

**Use of isoacteoside in preventing or treating amyloid beta peptide-associated diseases or conditions,
and method for inhibiting formation, accumulation or aggregation of amyloid beta peptides**

Description

CROSS-REFERENCE TO RELATED APPLICATIONS

5 [0001] This application claims priority benefit from US Provisional Application No. 61/355,169, filed June 16, 2010.

BACKGROUND OF THE INVENTION

1. Field of the Invention

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[0002] The present invention generally relates to the use of isoacteoside or a pharmaceutically acceptable salt thereof in preventing or treating amyloid β peptide (amyloid β peptide; A β) associated diseases or conditions.

2. Description of the Prior Art

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[0003] Alzheimer's disease is considered to be related to accumulation of peptides of 39-43 amino acids, and such peptides are called as amyloid β peptides, which are hydrolysis products of amyloid precursor protein (APP). Among the A β , A β 1-40 is the most abundant form, while A β 1-42 is more toxic to neurons and highly fibrillogenic and is considered as the most relevant A β form to Alzheimer's disease. A β monomers can form soluble A β oligomers through oligomerization, and further form insoluble fibrils or senile plaques extracellularly.

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[0004] It is generally considered that A β -associated diseases or conditions comprise Down syndrome, hereditary cerebral hemorrhage with amyloid (HCHWA) Dutch, Parkinsonism-dementia complex on Guam (Clinical Neurology and Neurosurgery (1990) 92: 305-310); Cerebral amyloid angiopathy (J. Neuropath. Exp. Neuro. (2002) 61: 282-293); inclusion body myositis (Neurology (2006) 66: 65-68); frontotemporal dementia (Neuroreport (2002) 13-5: 719-723); age-related macular degeneration (Experimental Eye Research (2004) 78: 243-256); Pick's disease (Neuroscience Letters (1994) 171: 63-66), and others.

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[0005] A β -associated diseases or conditions as described are generally related to formation, accumulation or aggregation of A β , leading to abnormal quantity of A β or A β aggregates present in organisms caused by congenital factors (e.g., inheritance) or acquired factors (e.g., aging or environmental effects).

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[0006] It is generally believed that inhibition of A β formation, accumulation or aggregation can be used as an approach for effectively preventing or treating Alzheimer's disease or other A β -associated diseases or conditions.

SUMMARY OF THE INVENTION

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[0007] It is the object of the present invention to provide an active ingredient for use in preventing or treating a disease or condition associated with amyloid β peptides in an individual, wherein said disease or condition is mild cognitive impairment, Lewy body dementia, Down syndrome, Hereditary cerebral hemorrhage with amyloid (HCHWA) Dutch, Parkinsonism-dementia complex on Guam, Cerebral amyloid angiopathy, inclusion body myositis, frontotemporal dementia, age-related macular degeneration, or Pick's disease. Such active ingredient can be used as an additive in food, drinks, chewing gums, patches or skin care products.

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[0008] Said object is achieved by isoacteoside or a pharmaceutically acceptable salt thereof for use according to appended claim 1.

[0009] Preferred embodiments of the present invention are set forth in the dependent claims.

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[0010] To better understand the above and other objects, features and advantages of the present invention, the present invention will be described in detail below taken from the examples with reference to the annexed drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011]

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Fig. 1 shows the percentage level of extracellular A β 1-40 of each well, the results indicating the test sample D (isoacteoside) possesses significant activity on reducing extracellular A β 1-40 accumulation;

Fig. 2 shows the percentage level of intracellular A β 1-40 of each well;

Fig. 3 shows the effects of the test samples on APP expression;

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Fig. 4 shows the effects of the test samples on A β 1-40 degradation

Fig. 5 shows the effects of the test samples on A β 1-40 oligomerization;

Fig. 6 illustrates the processes of formation, clearance and aggregation of A β ;

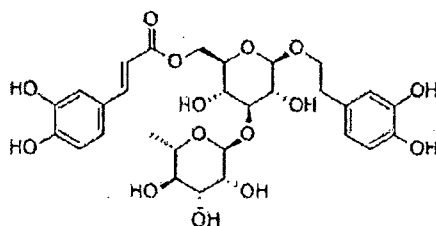
Fig. 7 illustrates the experimental schedule for animal studies;

Fig. 8 shows the effects of the test samples on the exploration behavior performance of the rats;
 Fig. 9 shows the effects of the test samples on the passive avoidance response performance of the rats;
 Fig. 10 shows the effects of the test samples on the water maze spatial performance of the rats;
 Fig. 11 shows the effects of the test samples on the water maze probe test performance of the rats;
 Fig. 12A-E shows the immunohistochemical staining results of the rats;
 Fig. 13A shows the levels of acetylcholine in the brain cortex and hippocampus of the rats;
 Fig. 13B shows the levels of choline in the brain cortex and hippocampus of the rats;
 Fig. 14A shows the activities of acetylcholinesterase in the brain cortex of the rats; and
 Fig. 14B shows the activities of acetylcholinesterase in the brain hippocampus of the rats.

DETAILED DESCRIPTION

[0012] Various diseases caused by A β have a common feature: formation of A β aggregates. These A β aggregates present in shapes such as fibrils or plaques, and deposit in systems, organs, tissues or body fluids of organisms, causing various diseases or conditions. It is therefore supposed that inhibition of A β formation, accumulation or aggregation can be used as an approach for effectively preventing or treating A β -associated diseases or conditions.

[0013] In view of the above, the present specification discloses using isoacteoside having a structure shown below or a pharmaceutically acceptable salt thereof as an active ingredient for inhibiting (e.g., reducing or preventing) A β formation, accumulation or aggregation, and in particular A β extracellular formation, accumulation or aggregation.



[0014] U.S. patent 7,087,252 B2 discloses a medicinal preparation, which comprises 10-70 weight percent of echinacoside and 1-40 weight percent of acteoside, prepared from fleshy stems of *Cistanche tubulosa* (Schenk) Wight, and is provided against senile dementia. Isoacteoside and various other phenylethanoid glycosides are known to be included in the medicinal preparation.

[0015] Liu Feng-Xia et al. ("The effects of glycosides of cistanche on learning and memory in beta-amyloid peptide induced Alzheimers disease in mice and its mechanism", Biosciences Information Service, Philadelphia, PA, US; May 2006) describe the protective effects of glycosides of cistance on an animal model of Alzheimer's disease.

[0016] In the present invention, hydrates or other solvates of isoacteoside are deemed as functional equivalents of isoacteoside.

[0017] When a pharmaceutically acceptable salt of isoacteoside is administered to an individual, the pharmaceutically acceptable salt generally provides equivalent or similar therapeutic effects as isoacteoside, and is physiologically tolerable without causing adverse side effects such as allergy. The pharmaceutically acceptable salt of isoacteoside may comprise but is not limited to iron, calcium, and magnesium salts.

[0018] The term "prevent" used herein means avoiding or delaying occurrence of a disease or condition in organisms. The term "treat" used herein means slowing or stopping progress of a disease or condition, or making an individual return back to his improved or normal status.

[0019] The term "amyloid β peptide (A β)-associated diseases or conditions" generally refers to those diseases or conditions that occur relating to formation, accumulation or aggregation of A β , and particularly refers to the diseases or conditions that are caused by A β . When abnormal formation, accumulation or aggregation is found in a certain proportion of individuals with certain diseases or conditions, the diseases or conditions can be considered as being associated with A β . In addition, when A β aggregates somewhere that is close to occurrence of pathological features affected in certain diseases or conditions, the diseases or conditions can be also considered as being associated with A β .

[0020] The active ingredient for A β inhibition can be used as an additive in food or drinks to facilitate the use of inhibiting formation, accumulation or aggregation of A β .

[0021] The drugs or pharmaceutical compositions for use in treating A β -associated diseases or conditions, which comprise isoacteoside or the pharmaceutically acceptable salt thereof as an active ingredient, can comprise suitable carriers, diluents or excipients, and can be present in powders, granules, tablets, troches, pills, capsules, solutions, suspensions, creams, ointments, gels, aerosols, suppositories, patches or any other desired forms. The drugs or pharmaceutical combinations described above can be administered via oral, topical, parenteral, dermal, intranasal, ocular,

intraocular or other routes.

[0022] In the context, the term "or" is generally defined as "and/or", unless it is otherwise specified.

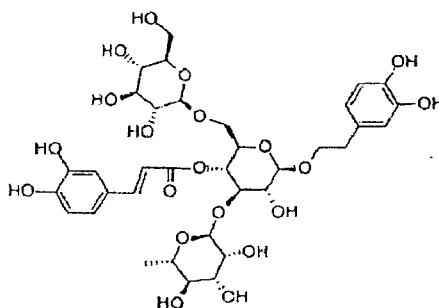
[0023] Along with the aging of the population, dementia, which is related to aging, has become one of the major concerns in the present medical researches related to senile diseases. Alzheimer's disease is the most common type of dementia. Alzheimer's disease is a chronic progressive neurodegenerative disease, characterized in that patients gradually lose their cognition ability and show abnormal behavior, followed by a possible loss of verbal and motion abilities, and thus Alzheimer's disease easily makes a great impact on the patient's and his/her family's life qualities. In view of the trend of the number of patients suffering Alzheimer's disease gradually increasing, causing severe burdens on the patient's family and the society, the present invention selects the amyloid beta peptides ($A\beta$) which are more related to Alzheimer's disease for carrying out experiments.

Table 1: Test samples

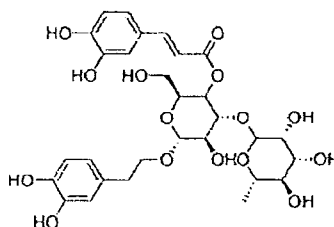
Symbol	Test sample	Purity (%)	Source
A	Preparation containing phenylethanoid glycosides	-	available from Tianlife® (referring to the preparation disclosed in US 7087252B2)
B	Echinacoside	99	Sinphar Lab.
C	Acteoside	97	Sinphar Lab.
D	Isoacteoside	97	Sinphar Lab.

[0024] The chemical structures of echinacoside, acteoside and isoacteoside are shown below:

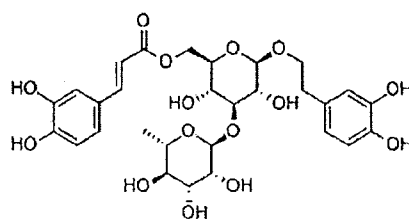
Echinacoside:



Acteoside:



Isoacteoside:



Example 1: Neuroblastoma cell culture

[0025] Wild-type human neuroblastoma cells (SH-SY5Y) were cultured in Eagle's Minimum essential Medium (EMEM) / Ham's F12 medium (1:1 mixture) (containing 10% FBS, 10 units/ml penicillin, 10 µg/ml Streptomycin). Wild-type mouse neuroblastoma Neuro-2a cells were cultured in minimum essential medium (MEM) (containing 10% FBS, 10 units/ml penicillin, 10 µg/ml Streptomycin).

Example 2: The effect of each test sample on extracellular Aβ1-40 accumulation

[0026] The medium of the wild-type human neuroblastoma SH-SY5Y cells in Example 1 were switched into chemical defined medium (EMEM/F12 medium (Cat.No.12500-062), Hepes 5mM, Glucose 0.6%, NaHCO₃ 3mM, Glutamine 2.5 mM, Insulin 25 µg/ml, Transferin 100 µg/ml, Progesterone 20 nM, Putrescine 60 µM, Sodium selenite 30 nM, Heparin 2 µg/ml). Each well contained 1x10⁵ SH-SY5Y cells in 300 µl of culture medium. Thirty minutes later, each well was treated with the test samples A-D given in Table 1 respectively at a concentration of 50 µg/ml for 24 hours. After that, the level of Aβ1-40 in the medium of each well was analyzed by Human Aβ1-40 immunoassay kits (Catalog # KHB3482 Invitrogen).

[0027] Human neuroblastoma SH-SY5Y cells cause extracellular accumulation of Aβ. Fig. 1 shows the percent level of Aβ1-40 in the medium of each SH-SY5Y well, based on the percentage level in Vehicle control group which is not treated with any test sample. The results were shown in mean ± standard deviation (SD) form. Significant difference between the Vehicle control group and the test sample-treated groups were indicated by *, P<0.05, **, P<0.01, and ***, P<0.001.

[0028] Referring to Fig. 1, in comparison with the Vehicle control group, the test samples A (preparation containing phenylethanoid glycosides) and C (acteoside) reduced the level of Aβ1-40 by about 20%, and D (isoacteoside) reduced the level of Aβ1-40 by about 47.44 ± 16.62%. The results in Fig. 1 suggest that the test sample D (isoacteoside) possesses significant activity on reducing extracellular Aβ1-40 accumulation.

Example 3: The effect of each test sample on intracellular Aβ1-40 accumulation

[0029] Each well contained 1x10⁵ SH-SY5Y cells in 300 µl of culture medium. After the SH-SY5Y cells of each well were individually treated with the test samples A-D at a concentration of 50 µg/ml, cell homogenate was individually prepared from cells of each well, and the amount of Aβ1-40 in the medium of each well was determined with Human Aβ1-40 immunoassay kits (Catalog # KHB3482 Invitrogen).

[0030] Fig. 2 shows the percentage level of intracellular Aβ1-40 of SH-SY5Y well, based on the Vehicle control group which is not treated with any test sample. Referring to Fig. 2, it is indicated that the test samples A-D do not significantly cause intracellular accumulation of Aβ.

Example 4: The effect of each test sample on APP expression

[0031] Each well contained 1x10⁵ SH-SY5Y cells in 300 µl of culture medium. After the SH-SY5Y cells of each well were individually treated with the test samples A-D at a concentration of 50 µg/ml for 24 hours, the cells were homogenized in homogenize buffer (50 mM Hepes pH 7.5, 1 mM EDTA, 150 mM NaCl, 1% NP-40, 1 mM PMSF, 5 µg/ml aprotinin, 10 µg/ml leupeptin). Cell debris was removed by centrifugation. Proteins were separated by SDS-polyacrylamide gel electrophoresis and were then transferred to PVDF membrane, followed by evaluating holoAPP amounts by immunoblot (antiAβ1-17, 6E10 or anti-APP C-terminal antibody)

[0032] Fig. 3 shows the effects of the test samples A-D on APP expression in the cells, expressing as percentage, based on the Vehicle control group which is not treated with any test sample. As shown in Fig. 3, it is seen that the test samples A-D do not down regulate APP expression.

Example 5: The effect of each test sample on extracellular A β 1-40 degradation

[0033] Mouse neuroblastoma Neuro-2a cells will release A β -degrading enzymes in the chemical defined medium, but will not produce detectable amount of A β in the medium. The Neuro 2a cells were incubated in the medium for 24 hours, and then the medium were drawn out without cells. The medium were individually treated with the test samples A-D at a concentration of 50 μ g/ml and 10 ng synthetic A β 1-40 for 24 hours, and then the effects of each test sample on promoting the enzymes in the medium were examined. The remaining A β 1-40 amount of each well was analyzed by Human A β 1-40 Immunoassay kits (Catalog # KHB3482 Invitrogen), expressing as percentage, as compared with the Vehicle control group. Referring to Fig. 4, the test sample D (isoacteoside) does not accelerate A β degradation.

Example 6: The effect of each test samples on A β 1-42 oligomerization

[0034] Dried Human A β 1-42 was taken out from the refrigerator and equilibrated to room temperature. A β 1-42 was dissolved in 1,1,1,3,3,3-Hexa-fluoro-2-propanol (HFIP) to a concentration of 1mM, and was then placed at room temperature for one hour. The A β 1-42/HFIP solution was aliquoted by Hamilton syringe, and was then dried under a stream of nitrogen gas, followed by storing at a temperature of -20°C. A β 1-42 treated with HFIP was dissolved in PBS, and was vibration-incubated with treatment of each test sample at a concentration of 50 μ g/ml and at 4°C for 24 hours to prepare A β 1-42 oligomers. The level of A β 1-42 oligomerization was analyzed by thioflavin T fluorescence (Ex=450 nm, Em=482 nm).

[0035] Fig. 5 shows the effects of the test samples A-D on A β 1-42 oligomerization in percentage. Referring to Fig. 5, the test samples A (preparation containing phenylethanoid glycosides), B (echinacoside), C (acteoside) and D (isoacteoside) were found to possess activities on inhibiting A β 1-42 oligomerization, wherein isoacteoside (D) was able to significantly inhibit A β 1-42 oligomerization by $98.93 \pm 1.70\%$. That is to say, isoacteoside (D) can significantly inhibit A β 1-42 oligomerization, and further inhibit A β from forming fibrils or senile plaques.

[0036] Fig. 6 illustrates the processes of formation, clearance and aggregation of A β (Pharmacology & Therapeutics (2005) 108: 131). It is shown that the extracellular A β accumulation (1) in the medium will be affected by intracellular A β accumulation (2), APP expression (3), A β degradation (4), A β oligomerization (5) and A β formation (6). According to the results from cell studies in Examples 2 to 6, as compared to the test samples A-C, isoacteoside (D) is effective in reducing the extracellular A β accumulation (1) in the medium and is able to significantly inhibit A β oligomerization (5), which is supposed to inhibit A β aggregation in advance. Besides, the effect of isoacteoside (D) on reducing extracellular A β accumulation (1) is not due to isoacteoside enhancing the intracellular A β accumulation (2), reducing the APP expression (3) of the cells, or enhancing extracellular A β degradation (4). It is therefore suggested that the effect of isoacteoside (D) is directly on the reducing of A β formation.

[0037] In summary, isoacteoside (D) or its pharmaceutically acceptable salts can be used as an active ingredient for inhibiting formation, accumulation or aggregation of A β . Therefore, it is expected that isoacteoside (D) can be provided for effectively inhibiting neuronal damage or apoptosis caused by A β , and further for retaining, improving or restoring learning or memory abilities. In addition, according to those results above, isoacteoside (D) or the pharmaceutically acceptable salts thereof can be provided for preventing or treating A β 3-associated diseases or conditions, as well as for inhibiting formation, accumulation or aggregation of A β .

[0038] The described A β -associated diseases or conditions are mild cognitive impairment, Lewy body dementia, Down syndrome, hereditary cerebral hemorrhage with amyloid (HCHWA) Dutch, Parkinsonism-dementia complex on Guam, Cerebral amyloid angiopathy, inclusion body myositis, frontotemporal dementia, age-related macular degeneration and Pick's disease. In addition, even though the described A β is exemplified by A β 1-40 at most or highly fibrillogenic A β 1-42, the A β can also comprise other peptide fragments.

[0039] Among the test samples above, isoacteoside (D) possesses excellent effects on reducing the formation, accumulation or aggregation of A β . In the following Examples 7 to 11 illustrated below, male Sprague-Dawley (SD) rats, weighed 250-300 gm, were obtained from BioLASCO Taiwan Co. Ltd.. The SD rats were infused intracerebroventricularly with A β 1-42 to cause neuronal damage, affecting their memory and learning ability, and leading to form senile plaque-like aggregates in the rat's brain, being used as an animal model of Alzheimer's disease. Alzheimer's disease animal model induced by A β can be referred to, for example, Nabeshima et al. (Neuroscience Letters (1994) 170: 63-66) (British Journal of Pharmacology (1999) 126: 235-244).

[0040] The rats were anesthetized for implantation of an infusion cannula into the left cerebral ventricle thereof. After sewing up the incisions, the rats were returned to their cages. Fig. 7 illustrates the experimental schedule for animal studies. The exploration behavior tests were carried out on day 7, the passive avoidance learning tests were carried out on day 8 and 9, the water maze tests were carried on day 10 to 13, and the probe tests were carried out on day 14, after the start of A β 1-42 infusion. The experimental groups were shown in Table 2. The Sham group was treated with TFA solution (64.9% sterile ddH₂O, 35% acetonitrile, 0.1 % trifluoroacetic acid), and the other experimental groups were treated with 0.5 μ l of A β 1-42 dissolved in TFA solution per hour, corresponding to 300 pmol/12 μ l of A β 1-42 (Tocris

1428 ; MW : 4514.08) per day. The test samples were administered orally to the rats, 1 hour before the tests (i.e., the exploration behavior tests, the passive avoidance learning tests, and the water maze tests) throughout the experimental period. Table 2 shows the conditions for each experimental group, wherein D is isoacteoside shown in Table 1, and Aricept is a commercial drug for treating dementia such as Alzheimer's disease. All the test samples were prepared freshly with deionized distilled water (ddH₂O) every day, and were administered orally by stomach tube.

[0041] In Examples 7 to 11, the results were shown in mean $\pm$ standard deviation (SD) form. Significant difference between the A β 1-42 control group and the other experimental groups were indicated by *, P<0.05, **, P<0.01, and ***, P<0.001.

[0042] All rats were sacrificed and their brains were removed, cut into slices and stained. Neurotransmitters were also tested.

Groups	Inducer (intracerebroventricular infusion)	Test sample (administered orally by stomach tube)	Rats
Sham group	TFA solution	ddH ₂ O	12
A β 1-42 control group	A β 1-42		12
D 2.5 mg/kg		D 2.5 mg/kg	12
D 5 mg/kg		D 5 mg/kg	12
Aricept		Aricept 0.75 mg/kg	12

Example 7: Exploration behavior tests and apparatus

[0043] An exploration behavior test apparatus consisted of a box (40cm x 40cm x 40cm) and a stainless steel bottom board with 16 holes of a diameter of 3cm, wherein these holes were spaced 4 cm apart and were at a distance of 7 cm from the side edge. The entry counts of each rat were recorded for 10 minutes.

[0044] Fig. 8 shows results of the exploration behavior tests performed by the rats according to Table 2. Referring to Fig. 8, intracerebroventricular infusion of A β 1-42 decreases the exploratory activities of the rats, and the test sample D (isoacteoside) and Aricept are effective in improving the exploratory activities of the rats, wherein the test sample D (isoacteoside) 5mg/kg shows better effects than Aricept 0.75mg/kg.

Example 8: Passive avoidance response tests

[0045] A passive avoidance apparatus is consisted of a light compartment, a dark compartment and a guillotine door between the two compartments.

[0046] Each rat was placed in the light compartment while the guillotine door was open. The rats, which entered into the dark compartment within 90 seconds, were selected to be tested in this experiment.

[0047] On the training trial, each of the selected rats was individually put in the light compartment while the guillotine door was open. Upon entering the dark compartment, the guillotine door was closed, and an electrical shock was given to the rat in the dark compartment through the floor for five seconds. After that, the rat was removed from the dark compartment and was put back into its home cage.

[0048] Twenty four hours after the training trial, the rats were individually put in the light compartment while the guillotine door was open, and the step-through latency (STL) of each rat was recorded.

[0049] Fig. 9 shows the passive avoidance response results of the rats according to Table 2. Referring to Fig. 9, intracerebroventricular infusion of A β 1-42 causes a significant impairment of the passive avoidance learning response for the rats, and the test sample D (isoacteoside) and Aricept reverse the passive avoidance learning impairment of the rats caused by intracerebroventricular infusion of A β 1-42. As shown in Fig. 9, the test sample D (isoacteoside) 2.5 mg/kg shows equivalent effect with Aricept 0.75mg/kg, and the test sample D (isoacteoside) 5 mg/kg shows better effects than Aricept 0.75mg/kg.

Example 9: Water maze tests

[0050] In this example, the water maze pool was divided into four quadrants, and a hidden platform was located in the fourth quadrant and was submerged 1.0 cm below the surface of water. Each rat was given two trials with an interval of four hours per day, two minutes for each trial to find the hidden platform. The first trial was performed for the spatial performance test. Each rat was allowed to swim a maximum of 120 seconds to find the hidden platform. When successful,

the rat was allowed a 30-second rest period on the platform. If unsuccessful within the aborted time period, the rat was given a score of 120 seconds and then put on the platform, allowing a 30-second rest period. The spatial performance tests were performed for four consecutive days. On the fifth day, the hidden platform was taken away from the water maze pool, and each rat was placed in the first quadrant and was given a swimming period of 60 seconds. The time for each rat spent in the quadrant where the hidden platform located was recorded during the probe test.

[0051] Fig. 10 shows results of the spatial performance tests performed by the animals in Table 2, and Fig. 11 shows results of the probe tests performed by the animals in Table 2. Referring to Fig. 10 and Fig. 11, it is shown that deficits of the water maze spatial performance and the probe test caused by A β 1-42 can be improved by the test sample D (isoacteoside) and Aricept, and the test sample D (isoacteoside) 5 mg/kg shows better effects than Aricept 0.75mg/kg.

Example 10: Immunohistochemical staining of rat's brain

[0052] After the water maze performance tests, all rats were decapitated and their brains were rapidly removed from the skull, and then immunohistochemical staining for A β 1-42 in the hippocampus section of their brains. Fig. 12A-E shows immunohistochemical staining results. In Fig. 12B, a number of plaques were found in the brain of the rat continuously infused with A β 1-42. In Fig. 12C and Fig. 12D, few plaques were found in the brain of the rat treated with the test sample D (isoacteoside) 2.5 mg/kg, and almost no plaques were found in the brain of the rat treated with the test sample D (isoacteoside) 5mg/kg. Comparing Fig. 12C to Fig. 12E, it is obvious that the plaques in the brain of the rat treated with the test sample D (isoacteoside) were less than that in the brain of the rat treated with Aricept. According to the results, the test sample D (isoacteoside) is effective in inhibiting A β forming or cleaning plaques from aggregation.

Example 11: Neurotransmitters levels in cortex and hippocampus of rats

[0053] After the water maze performance tests, all rats were decapitated and their brains were rapidly removed from the skull. According to Glowinski and Iversen, the brain cortex and hippocampus were separated, and then each cortex and hippocampus were weighed and were homogenized by addition of 50 mM Na-phosphate buffer (pH 7.8) for analyzing the concentrations of neurotransmitters and the levels of enzymes.

[0054] Table 3 and Table 4 respectively show concentrations of neurotransmitter dopamine (DA) and its metabolites (DOPAC and HVA) in brain cortex and hippocampus of the rats according to Table 2. Referring to Table 3 and Table 4, A β 1-42 decreases DA levels in the cortex and hippocampus of the rats, and the test sample D (isoacteoside) and Aricept reverse the decrease in DA levels in the cortex and hippocampus of rats caused by A β 1-42.

Table 3: DA levels and its metabolites DOPAC and HVA levels in the brain cortex of the rats according to Table 2

	DOPAC	HVA	DA
Sham	16.75 $\pm$ 1.70*	3.71 $\pm$ 0.58	3.92 $\pm$ 0.4**
Vehicle	11.66 $\pm$ 1.13	2.76 $\pm$ 0.48	2.33 $\pm$ 0.28
D 2.5 mg/kg	12.53 $\pm$ 3.33	2.92 $\pm$ 0.58	2.67 $\pm$ 0.43
D 5 mg/kg	13.80 $\pm$ 3.03	2.74 $\pm$ 0.69	3.84 $\pm$ 0.47*
Aricept	10.49 $\pm$ 2.38	2.42 $\pm$ 0.78	3.10 $\pm$ 0.46

Table 4: DA levels and its metabolites DOPAC and HVA levels in the brain hippocampus of the rats according to Table 2

	DOPAC	HVA	DA
Sham	5.87 $\pm$ 0.92	3.68 $\pm$ 0.41	5.36 $\pm$ 0.75***
Vehicle	5.13 $\pm$ 0.80	3.39 $\pm$ 0.36	1.00 $\pm$ 0.21
D 2.5 mg/kg	4.98 $\pm$ 1.15	3.68 $\pm$ 0.46	5.41 $\pm$ 1.30***
D 5 mg/kg	4.13 $\pm$ 0.45	3.29 $\pm$ 0.25	4.07 $\pm$ 0.59***
Aricept	3.19 $\pm$ 1.00	2.45 $\pm$ 0.33	1.33 $\pm$ 0.37

[0055] Fig. 13A shows the levels of neurotransmitter acetylcholine in the brain cortex and hippocampus of the rats according to Table 2, and Fig. 13B shows the levels of choline in the brain cortex and hippocampus of the rats according to Table 2. Referring to Fig 13A and Fig. 13B, A β 1-42 decreases acetylcholine levels in the brain cortex and hippocampus of the rats, and the test sample D (isoacteoside) and Aricept reverse the decrease in acetylcholine levels in the brain cortex and hippocampus of rats caused by A β 1-42, wherein the test sample D (isoacteoside) 5mg/kg is particularly potent.

[0056] Fig. 14A and Fig. 14B show the activities of acetylcholinesterase in the brain cortex and hippocampus of the

rats according to Table 2 respectively. Referring to Fig 14A and Fig. 14B, A β 1-42 increases acetylcholinesterase activities in the brain cortex and hippocampus of the rats, and the test sample D (isoacteoside) and Aricept reverse the increase in acetylcholinesterase activities in the brain cortex and hippocampus of rats caused by A β 1-42. Since

[0057] A β aggregates cause cell damage, and further lead to various diseases or conditions. According to the results from cell studies in Examples 2 to 6, isoacteoside (D) is effective in inhibiting formation, accumulation or aggregation of A β , further preventing A β from forming aggregates, and thus isoacteoside can be used in preventing or treating Alzheimer's disease or other A β -associated diseases or conditions.

[0058] The results from animal studies in Examples 7 to 9 show that isoacteoside possesses significant effects on improving memory or learning deficits induced by A β . According to Example 10, isoacteoside is effective in inhibiting A β forming plaques by aggregation or in cleaning plaques. Besides, according to Example 11, isoacteoside can reverse the decrease in neurotransmitter levels caused by A β 1-42, and therefore it is effective in improving deficits of memory or learning abilities. The above cell and animal studies results indicate that isoacteoside or its equivalent pharmaceutically acceptable salt can be administered to a person in a daily therapeutically effective amount of 0.2 mg/kg to 4 mg/kg (i.e., a recommended dosage of an adult weighted 60 kg of 12 mg - 240 mg), and used in preventing or treating a disease or condition associated with amyloid β peptides in an individual, wherein said disease or condition is mild cognitive impairment, Lewy body dementia, Down syndrome, Hereditary cerebral hemorrhage with amyloid (HCHWA) Dutch, Parkinsonism-dementia complex on Guam, Cerebral amyloid angiopathy, inclusion body myositis, frontotemporal dementia, age-related macular degeneration, or Pick's disease.

Claims

1. Isoacteoside or a pharmaceutically acceptable salt thereof for use in preventing or treating a disease or condition associated with amyloid β peptides in an individual, wherein said disease or condition is mild cognitive impairment, Lewy body dementia, Down syndrome, hereditary cerebral hemorrhage with amyloid (HCHWA) Dutch, Parkinsonism-dementia complex on Guam, Cerebral amyloid angiopathy, inclusion body myositis, frontotemporal dementia, age-related macular degeneration, or Pick's disease.
2. The isoacteoside or a pharmaceutically acceptable salt thereof for use according to claim 1, wherein the isoacteoside or a pharmaceutically acceptable salt thereof is used in a dosage equivalent to 0.2 mg - 4.0 mg of isoacteoside or a pharmaceutically acceptable salt thereof per kg of body weight per day.
3. The isoacteoside or a pharmaceutically acceptable salt thereof for use according to claim 1 or 2 as an additive in food, drinks, chewing gums, patches or skin care products.