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(54) Title: A PHARMACEUTICAL COMPOSITION COMPRISING THE EXTRACT OF SORBUS AMURENSIS KOEHNE FOR TREATING OR PREVENTING CEREBROVASCULAR SYSTEM DISEASE

(57) Abstract: The present invention relates to a composition comprising the crude extract of Sorbus amurensis KOEHNE, as active ingredients for the treatment and prevention of cerebrovascular disease. The extracts have potent relaxing activity of vascular endothelial cell by increasing endothelium derived NO production, therefore it can be used as the therapeutics or health care food for treating and preventing cerebrovascular diseases.
Description

A PHARMACEUTICAL COMPOSITION COMPRISING THE EXTRACT OF SORBUS AMURENSIS KOEHNE FOR TREATING OR PREVENTING CEREBROVASCULAR SYSTEM DISEASE

Technical Field

[1] The present invention relates to the extract of Sorbus amurensis having treating and preventing activity for cerebrovascular disease.

Background Art

[2] The action mechanism of controlling the contraction and relaxation of vascular smooth muscle are various. Among the mechanism, endothelium-derived relaxing factor (EDRF), i.e., Nitric Oxide (NO), plays an important role in the mechanism. Nitric oxide is formed by hydrolyzing L-arginine substrate by the action of nitric oxide synthase (NOS) and is reproduced in vascular endothelial cell. There are three kinds of isoform of NOS, i.e., (1) brain type NOS (bNOS, nNOS, NOS I) which plays a role of synthesizing NO as a neurotransmitter and is found at first in brain, (2) inducible NOS (iNOS, NOS II) which synthesize NO mainly acting on immune system, and (3) endothelium cell type NOS (ecNOS, eNOS, NOS III) which is mainly distributed in vascular endothelial cell and acts on the relaxation of blood vessel. It is activated by acetylcholine and it relax blood vessel. The NO activates guanylate cyclase enzyme in cytoplasm, increases the production of cGMP resulting in relaxing vascular smooth muscle through signal transduction system (Arnold W. P., Mittal C. K., et al., Proc. Nat'l. Acad. Sci. USA, 74(8), pp3203-307, 1977).

[3] Endothelium-derived relaxing factor is synthesized and released at normal state in endothelial cell and the synthesis and the release of them are promoted by various agonists such as acetylcholine, histamine, substance P, isopropanol etc.

[4] There have been several reports on a sort of oriental medicine or crude drug promoting NO production in vascular endothelial cell resulting in relaxation blood vessel till now as follows; for example, Kuramochi T. et al report that the hot water extract of Uncaria rhynchophylla JACKS shows increasing effect on the vasodilation in dose dependent manner (Kuramochi T. et al., Life Sci., 54(26), pp2061-2069, 1994); Goto et al report that the hot water extract of Uncaria rhynchophylla JACKS shows lowering activity of hypertension and relaxing effect on blood vessel in pectoral
arteriae aorta; Chen Z. Y. et al report that the ethanol soluble extract of *Crataegus fructus* has relaxing activity of mesentery artery dependently with the concentration of NO (Chen Z. Y. et al., *Life Sci.*, 63(22), pp1983-191, 1998). Moreover, it has been reported that the extract of various crude drugs such as mistletoe, gingko leave, lede-bouriella root, angelica radix, and *Leonurus sibiricus* shows vascular relaxing activity and the active ingredients isolated therefrom showing potent relaxing activity are procyanidine, flavonoid, glycoside and sesquiterpene lactone compounds (Nishida S et al., *Life Sci.*, 72(23), pp2659-67, 2003; Lee T. H. et al., *Planta Med.*, 68(6), pp492-6, 2002; Yuzurihara M. et al., *Eur. J. Pharmacol.*, 444(3), pp183-9, 2002).

[5] There have been known that those vascular relaxation activities are closely correlated with the treatment or prevention of hypertension, arteriosclerosis, cerebrovascular disease, heart disease as well as renal failure and impotence. (Simon AC et al., *J. Cardiovasc. Pharmacol.* 19 Suppl 5, pp.S11-20, 1992).

[6] Accordingly, there have been investigated to develop a medicine or health care food to treat or prevent above described various diseases by finding potent inhibitors for the production of NO till now.


[9] However, there has been not reported or disclosed on the therapeutic effect for cerebrovascular disease of *Sorbus amurensis* KOEHNE in any of above cited literatures, the disclosures of which are incorporated herein by reference.

[10] To investigate and confirm the treating and preventing effect on cerebrovascular disease of *Sorbus amurensis* KOEHNE, the inventors of the present invention have in-
tensively carried out biological experiments and finally completed present invention by confirming that the extract has treating and preventing activity on cerebrovascular disease.

[11] These and other objects of the present invention will become apparent from the detailed disclosure of the present invention provided hereinafter.

Disclosure

[12] The present invention provides a pharmaceutical composition comprising the crude extract or non-polar solvent soluble extract of *Sorbus amurensis* KOEHNE, as an active ingredient for the treatment and prevention of cerebrovascular disease by relaxing endothelial cell.

[13] Above described crude extract comprises the extract prepared by extracting plant material with water, lower alcohols such as methanol, ethanol, preferably methanol and the like, or the mixtures thereof.

[14] Above described non-polar solvent soluble extract can be prepared by extracting above crude extract with non-polar solvent, for example, hexane, ethyl acetate or dichloromethane, preferably ethyl acetate.

[15] The present invention also provides a use of above extract for the preparation of pharmaceutical composition to treat and prevent cerebrovascular disease.

[16] The present invention also provides a health care food or food additives comprising above extract for the prevention or alleviation of cerebrovascular disease.

[17] Accordingly, it is an object of the present invention to provide a pharmaceutical composition comprising the crude extract or non-polar solvent soluble extract of *Sorbus amurensis* KOEHNE, as active ingredients for the treatment and prevention of cerebrovascular disease by relaxing endothelial cell.

[18] The term 'crude extract' disclosed herein comprises the extract prepared by extracting plant material with water, lower alcohols such as methanol, ethanol, preferably methanol and the like, or the mixtures thereof.

[19] The term 'non-polar solvent soluble extract' disclosed herein can be prepared by extracting above crude extract with non-polar solvent, for example, hexane, ethyl acetate or dichloromethane, preferably ethyl acetate.

[20] It is an object of the present invention to provide a use of a crude extract or non-polar solvent soluble extract of *Sorbus amurensis* KOEHNE for the preparation of therapeutic agent for the treatment and prevention of cerebrovascular disease by relaxing endothelial cell in human or mammal.

[21] It is an object of the present invention to provide a method of treating or preventing
cerebrovascular disease by relaxing endothelial cell in a mammal including human comprising administering to said mammal an effective amount of crude extract or non-polar solvent soluble extract of Sorbus amurensis KOEHNE, together with a pharmaceutically acceptable carrier thereof.

[22] It is another object of the present invention to provide a health care food or food additives comprising a crude extract or non-polar solvent soluble extract of Sorbus amurensis KOEHNE, together with a sitologically acceptable additive for the prevention and alleviation of cerebrovascular disease.

[23] The term 'cerebrovascular disease' disclosed herein comprises various brain diseases such as transient ischemic attack (TIA), thrombotic cerebral infarction, hemodynamic cerebral infarction, hiatal infarction, cerebral hemorrhage hypertension and the like.

[24] The pharmaceutical composition of the present invention can contain about 0.01 ~ 50 % by weight of the above extract based on the total weight of the composition.

[25] The health care food of the present invention comprises the above extract as 0.01 to 80 %, preferably 1 to 50 % by weight based on the total weight of the composition.

[26] Above health care food can be contained in health care food, health beverage etc, and may be used as powder, granule, tablet, chewing tablet, capsule, beverage etc.

[27] An inventive crude extract or non-polar solvent soluble extract of Sorbus amurensis KOEHNE may be prepared in accordance with the following preferred embodiment.

[28] Hereinafter, the present invention is described in detail.

[29] An inventive crude extract or non-polar solvent soluble extract of Sorbus amurensis KOEHNE can be prepared in detail by following procedures,

[30] The inventive crude extract of Sorbus amurensis KOEHNE can be prepared by follows; Sorbus amurensis KOEHNE is dried, cut, crushed and mixed with 5 to 25-fold, preferably, approximately 10 fold volume of distilled water, lower alcohols such as methanol, ethanol, butanol and the like, or the mixtures thereof, preferably methanol; the solution is treated with the solvent at the temperature ranging from 20 to 100 °C, preferably from 60 to 100 °C, for the period ranging from 1 to 24 hours with extraction method by the extraction with hot water, cold water, reflux extraction, or ultra-sonication extraction with 1 to 5 times, preferably 2 to 3 times, consecutively; the residue is filtered to obtain the supernatant to be concentrated with rotary evaporator, at the temperature ranging from 20 to 100 °C, preferably from 50 to 70 °C and then dried by vacuum freeze-drying, hot air-drying or spray drying to obtain dried crude
extract powder of *Sorbus amurensis* KOEHNE which can be soluble in water, lower alcohols, or the mixtures thereof.

[31] Additionally, polar solvent soluble and non-polar solvent soluble extract of present invention can be prepared by following procedure; the crude extract prepared by above step, is suspended in water, and then is mixed with 1 to 100-fold, preferably, 1 to 5-fold volume of non polar solvent such as ethyl acetate, chloroform, hexane and the like; the non-polar solvent soluble layer is collected to obtain non-polar solvent soluble extract of the present invention and remaining polar solvent soluble layer is collected to obtain polar solvent soluble extract of the present invention which is soluble in water, lower alcohols, or the mixtures thereof. Also, above described procedures may be modified or subjected to further step to fractionate or to isolate more potent fractions or compounds by conventional procedure well-known in the art, for example, the procedure disclosed in the literature (Harborne J. B. Phytochemical methods: A guide to modern techniques of plant analysis, 5th Ed. pp6-7, 1998).

[32] To investigate the effect of *Sorbus amurensis* KOEHNE on the vasodilation experiments and to confirm whether the crude extract and non-polar solvent soluble extract play an important role in inhibiting NO production and relaxing endothelial cell or not, and then it is confirmed that the crude extract and non-polar solvent soluble extract increase the NO production, and thus increase the relaxation of endothelial cell.

[33] In accordance with another aspect of the present invention, there is provided a pharmaceutical composition comprising the crude extract or non-polar solvent soluble extract of *Sorbus amurensis* KOEHNE prepared by above preparation method for the treatment and prevention of cerebrovascular disease as active ingredients.

[34] It is another object of the present invention to provide a method of treaty and preventing cerebrovascular disease comprising administering a pharmaceutical composition comprising said extract prepared by above preparation method to said mammals including human for treating cerebrovascular disease.

[35] The inventive composition for treating and preventing vascular contraction by inhibiting NO production may comprises above extracts as 0.01 ~ 50 % by weight based on the total weight of the composition.

[36] The inventive composition may additionally comprise conventional carrier, adjuvants or diluents in accordance with a using method well known in the art. 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 ).
[37] Hereinafter, the following formulation methods and excipients are merely exemplary and in no way limit the invention.

[38] 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.

[39] For example, the compositions of the present invention can be dissolved in oils, propylene glycol or other solvents that 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 extract of the present invention can be formulated in the form of ointments and creams.

[40] Pharmaceutical formulations containing present 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), or injectable preparation (solution, suspension, emulsion).

[41] The 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.

[42] The desirable dose of the inventive extract varies depending on the condition and the weight of the subject, severity, drug form, route and a 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 10 g/kg, preferably, 1 to 3 g/kg by weight/day of the inventive extract or compounds of the present invention. The dose may be administered in single or divided into several times per day. In terms of composition, the amount of inventive extract should be present between 0.01 to
50% by weight, preferably 0.5 to 40% by weight based on the total weight of the composition.

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, intracutaneous, intrathecal, epidural or intracerebroventricular injection.

Also, the present invention provide a composition of the health care food beverage for the prevention and improvement of cerebrovascular disease adding above described extracts 0.01 to 80% by weight, amino acids 0.001 to 5% by weight, vitamins 0.001 to 2% by weight, sugars 0.001 to 20% by weight, organic acids 0.001 to 10% by weight, sweetener and flavors of proper amount.

Above described extract of the present invention can be added to food and beverage for the prevention and improvement of cerebrovascular disease.

To develop for health care food, examples of addable food comprising above extracts of the present invention are various food, beverage, gum, vitamin complex, health improving food and the like, and can be used as power, granule, tablet, chewing tablet, capsule or beverage etc.

Also, the extract of the present invention will be able to prevent and improve cerebrovascular disease by adding to child and infant food, such as modified milk powder, modified milk powder for a growth period, modified food for a growth period.

Above described composition therein can be added to food, additive or beverage, wherein the amount of above described extract in food or beverage may generally range from about 0.1 to 80 w/w %, preferably 1 to 50 w/w % of total weight of food for the health care food composition and 1 to 30 g, preferably 3 to 10 g on the ratio of 100 ml of the health beverage composition.

Providing that the health beverage composition of present invention contains above described extract 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 levavidoside 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 100 ml of present beverage composition.

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. Examples of addable food comprising aforementioned extract therein are various food, beverage, gum, vitamin complex, health improving food and the like.

The inventive composition may additionally comprise one or more than one of organic acid, such as citric acid, fumaric acid, adipic acid, lactic acid, malic acid; phosphate, such as phosphate, sodium phosphate, potassium phosphate, acid pyrophosphate, polyphosphate; natural anti-oxidants, such as polyphenol, catechin, alpha-tocopherol, rosemary extract, vitamin C, green tea extract, licorice root extract, chitosan, tannic acid, phytic acid etc.

The above extract of Sorbus amurensisKOEHNE may be 20 to 90 % high concentrated liquid, power, or granule type.

Similarly, the above extract of Sorbus amurensisKOEHNE can comprise additionally one or more than one of lactose, casein, dextrose, glucose, sucrose and sorbitol.

Inventive extract of the present invention have no toxicity and adverse effect therefore; they can be used with safe.

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.

**Description Of Drawings**

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:
Fig 1 shows the vascular relaxing activity according to respective solvent soluble extract, i.e., soluble n-hexane soluble extract, ethylacetate soluble extract and n-butanol extract of *Sorbus amurensis* KOEHNE for 100 ?g/ml.

Fig 2 shows the vascular relaxing activity according to each concentration of n-butanol soluble extract of *Sorbus amurensis* KOEHNE.

**Mode for Invention**

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.

The present invention is more specifically explained by the following examples. However, it should be understood that the present invention is not limited to these examples in any manner.

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

**Example 1. Preparation of the crude extract of *Sorbus amurensis* KOEHNE**

1.2 kg of dried cortex of *Sorbus amurensis* KOEHNE was macerated to powder, mixed with 5 L of methanol and the mixture was extracted 3 times at room temperature for three weeks, repeatedly. And the extract was filtered with filter paper (Whatman Co., U.S.A.). The filtrates were pooled and concentrated by rotary evaporator (N-1000, Eyela Co. Japan) at 55 ~ 65 °C under reduced pressure and dried with freezing dryer (Speed Spec 3000, Bio-Rad, U.S.A.) to obtain 63.2 g of dried crude extract of *Sorbus amurensis* KOEHNE.

**Example 2. Preparation of polar solvent and non-polar solvent soluble extract of *Sorbus amurensis* KOEHNE.**

2-1. Preparation of *n*-hexane soluble extract

63.2 g of crude extract of *Sorbus amurensis* KOEHNE, prepared in Example 1 was suspended in 1 liter of distilled water and the suspension was mixed with 1 liter of *n*-hexane vigorously to divide into *n*-hexane soluble fraction and water-soluble fraction. *n*-hexane soluble fraction was collected and the residual solution was subjected to the *n*-hexane extraction again. Above-described procedure was repeated 3 times.

*n*-hexane soluble fraction was evaporated *in vacuo* to give 11.7 g of *n*-hexane soluble extract of *Sorbus amurensis* KOEHNE.

2-2. Preparation of ethylacetate soluble extract

Water-soluble fraction of *Sorbus amurensis* KOEHNE in Example 2-1 was mixed
with equivalent volume of ethylacetate vigorously to divide into ethylacetate soluble fraction and water-soluble fraction. The ethylacetate soluble fraction was collected and the residual solution was subjected to the ethylacetate extraction again. Above-described procedure was repeated 3 times.

Boiled ethylacetate soluble fraction was evaporated in vacuo to give 15.3g of ethylacetate soluble extract of Sorbus amurensis KOEHNE.

2-3. Preparation of n-butanol and water-soluble extract

Water-soluble fraction of Sorbus amurensis KOEHNE in Example 2-2 was mixed with equivalent volume of n-butanol vigorously to divide into n-butanol soluble fraction and water-soluble fraction. n-butanol soluble fraction was collected and the residual solution was subjected to the n-butanol extraction again. Above-described procedure was repeated 3 times.

n-butanol soluble fraction and water-soluble fraction were respectively evaporated in vacuo to give 17.2g of n-butanol soluble extract and 12.4g of water-soluble extract of Sorbus amurensis KOEHNE.

Experimental Example 1. Determination of vascular relaxation activity

1-1. Reagent and Instrument

Phenylephrine HCl, L-NAME (Nitroarginine methyl ester), methylene blue, ODQ, indomethacin, glibenclamide, TEA, verapamil, atropine, propranolol and yohimbine etc were procured from Sigma Chemical Co. (St. Louis, USA) and Y-27632 2HCl (trans-4-[(1R)-1-aminoethyl]-N-4-pyrindinylcyclohexane carbamidide dihydrochloride) and Zaprinast (2-(2-propyloxyphenyl)-8-azapurin-6-one) were from Tocris Cookson Ltd. (Bristol, Great Britain).

1-2. Preparation of Experimental Sample

To evaluate the efficacy of Sorbus amurensis KOEHNE on the relaxation of vascular tissue, following procedure was performed by modifying the method disclosed in the literature.

Healthy Sprague-Dawley white mouse weighing from 250 to 300g was killed with decapitation. The thoracic aorta of the rat was isolated and isolated aorta was immersed in cold Krebs solution (pH 7.4) containing 118mM of NaCl, 47mM of KCl, 1.1mM of MgSO$_4$ , 1.2mM KH$_2$PO$_4$ , 1.5mM of CaCl$_2$ , 25mM of NaHCO$_3$ and 10mM of glucose. And the connective tissue and fat attached thereto were removed and the preparation was cut to the slices in the width of about 3 mm.

The slice of pectoral arteriae aorta was fixed in Krebs solution (pH 7.4) saturated
with 95% O\textsubscript{2} and 5% CO\textsubscript{2} gas mixture at 37 °C and the relaxation effect of the slice was determined by Grass physiograph (Model 7E, USA) equipped with force-displacement transducer (Grass FT03, USA) transforming isometric tension.

At first, the slice was artificially to be contracted using by 1x 10\textsuperscript{-6} M of phenylephrine, to be relaxed by 1x 10\textsuperscript{-6} M of acetylcholine and the stability of vascular endothelial cell was determined by washing with Krebs solution. For determining the relaxing activity of samples, after the contraction of the slice with phenetphrine, various concentration of the extract of Sorbus amurensis KOEHNE ranging from 1x 10\textsuperscript{-6} g/ml to 1x 10\textsuperscript{-4} g/ml were treated to observe their relaxing activities in dose dependent manner. In vascular endothelium independent experiment, the contraction and the relaxation of the cell using by respective phenylephrine and Ach were performed after removing endothelial cell by small cotton pellet and further steps were proceed by confirming the removal of the cell.

1-3. Preparation of rat aorta

The animal breeding procedures were performed in strict accordance with the National Institutes of Healthy Guidelines for the Care and Use of laboratory Animals and were approved by the Institutional Animal Care and Utilization Committee. Male Sprague-Dawley rats were purchased from Korean Experimental Animals Co. (Daejeon, Korea). The rats weighing 250 - 300 g were sacrificed by decapitation. The thoracic aortas were rapidly and carefully dissected and placed into ice-cold Krebs solution (pH 7.4) containing 118 mmol/L NaCl, 47 mmol/L KCl, 1.1 mmol/L MgSO\textsubscript{4}, 1.2 mmol/L KH PO\textsubscript{4}, 1.5 mmol/L CaCl\textsubscript{2}, 25 mmol/L NaHCO\textsubscript{3}, and 10 mmol/L Glucose. The aortas were performed in free of connective tissue and fat, and then cut into rings having width of approximately 3 mm. All dissecting procedures were performed with extreme care to protect the endothelium from inadvertent damage. In some aortic rings, the endothelial layer was mechanically removed by gently rubbing the luminal surface of the aortic ring back and forth several times with plastic tubing. Endothelial integrity or functional removal was verified by the presence or absence, respectively, of the relaxant response to 3 x 10\textsuperscript{-6} M acetylcholine on phenylephrine (3 x 10\textsuperscript{-6} M) contracted vessels.

Record of isometric vascular tone was disclosed in the literature (Gray DW et al., \textit{Br J Pharmacol} 107: 691-6, 1992).

The aortic rings were suspended by means of two L-shape stainless-steel wires inserted into lumen in a tissue bath containing Krebs solution (pH 7.4) at 37 °C, while being continuously bubbled with 95% O\textsubscript{2} -5% CO\textsubscript{2}. The baseline load placed on the
aortic rings was 2.0 g, and the changes in isometric tension were recorded using a force-displacement transducer (Grass FT 03, Quincy, MA, USA) connected to a Grass polygraph recording system (Model 7E). In the first set of experiments, the aortic rings were contacted with phenylephrine (3 x 10^{-6} M) to obtain maximal response. Once the maximal response to phenylephrine had been obtained, the aortic rings were washed every 20 min with Krebs solution until the tension returned to the basal level. The concentration-dependent response curve to acetylcholine (Ach) (10^{-9} - 10^{-5} M) was performed as a positive control in endothelium-intact aortic rings contracted by 3 x 10^{-6} M phenylephrine. The rings were then exposed to various drugs for 30 min, and then aortic relaxation was carried out by cumulative addition of n-butanol extract of *Sorbus amurensis* KOEHNE. The effect of vehicle, i.e., less than 0.2% dimethylsulfoxide (DMSO), was also tested. After each test, the aortic rings were washed three times with fresh Krebs solution and allowed for 30 min to equilibrate.

As can be seen in the results as shown in Fig. 1 to 2, Fig 1 shows the vascular relaxing activity according to respective solvent soluble extract. The extract, i.e., soluble n-hexane soluble extract, ethylacetate soluble extract and n-butanol extract of *Sorbus amurensis* KOEHNE shows potent relaxing activity on endothelial cell by 395 ± 1.5%, 71.1 ± 415%, and 57.8 ± 6.2%, in respective concentration of 100 μg/ml respectively, which shows that ethylacetate soluble extract shows most potent activity among them. Fig 2 shows the vascular relaxing activity of various concentration of n-butanol soluble extract of *Sorbus amurensis* KOEHNE, which shows that the relaxing activity of endothelial cell in dose dependent manner. However, those activities were disappeared by removing vascular endothelial cell or inhibiting NO system, therefore, it is confirmed that n-butanol soluble extract shows relaxing activity of n-butanol soluble extract of *Sorbus amurensis* KOEHNE is dependent on the endothelial cell and NO system.

1-4. Statistics Treatment

The significance of experimental result was determined through student t-test and one-way ANOVA test and acknowledged where the standard deviation (σ) value is less than 0.05 statistically. The expression of test value was shown as Mean ± SE in the experiment.

Experimental Example 2. Toxicity test

Methods (1)

The acute toxicity tests on ICR mice (mean body weight 25 ± 5g) and Sprague-Dawley rats (235 ± 10g, Jung-Ang Lab Animal Inc.) were performed using the extract
of the Example 1. Four group consisting of 10 mice or rats was administrated orally with 100mg/kg, 250mg/kg, 500mg/kg and 1000mg/kg of test sample or solvents (0.2 ml, i.p.) respectively and observed for 2 weeks.

Methods (2)

The acute toxicity tests on ICR mice (mean body weight 25 ± 5g) and Sprague-Dawley rats (235 ± 10g, Jung-Ang Lab Animal Inc.) were performed using the extract of the Example 1. Four group consisting of 10 mice or rats was administrated intraperitoneally with 25mg/kg, 50mg/kg, 100mg/kg, and 200mg/kg of test sample or solvents (0.2 ml, i.p.), respectively, and observed for 24 hours.

Results

There were no treatment-related effects on mortality, clinical signs, body weight changes and gross findings in any group or either gender. These results suggested that the extract prepared in the present invention were potent and safe.

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.

Preparation of powder

- Dried powder of Example 1 : 50mg
- Lactose : 100mg
- Talc : 10mg
- Powder preparation was prepared by mixing above components and filling sealed package.

Preparation of tablet

- Dried powder of Example 1 : 50mg
- Corn Starch : 100mg
- Lactose : 100mg
- Magnesium Stearate : 2mg
- Tablet preparation was prepared by mixing above components and entabletting.

Preparation of capsule

- Dried powder of Example 1 : 50mg
- Corn starch : 100mg
- Lactose : 100mg
- Magnesium Stearate : 2mg
- Tablet preparation was prepared by mixing above components and filling gelatin capsule by conventional gelatin preparation method.
Preparation of injection

Dried powder of Example 1 .......................... 50mg
Distilled water for injection ......................... optimum amount
PH controller ........................................ optimum amount

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

Preparation of liquid

Dried powder of Example 1 .......................... 0.1~80g
Sugar .................................................. 5~10g
Citric acid ............................................. 0.05~0.3%
Caramel ................................................ 0.005~0.02%
Vitamin C .............................................. 0.1~1%
Distilled water ....................................... 79-94%
CO_2 gas ............................................... 0.5~0.8%

Liquid preparation was prepared by dissolving active component, filling all the components and sterilizing by conventional liquid preparation method.

Preparation of health care food

Extract of Example 1 ................................. 1000mg
Vitamin mixture ........................................ optimum amount
Vitamin A acetate ..................................... 70?g
Vitamin E .............................................. 1.0mg
Vitamin B_1 ........................................... 0.13mg
Vitamin B_2 ........................................... 0.15mg
Vitamin B_6 ........................................... 0.5mg
Vitamin B_12 .......................................... 0.2 ?g
Vitamin C .............................................. 10mg
Biotin .................................................. 10 ?g
Amide nicotinic acid .................................. 1.7mg
Folic acid ............................................. 50 ?g
Calcium pantothenic acid ............................ 0.5mg
Mineral mixture ...................................... optimum amount
Ferrous sulfate ........................................ 1.75mg
Zinc oxide ............................................. 0.82mg
Magnesium carbonate ............................... 25.3mg
[146] Monopotassium phosphate .......................... 15mg
[147] Dicalcium phosphate ................................. 55mg
[148] Potassium citrate ........................................ 90mg
[149] Calcium carbonate ...................................... 100mg
[150] Magnesium chloride ..................................... 248mg
[151] 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.

[152] Preparation of health beverage
[153] Extract of Example 1 ................................. 1000mg
[154] Citric acid .................................................. 1000mg
[155] Oligosaccharide ............................................ 100g
[156] Apricot concentration ................................. 2g
[157] Taurine ...................................................... 1g
[158] Distilled water ............................................. 900 ml
[159] 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 1000 ml ample and sterilizing by conventional health beverage preparation method.

[160] 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.

Industrial Applicability

[161] As described in the detailed description of the present invention, the crude extract or non-polar solvent soluble extract of Sorbus amurensis KOEHN, show potent relaxing activity of vascular endothelial cell by increasing the synthesis of endothelium-derived NO, therefore, it can be used as the therapeutics or health care food for treating and preventing cerebrovascular disease.
Claims

1. A pharmaceutical composition comprising the crude extract of *Sorbus amurensis* KOEHNE, as an active ingredients for the treatment and prevention of cerebrovascular disease.

2. The pharmaceutical composition according to claim 1 wherein said crude extract is extracted with the solvent selected from the group consisting of water, lower alcohol and the mixture thereof.

3. A pharmaceutical composition comprising a non-polar solvent soluble extract of *Sorbus amurensis* KOEHNE, as an active ingredients for the treatment and prevention of cerebrovascular disease.

4. The pharmaceutical composition according to claim 3 wherein said non-polar solvent soluble extract is extracted with non-polar solvent selected from the group consisting of hexane, ethyl acetate, dichloromethane and the mixture thereof.

5. The pharmaceutical composition according to claim 4 wherein said non-polar solvent soluble extract is extracted with ethyl acetate.

6. The pharmaceutical composition according to claim 1 or 3 wherein said cerebrovascular disease comprises various brain diseases such as transient ischemic attack (TIA), thrombotic cerebral infarction, hemodynamic cerebral infarction, hialtal infarction, cerebral hemorrhage hypertension and the like.

7. The pharmaceutical composition according to any one claims 1 to 6 wherein said composition contains the extract in the amount ranging from 0.01 to 50% by weight based on the total weight of the composition.

8. A health care food comprising the crude extract or non-polar solvent soluble extract of *Sorbus amurensis* KOEHNE as set forth in claim 1 or 3, together with a sitologically acceptable additive for the prevention and improvement of cerebrovascular disease.

9. The health care food according to claim 8 wherein said health care food is provided as powder, granule, tablet, chewing tablet, capsule or beverage type.
A. CLASSIFICATION OF SUBJECT MATTER

IPC7 A61K 35/78

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K 35/78, A23L 1/29, A23F 3/16

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
KOREAN PATENTS AND APPLICATIONS FOR INVENTIONS SINCE 1975

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubMed on-line

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
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<tbody>
<tr>
<td>A</td>
<td>LEE, MK et al. 'Anticancer effect of Sorbus commixta Hedl extracts' In; Korean J. Medicinal Crop Sci. 2002; 10(5): 403-8</td>
<td>1-9</td>
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<tr>
<td>A</td>
<td>KR 0181168 B1 (NAM JH et al.), 01 February 1999 See abstract</td>
<td>1-9</td>
</tr>
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</table>

☐ Further documents are listed in the continuation of Box C.  ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of 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

Name and mailing address of the ISA/KR
Korean Intellectual Property Office
920 Dunsan-dong, Seo-gu, Daejeon 302-701, Republic of Korea
Facsimile No. 82-42-472-7140

Authorized officer
YEO, Ho Sup
Telephone No. 82-42-481-5627
## INTERNATIONAL SEARCH REPORT

### Box No. II  Observations where certain claims were found unsearchable (Continuation of item 2 of 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. □ Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:

2. □ Claims 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. □ Claims 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:

Claims 1-7 are directed to a pharmaceutical composition, and claims 8-9 are directed to a health care food.

Although claims 1-7 and 8-9 are relevant to the composition comprising the same active ingredient, there is no technical relationship among a pharmaceutical composition and health care food.

Hence, the application contains the following separate groups of inventions not so linked as to form a single general inventive concept (PCT Rule 13.1):

i) Claims 1-7
ii) Claims 8-9

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

2. X As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any addition fee.

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 claims 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 claims Nos.:

### Remark on Protest

□ The additional search fees were accompanied by the applicant's protest.

□ No protest accompanied the payment of additional search fees.
<table>
<thead>
<tr>
<th>Patent document cited in search report</th>
<th>Publication date</th>
<th>Patent family member(s)</th>
<th>Publication date</th>
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<tr>
<td>KR 0181168 B1</td>
<td>01/02/1999</td>
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