

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

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
6 October 2011 (06.10.2011)

PCT

(10) International Publication Number
WO 2011/122805 A2

(51) International Patent Classification:

A61K 36/8962 (2006.01) **A61P 9/12** (2006.01)
A61P 1/16 (2006.01) **A61P 3/00** (2006.01)

(21) International Application Number:

PCT/KR2011/002094

(22) International Filing Date:

25 March 2011 (25.03.2011)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

10-2010-0029984 1 April 2010 (01.04.2010) KR

(71) Applicant (for all designated States except US): **SNU R&DB FOUNDATION** [KR/KR]; #56-1, San, Sillim-dong, Gwanak-gu, Seoul 151-015 (KR).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **KIM, Sang Geon** [KR/KR]; #102-206, Gangchon Apt., 402, Ichon-dong, Youngsan-gu, Seoul 140-030 (KR). **RYU, Jae Ha** [KR/KR]; #601-402, Mokdong Apt., 911, Mok-dong, Yangcheon-gu, Seoul 158-050 (KR). **JEON, Ra Ok** [KR/KR]; #3-1105, Gaepowoosung Apt., Daechi 1-dong, Gangnam-gu, Seoul 135-281 (KR). **HAN, Chang Yeob** [KR/KR]; #302, 1642-19, Inhun-dong, Gwanak-gu, Seoul 151-061 (KR).

(74) Agent: **SHIN, Dong In**; #503, V-Charmant Bldg., 1601-1, Seocho 3-dong, Seocho-gu, Seoul 137-953 (KR).

(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, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, 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, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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

Published:

- without international search report and to be republished upon receipt of that report (Rule 48.2(g))
- with sequence listing part of description (Rule 5.2(a))

(54) Title: A COMPOSITION COMPRISING AJOENE FOR PREVENTING OR TREATING A DISEASE CAUSED BY OVEREXPRESSION OF LXR-ALPHA

(57) Abstract: The present invention is related to a composition comprising the ajoene -abundant garlic extract or ajoene isolated therefrom as an active ingredient for treating or preventing the disease caused by over-expression or hyper-activation of LXR-alpha or SREBP-1, together with a pharmaceutically acceptable carrier.



WO 2011/122805 A2

Description

Title of Invention: A COMPOSITION COMPRISING AJOENE FOR PREVENTING OR TREATING A DISEASE CAUSED BY OVEREXPRESSION OF LXR-ALPHA

Technical Field

- [1] The present invention is related to a composition comprising ajoene for preventing and treating a disease caused by over-expression of LXR-alpha and the use thereof.

[2]

Background Art

- [3] LXR (liver X receptor), a nuclear hormone receptor, plays an important role in controlling a genetic transcription of several genes involved in cholesterol metabolism and homeostasis such as apoE, ABCA1, ABCG1, ABCG5, ABCG8, cholesterol 7alpha-hydroxylase and scavenger receptor class B type I (Biochem. Biophys. Res. Comm., 2000, 274: pp794-802) and LXR controls lipid metabolism by directly acting on SREBP-1c gene (Mol. Cell. Biol., 2001, 21:pp2991-3000).
- [4] LXR exists as two isoforms, LXR-alpha and LXRbeta, of which LXR-alpha mainly is distributed in liver and LXRbeta in the most of organs. In particular, the LXR-alpha is activated by the action of oxysterols, highly abundant saccharides and synthetic ligands such as T0901017, GW3965 et al and in charge of controlling the expression of genes involved in adipogenesis and cholesterol homeostasis. LXR-alpha potently increases the expression and activity of SREBP-1c, a key transcription factor controlling gene expression of adipogenesis functional by acting as a lipid sensor in hepatic adipogenesis, which stimulates hepatic fatty acid synthesis and increases the level of blood triglyceride in hepatic tissue.
- [5] The activation of LXR-alpha causing to non-alcoholic hepatic lipidosis, can be classified into two pathways, i.e., SREBP-1c-dependent pathway and SREBP-1c-independent pathway. SREBP-1c-dependent fatty liver appears by up-regulating the adipogenesis gene expression through the transcription of activation of LXR-alpha-mediated SREBP-1c. In a while, SREBP-1c-independent fatty liver appears by increasing the expression of CD36 protein, a transporter of free fatty acid, which stimulates fatty acid mobilization to liver tissue.
- [6] It has been reported that LXR-alpha plays important roles in controlling the rennin secretion in kidney. LXR-alpha and LXR-beta are abundantly expressed in juxta-glomerular cell producing rennin and T0901017 or GW3965, a LXR-alpha agonists, increases mRNA expression of rennin in kidney and then increases blood rennin activity (J. Clin. Invest., 2005, 115:pp1913-1922). Excessive increase of blood rennin

gives rises to hyperreninemia resulting in hypertension and aldosteronism.

- [7] LXR inhibitor has been reported to be useful in treating ALD since LEXR controls the gene expression of ABCD2 involved in ALD (adrenoleukodystrophy) disease, a rare disease damaging neuronal cell through VLCFA's entering into brain (J. Biol. Chem., 2005, 280: pp41243-41251).
- [8] SREBP (Sterol Response Element Binding Protein) is a protein bound to SRE (Sterol Response Element), a transcription regulating genetic moiety controlled by sterol and exists as three isoforms, i.e., SREBP-1 and SREBP-1c transcribed by identical gene and SREBP-2 expressed by the other gene. SREBP-1c is a transcription factor controlling the transcription of genes mainly involved in adipogenesis while SREBP-2 in cholesterol synthesis.
- [9] The gene expression of SREBP-1 and SREBP-2 is increased at kidney with aging and then the lipogenesis and the accumulation of triglyceride and cholesterol in kidney are increased, which plays important roles in glomerulosclerosis, proteinuria and nephropathy (Kidney Int., 2005, 68(6); pp2608-2620).
- [10] SREBP-1c targeting the catalyzing enzymes of fatty acid synthetic pathway such as FAS (fatty acid synthase), ACC (acetyl CoA carboxylase), SCD (Stearoyl CoA desaturase) etc in chage of adipogenesis in liver, is reported as a key factor of alcoholic or nonalcoholic liver steatosis (Int. J. Mol. Med., 2008, 21(4): pp507-511).
- [11] Ajoene is stable unsaturated disulfide compound produced from the sulfoxide rearrangement of allicin. Allin is transformed into allylsulfenic acid by the action of allinase enzyme stored in the vesicle of garlic, which results in forming two allicin. The ajoene compound having allyl vinyl disulfide moiety has been reported to have various physiological activities, for example, anti-tumor, anti-bacterial and anti-platelets aggregation activities (Prostaglandins Leukot Essent Fatty Acids, Vol.49 (2), pp587-595, 1993), immune-modulating activity, hangover treating activity etc (Korea Patent Publication No. 10-2207-0017452).
- [12]
- [13] However, there has been not reported or disclosed about the therapeutic effect of ajoene or ajoene-abundant extract of garlic on the disease caused by over-expression of LXR-alpha or diabetes in any of above cited literatures, the disclosures of which are incorporated herein by reference.
- [14]
- [15] Therefore, the present inventors have endeavored to find the novel inhibitor for treating the disease caused by over-expression of LXR-alpha or SREBP-1, such as alcoholic or nonalcoholic liver steatosis, diabetes, fatty liver, hypertriglyceridemia, hyperreninemia, rennin-induced hypertension, aldosteronism, adrenoleukodystrophy, glomerulosclerosis, proteinuria, nephropathy etc and to study the pharmacological

effect of garlic extract or ajoene isolated therefrom through various *in vitro* test and animal model tests, for example, inhibitory effect on the gene expression of LXR-alpha, the activation of LXR-alpha protein, the gene expression of SREBP-1, the activation of SREBP-1 using by HepG2 hepatic cell line as well as on the over-expressed LXR-alpha induced by high fat diet, the over-expressed SREBP-1 induced by high fat diet, the triglyceride content in hepatic cell, morphological change in hepatic cell, the weight-gain induced by high fat diet, and the increased blood glucose level induced by high fat diet etc using by male C57BL/6 mice animal model.

- [16] Finally, the present inventors have found that the ajoene-abundant garlic extract or ajoene isolated therefrom is effective in treating and preventing the disease caused by over-expression or hyper-activation of LXR-alpha or SREBP-1.

[17]

Disclosure of Invention

Technical Problem

- [18] According to one aspect, the present invention also provides a use of the ajoene-abundant garlic extract or ajoene isolated therefrom for the manufacture of medicament employed for treating or preventing the disease caused by over-expression or hyper-activation of LXR-alpha or SREBP-1 in human or mammal.
- [19] The present invention also provides a method for treating the disease caused by over-expression or hyper-activation of LXR-alpha or SREBP-1 in human or mammal comprising administering to said mammal an effective amount of above-mentioned extract or the compound isolated therefrom, together with a pharmaceutically acceptable carrier thereof.
- [20] The present invention provides a pharmaceutical composition comprising the ajoene-abundant garlic extract or ajoene isolated therefrom as an active ingredient for treating or preventing the disease caused by over-expression or hyper-activation of LXR-alpha or SREBP-1.
- [21] The present invention also provides a health functional food comprising the above-described extract or the compound isolated therefrom for the prevention or improvement of the disease caused by over-expression or hyper-activation of LXR-alpha or SREBP-1 as an active ingredient in an amount effective to preventing and improving the disease caused by over-expression or hyper-activation of LXR-alpha or SREBP-1, together with a sitologically acceptable additive.

[22]

Solution to Problem

- [23] Accordingly, it is an object of the present invention to provide a pharmaceutical composition comprising the ajoene-abundant garlic extract or ajoene isolated therefrom as

an active ingredient for treating or preventing the disease caused by over-expression or hyper-activation of LXR-alpha or SREBP-1, together with a pharmaceutically acceptable carrier.

[24]

[25] It is another object of the present invention to provide an inhibitor of the over-expression of LXR-alpha or SREBP-1, comprising the ajoene-abundant garlic extract or ajoene isolated therefrom as an active ingredient.

[26] It is another object of the present invention to provide a use of the ajoene-abundant garlic extract or ajoene isolated therefrom for the manufacture of medicament employed for treating or preventing the disease caused by over-expression or hyper-activation of LXR-alpha or SREBP-1 in human or mammal.

[27]

[28] It is the other object of the present invention to provide a method for treating the disease caused by over-expression or hyper-activation of LXR-alpha or SREBP-1 in human or mammal comprising administering to said mammal an effective amount of ajoene-abundant garlic extract or ajoene isolated therefrom, together with a pharmaceutically acceptable carrier thereof.

[29]

[30] The term "the ajoene-abundant garlic extract" disclosed herein comprise the extract of garlic including *Allium sativum* LINN, *Allium scorodorpasum* var. *viviparum* Regel or and the like, preferably, *Allium sativum* LINN, for example, which can be prepared by the procedure comprising the steps: washing and crushing garlic to pastes to be left alone for the period ranging from 1 hour to 48 hours, preferably, 2 hours to 28 hours at the temperature ranging from 0°C to 100°C, preferably, from room temperature to 50°C as the 1st fermentation step and subsequently, left alone for the period ranging from 1 hour to 48 hours, preferably, 2 hours to 28 hours at the temperature ranging from 0°C to 100°C, preferably, from 25°C to 50°C as the 2nd fermentation step in order to produce ajoene from alliin in garlic at the 1st step; subjecting to extraction with 5 to 20-fold, preferably, 10 to 15-fold volume of distilled water, alcohols such as methanol, ethanol and the like, or the mixtures thereof, preferably, water and ethanol mixture solvent at the temperature ranging from 0°C to 100°C, preferably, from room temperature to 50°C for the period ranging from 1 hour to 48 hours, preferably, 2 hours to 28 hours to obtain garlic extract at the 2nd step; concentrating the solution under vacuo and adding water thereto at the 3rd step; subjecting to fractionation with non-polar solvent selected from hexane, ethylacetate, chloroform, dimethylenchloride or diethylether, preferably, diethylether to afford non-polar solvent-soluble fraction; subjecting to silicagel column chromatography eluting with mixture solvent system (hexane/ethylacetate or n-hexane/acetone) to obtain ajoene-abundant extract of the

present invention.

[31]

[32] In accordance with one aspect of the present invention, there provided a health functional food comprising a ajoene-abundant garlic extract or ajoene isolated therefrom for the prevention or improvement of the disease caused by over-expression or hyper-activation of LXR-alpha or SREBP-1 an active ingredient in an amount effective to prevent and improve the disease caused by over-expression or hyper-activation of LXR-alpha or SREBP-1, together with a sitologically acceptable additive.

[33]

[34] The term “the disease caused by over-expression or hyper-activation of LXR-alpha or SREBP-1” disclosed herein comprises alcoholic or nonalcoholic liver steatosis, diabetes, fatty liver, hypertriglyceridemia, hyperreninemia, rennin-induced hypertension, aldosteronism, adrenoleukodystrophy, glomerulosclerosis, proteinuria, nephropathy etc, preferably, alcoholic or nonalcoholic liver steatosis, diabetes, fatty liver.

[35]

[36] The garlic, which can be used in the present invention, but not intent to limit thereto, include the same genus plants which would be apparent to those skilled in the art and have be used for identical or similar purpose and can be substituted for the prevention and treatment of purposed diseases.

[37]

[38] The pharmaceutical composition for treating purposed diseases could contain about 0.01 to 95 w/w%, preferably 0.5 to 50 w/w% of inventive extract or compound of present invention based on the total weight of the composition.

[39]

[40] An inventive extract and compound may be prepared in accordance with the following preferred embodiment.

[41]

[42] For the present invention, the above-described extract of garlic or ajoene isolated therefrom can be prepared by following procedure;

[43] For example, the garlic including *Allium sativum* LINN, *Allium scorodorpasum* var. *viviparum* Regel or and the like, preferably, *Allium sativum* LINN, is washed and crushed to be pastes to be left alone for the period ranging from 1 hour to 48 hours, preferably, 2 hours to 28 hours at the temperature ranging from 0°C to 100°C, preferably, from room temperature to 50°C as the 1st fermentation step and subsequently, left alone for the period ranging from 1 hour to 48 hours, preferably, 2 hours to 28 hours at the temperature ranging from 0°C to 100°C, preferably, from 25°C to 50°C as the 2nd fermentation step in order to produce ajoene from alliin in garlic at the

1st step; subjected to extraction with 5 to 20-fold, preferably, 10 to 15-fold volume of distilled water, alcohols such as methanol, ethanol and the like, or the mixtures thereof, preferably, water and ethanol mixture solvent at the temperature ranging from 0°C to 100°C, preferably, from room temperature to 50°C for the period ranging from 1 hour to 48 hours, preferably, 2 hours to 28 hours to obtain garlic extract at the 2nd step; is concentrated under vacuo and added with water at the 3rd step; subjected to fractionation with non-polar solvent selected from hexane, ethylacetate, chloroform, dimethylenechloride, or diethylether, preferably, diethylether to afford non-polar solvent-soluble fraction; subjected to silicagel column chromatography eluting with mixture solvent system (hexane/ethylacetate or n-hexane/acetone) to obtain ajoene-abundant extract of the present invention.

[44] To obtain pure ajoene of the present invention, the ajoene-abundant extract is subjected to further purification process such as reverse phase HPLC, Silicagel column chromatography or re-crystallization method to obtain ajoene of the present invention or synthesized from the method well-known in the art, for example the preparation method disclosed in the literature (Bioorg. Med. Chem. Let., 2008, 18: pp5277-5279).

[45]

[46] It is another object of the present invention to provide a process for preparing the above-described extract of garlic and ajoene isolated therefrom as described above for the preparation of composition effective in treating or preventing the disease caused by over-expression or hyper-activation of LXR-alpha or SREBP-1.

[47] It is the other object of the present invention to provide a method for preparing ajoene-abundant extract from the extract of garlic comprising the steps consisting of; washing and crushing garlic to pastes to be left alone for the period ranging from 1 hour to 48 hours, preferably, 2 hours to 28 hours at the temperature ranging from 0°C to 100°C, preferably, from room temperature to 50°C as the 1st fermentation step and subsequently, left alone for the period ranging from 1 hour to 48 hours, preferably, 2 hours to 28 hours at the temperature ranging from 0°C to 100°C, preferably, from 25°C to 50°C as the 2nd fermentation step in order to produce ajoene from alliin in garlic at the 1st step; subjecting to extraction with 5 to 20-fold, preferably, 10 to 15-fold volume of distilled water, alcohols such as methanol, ethanol and the like, or the mixtures thereof, preferably, water and ethanol mixture solvent at the temperature ranging from 0°C to 100°C, preferably, from room temperature to 50°C for the period ranging from 1 hour to 48 hours, preferably, 2 hours to 28 hours to obtain garlic extract at the 2nd step; concentrating the solution under vacuo and adding water thereto at the 3rd step; subjecting to fractionation with non-polar solvent selected from hexane, ethylacetate, chloroform, dimethylenechloride, or diethylether, preferably, diethylether to afford non-polar solvent-soluble fraction; subjecting to silicagel column chromatography

eluting with mixture solvent system (hexane/ethylacetate or n-hexane/acetone) to obtain ajoene-abundant extract of the present invention.

[48]

[49] The inventive compound can be transformed into their pharmaceutically acceptable salt and solvates by the conventional method well known in the art. For the salts, acid-addition salt thereof formed by a pharmaceutically acceptable free acid thereof is useful and can be prepared by the conventional method. For example, after dissolving the compound in the excess amount of acid solution, the salts are precipitated by the water-miscible organic solvent such as methanol, ethanol, acetone or acetonitrile to prepare acid addition salt thereof and further the mixture of equivalent amount of compound and diluted acid with water or alcohol such as glycol monomethylether, can be heated and subsequently dried by evaporation or fiLXRated under reduced pressure to obtain dried salt form thereof.

[50]

[51] As a free acid of above-described method, organic acid or inorganic acid can be used. For example, organic acid such as methansulfonic acid, p-toluensulfonic acid, acetic acid, trifluoroacetic acid, citric acid, maleic acid, succinic acid, oxalic acid, benzoic acid, lactic acid, glycolic acid, gluconic acid, galacturonic acid, glutamic acid, glutaric acid, glucuronic acid, aspartic acid, ascorbic acid, carbonylic acid, vanillic acid, hydroiodic acid and the like, and inorganic acid such as hydrochloric acid, phosphoric acid, sulfuric acid, nitric acid, tartaric acid and the like can be used herein.

[52]

[53] Further, the pharmaceutically acceptable metal salt form of inventive compound may be prepared by using base. The alkali metal or alkali-earth metal salt thereof can be prepared by the conventional method, for example, after dissolving the compound in the excess amount of alkali metal hydroxide or alkali-earth metal hydroxide solution, the insoluble salts are filtered and remaining fiLXRRate is subjected to evaporation and drying to obtain the metal salt thereof. As a metal salt of the present invention, sodium, potassium or calcium salt are pharmaceutically suitable and the corresponding silver salt can be prepared by reacting alkali metal salt or alkali-earth metal salt with suitable silver salt such as silver nitrate.

[54]

[55] The pharmaceutically acceptable salt of the compound comprise all the acidic or basic salt which may be present at the compounds, if it does not indicated specifically herein. For example, the pharmaceutically acceptable salt of the present invention comprise the salt of hydroxyl group such as the sodium, calcium and potassium salt thereof; the salt of amino group such as the hydrogen bromide salt, sulfuric acid salt, hydrogen sulfuric acid salt, phosphate salt, hydrogen phosphate salt, dihydrophosphate

salt, acetate salt, succinate salt, citrate salt, tartarate salt, lactate salt, mandelate salt, methanesulfonate(mesylate) salt and p-toluenesulfonate (tosylate) salt etc, which can be prepared by the conventional method well known in the art.

[56]

[57] It is still another object of the present invention to provide a pharmaceutical composition comprising the pulverized form, extracted form or dried extract form of above crude drug extract obtained by above described process as an active ingredient for preventing and treating the disease caused by over-expression or hyper-activation of LXR-alpha or SREBP-1.

[58]

[59] The inventive composition of the present invention significantly inhibited the gene expression of LXR-alpha, the activation of LXR-alpha protein, the gene expression of SREBP-1, the activation of SREBP-1 using by HepG2 hepatic cell line as well as on the over-expressed LXR-alpha induced by high fat diet, the over-expressed SREBP-1 induced by high fat diet, the triglyceride content in hepatic cell, morphological change in hepatic cell, the weigh-gain induced by high fat diet, and the increased blood glucose level induced by high fat diet etc using by male C57BL/6 mice animal model when the inventive extract or compound of the present invention was orally and intravenously administrated thereto. When the oral acute toxicity of the extract was tested, the extract had no apparent effect on mortality, clinical signs, body weight changes, and gross findings at necropsy.

[60]

[61] The pharmaceutical composition for treating purposed diseases could contain about 0.01 to 99.9 w/w%, preferably 0.1 to 90 w/w% of the above crude drug composition of present invention based on the total weight of the composition.

[62]

[63] The inventive composition may additionally comprise conventional carrier, adjuvants or diluents in accordance with a using method. It is preferable that said carrier is used as appropriate substance according to the usage and application method, but it is not limited. Appropriate diluents are listed in the written text of Remington's Pharmaceutical Science (Mack Publishing co, Easton PA).

[64]

[65] Hereinafter, the following formulation methods and excipients are merely exemplary and in no way limit the invention.

[66]

[67] The composition according to the present invention can be provided as a pharmaceutical composition containing pharmaceutically acceptable carriers, adjuvants or diluents, e.g., lactose, dextrose, sucrose, sorbitol, mannitol, xylitol, erythritol, maltitol,

starches, acacia rubber, alginate, gelatin, calcium phosphate, calcium silicate, cellulose, methyl cellulose, polyvinyl pyrrolidone, water, methylhydroxy benzoate, propylhydroxy benzoate, talc, magnesium stearate and mineral oil. The formulations may additionally include fillers, anti-agglutinating agents, lubricating agents, wetting agents, flavoring agents, emulsifiers, preservatives and the like. The compositions of the invention may be formulated so as to provide quick, sustained or delayed release of the active ingredient after their administration to a patient by employing any of the procedures well known in the art.

[68]

[69] For example, the compositions of the present invention can be dissolved in oils, propylene glycol or other solvents which are commonly used to produce an injection. Suitable examples of the carriers include physiological saline, polyethylene glycol, ethanol, vegetable oils, isopropyl myristate, etc., but are not limited to them. For topical administration, the compounds of the present invention can be formulated in the form of ointments and creams.

[70]

[71] Pharmaceutical formulations containing inventive composition may be prepared in any form, such as oral dosage form (powder, tablet, capsule, soft capsule, aqueous medicine, syrup, elixirs pill, powder, sachet, granule), or topical preparation (cream, ointment, lotion, gel, balm, patch, paste, spray solution, aerosol and the like), suppository, or sterile injectable preparation (solution, suspension, emulsion).

[72]

[73] The inventive composition of the present invention in pharmaceutical dosage forms may be used in the form of their pharmaceutically acceptable salts, and also may be used alone or in appropriate association, as well as in combination with other pharmaceutically active compounds.

[74]

[75] The desirable dose of the inventive composition varies depending on the condition and the weight of the subject, severity, drug form, route and period of administration, and may be chosen by those skilled in the art. However, in order to obtain desirable effects, it is generally recommended to administer at the amount ranging 0.01-10g/kg, preferably, 1 to 5g/kg by weight/day of the inventive composition of the present invention. The dose may be administered in a single or multiple doses per day. In terms of composition, the crude drug composition should be present between 0.01 to 80% by weight, preferably 0.5 to 50% by weight based on the total weight of the composition.

[76]

[77] The pharmaceutical composition of present invention can be administered to a

subject animal such as mammals (rat, mouse, domestic animals or human) via various routes. All modes of administration are contemplated, for example, administration can be made orally, rectally or by intravenous, intramuscular, subcutaneous, intra-cutaneous, intrathecal, epidural or intracerebroventricular injection.

[78]

[79] In accordance with one aspect of the present invention, there provided a health functional food comprising the above extract or the compound for the prevention or improvement of the disease caused by over-expression or hyper-activation of LXR-alpha or SREBP-1 as an active ingredient in an amount effective to preventing and improving the disease caused by over-expression or hyper-activation of LXR-alpha or SREBP-1, together with a sitologically acceptable additive.

[80]

[81] The crude drug composition of inventive health functional food is used in the form of pulverized form thereof, extracted form therefrom or dried extract form thereof.

[82] Accordingly, it is the other object of the present invention to provide a functional health food comprising the above extract or the compound for the prevention or improvement of the disease caused by over-expression or hyper-activation of LXR-alpha or SREBP-1.

[83]

[84] The term “a functional health food” defined herein is “the functional food having enhanced functionality such as physical functionality or physiological functionality by adding the extract of the present invention to conventional food to prevent or improve aimed disease in human or mammal”.

[85]

[86] It is the other object of the present invention to provide a health functional food comprising the above extract or the compound, together with a sitologically acceptable additive for the prevention and alleviation of aimed disease.

[87]

[88] The term “a health functional food” defined herein is the food containing inventive extract of the present invention showing no specific intended effect but general intended effect in a small amount of quantity as a form of additive or in a whole amount of quantity as a form of capsule, pill, tablet etc.

[89]

[90] The term “a sitologically acceptable additive” defined herein is “any substance the intended use which results or may reasonably be expected to result-directly or indirectly-in its becoming a component or otherwise affecting the characteristics of any food” for example, thickening agent, maturing agent, bleaching agent, sequesterants, humectant, anticaking agent, clarifying agents, curing agent, emulsifier, stabilizer,

thickner, bases and acid, foaming agents, nutrients, coloring agent, flavoring agent, sweetner, preservative agent, antioxidant, etc, which had been well-known in the art.

[91] If a substance is added to a food for a specific purpose in that food, it is referred to as a direct additive and indirect food additives are those that become part of the food in trace amounts due to its packaging, storage or other handling.

[92]

[93] Above described health foods can be contained in food, health beverage, dietary therapy etc, and may be used as a form of powder, granule, tablet, chewing tablet, capsule, beverage etc for preventing or improving aimed disease.

[94]

[95] The health functional food composition for preventing and improving purposed diseases could contain about 0.01 to 95 w/w%, preferably 0.5 to 80 w/w% of the above inventive composition of present invention based on the total weight of the composition.

[96]

[97] Above described composition therein can be added to food, additive or beverage for prevention and improvement of purposed diseases. For the purpose of preventing and improving purposed diseases, wherein, the amount of above described crude drug composition in food or beverage may generally range from about 0.1 to 15 w/w %, preferably 1 to 10 w/w % of total weight of food for the health food composition and 1 to 30 g, preferably 3 to 10 g on the ratio of 100ml of the health beverage composition.

[98]

[99] Providing that the health beverage composition of present invention contains above described crude drug composition as an essential component in the indicated ratio, there is no particular limitation on the other liquid component, wherein the other component can be various deodorant or natural carbohydrate etc such as conventional beverage. Examples of aforementioned natural carbohydrate are monosaccharide such as glucose, fructose etc; disaccharide such as maltose, sucrose etc; conventional sugar such as dextrin, cyclodextrin; and sugar alcohol such as xylitol, and erythritol etc. As the other deodorant than aforementioned ones, natural deodorant such as taumatin, stevia extract such as leauidoside A, glycyrrhizin et al., and synthetic deodorant such as saccharin, aspartam et al., may be useful favorably. The amount of above described natural carbohydrate is generally ranges from about 1 to 20 g, preferably 5 to 12 g in the ratio of 100ml of present beverage composition.

[100]

[101] The other components than aforementioned composition are various nutrients, a vitamin, a mineral or an electrolyte, synthetic flavoring agent, a coloring agent and improving agent in case of cheese, chocolate et al., pectic acid and the salt thereof,

alginic acid and the salt thereof, organic acid, protective colloidal adhesive, pH controlling agent, stabilizer, a preservative, glycerin, alcohol, carbonizing agent used in carbonate beverage et al. The other component than aforementioned ones may be fruit juice for preparing natural fruit juice, fruit juice beverage and vegetable beverage, wherein the component can be used independently or in combination. The ratio of the components is not so important but is generally range from about 0 to 20 w/w % per 100 w/w % present composition.

[102]

[103] Examples of addable food comprising aforementioned crude drug composition therein are various food, beverage, gum, vitamin complex, health improving food and the like.

[104]

[105] It will be apparent to those skilled in the art that various modifications and variations can be made in the compositions, use and preparations of the present invention without departing from the spirit or scope of the invention.

[106]

[107]

Advantageous Effects of Invention

[108] The inventive extract or compound significantly inhibited the gene expression of LXR-alpha, the activation of LXR-alpha protein, the gene expression of SREBP-1, the activation of SREBP-1 using by HepG2 hepatic cell line as well as on the over-expressed LXR-alpha induced by high fat diet, the over-expressed SREBP-1 induced by high fat diet, the triglyceride content in hepatic cell, morphological change in hepatic cell, the weigh-gain induced by high fat diet, and the increased blood glucose level induced by high fat diet. The inventive compositions according to the present invention are useful in the prevention and treatment of the liver diseases and can be used as safe and efficient hepato-protective agent.

[109]

Brief Description of Drawings

[110] The above and other objects, features and other advantages of the present invention will more clearly understood from the following detailed description taken in conjunction with the accompanying drawings, in which;

[111]

[112] Fig. 1 shows the inhibition effect on LXR-alpha expression (Con: control, **: comparison with control group $p < 0.01$, #: comparison with the group treated with T0901317 only, $p < 0.05$);

[113] Fig. 2 shows the inhibition effect on LXRE (Con: control, **: comparison with

control group $p < 0.01$, #: comparison with the group treated with T0901317 only, $p < 0.05$, ##: comparison with the group treated with T0901317 only, $p < 0.01$);

Fig. 3 shows the inhibition effect on SREBP-1 expression (Con: control, **: comparison with control group $p < 0.01$, #: comparison with the group treated with T0901317 only, $p < 0.05$, ##: comparison with the group treated with T0901317 only, $p < 0.01$);

Fig. 4 represents the inhibition effect on increased LXR expression in hepatic tissue caused by high fat diet (ND: normal diet, HFD: High fat diet, Ajo: ajoene, **: comparison with ND group $p < 0.01$, #: comparison with the HFD group only, $p < 0.05$, ##: comparison with the HFD group only, $p < 0.01$);

Fig. 5 represents the inhibition effect on increased triglyceride level in hepatic tissue caused by high fat diet (ND: normal diet, HFD: High fat diet, Ajo: ajoene, **: comparison with ND group $p < 0.01$, #: comparison with the HFD group only, $p < 0.05$, ##: comparison with the HFD group only, $p < 0.01$);

Fig. 6 represents the treatment protocol of ajoene and the inhibition effect on weight gain change caused by high fat diet (ND: normal diet, HFD: High fat diet, Ajo: ajoene, **: comparison with ND group $p < 0.01$, #: comparison with the HFD group only, $p < 0.05$, ##: comparison with the HFD group only, $p < 0.01$);

Fig. 7 presents the inhibition effect on the blood glucose level of the mice caused by high fat diet (ND: normal diet, HFD: High fat diet, Ajo: ajoene, **: comparison with ND group $p < 0.01$, #: comparison with the HFD group only, $p < 0.05$, ##: comparison with the HFD group only, $p < 0.01$);

Best Mode for Carrying out the Invention

The following Examples and Experimental Examples are intended to further illustrate the present invention without limiting its scope.

Mode for the Invention

Example 1. Preparation of ajoene-abundant extract of garlic (ARF)

2kg of garlic purchased from Kyung-dong market located in Seoul were washed, left alone at room temperature for 12 hours as the 1st fermentation step and subsequently, 37°C for 5 hours as the 2nd fermentation step. The fermented garlic was extracted with 80% ethanol at room temperature for 24 hours and the extract was concentrated in vacuo. The concentrates were dispersed with distilled water and fractionated into diethylether to afford 5.2g of ethylacetate soluble fraction. The fraction was further

purified with silicagel column chromatography using by n-hexance/ethylacetate and n-hexnane/acetone solvent system to afford 120mg of ajoene-abundant extract of garlic (designated as ARF hereinafter).

[125]

[126] Example 2. Preparation of ajoene compound from garlic extract

[127] 120mg of the ajoene-abundant extract of garlic was further purified by Reverse-phase HPLC (C-18, 10x300mm; 70% methanol; 2.0ml/min; UV 254nm) to afford 53mg of ajoene compound, which was confirmed by comparing the spectral analysis such as MS and H1-NMR spectroscopy with that disclosed in literature.

[128]

[129] Example 3. Preparation of ajoene compound with synthetic method

[130] Ajoene compound was chemically synthesized by the method disclosed in the literature (Bioorg. Med. Chem. Lett., 2008, 18:pp5277-5279) as follows:

[131] Allylpropargyl sulfide was synthesized from ally thiol as a starting material through propargylation and vinylthioacetate was synthesized through regioselective radical addition in the presence with azobisisobutyronitrile (AIBN). Resulting thiolacetate was subjected to deprotection and sulfenylation using by S-allyl-p-toluene sulfonyl thioate to afford vinyl disulfide. The disulfide was oxidized with m-CPBA (meta-chloroperoxybenzoic acid) to afford ajoene compound, which was confirmed by comparing the spectral analysis such as MS and H1-NMR spectroscopy with that disclosed in literature.

[132]

[133] Reference Example. Preparation of Experiment

[134]

[135] 1-1. Reagent and experimental animals

[136] Male C57BL/6 mice (25 to 30g) were purchased from Charles River Orient Co. (Seoul, Korea) and used in the experiment and were allowed to access to feed and drinking water. All animals were maintained in a controlled environment with temperatures at 22±2°C and humidity at 55±5% with 12 hours of light and dark cycles for at least one week prior to use.

[137] The morphological changes of mice have been checked once a week and the mice were divided into two groups, one group fed with high fat feed (Dyets Inc., Bethlehem) to induce fatty liver and another group with normal feed. The mice were bred for 8 weeks and ajoene diluted with 40% PEG #400 [PEG400: water=4:6] was administrated into each group consisting of 8 mice for 4 weeks (10mg or 30mg/kg).

[138]

[139] 1-2. Real time-RT PCR

[140] cDNA was obtained from 2 microgram of total RNA extracted from mouse liver,

d(T)₁₆ primer and AMV reverse transcriptase. The relative contents of genes was quantitatively determined according to realtime RT-PCR method using by CyBrgreen dye and real time RT-PCR was performed by using Light-cycler2.0 (Roche,Mannheim,Germany).

[141] PCR was performed according the manufacture's manual and relative amount of each gene was analyzed using Light-cycler software 4.0 program.

[142]

[143] 1-3. Western-blot analysis

[144] SDS-PAGE (Sodium dodecylsulfate-polyacrylamide gel electrophoresis) was performed by using Might Smal II SE 250 apparatus according to the method well-known in the art (Laemmli UK method, 1970).

[145] Dissolution fraction of each sample was diluted in sample dilution buffer containing 63mM Tris (pH. 6.8), 10% glycerol, 2% SDS, 0.0013% bromophenol blue, and 5% beta-mercaptoethanol and subjected to electrophoresis using 7.5% and 9% gel in electrode buffer (containing 15g of Tris, 72g of glycine and 5g of SDS in 1 L). The finished gel was electroblotted onto nitrocellulose membrane in buffer [25mM Tris, 192mM glycine and 20% v/v methanol, pH 8.3] with 190mAmps for 1 hour. Anti-SREBP-1 was reacted as a primary antibody and horse radish peroxidase-conjugated goat anti-rabbit IgG was reacted for 1 hour as a secondary antibody. The result was developed using by ECL chemi-luminescence system (Amersham, Gaithersburg, MA) and the homogeneity of protein content between each sample was confirmed using by anti-beta-actin antibody (Sigma St. Louis, MO).

[146]

[147] 1-4. Statistics

[148] All the result was analyzed by using pharmacological calculation. The significance between the test groups was evaluated by ANOVA (one-way analysis of variance; Fisher R.A., statistical Methods for Research Workers, Edinburgh: Oliver & Boyd., 1925) and determined by Newmann-Keuls test method (*p<0.05, **p<0.01; Norman GR et al., Biostatistics: The Bare Essentials, 2000).

[149]

[150] Experimental Example 1. Inhibition effect on LXR-alpha expression

[151] In order to investigate the inhibitory effect of the inventive extract and compound obtained in Examples on LXR-alpha expression, following experiment was performed in the procedure.

[152] 10 micromole and 30 micromole Ajoene were pretreated to HepGe cell line, a representative hepatic cell line for 1 hour and 10 micromole T0901317, a LXR-alpha activator was treated thereto for 12 hours. mRNA was isolated from the hepatic cell line. cDNA was synthesized through RT-PCR method and realtime PCR was

performed using by specific primers (Human LXR, sense:
5'-GATCGAGGTGATGCTTCTGGAG-3'(SEQ. I.D.1); anti-sense:
5'-CCCTGCTTTGGCAAAGTCTTC-3'(SEQ. I.D.2)).

[153] At the result, the relative mRNA level in the group treated with 10 micromole and 30 micromole ajoene is sharply reduced to 1.9 fold and 1.8 fold comparing with the increased mRNA expression of LXR-alpha respectively, whereas that with T091317 is increased to 3.0 fold.

[154] Accordingly, it has confirmed that the ajoene potently inhibited the gene expression of LXR-alpha (*See Fig.1*).

[155]

[156] Experimental Example 2. Inhibition effect on LXR-alpha activation

[157] In order to investigate the inhibitory effect of the inventive extract and compound obtained in Examples on the LXR-alpha activation, following experiment was performed in the procedure.

[158] There have been reported that LXR-alpha is bound to specific region existing in target gene promoter (LXRE) to regulate the gene expression. Luciferase reporter gene assay was performed to observe whether LXRE binding affinity is changed or not.

[159] LXRE comprising vector was transfected to HepG2 cell using by lipofectamine (Invitrogen, USA). 12 hours after the transfection, 10 micromole and 30 micromole ajoene (Ajo) were pretreated to HepG2 cell respectively, for 1 hour and 10 micromole T0901317, LXR-alpha activator was pretreated for 12 hours. Cell lysate was obtained using by passive lysis buffer (Promega, USA) and Luciferase activity was determined using by Luminometer.

[160] At the result, the relatively increased activity of Luciferase induced by T0901317 treatment to 2.3 fold comparing that of negative control was significantly inhibited to 1.8 fold and 1.3 fold by the treatment with 10 micromole and 30 micromole ajoene respectively.

[161] Accordingly, it has confirmed that the ajoene potently inhibited the activity of LXR-alpha (*See Fig.2*).

[162]

[163] Experimental Example 3. Inhibition effect on the gene expression and activity of SREBP-1

[164] In order to investigate the inhibitory effect of the inventive extract and compound obtained in Examples on the gene expression and activity of SREBP-1, following experiment was performed in the procedure.

[165] The expression of mRNA and protein of SREBP-1 in case with the group treated with T0901317 and the group treated with T091317 and ajoene, was compared. 10 micromole and 30 micromole Ajoene were pretreated to HepGe cell line, for 1 hour

and 10 micromole T0901317, a LXR-alpha activator was treated thereto for 12 hours. Protein was isolated and the protein change of SREBP-1 was determined by Western blotting method. mRNA was isolated from the hepatic cell line. cDNA was synthesized through RT-PCR method and realtime PCR was performed using by specific primers (Human SREBP-1, sense: 5'-CGACATCGAAGACATGCTTCAG-3'(SEQ. I.D.3); ant-sense: 5'-GGAAGGCTTCAAGAGAGGAGC-3'(SEQ. I.D.4)).

[166] At the result, the expression of mRNA and protein of SREBP-1, a cellular protein regulating adipogenesis gene expression in the group treated with 10 micromole and 30 micromole ajoene is sharply reduced to 2.8 fold and 2.4 fold comparing with the increased mRNA expression of SREBP-1 respectively, whereas that with T091317 is increased to 4.8 fold.

[167] Accordingly, it has confirmed that the ajoene potently inhibited the gene expression of SREBP-1 (*See Fig.3*).

[168]

[169] Experimental Example 4. Inhibition effect on increased LXR-alpha expression with high fat diet

[170] In order to investigate the inhibitory effect of the inventive extract and compound obtained in Examples on the increased LXR-alpha expression caused by high fat diet, following experiment was performed in the procedure.

[171] The expression of LXR-alpha, a cellular lipid sensor, was analyzed by real time PCR using by specific primer after cDNA synthesis. The mice were bred with high fat feed and normal feed for 8 weeks and the LXR-alpha expression of hepatic cell in the group treated with high fat feed and ajoene (10 or 30 mg/kg, 5 times/week) for 4 weeks was determined.

[172] mRNA was isolated from the hepatic cell line. cDNA was synthesized through RT-PCR method and realtime PCR was performed using by specific primers (mouse LXR, sense: 5'-TGCCATCAGCATCTTCTCTG-3'(SEQ. I.D.5); anti-sense: 5'-GGCTCACCAGCTTCATTAGC-3'(SEQ. I.D.6)).

[173] Relative LXR-alpha mRNA in the group treated with high fat feed or both of high fat feed and ajoene was shown where that in the group treated with normal diet (ND) is set to 1.

[174]

[175] At the result, the increased LXR-alpha expression in the group treated with high fat diet was sharply reduced to 1.3 fold by the treatment with ajoene (30mg/kg) whereas relative mRNA level of the group treated with high fat diet had been increased to 2.1 fold ($p<0.01$).

[176] Accordingly, it has confirmed that the ajoene potently inhibited the increased gene expression of LXR-alpha caused by high fat diet (*See Fig.4*).

[177]

[178] Experimental Example 5. Inhibition effect on increased SREBP-1 expression with high fat diet

[179] In order to investigate the inhibitory effect of the inventive extract and compound obtained in Examples on the increased SREBP-1 expression caused by high fat diet, following experiment was performed in the procedure.

[180] The expression of SREBP-1 was analyzed according to the similar method in Experimental Example 3.

[181] mRNA was isolated from the hepatic cell line. cDNA was synthesized through RT-PCR method and realtime PCR was performed using by specific primers (mouse SREBP-1, sense: 5'-AACGTCACCTCCAGCTAGAC-3'(SEQ. I.D.7); anti-sense: 5'-CCACTAAGGTGCCTACAGAGC-3'(SEQ. I.D.8)).

[182] Relative SREBP-1 mRNA in the group treated with high fat feed or both of high fat feed and ajoene was shown where that in the group treated with normal diet (ND) is set to 1.

[183]

[184] At the result, the increased SREBP-1 mRNA expression in the group treated with high fat diet was sharply reduced to 2.0 and 1.6 fold by the treatment with ajoene (10mg/kg and 30mg/kg) respectively, whereas relative mRNA level of the group treated with high fat diet had been increased to 3.4 fold ($p < 0.01$).

[185] Accordingly, it has confirmed that the ajoene potentially inhibited the increased gene expression of SREBP-1 caused by high fat diet (*See Fig.4*).

[186]

[187] Experimental Example 6. Inhibition Effect on increased level of triglyceride in hepatic tissue with high fat diet

[188] In order to investigate the inhibitory effect of the inventive extract and compound obtained in Examples on the increased level of triglyceride in hepatic tissue caused by high fat diet, following experiment was performed in the procedure.

[189] The triglyceride level in hepatic tissue is an indicator of fatty liver. The mice were bred for 8 weeks with high fat diet and normal diet and ajoene was administrated the mice for final 4 weeks 5 times a week, at the dose of 10mg/kg and 30mg/kg. The triglyceride level in hepatic tissue caused by with high fat feed was observed.

[190]

[191] At the result, the triglyceride level of hepatic tissue in the group treated with 10mg/kg and 30mg/kg of ajoene was sharply reduced to 36mg/g (liver) and 31mg/g(liver) respectively, whereas that of the group treated with high fat diet and normal diet had been increased to 61mg/g (liver) and 27mg/g (liver), respectively.

[192] Accordingly, it has confirmed that the ajoene potentially inhibited the triglyceride level

of hepatic tissue caused by high fat diet (*See Fig.5*).

[193]

[194] Experimental Example 7. Inhibition effect on morphological change of hepatic tissue with high fat diet

[195] In order to investigate the inhibitory effect of the inventive extract and compound obtained in Examples on the morphological change of hepatic tissue caused by high fat diet, following experiment was performed in the procedure.

[196] The hepatic tissues prepared in Experimental Example 5 were fixed with 10% formalin solution, dehydrated and embedded with paraffin. The hepatic tissue was sliced to 4 micrometer of width to be stained with dye (Oil Red O). The morphological change of hepatic tissue was observed by optical spectroscopy.

[197] At the result, the stained area of the hepatic tissue in the group treated with ajoene (Ajo) and high fat diet (HFD) was sharply reduced whereas that of the group treated with high fat diet (HFD+vehicle) was very large with red color.

[198] Accordingly, it has confirmed that the ajoene potently reduced the adipocytic cells increased by high fat diet.

[199]

[200] Experimental Example 8. Inhibition effect on weight gain with high fat diet

[201] In order to investigate the inhibitory effect of the inventive extract and compound obtained in Examples on the weight gain of mice caused by high fat diet, following experiment was performed in the procedure.

[202] The mice were bred for 8 weeks with high fat diet (4 animals) and normal diet (4 animals) and ajoene was administrated the mice for final 4 weeks 5 times a week, at the dose of 10mg/kg and 30mg/kg. The weight gain of each mouse caused by high fat feed was observed.

[203]

[204] At the result, the weight gain in the group treated with ajoene (Ajo) and high fat diet (HFD) was sharply reduced.

[205] Accordingly, it has confirmed that the ajoene potently reduced the weight gain of the mice increased by high fat diet (*See Fig.6*).

[206]

[207] Experimental Example 9. Inhibition effect on blood glucose level increased by high fat diet

[208] In order to investigate the inhibitory effect of the inventive extract and compound obtained in Examples on the increased level of blood glucose caused by high fat diet, following experiment was performed in the procedure.

[209] The mice were bred for 8 weeks with high fat diet (4 animals) and normal diet (4 animals) and ajoene was administrated the mice for final 4 weeks 5 times a week, at

the dose of 10mg/kg and 30mg/kg. 0.01 ml of blood sample was collected from the tail vein of each mouse caused by high fat feed and the blood glucose level was determined by kit (Accucheck blood glucose monitoring apparatus, Roche Co.).

[210]

[211] At the result, the blood glucose level in the group treated with ajoene (10mg/kg and 30mg/kg) and high fat diet (HFD) was sharply reduced to 102 mg/dl and 92mg/dl respectively, whereas that of the group treated with high fat diet had been increased to 162mg/dl.

[212] Accordingly, it has confirmed that the ajoene potently reduced the blood glucose level of the mice increased by high fat diet (*See* Fig.7).

[213]

[214] Hereinafter, the formulating methods and kinds of excipients will be described, but the present invention is not limited to them. The representative preparation examples were described as follows.

[215]

[216] Preparation of powder

[217] Ajoene 25mg

[218] Corn Starch 20mg

[219] Lactose 30mg

[220] Magnesium stearate optimum amount

[221] Powder preparation was prepared by mixing above components and filling sealed package.

[222]

[223] Preparation of tablet

[224] Ajoene 100mg

[225] Corn Starch 10mg

[226] Lactose 50mg

[227] Magnesium stearate optimum amount

[228] Tablet preparation was prepared by mixing above components and entabletting.

[229]

[230] Preparation of capsule

[231] ARF 10mg

[232] Crystalline cellulose 3mg

[233] Lactose 14.8mg

[234] Magnesium stearate 0.2 mg

[235] Tablet preparation was prepared by mixing above components and filling gelatin capsule by conventional gelatin preparation method.

[236]

[237] Preparation of injection

[238] Ajoene 10mg

[239] Mannitol 180mg

[240] Na₂HPO₄-12H₂O 26mg

[241] Distilled water for injection 1974mg

[242] Injection preparation was prepared by dissolving active component, controlling pH to about 7.5 and then filling all the components in 2ml ample and sterilizing by conventional injection preparation method.

[243]

[244] Preparation of liquid

[245] Ajoene 20mg

[246] Sugar 20g

[247] Mannitol 5g

[248] Distilled water optimum amount

[249] Liquid preparation was prepared by dissolving active component, and then filling all the components in 1000ml ample and sterilizing by conventional liquid preparation method.

[250]

[251] Preparation of health food

[252] Ajoene 1000mg

[253] Vitamin mixture optimum amount

[254] Vitamin A acetate 70mg

[255] Vitamin E 1.0mg

[256] Vitamin B₁ 0.13mg

[257] Vitamin B₂ 0.15mg

[258] Vitamin B₆ 0.5mg

[259] Vitamin B₁₂ 0.2mg

[260] Vitamin C 10mg

[261] Biotin 10mg

[262] Amide nicotinic acid 1.7mg

[263] Folic acid 50mg

[264] Calcium pantothenic acid 0.5mg

[265] Mineral mixture optimum amount

[266] Ferrous sulfate 1.75mg

[267] Zinc oxide 0.82mg

[268] Magnesium carbonate 25.3mg

[269] Monopotassium phosphate 15mg

[270] Dicalcium phosphate 55mg

- [271] Potassium citrate 90mg
[272] Calcium carbonate 100mg
[273] Magnesium chloride 24.8mg
[274] The above mentioned vitamin and mineral mixture may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the present invention.

[275]

[276] Preparation of health beverage

- [277] ARF 1000mg
[278] Vitamin C 15g
[279] Vitamin E(powder) 100g
[280] Vitamin A 0.2g
[281] Vitamin B₁ 0.25g
[282] Vitamin B₂ 0.3g
[283] Amide nicotinic acid 3.5g
[284] Zinc oxide 3.5g
[285] Ferrous lactate 19.75g
[286] Distilled water optimum amount
[287] Health beverage preparation was prepared by dissolving active component, mixing, stirred at 85°C for 1 hour, filtered and then filling all the components in 1000ml ample and sterilizing by conventional health beverage preparation method.

[288]

- [289] The invention being thus described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the present invention, and all such modifications as would be obvious to one skilled in the art are intended to be included within the scope of the following claims.

[290]

Industrial Applicability

- [291] As described in the present invention, the inventive extract or compound significantly inhibited the gene expression of LXR-alpha, the activation of LXR-alpha protein, the gene expression of SREBP-1, the activation of SREBP-1 using by HepG2 hepatic cell line as well as on the over-expressed LXR-alpha induced by high fat diet, the over-expressed SREBP-1 induced by high fat diet, the triglyceride content in hepatic cell, morphological change in hepatic cell, the weigh-gain induced by high fat diet, and the increased blood glucose level induced by high fat diet. The inventive compositions according to the present invention are useful in the prevention and treatment of the liver diseases and can be used as safe and efficient hepato-protective

agent.

[292]

Sequence Listing Free Text

[293] SEQ.I.D.1 5'-GATCGAGGTGATGCTTCTGGAG-3'

[294] SEQ.I.D.2 5'-CCCTGCTTTGGCAAAGTCTTC-3'

[295] SEQ.I.D.3 5'-CGACATCGAAGACATGCTTCAG-3'

[296] SEQ.I.D.4 5'-GGAAGGCTTCAAGAGAGGAGC-3'

[297] SEQ.I.D.5 5'-TGCCATCAGCATCTTCTCTG-3'

[298] SEQ.I.D.6 5'-GGCTCACCAGCTTCATTAGC-3'

[299] SEQ.I.D.7 5'-AACGTCACCTCCAGCTAGAC-3'

[300] SEQ.I.D.8 5'-CCACTAAGGTGCCTACAGAGC-3'

[301]

[302]

[303]

Claims

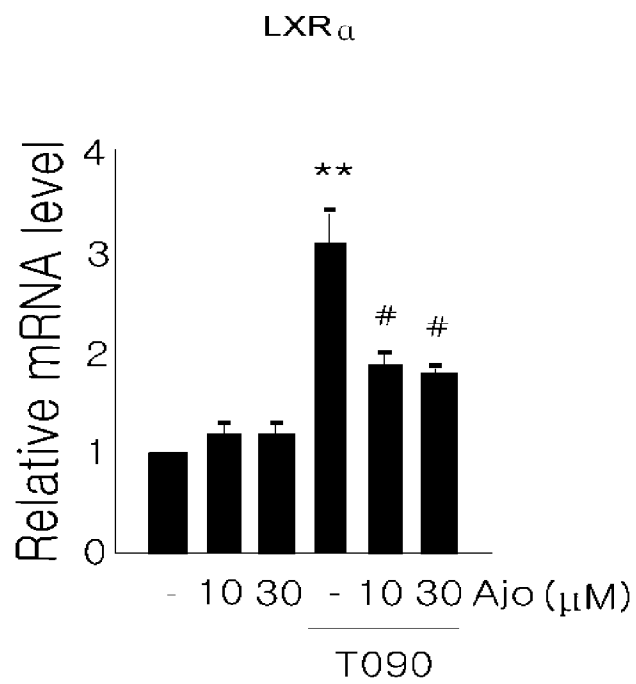
- [Claim 1] A pharmaceutical composition comprising the ajoene-abundant garlic extract or ajoene isolated therefrom as an active ingredient for treating or preventing the disease caused by over-expression or hyper-activation of LXR-alpha or SREBP-1, together with a pharmaceutically acceptable carrier.
- [Claim 2] The pharmaceutical composition according to claim 1, wherein said garlic is *Allium sativum* LINN or *Allium scorodorpasum* var. *viviparum* Regel .
- [Claim 3] The pharmaceutical composition according to claim 1, wherein said ajoene-abundant garlic extract is prepared by the procedure consisting of the steps; washing and crushing garlic to pastes to be left alone for the period ranging from 1 hour to 48 hours at the temperature ranging from 0°C to 100°C as the 1st fermentation step and subsequently, left alone for the period ranging from 1 hour to 48 hours at the temperature ranging from 0°C to 100°C as the 2nd fermentation step in order to produce ajoene from alliin in garlic at the 1st step; subjecting to extraction with 5 to 20-fold volume of distilled water, alcohols such as methanol, ethanol and the like, or the mixtures thereof at the temperature ranging from 0°C to 100°C for the period ranging from 1 hour to 48 hours to obtain garlic extract at the 2nd step; concentrating the solution under vacuo and adding water thereto at the 3rd step; subjecting to fractionation with non-polar solvent selected from hexane, ethylacetate, chloroform, dimethylene chloride, or diethyl ether to afford non-polar solvent-soluble fraction; subjecting to silicagel column chromatography eluting with mixture solvent system (hexane/ethylacetate or n-hexane/acetone) to obtain ajoene-abundant extract.
- [Claim 4] The pharmaceutical composition according to claim 1, wherein said disease caused by over-expression or hyper-activation of LXR-alpha or SREBP-1 is selected from alcoholic or nonalcoholic liver steatosis, diabetes, fatty liver, hypertriglyceridemia, hyperreninemia, rennin-induced hypertension, aldosteronism, adrenoleukodystrophy, glomerulosclerosis, proteinuria or nephropathy etc, preferably, alcoholic or non-alcoholic liver steatosis, diabetes, fatty liver.
- [Claim 5] The pharmaceutical composition according to claim 4, wherein said disease caused by over-expression or hyper-activation of LXR-alpha or

SREBP-1 is selected from alcoholic or nonalcoholic liver steatosis, diabetes or fatty liver.

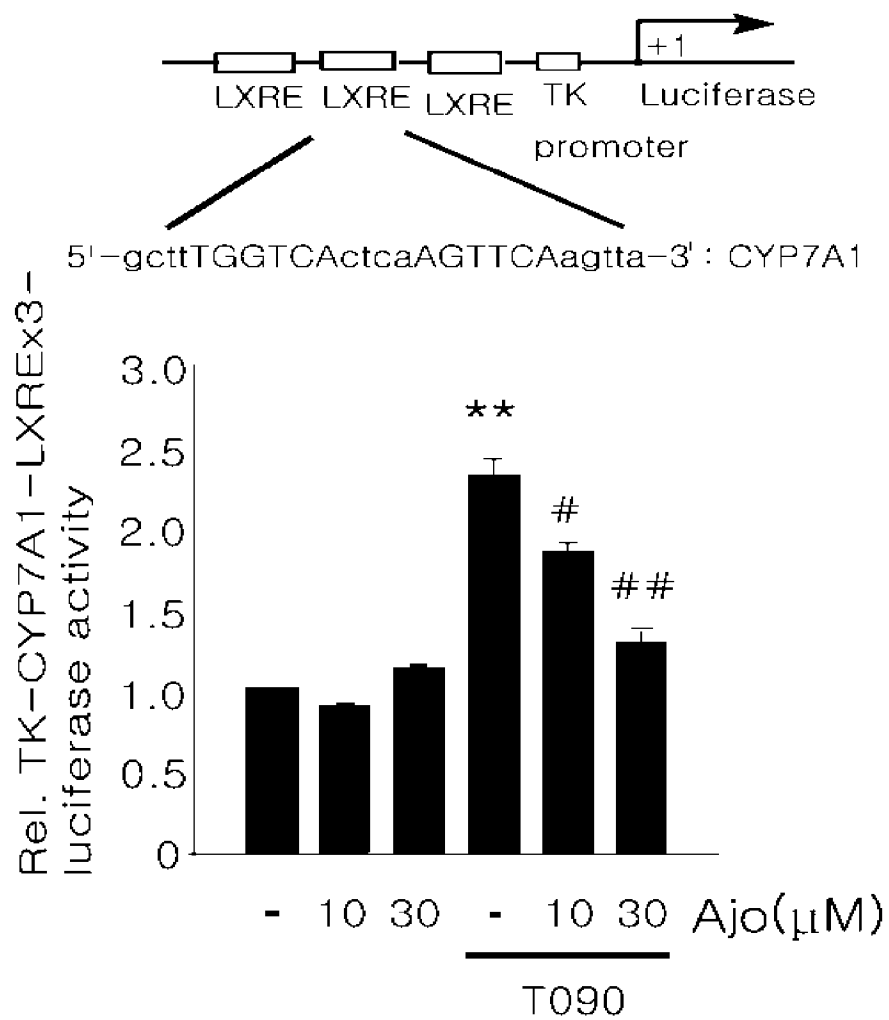
- [Claim 6] An inhibitor of the over-expression of LXR-alpha or SREBP-1, comprising the ajoene-abundant garlic extract or ajoene isolated therefrom as an active ingredient.
- [Claim 7] A use of the ajoene-abundant garlic extract or ajoene isolated therefrom for the manufacture of medicament employed for treating or preventing the disease caused by over-expression or hyper-activation of LXR-alpha or SREBP-1 in human or mammal.
- [Claim 8] A method a method for treating the disease caused by over-expression or hyper-activation of LXR-alpha or SREBP-1 in human or mammal comprising administering to said mammal an effective amount of ajone-abundant garlic extract or ajoene isolated therefrom, together with a pharmaceutically acceptable carrier thereof.
- [Claim 9] A health functional food comprising the above extract or the compound for the prevention or improvement of the disease caused by over-expression or hyper-activation of LXR-alpha or SREBP-1 as an active ingredient in an amount effective to preventing and improving the disease caused by over-expression or hyper-activation of LXR-alpha or SREBP-1, together with a sitologically acceptable additive.
- [Claim 10] The health functional food of claim 9, wherein said health functional food is provided as powder, granule, tablet, capsule or beverage type.
- [Claim 11] A method for preparing ajoene-abundant extract from the extract of garlic comprising the steps consisting of; washing and crushing garlic to pastes to be left alone for the period ranging from 1 hour to 48 hours at the temperature ranging from 0°C to 100°C as the 1st fermentation step and subsequently, left alone for the period ranging from 1 hour to 48 hours at the temperature ranging from 0°C to 100°C, as the 2nd fermentation step in order to produce ajoene from alliin in garlic at the 1st step; subjecting to extraction with 5 to 20-fold volume of distilled water, alcohols such as methanol, ethanol and the like, or the mixtures thereof at the temperature ranging from 0°C to 100°C, for the period ranging from 1 hour to 48 hours to obtain garlic extract at the 2nd step; concentrating the solution under vacuo and adding water thereto at the 3rd step; subjecting to fractionation with non-polar solvent selected from hexane, ethylacetate, chloroform, dimethylene chloride or diethyl ether to afford non-polar solvent-soluble fraction; subjecting to silicagel column chromatography eluting with mixture solvent system

(hexane/ethylacetate or n-hexane/acetone) to obtain ajoene-abundant extract of the present invention.

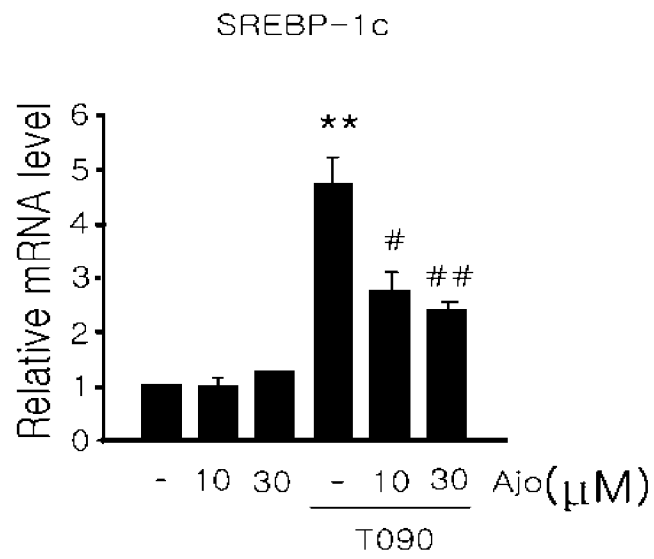
[Fig. 1]



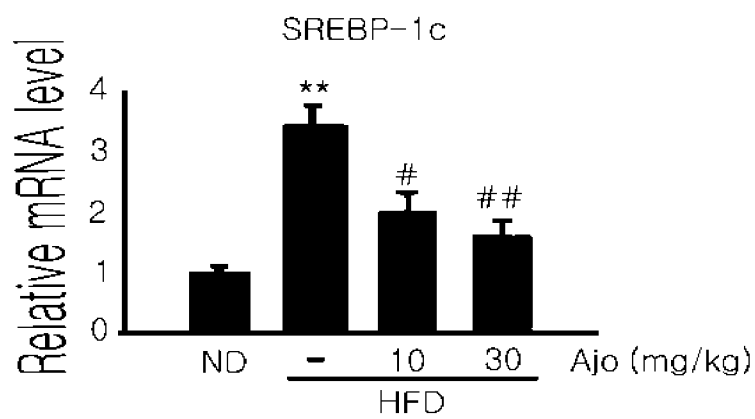
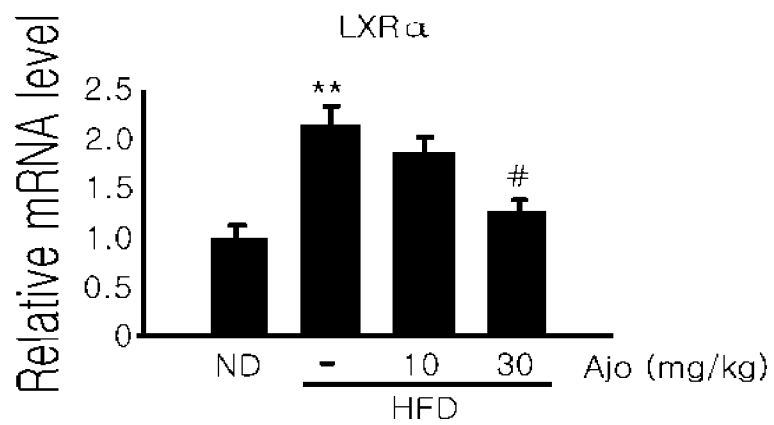
[Fig. 2]



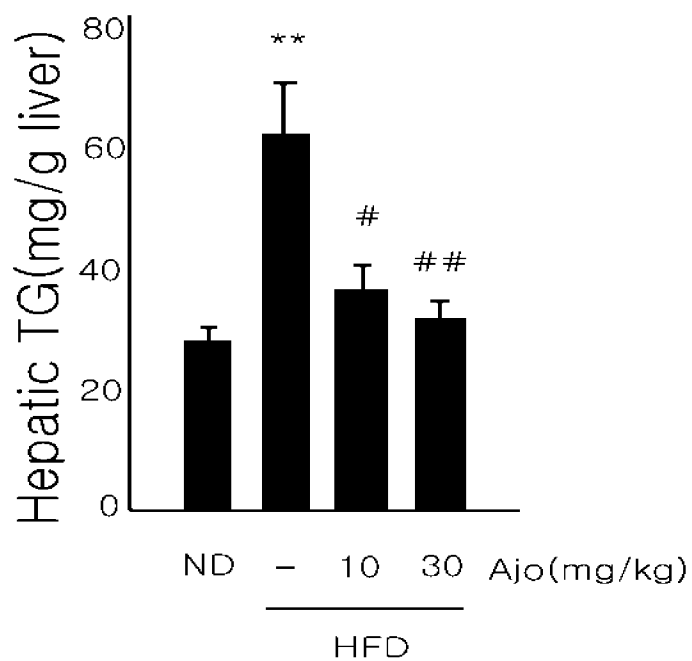
[Fig. 3]



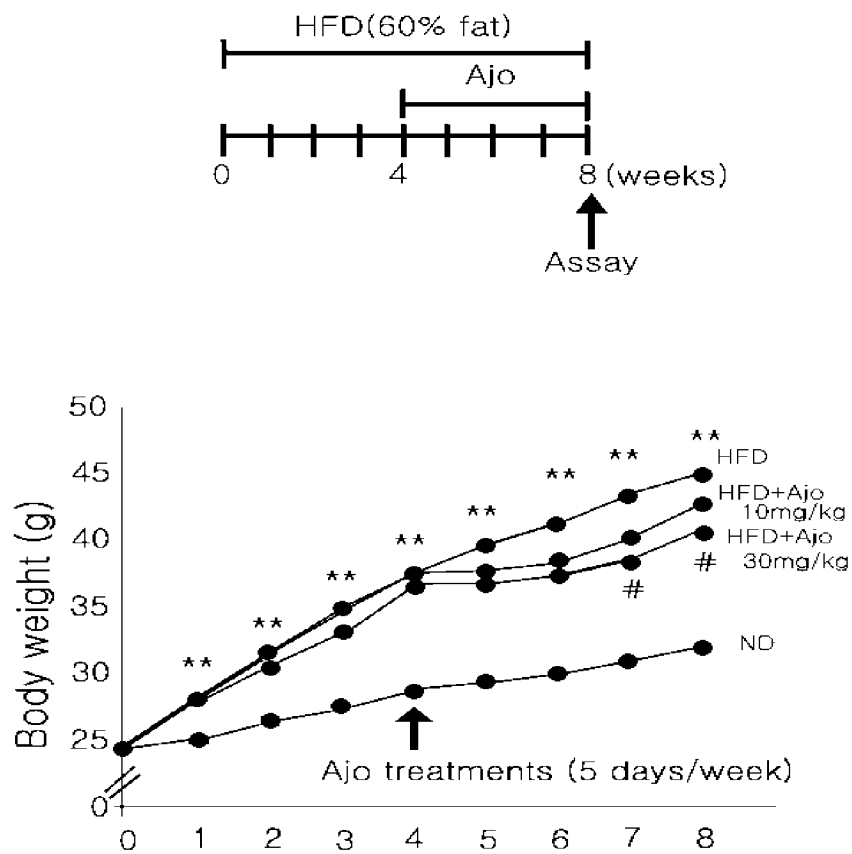
[Fig. 4]



[Fig. 5]



[Fig. 6]



[Fig. 7]

