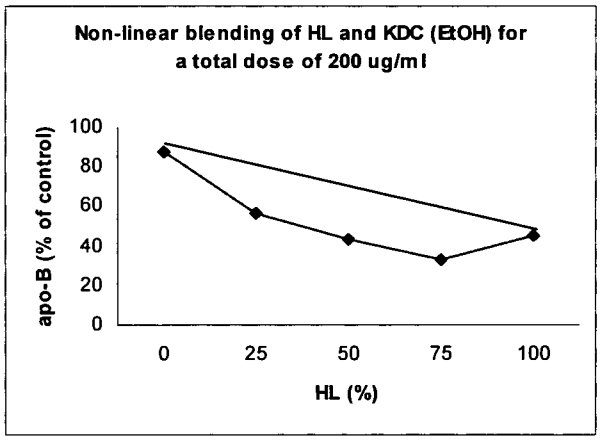

Non-linear blending of HL and KDC (EtOH) for a total dose of 200 ug/ml
apo-B (% of control)
100
80
60
40
20
0
0
25
50
75
100
HL (%)

FIG. 16

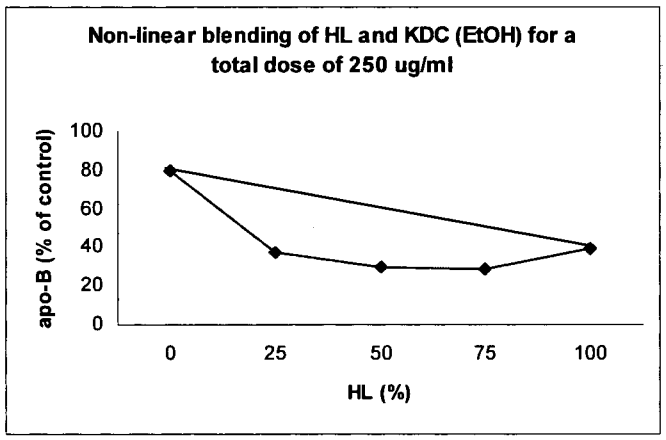

Non-linear blending of HL and KDC (EtOH) for a total dose of 250 ug/ml
apo-B (% of control)
100
80
60
40
20
0
0
25
50
75
100
HL (%)

FIG. 17

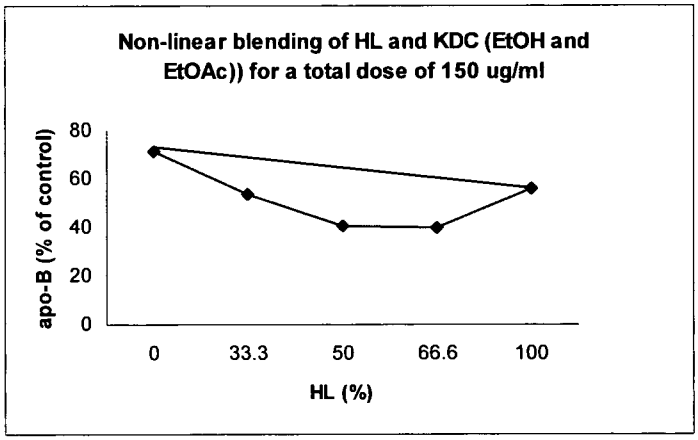

Non-linear blending of HL and KDC (EtOH and EtOAc)) for a total dose of 150 ug/ml
apo-B (% of control)
80
60
40
20
0
0
33.3
50
66.6
100
HL (%)

FIG. 18

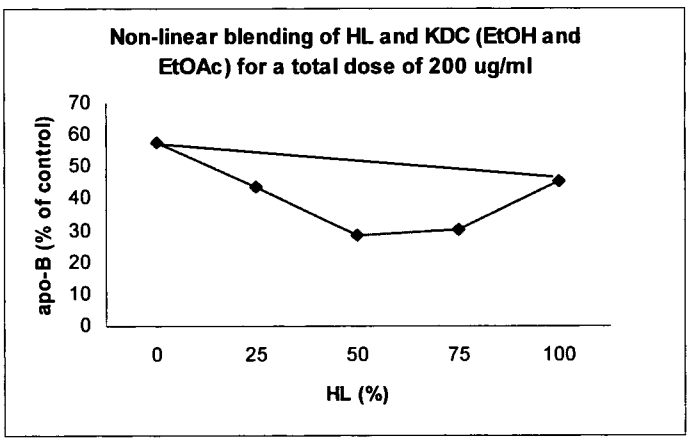

Non-linear blending of HL and KDC (EtOH and EtOAc) for a total dose of 200 ug/ml
apo-B (% of control)
70
60
50
40
30
20
10
0
0
25
50
75
100
HL (%)

FIG. 19

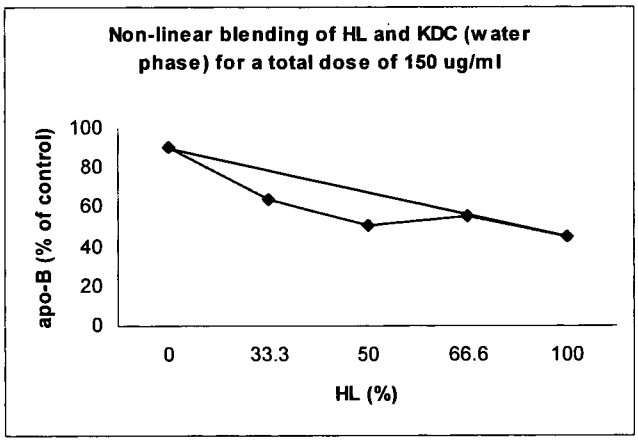

Non-linear blending of HL and KDC (water phase) for a total dose of 150 ug/ml
apo-B (% of control)
100
80
60
40
20
0
0
33.3
50
66.6
100
HL (%)

FIG. 20

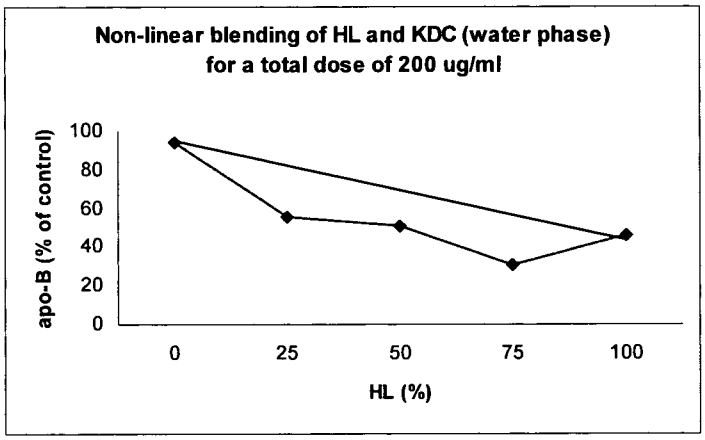

Non-linear blending of HL and KDC (water phase) for a total dose of 200 ug/ml
apo-B (% of control)
100
80
60
40
20
0
0
25
50
75
100
HL (%)

FIG. 21

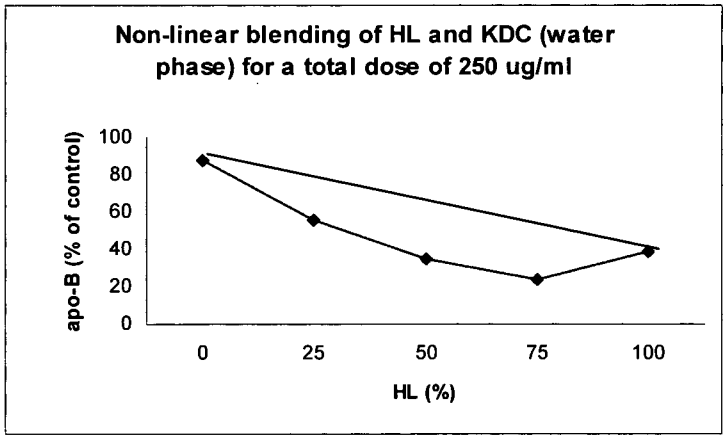

Non-linear blending of HL and KDC (water phase) for a total dose of 250 ug/ml
apo-B (% of control)
100
80
60
40
20
0
0
25
50
75
100
HL (%)

FIG. 22

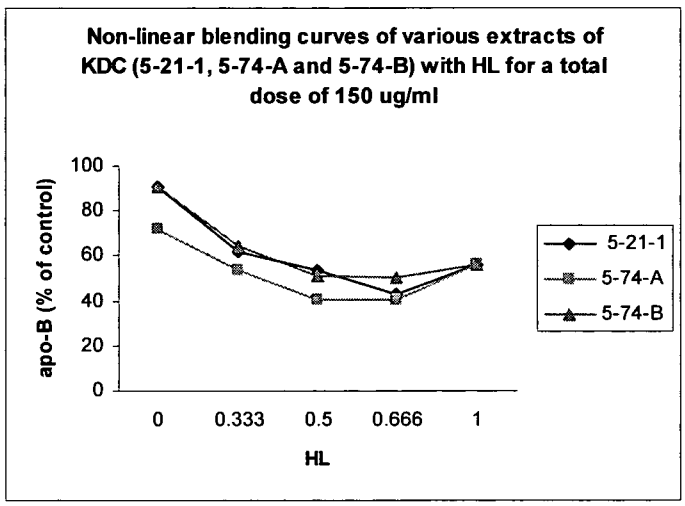

Non-linear blending curves of various extracts of KDC (5-21-1, 5-74-A and 5-74-B) with HL for a total dose of 150 ug/ml
apo-B (% of control)
100
80
60
40
20
0
0
0.333
0.5
0.666
1
HL
5-21-1
5-74-A
5-74-B

FIG. 23

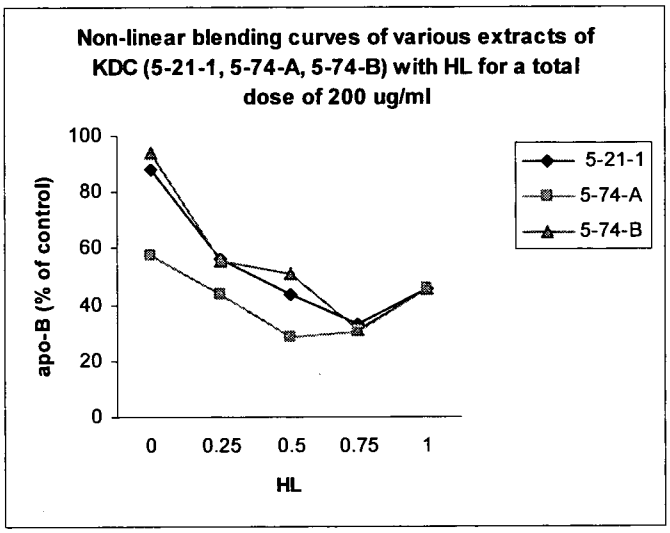

Non-linear blending curves of various extracts of KDC (5-21-1, 5-74-A, 5-74-B) with HL for a total dose of 200 ug/ml
apo-B (% of control)
100
80
60
40
20
0
0
0.25
0.5
0.75
1
HL
5-21-1
5-74-A
5-74-B

FIG. 24

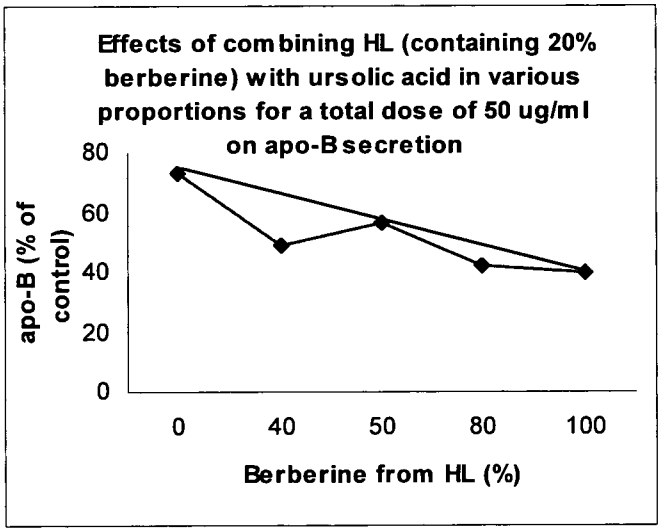

Effects of combining HL (containing 20% berberine) with ursolic acid in various proportions for a total dose of 50 ug/ml on apo-B secretion
apo-B (% of control)
80
60
40
20
0
0
40
50
80
100
Berberine from HL (%)

FIG. 25

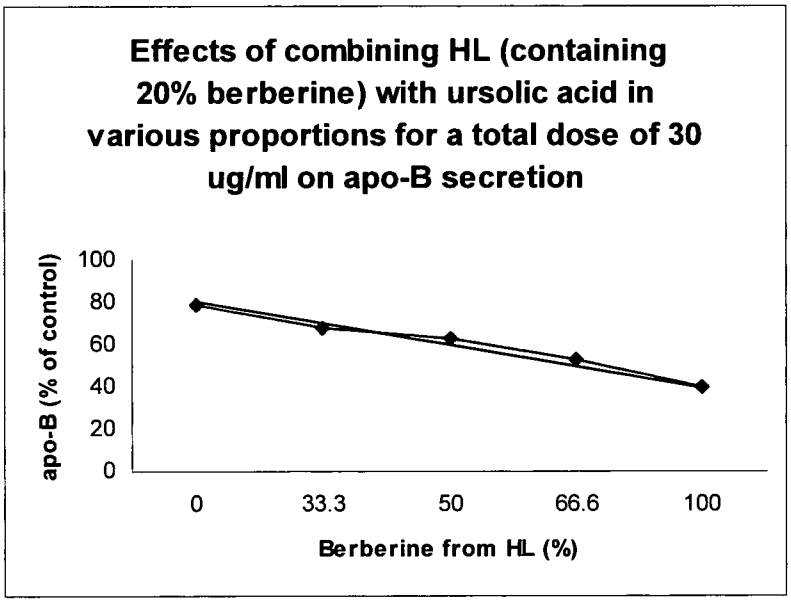

Effects of combining HL (containing 20% berberine) with ursolic acid in various proportions for a total dose of 30 ug/ml on apo-B secretion
apo-B (% of control)
100
80
60
40
20
0
0
33.3
50
66.6
100
Berberine from HL (%)

FIG. 26

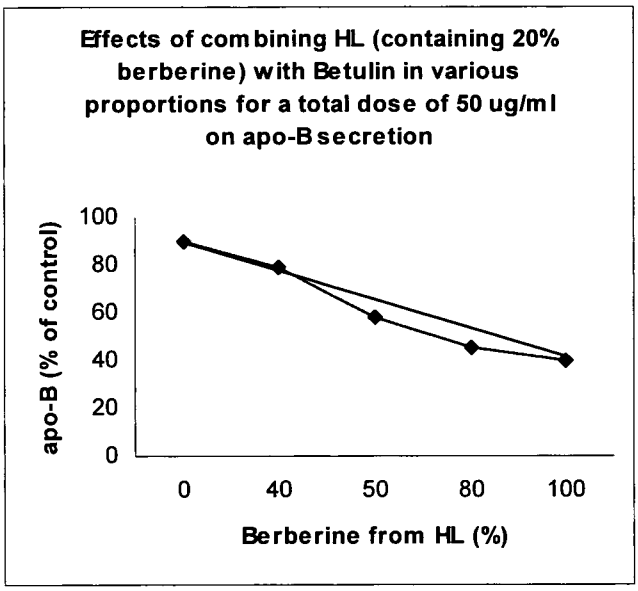

Effects of combining HL (containing 20% berberine) with Betulin in various proportions for a total dose of 50 ug/ml on apo-B secretion
apo-B (% of control)
100
80
60
40
20
0
0
40
50
80
100
Berberine from HL (%)

FIG. 27

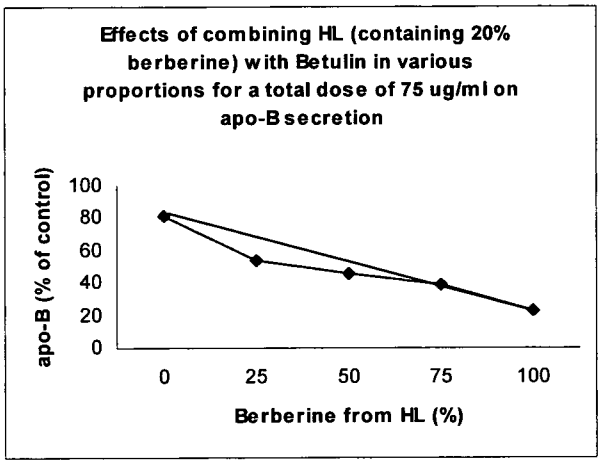

Effects of combining HL (containing 20% berberine) with Betulin in various proportions for a total dose of 75 ug/ml on apo-B secretion
apo-B (% of control)
100
80
60
40
20
0
0
25
50
75
100
Berberine from HL (%)

FIG. 28

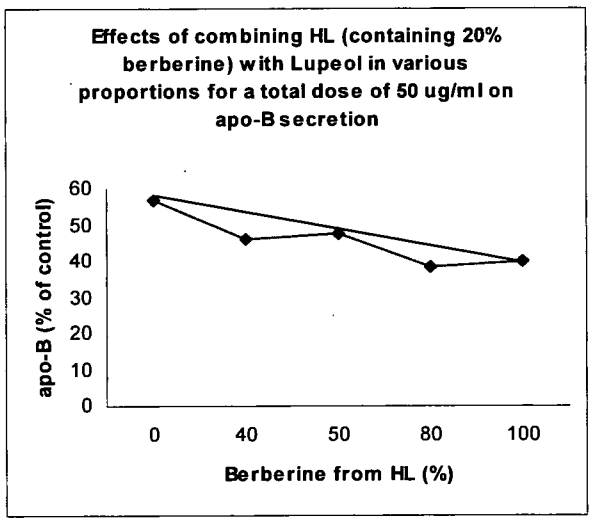

Effects of combining HL (containing 20% berberine) with Lupeol in various proportions for a total dose of 50 ug/ml on apo-B secretion
apo-B (% of control)
60
50
40
30
20
10
0
0
40
50
80
100
Berberine from HL (%)

FIG. 29

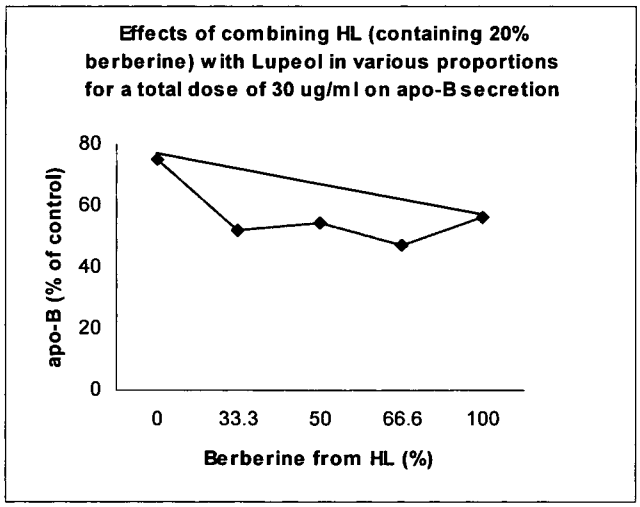

Effects of combining HL (containing 20% berberine) with Lupeol in various proportions for a total dose of 30 ug/ml on apo-B secretion
apo-B (% of control)
80
60
40
20
0
0
33.3
50
66.6
100
Berberine from HL (%)

FIG. 30

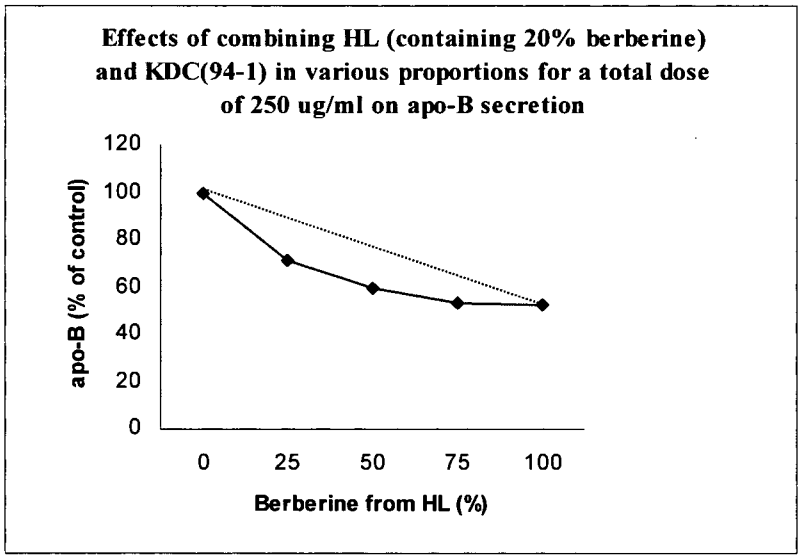

Effects of combining HL (containing 20% berberine) and KDC(94-1) in various proportions for a total dose of 250 ug/ml on apo-B secretion
apo-B (% of control)
120
100
80
60
40
20
0
0
25
50
75
100
Berberine from HL (%)

FIG. 31

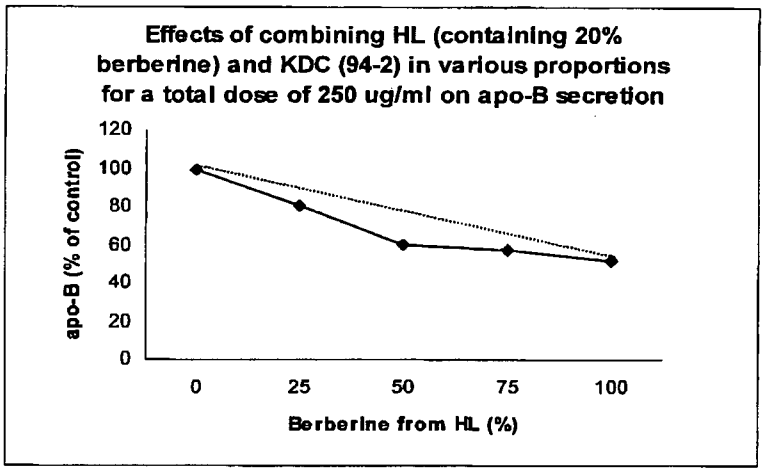

Effects of combining HL (containing 20% berberine) and KDC (94-2) in various proportions for a total dose of 250 ug/ml on apo-B secretion
apo-B (% of control)
120
100
80
60
40
20
0
0
25
50
75
100
Berberine from HL (%)

FIG. 32

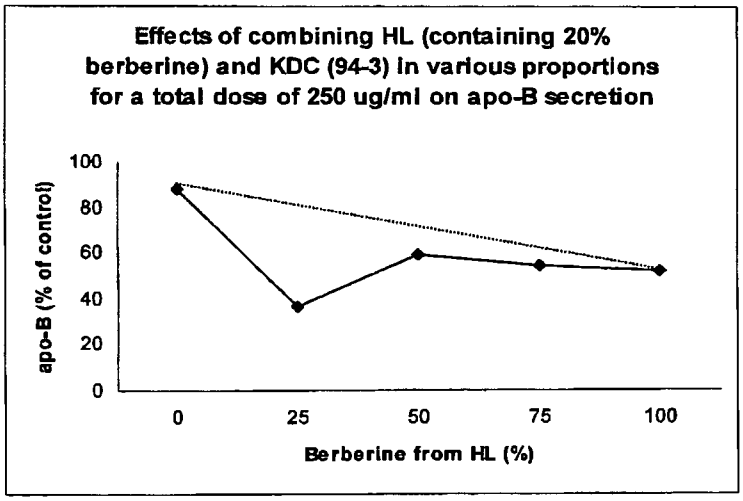

Effects of combining HL (containing 20% berberine) and KDC (94-3) in various proportions for a total dose of 250 ug/ml on apo-B secretion
apo-B (% of control)
100
80
60
40
20
0
0
25
50
75
100
Berberine from HL (%)

FIG. 33

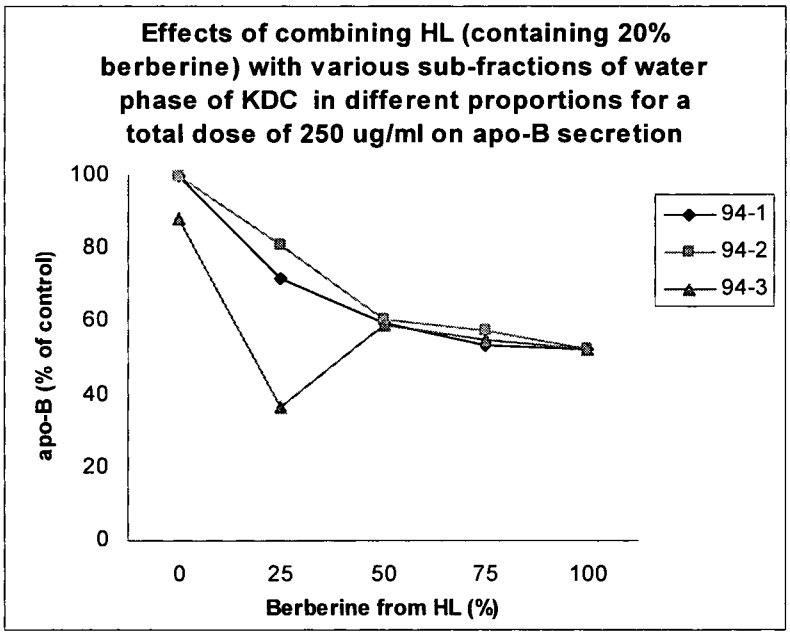

Effects of combining HL (containing 20% berberine) with various sub-fractions of water phase of KDC in different proportions for a total dose of 250 ug/ml on apo-B secretion
apo-B (% of control)
100
80
60
40
20
0
0
25
50
75
100
Berberine from HL (%)
94-1
94-2
94-3

FIG. 34

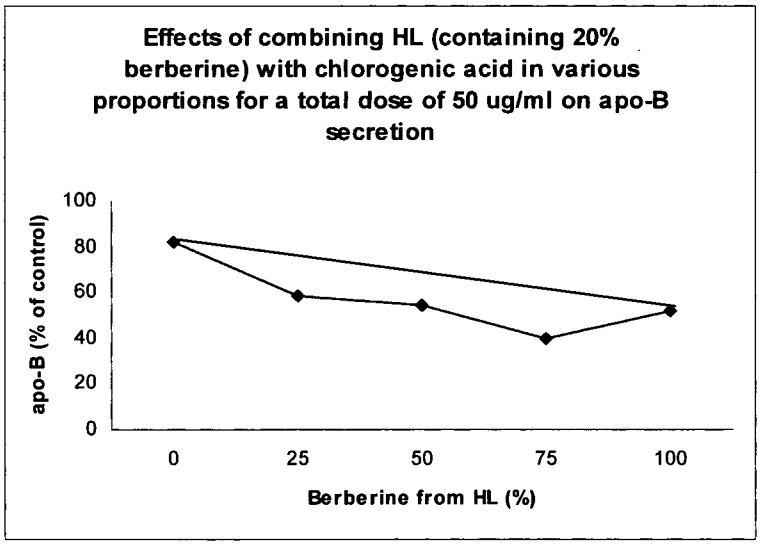

Effects of combining HL (containing 20% berberine) with chlorogenic acid in various proportions for a total dose of 50 ug/ml on apo-B secretion
apo-B (% of control)
100
80
60
40
20
0
0
25
50
75
100
Berberine from HL (%)

FIG. 35

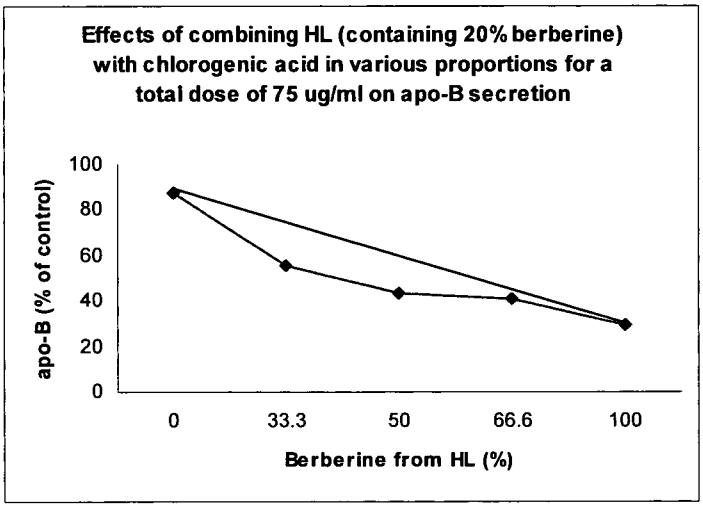

Effects of combining HL (containing 20% berberine) with chlorogenic acid in various proportions for a total dose of 75 ug/ml on apo-B secretion
apo-B (% of control)
100
80
60
40
20
0
0
33.3
50
66.6
100
Berberine from HL (%)

FIG. 36

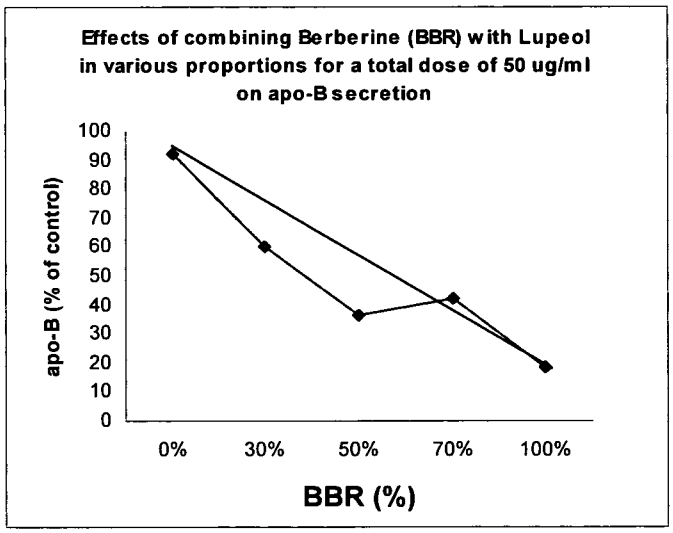

Effects of combining Berberine (BBR) with Lupeol in various proportions for a total dose of 50 ug/ml on apo-B secretion
apo-B (% of control)
100
90
80
70
60
50
40
30
20
10
0
0%
30%
50%
70%
100%
BBR (%)

FIG. 37

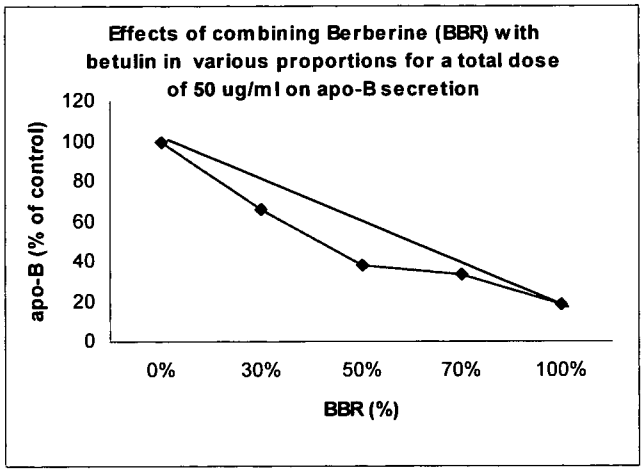

Effects of combining Berberine (BBR) with betulin in various proportions for a total dose of 50 ug/ml on apo-B secretion
apo-B (% of control)
120
100
80
60
40
20
0
0%
30%
50%
70%
100%
BBR (%)

FIG. 38

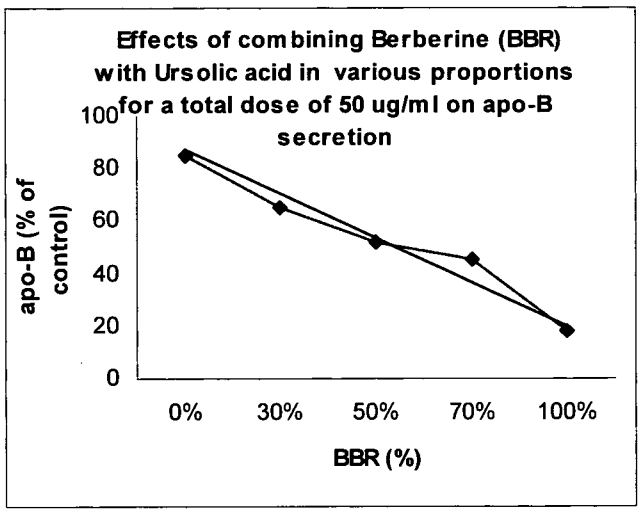

Effects of combining Berberine (BBR) with Ursolic acid in various proportions for a total dose of 50 ug/ml on apo-B secretion
apo-B (% of control)
100
80
60
40
20
0
0%
30%
50%
70%
100%
BBR (%)

FIG. 39

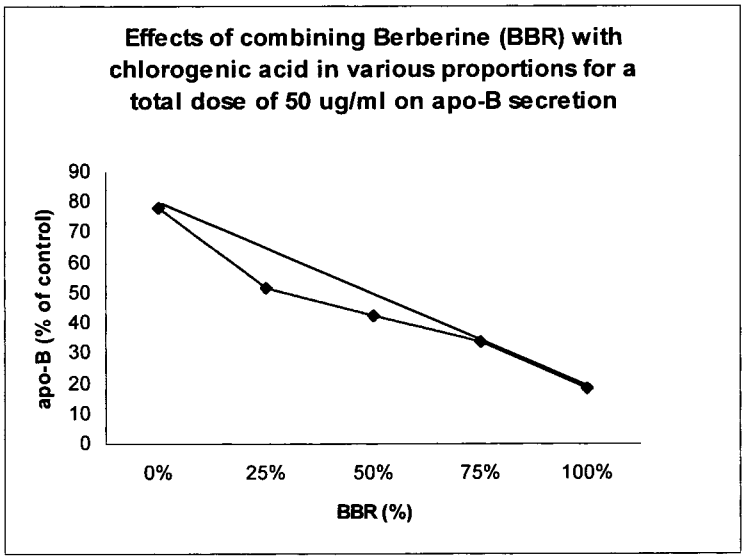
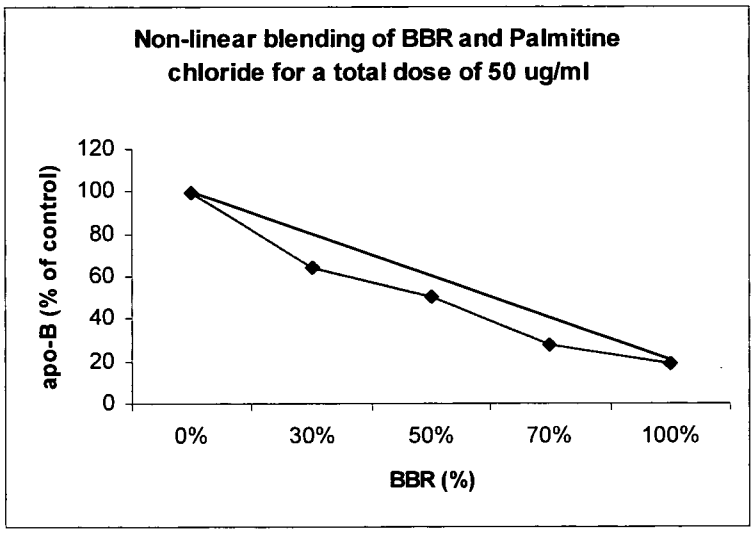

Effects of combining Berberine (BBR) with chlorogenic acid in various proportions for a total dose of 50 ug/ml on apo-B secretion
apo-B (% of control)
90
80
70
60
50
40
30
20
10
0
0%
25%
50%
75%
100%
BBR (%)

FIG. 40


Non-linear blending of BBR and Palmitine chloride for a total dose of 50 ug/ml
apo-B (% of control)
120
100
80
60
40
20
0
0%
30%
50%
70%
100%
BBR (%)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2009/005461

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons :

1. [X] Claim Nos. : 8, 9, 35-51 and 56
because they relate to subject matter not required to be searched by this Authority, namely :

Claims 8, 9, 35-51 and 56 are directed to a method for treatment of the human or animal body by surgery or therapy, which the International Search Authority is not required to search under Rule 39.1(iv). Regardless, this Authority has established a search report based on the alleged effect or use of the product defined in these claims.

2. [ ] Claim Nos. :
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically :

3. [ ] Claim Nos. :
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows :

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos. :

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos. :

Remark on Protest
[ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
[ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2009/005461

A. CLASSIFICATION OF SUBJECT MATTER
IPC: ***A61K 36/718*** (2006.01) , ***A61K 31/473*** (2006.01) , ***A61K 36/638*** (2006.01) , ***A61K 36/734*** (2006.01) , ***A61P 3/06*** (2006.01) , ***A61P 9/00*** (2006.01)
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC: ***A61K 36/718*** (2006.01) , ***A61K 31/473*** (2006.01) , ***A61K 36/638*** (2006.01) , ***A61K 36/734*** (2006.01) , ***A61P 3/06*** (2006.01) , ***A61P 9/00*** (2006.01)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
Scopus, PubMed, Agricola, Delphion, Canadian Patent Database, Keywords: Huanglian, HL, Coptis, berberine, BBR, Ku Ding Cha, KDC, Kuding tea, bitter tea, Ilex, Ligustrum, Hawthorn, Crataegus, lipid lowering composition, apo-B, Afexa Life Sciences Inc., Shan, Ling, Goel, Qi, Wang

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO06029577 A1 (JIANG J-D. et al.) 23 March 2006 (23-03-2006) (see whole document)	1-57
Y	FUKUDA T. et al. Two new monoterpene glycosides from ku-ding-cha. Inhibitors of acyl-CoA:cholesterol acyltransferase (ACAT) CHEMICAL AND PHARMACEUTICAL BULLETIN (TOKYO) November 1996 44:2173-2176 (see whole document)	1-57

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents :
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 August 2009 (10-08-2009)	3 September 2009 (03-09-2009)

Name and mailing address of the ISA/CA	Authorized officer
Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 001-819-953-2476	Sarita Chaudhary 819- 934-7926

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO07090289 A1 (WANG Y. and ZIDICHOUSKI J.) 16 AUGUST 2007 (163-08-2007) (see whole document)	1-57
A	KONG W. et al. Berberine is a novel cholesterol-lowering drug working through a unique mechanism distinct from statins. NATURE MEDICINE December 2004 10:1344-1351 (see whole document)	
A	LAU K. M. et al. Anti-oxidative, anti-inflammatory and hepato-protective effects of Ligustrum robustum. JOURNAL OF ETHNOPHARMACOLOGY 2002 83:63-71 (see whole document)	

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/IB2009/005461

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
WO06029577 A1	23-03-2006	US20080081781 A1	03-04-2008
		US20060223838 A1	05-10-2006
		NO20071930 A	12-06-2007
		MX2007003023	16-01-2008
		JP2008513382 T2	01-05-2008
		EP1796666 A1	20-06-2007
		CN11102768 A	09-01-2008
		CN1759834 A	19-04-2006
		CA2620208 AA	23-03-2006
		BR10515393 A	22-07-2008
		AU5284528 AA	23-03-2006
WO07090289 A1	16-08-2007	EP1983989 A1	29-10-2008
		CA2640414 AA	16-08-2007