Title: COMPOSITION FOR PREVENTION AND TREATMENT OF THE DEMENTIA

Abstract: Disclosed is a composition for prevention and treatment of neurodegenerative diseases associated with learning ability, memory retention and cognitive ability. In particular, disclosed is a composition for the prevention and treatment of dementia comprising a hot-water soluble extract of a herbal mixture (so-called 'Koo Gi Ji Hwang Tang') including steamed Rehmannia Radix, Lycium Fructus, Discocere Rizhoma, Cornus Fructus, Hoelen, Moutan Cortex Radicis and Alismatis Rizhoma.
Published: without international search report and to be republished upon receipt of that report
[DESCRIPTION]

[Invention Title]
COMPOSITION FOR PREVENTION AND TREATMENT OF THE DEMENTIA

5 [Technical Field]
The present invention relates, in general, to a composition for prevention and treatment of neurodegenerative diseases associated with learning ability, powers of memory and cognitive ability and, more particularly, to a composition for the prevention and treatment of dementia comprising a hot-water soluble extract of a herbal mixture (so-called 'Koo Gi Ji Hwang Tang') including Steamed Rehmannia Radix, Lycium Fructus, Discoreae Rhizoma, Cornus Fructus, Hoelen, Moutan Cortex Radicis and Alismatis Rhizoma.

15 [Background Art]
Oriental herbs contain lots of useful substances, which have been used to treat a variety of neurodegenerative diseases, and it has been reported that oriental herbs function to suppress the expression of neurodegenerative diseases.

Also, a lot of research suggests that traditional herbal medicines have the potential to prevent the
pathological consequences of neurodegenerative diseases.

Therefore, many researchers have attempted to treat neurodegenerative diseases, especially dementia, by using such herbal medicines.

Also, the present inventors have made continuous attempts to treat dementia using herbal medicine, and found that, from the fact that an extract, which is obtained by extracting herbs selected from the group consisting of Steamed Rehmannia Radix, Lycium Fructus, Discordae Rhizoma, Cornus Fructus, Hoelen, Moutan Cortex Radicis and Alismatis Rhizoma with alcohol or an aqueous alcohol solution, is effective in improving the memory retention, the herbal alcohol extract may be used to prevent and treat dementia or Alzheimer's disease since memory loss is one of the characteristics of patients suffering from dementia or Alzheimer's disease (Korean Patent No. 491429).

However, memory loss is only one of the characteristics of patients suffering from dementia or Alzheimer’s disease, and it is impossible to prevent and treat dementia by merely improving the memory retention of the patients. Therefore, the present inventors have attempted to develop herbal medicines that are able to treat the underlying causes of dementia.

That is, although the pathological mechanism of how dementia develops is not fully understood, the present inventors have attempted to develop herbal medicine
components that can indirectly suppress the reduction in choline acetyltransferase activity and simultaneously prevent the loss of learning ability, cognitive ability and memory retention from the fact that the activity of choline acetyltransferase (ChAT), which synthesizes acetylcholine, is more reduced in a dementia patient’s brain than in a normal human brain. Then, the present inventors have found that a hot-water soluble extract of an herbal mixture (so-called Koo Gi Ji Hwang Tang), including Steamed Rehmannia Radix, Lycium Fructus, Discocreae Rhizoma, Cornus Fructus, Hoelen, Moutan Cortex Radicis and Alismatis Rhizoma, showed the above-mentioned therapeutic effect. As a result, the present invention was completed on the basis of the above facts.

[Disclosure]

[Technical Problem]

Accordingly, the present invention has been made keeping in mind the above problems occurring in the prior art, and an object of the present invention is to provide a pharmaceutical composition capable of preventing and treating dementia or Alzheimer’s disease by suppressing the loss of learning and cognitive abilities, the failure of memory, and the loss of activity of choline acetyltransferase.

Another object of the present invention is to provide
a health supplement food capable of preventing and treating dementia or Alzheimer's disease.

[Technical Solution]

In order to accomplish the above objects, the present invention provides a pharmaceutical composition for prevention and treatment of dementia comprising a hot-water soluble extract of an herbal mixture as an effective component, wherein the herbal mixture includes Steamed Rehmannia Radix, Lycium Fructus, Discoraeae Rhizoma, Cornus Fructus, Hoelen, Moutan Cortex Radicis, and Alismatis Rhizoma in a weight ratio (w/w) of 30 to 40 : 30 to 40 : 10 to 20 : 10 to 20 : 5 to 10 : 5 to 10 : 5 to 10.

Also, the present invention provides a health supplement for prevention and treatment of dementia comprising a hot-water soluble extract of an herbal mixture and a sitologically acceptable food supplement, wherein the herbal mixture includes Steamed Rehmannia Radix, Lycium Fructus, Discoraeae Rhizoma, Cornus Fructus, Hoelen, Moutan Cortex Radicis, and Alismatis Rhizoma in a weight ratio (w/w) of 30 to 40 : 30 to 40 : 10 to 20 : 10 to 20 : 5 to 10 : 5 to 10.

Hereinafter, preferred embodiments of the present invention will be described in detail referring to the accompanying drawings. Prior to the description, it should be understood that the terms used in the specification and
appended claims should not be construed as limited to
general and dictionary meanings, but should be interpreted
based on the meanings and concepts corresponding to
technical aspects of the present invention on the basis of
the principle that the inventor is allowed to define terms
appropriately to provide the best explanation.

[Advantageous Effects]

According to the present invention, the composition
according to one exemplary embodiment of the present
invention may be useful to be taken as medicine for an
extended time period for the purpose of the prevention of
dementia since the composition includes as an effective
component the herbal medicine extract which has neither
toxicity nor side effects.

Also, the composition according to one exemplary
embodiment of the present invention does not cause toxic
side effects even when people take it as medicine for an
extended time period or take excessive amounts of the
medicine since the composition comprises herbal components
in an effective amount and is formulated into a final
product that may be easily used by the general public.
Therefore, the composition may be useful in preventing and
continuously treating dementia by allowing anyone to take
the medicine as a therapeutic composition or health foods.

Furthermore, the composition according to one
exemplary embodiment of the present invention may be usefully used as a functional food or health food for researchers and the general public since the composition improves learning ability, cognitive ability, memory retention, etc.

[Description of Drawings]

FIG. 1 is a graph illustrating the comparison of cognitive abilities of four groups of rats in a Morris water maze experiment. Here, each maze experiment on each group is conducted 4 times a day for 6 days to calculate an average of moving/swimming time (Efficiency under comparison with corresponding data of normal groups: *p < 0.05; Efficiency under comparison with corresponding data of a TMT-treated group: #p < 0.05; and Efficiency under comparison with corresponding data of a TMT-treated group: ###p < 0.01).

FIG. 2 is a graph illustrating the comparison of cognitive abilities of four groups of rats in a Morris water maze experiment. Here, an average of platform/swimming time per experiment is represented as a percentage (Efficiency under comparison with corresponding data of a TMT-treated group: ###p < 0.001).

FIG. 3 is a photograph illustrating the distribution of ChAT immunoreactive cells in Hippocampus regions, CA1 and CA3, of the normal TMT, TMT+LF and TMT+GJT groups after
surgery has been performed on the rats on the seventh day of the Morris water maze experiment.

FIG. 4 is a photograph illustrating the distribution of PKC immunoreactive cells in Hippocampus regions, CA1 and CA3, of the normal TMT, TMT+LF and TMT+GJT groups after surgery has been performed on the rats on the seventh day of the Morris water maze experiment.

[Best Mode]

Hereinafter, embodiments of the present invention will be described in detail with reference to the accompanying drawings.

The composition according to one exemplary embodiment of the present invention includes, as an effective component, a hot-water soluble extract of an herbal mixture (so-called 'Koo Gi Ji Hwang Tang') including Steamed Rehmannia Radix, Lycium Fructus, Discocerae Rhizoma, Cornus Fructus, Hoelen, Moutan Cortex Radicis and Alismatis Rhizoma. Here, the respective herbal medicine components of the Koo Gi Ji Hwang Tang have the following effects.

That is, Steamed Rehmannia Radix (Rehmannia glutinosa (Graepner)Linderm) has pharmacological effects including a hypoglycemic effect, cardiotonic and diuretic effects on the circulatory system, a hepatoprotective effect and an antibacterial effect, etc.

Lycium Fructus (Lycium chinense MULL.) has a non-
specific immune stimulant effect. Thus, Lycium Fructus significantly enhances the phagocytosis of the reticuloendothelial system, and a regulatory effect. At the same time, a soup obtained by boiling Lycium Fructus has a hematopoietic effect and acts as a biostimulant to highly increase the number of eggs when chickens are fed with the biostimulant. Also, it has pharmacological effects such as hypotensive and hypoglycemic effects, reduction in serum cholesterol level, an anti-fatty liver effect, a growth-stimulating effect, cancer cell proliferation inhibitive activity and an increase in the weight of the uterus, etc.

Discorea Rhizoma (Discorea batatas DSCHE.) has pharmacological effects such as a hypoglycemic effect, excellent harmonic effects in the intestinal tract of the digestive system, and extends the lifespan because of its antioxidative effects, etc.

Cornus Fructus (Cornus officinalis SIEB. et Zucc.) has pharmacological effects such as a hypoglycemic effect, a diuretic effect, an antihypertensive effect, an effect on the suppressions of Staphylococcus sp. and Shigella sp., inhibitive activity on the proliferation of ascites cancer, a lymphoproliferative effect on the immune system, an effect on the inhibition of platelet aggregation, an effect on an increase in contractile force of heart muscle, etc.

Hoelen (Poria cocos(FR.)WOLF) has pharmacological effects such as a diuretic effect, a hypoglycemic effect,
an effect on an increase in contractile force of the heart muscle, an immune stimulant effect, etc.

*Moutan Cortex Radicis* (*Paeonia suffruticosa A. NER)* has pharmacological effects such as alleviation of pain, relief, removal of fever, an anticonvulsive effect, an antiinflammatory effect, an antithrombogenic effect, an antiallergic effect, an effect on the suppression of gastric juice secretion, etc.

*Alismatis Rhizoma* (*Alisma canaliculatum A. Bgr. et BoccH*) has pharmacological effects such as a diuretic effect, an effect on the improvement of tinnitus and worsening eye sight, etc.

The composition for prevention and treatment of dementia according to one exemplary embodiment of the present invention includes a hot-water soluble extract as an effective component, wherein the hot-water soluble extract is prepared by mixing Steamed Rehmannia Radix, Lycium Fructus, Discordae Rhizoma, Cornus Fructus, Hoelen, Moutan Cortex Radicis and Alismatis Rhizoma, each of which has the above-mentioned pharmacological effects, in a weight ratio (w/w) of 30 to 40 : 30 to 40 : 10 to 20 : 10 to 20 : 5 to 10 : 5 to 10 : 5 to 10 and extracting the herbal mixture with hot water.

The method for obtaining a herbal medicine extract according to one exemplary embodiment of the present invention is described in more detail, as follows.
A hot-water soluble extract is prepared by adding impurity-free distilled water, particularly deionized water, to the herbal mixture and extracting the herbal mixture at 80 to 100°C for 1 to 3 hours.

In this case, when the hot-water extraction is conducted at a temperature below 80°C for a time period shorter than 1 hour, the effective components are not easily eluted from the herbal mixture, whereas when the hot-water extraction is conducted at a temperature above 100°C for a time period longer than 3 hours, the effective components may be discomposed. Therefore, it is preferred to conduct the hot-water extraction within the given ranges of temperature and time. Meanwhile, the hot-water extraction may be conducted using reflux extraction or vacuum distillation, and the reflux extraction is preferred.

The herbal medicine extract according to one exemplary embodiment of the present invention may be used in the field of various applications by extracting the herbal mixture with hot water, followed by filtering and concentrating the extract, or freeze-drying the concentrated extract.

The present invention provides a pharmaceutical composition including, as an effective component, the herbal medicine extract prepared according to the method, wherein the composition is effective in preventing and
treating diseases such as dementia.

The composition according to one exemplary embodiment of the present invention preferably includes 0.01 to 99.9% by weight, and more preferably 0.1 to 50% by weight of the herbal medicine extract. However, the content of the herbal medicine extract may be varied according to the condition of the patient, the severity of disease, and the kind and progress of disease, but the present invention is not particularly limited thereto.

The composition comprising the herbal medicine extract according to one exemplary embodiment of the present invention may further include a suitable carrier, vehicle and diluent, all of which are widely used in the preparation of pharmaceutical compositions.

The composition according to one exemplary embodiment of the present invention may be formulated into oral formulations such as powders, granules, tablets, capsules, a suspension, an emulsion, a syrup and an aerosol, and external preparations, and also may be formulated in the form of a suppository and sterile injection. The carrier, vehicle and diluent that may be included in the composition comprising the herbal medicine extract includes, but is not particularly limited to, lactose, dextrose, sucrose, sorbitol, mannitol, xylitol, erythritol, maltitol, starch, acacia gum, alginate, gelatin, calcium phosphate, calcium silicate, cellulose, methyl cellulose, microcrystalline
cellulose, polyvinyl pyrrolidone, water, methyl hydroxybenzoate, propyl hydroxybenzoate, talc, magnesium stearate and mineral oils. When the composition is formulated, the diluent or vehicle such as a filler, an extender, a binder, a wetting agent, a disintegrant and a surfactant is generally used. A solid formulation for oral delivery includes a tablet, a pill, powders, granules, a capsule, and the like, and the solid formulation is prepared by mixing at least one vehicle, for example starch, calcium carbonate, sucrose or lactose and gelatin, with the hot-water soluble extract. In addition to the simple vehicles, lubricants such as magnesium, styrate and talc are also used herein.

A liquid formulation for oral delivery includes a suspension, a solution, an emulsion, a syrup, etc. In addition to the generally used diluent (i.e. water, liquid paraffin) a variety of vehicles, for example a wetting agent, a sweetener, an aromatic, a preservative and the like may be included in the liquid formulation.

A formulation for parenteral delivery includes a sterile aqueous solution, an insoluble solvent, a suspension, an emulsion, a freeze dryer, a suppository, etc. Vegetable oils such as propylene glycol, polyethylene glycol and olive oil, and injectable ester such as ethyl oleate may be used as the insoluble solvent and the suspension, respectively. Basic materials of the
suppository that may be used herein comprise witepsol, macrogol, Tween 61, cacao butter, sevum laurinum, glycerogelatin, etc.

A composition may, for example, be administered in a dose equal to or 2, 3 or 4 times higher than the single dosage, or be administered in a dose 1/2, 1/3 or 1/4 times lower than the single dosage. Preferably, the single dosage refers to a dose in which an effective drug is administered once. In general, the single dosage corresponds to the equivalent dose, or a dose 1/2, 1/3 or 1/4 times lower than the daily dosage.

The desirable dose of the composition of the present invention may be suitably varied according to the condition and body weight of a patient, the severity of disease, the dosage form, the route and time of administration, but may be easily selected by those skilled in the art. However, to show desirable effects, the extract of the present invention is administered daily in a dose of 100 to 800 mg/kg, and preferably 100 to 600mg/kg. The administration may be conducted once or twice a day, or may be conducted several times, and preferably 1 to 6 times.

The herbal medicine extract of the present invention is orally and intraperitoneally administered, and subcutaneously injected to white rats to conduct a toxicity test. As a result, when the herbal medicine extract is intraperitoneally administered in a dose of at least 5
g/kg, the herbal medicine extract is proven to be safe at the minimum lethal dose (MLD) in the toxicity test.

Also, the present invention provides a health supplement for the treatment of dementia comprising the herbal medicine extract as an effective component. When the hot-water soluble extract of the present invention is used in food, the hot-water soluble extract may be used in the food, alone or in combinations with other food or food components. In this case, the hot-water soluble extract may be suitably used according to the conventional methods. Amount of mixed effective components may be suitably determined according to the reason for its use (preventions, health or therapeutic procedures). In general, in the preparation of foods or beverages, the extract of the present invention may be added in a content of 0.01 to 1 % by weight, and preferably 0.2 to 0.6 % by weight, based on the total weight of the extract materials. The extract of the present invention may be used in an effective dose, based on the effective dose of the pharmaceutical composition, but may be used in a dose lower than the dose range when patients are fed for an extended time period for the purpose of health and hygiene or for the purpose of health management. Also, the effective components may be used in a dose greater than the dose range since it is safe and is without any side effects.

There is no particular limitation on the kinds of the
food. Examples of the food to which the effective components may be added include, but are not particularly limited to, meat, sausage, bread, chocolate, candy, snacks, confectionery, pizza, instant noodles, other noodles, gum, dairy products including ice cream, various soups, beverages, teas, drinks, alcoholic drinks and vitamin cocktails. Also, the foods include folk remedies such as an antianaeemic agent, medicine to aid virility, a skin whitening agent and the like for the purpose of the above effects. Also, a variety of oriental medical remedies such as Choung Sim Gang Hwa Tang may also be used herein.

**[Mode for Invention]**

A better understanding of the present invention may be obtained through the following examples which are set forth to illustrate, but are not to be construed as the limit of the present invention.

**EXAMPLES**

**EXAMPLE 1: Preparation of Koo Gi Ji Hwang Tang Extract**

600 g of a powdered mixture of Steamed *Rehmannia Radix*, *Lycium Fructus*, *Discoreae Rhizoma*, *Cornus Fructus*, *Hoelen*, *Moutan Cortex Radicis* and *Alismatis Rhizoma*, which are mixed in a weight ratio as listed in the following
Table 1, was put into a flask with 1ℓ of deionized water, and extracted with hot water at 100°C for 1 hour under a reflux condition. The resulting powder mixture was filtered through gauze to obtain filtrate. The filtrate was concentrated through a vacuum filter (Eyela, Japan), and then freeze-dried to prepare an extract of a herbal mixture (referred to as ‘Koo Gi Ji Hwang Tang’). As a result, the dried extract was obtained in a yield of 120 g.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Content (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steamed Rehmannia Radix (Korea)</td>
<td>16</td>
</tr>
<tr>
<td>Lycium Fructus (Korea)</td>
<td>16</td>
</tr>
<tr>
<td>Discoreae Rhizoma (Korea)</td>
<td>8</td>
</tr>
<tr>
<td>Cornus Fuctus (Korea)</td>
<td>8</td>
</tr>
<tr>
<td>Hoelen (Korea)</td>
<td>4</td>
</tr>
<tr>
<td>Moutan Cortex Radicis (Korea)</td>
<td>4</td>
</tr>
<tr>
<td>Alismatis Rhizoma (Korea)</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
</tr>
</tbody>
</table>

REFERENCE EXAMPLE 1: Preparation for Experiment

<1-1> Preparation for animal test

Male Sprague-Dawley rats weighing 250 to 280g were used. These laboratory animals were purchased from SAMTAKO INC. (kyunggi-do, Korea). Before the experiment, the laboratory animals were adapted to a laboratory environment for at least one week. The laboratory animals were raised in private cages under light-controlled conditions (12/12-
hr light/dark cycles). The temperature in a breeding room was maintained to 23°C, and the laboratory animals were fed with animal feed and water without restrictions.

<1-2> Drug treatment

The laboratory animals were divided into four groups, and each group was treated with drugs.

(1) Normal group - a group that is not treated in any way with a drug. Hereinafter, this group is referred to as "Normal" or "Nor."

(2) TMT (Trimethyltin)-treated group - a group in which 6.0 mg/kg of TMT is dissolved in 0.9% saline and intraperitoneally administered to laboratory animals. Hereinafter, this group is referred to as "TMT" or "TMT+CON."

(3) TMT/herbal mixture extract-treated group - a group in which 6.0 mg/kg of TMT is dissolved in 0.9% saline and intraperitoneally administered to laboratory animals, the laboratory animals return to their own cages, and 400 mg/kg of the herbal mixture extract is dissolved in saline and orally administered to the laboratory animals for 2 weeks. Hereinafter, this group is referred to as "TMT+GJT."

(4) TMT/Lycium Fructus-treated group - a group that is treated in the same manner as in the TMT+GJT group, except that Lycium Fructus is used instead of the herbal mixture extract. Hereinafter, this group is referred to as
"TMT+LF."

EXPERIMENTAL EXAMPLE 1: Measurement of Spatial Learning Ability and Memory Retention Using a Morris Water Maze

A Morris water maze is a round water bath made of fiberglass-stacked material. The Morris water maze is painted white, and has a diameter of 200 cm and a height of 35 cm. Here, the water maze was filled with water up to a height of 21 cm, and the water temperature was maintained to 23±2°C. The water bath was divided into four quadrant regions in all directions at the same distance from the girth of the water bath. During the water maze test, a transparent escape platform with a diameter of 15 cm and a height of 20 cm was located apart at a distance of approximately 50 cm from a wall of the water bath, and positioned at a distance of 1.5 cm below the water level in the center of the northeast quadrant region. In order to check the movement of animals, marks that show directions in which the animals move were indicated outside the water bath. The marks remained intact during the water maze test. A CCD camera was installed in the wall positioned right above the water bath, and then connected to a video recorder and a tracking device (SMART; Pan-Lab, Barcelona, Spain) to automatically record the migrating route of the laboratory animals to a computer.
From the 15th day after TMT was administered, the laboratory animals were trained four times a day for 6 days so as to find an escape platform, and each test was conducted three times. After the escape platform was removed on the 7th day of the administration of TMT, each of the laboratory animals was subject to the water maze test to find the escape platform. During the water maze test, the laboratory animals were trained to find the hidden escape platform as described above. Each experiment to find an escape platform continued for up to 180 seconds, and the number of times when the laboratory animals found the escape platform under water were recorded. The results are shown in FIG. 1.

As shown in FIG. 1, it was revealed that the number of times when the laboratory animals found the escape platform was significantly reduced during the 6-week training period. However, the TMT-treated group has a very significant difference in time, compared to the normal group. This indicates that TMT damages memory power of the rats by destroying their cholinergic neurons. However, the TMT/herbal medicine extract-treated group had no significant time difference with respect to the normal group, but had a significant time difference with respect to the TMT-treated group for the time period from the 1st to the 5th day.

From the experimental results, it is obvious that the
memory power of the rats was recovered since use of the cholinergic neurons damaged by TMT was recouped by the administration of the composition of the present invention.

Meanwhile, laboratory animals were trained four times a day for 6 days under the same conditions as in the above-mentioned water maze test. Then, the escape platform was removed from the water bath on the 7th day of the administration, and the laboratory animals were set free in an opposite direction of the escape platform. Then, the time that the laboratory animals stayed in the quadrant region including the escape platform, and the other quadrant regions was measured for a total of 60 seconds. Then, the time that the laboratory animals stayed in each of the quadrant regions was calculated as a percentage of the total time. The results are shown in FIG. 2.

From the results as shown in FIG. 2, it was revealed that, when compared to the normal group, the TMT/herbal medicine extract-treated group showed no significant difference in the total percentage of the time that the laboratory animals stayed in the quadrant region including the escape platform, as measured on the day when the escape platform was removed on the 7th day of the administration and the water maze test was carried out. Also, it was revealed that the TMT/herbal medicine extract-treated group showed significant difference in the total percentage of time in respect to the TMT-treated group.
From the experimental results, it was revealed that the memory retention of the rats was recouped since the cholinergic neurons damaged by TMT were recovered by the administration of the composition of the present invention.

EXPERIMENTAL EXAMPLE 2: Test for Neurochemical Changes in Dementia Animal Model

A Hippocampus region of each rat was subject to choline acetyltransferase (ChAT) and protein kinase (PKC) immunohistochemical staining to observe cholinergic neurons and down-stream signal transduction-associated proteins.

That is, the laboratory animals were anesthetized with sodium pentobarbital (100mg/kg, i.p.) right after all the behavior tests were completed, and a 4% formalin fixative was perfused through the hearts of the rats, their brains were taken out, and fixed with the same fixative for 2 to 3 hours. Each extracted brain tissue was cut to a thickness of 30 μm along an inner middle region of dorsal and ventral Hippocampus by using a microtome (Leica, CM1850, Germany), and cultured with primary sheep polyclonal ChAT antibody and PKC antibody (Cambridge Research Biochemicals, Wilmington, DE, USA) at 4°C for 72 hours while being stirred continuously.

Then, the brain tissue was washed three times with PBST, and reacted at room temperature for 2 hours with
biotinylated anti-sheep serum (Vector Laboratories, Burlingame, CA, USA), which was diluted 200 times, in PBST supplemented with 2% rabbit serum. Then, the brain tissue was dipped in a Vectastain Elite ABC reagent (Vector) at room temperature for 2 hours, washed several times with PBS, strengthened with nickel chloride, and then developed using diaminobenzidine (DAB) as a coloring agent. Each brain tissue undergoing all the above-mentioned procedures was fixed on a gelatin-coated slide, covered with a cover glass while air was being removed from the gelatin-coated slide, and then observed under the microscope. The nerve cells of the ChAT- and PKC-immunoreactive nerve cell in the Hippocampus were counted at 200× magnifications using a microscope rectangle grid measuring 200 × 200 μm. The results are shown in FIGS. 3 and 4.

From the experimental results as shown in FIG. 3, it was revealed that ChAT was significantly recovered in the TMT+GJT group, compared to the TMT-treated group. Therefore, it was revealed that the rat cholinergic neurons damaged by TMT were recovered by the administration of the composition according to one exemplary embodiment of the present invention.

Also from the experimental results as shown in FIG. 4, it was revealed that PKC was significantly recovered in the TMT+GJT group, compared to the TMT-treated group. Therefore, it was revealed that the rat cholinergic neurons
damaged by TMT were recovered by the administration of the composition according to one exemplary embodiment of the present invention.

Form the experimental results of Experimental examples, it was revealed that the TMT treatment reduces the ChAT and PKC activities in the Hippocampal CA1 and CA2 regions. However, it was also revealed that the administration of the composition of the present invention compensates for the ChAT and PKC activities in the Hippocampal CA1 and CA2 regions by recovering the cholinergic neurons. In the water maze test which is an animal behavior test, the time it takes the laboratory animals to find an escape platform was significantly reduced for the time period from the 1st to the 5th day among the total 6 days. When the time the laboratory animals stayed in the quadrant region including the escape platform was measured after the removal of the escape platform on the 7th day of the administration, it was revealed that the time the laboratory animals stayed in the quadrant region was significantly increased in the TMT+GJT group, compared to the TMT-treated group. These experimental results indicate that the spatial learning ability and working memory retention of the laboratory animals in the above-mentioned water maze test were recovered since the cholinergic neurons in the Hippocampal regions damaged by TMT were recovered by the administration of the composition.
according to one exemplary embodiment of the present invention, which leads to the recovered activities of the ChAT and PKC as representative activity indexes. Therefore, the composition according to one exemplary embodiment of the present invention may be used to prevent and treat dementia since it recovers the impaired learning ability, memory power and cognitive ability of dementia patients.

**PREPARATION EXAMPLE 1: Preparation of Tablets**

100.0 mg of the extract of the herbal mixture prepared in Example 1, 90.0 mg of corn starch, 180 mg of lactose, 18.0 mg of L-hydroxypropyl cellulose, 5.0 mg of polyvinylpyrrolidone and a suitable amount of ethanol were homogeneously mixed, and the resulting mixture was granulated using a wet granulation method. Then, 1.8 mg of magnesium stearate was mixed with the granulated mixture, and the resulting granulated mixture was formulated into tablets (content: 600 mg per tablet).

**PREPARATION EXAMPLE 2: Preparation of Soft Capsules**

100.0 mg of the extract of the herbal mixture prepared in Example 1, 180.0 mg of bean oil, 40.0 mg of yellow wax, 128.0 mg of hydrogenated coconut palm oil, 20.5 mg of soybean phospholipid, 212.0 mg of gelatin, 50.0 mg of
glycerin (specific gravity: 1.24), 76.0 mg of d-sorbitol, 0.54 mg of methyl parahydroxybenzoate, 0.90 mg of propyl parahydroxybenzoate, 0.56 mg of methyl vanillin and a suitable amount of Yellow 203 were homogeneously mixed, and the resulting mixture was formulated into capsules according to the general provisions of the Korean Pharmacopeia (KP).

PREPARATION EXAMPLE 3: Preparation of Capsules

100.0 mg of the extract of the herbal mixture prepared in Example 1, 83.0 mg of corn starch, 175.0 mg of lactose and 2.0 mg of magnesium stearate were homogeneously mixed, and the resulting mixture was formulated into capsules so that one capsule could be filled with 360 mg of the herbal mixture.

PREPARATION EXAMPLE 4: Preparation of Food and Beverages

A food or beverage composition comprising as an effective component the herbal mixture prepared in Example 1 was prepared, as follows.

<4-1> Preparation of chewing gum

0.24 to 0.64% by weight of the herbal mixture prepared in Example 1, 20% by weight of gum base, 76.36 to
76.76% by weight of sugar, 1% by weight of fruit flavor and 2% by weight of water were homogeneously mixed, and the resulting mixture was formulated into chewing gum by using one of the conventional methods.

<4-2> Preparation of ice cream

0.24 to 0.64% by weight of the herbal mixture prepared in Example 1, 10.0% by weight of milk fat, 10.8% by weight of solid-not fat (SNF), 12.0% by weight of sugar, 3.0% by weight of starch syrup, 0.5% by weight of a stabilizer (span), 0.15% by weight of an aromatic (strawberry) and 63.31 to 62.91% by weight of water were homogeneously mixed, and the resulting mixture was formulated into ice cream by using one of the conventional methods.

<4-3> Preparation of a beverage

0.48 to 1.28 mg of the herbal mixture prepared in Example 1, 522 mg of honey, 5 mg of thiocytic acid amide, 10 mg of nicotinamide, 3 mg of riboflavin sodium hydrochloride, 2 mg of pyridoxine hydrochloride, 30 mg of inositol, 50 mg of orotic acid and 200 ml of water were homogeneously mixed, and the resulting mixture was formulated into a beverage by using one of the conventional methods.

Although the preferred embodiments of the present
invention have been disclosed for illustrative purposes, those skilled in the art will appreciate that various modifications, additions and substitutions are possible, without departing from the scope and spirit of the invention as disclosed in the accompanying claims.
[CLAIMS]

[Claim 1]

A pharmaceutical composition for prevention and treatment of dementia, comprising a hot-water soluble extract of a herbal mixture as an effective component, wherein the herbal mixture comprises Steamed *Rehmannia Radix*, *Lycium Fructus*, *Discoreae Rhizoma*, *Cornus Fructus*, *Hoelen*, *Moutan Cortex Radicis*, and *Alismatis Rhizoma* in a weight ratio (w/w) of 30 to 40 : 30 to 40 : 10 to 20 : 10 to 20 : 5 to 10 : 5 to 10 : 5 to 10.

[Claim 2]

The pharmaceutical composition according to claim 1, wherein the hot-water soluble extract of the herbal mixture comprises an extract prepared by adding water to the herbal mixture and extracting the herbal mixture at 80 to 100°C for 1 to 3 hours.

[Claim 3]

The pharmaceutical composition according to claim 1 or 2, wherein the composition is formulated in the form of a tablet, a pill, an extract, a solution, a powder, granules, an infusion, a capsule or an injectable solution.
[Claim 4]

A health supplement for prevention and treatment of dementia comprising a hot-water soluble extract of a herbal mixture and a sitologically acceptable food supplement, wherein the herbal mixture comprises steamed *Rehmannia Radix*, *Lycium Fructus*, *Discordiae Rhizoma*, *Cornus Fructus*, *Hoelen*, *Moutan Cortex Radicis*, and *Alismatis Rhizoma* in a weight ratio (w/w) of 30 to 40 : 30 to 40 : 10 to 20 : 10 to 20 : 5 to 10 : 5 to 10 : 5 to 10.

[Claim 5]

The health supplement according to claim 4, wherein the hot-water soluble extract of the herbal mixture comprises an extract prepared by adding water to the herbal mixture and extracting the herbal mixture at 80 to 100°C for 1 to 3 hours.

[Claim 6]

The health supplement according to claim 4 or 5, wherein the health supplement is formulated in gum, emulsion, confectionery beverage or tea form.
[Fig. 4]

NOR

TMT-CON

TMT-LF

TMT-GJT