

PHARMACEUTICAL COMPOSITION FOR TREATING INSOMNIA AND PREPARATION METHOD THEREOF

Field of the invention

[01] The present invention belongs to the field of Chinese herbals, relates to a pharmaceutical composition and preparation method thereof, wherein said pharmaceutical composition comprises Radix Polygoni Multiflori and/or extracts thereof, Semen Ziziphi Spinosae and/or extracts thereof and Fructus Mori and/or extracts thereof, and is useful in the treatment of insomnia.

Background of the invention

[02] Insomnia is a dissatisfying condition in terms of the quality and quantity of sleep which continues for quite a long time. Insomnia is a clinically common and frequently-occurring disease, and the clinical symptoms thereof manifest difficulty in falling asleep, ready to wake up from sleep, unable to sleep again after waking up, waking up too early in the morning, broken sleep, unable to sleep all night long, no-deep sleep or loss of sleep feeling, and may be accompanied with dreaminess. In addition, insomnia can induce discomfort after waking up, fatigue, reduction in efficiency of psychic activity or interference with social functions. It is suggested that about 20% of people at the age of 20 suffer from sleep disorder; which increases to 50% at the age of 55 and to 90% at the age of 80. As is indicated by the latest statistic data, more than 42.5% of people in China suffer from sleep disorder to different degrees; the morbidity of insomnia is up to 10%-20%. The results of "Sanofi-Aventis Sleep Survey" conducted by the Branch Society of Psychiatry, Chinese Medical Association in 2002 indicated that 25.9% people consider themselves suffering from sleep disorder, which started 2 years (median) ago. However, it is worth noting that, according to the athene scale, 45.4% of people do not have ideal quality of sleep, wherein 28% belongs to insomnia, and 17.4% belongs to suspicious insomnia (Xiaohong ZHANG, China Medical Tribune, October 10, 2002, Issue 831).

[03] In recent years, with the continuous improvement of living standards of the people and the increase of social competition pressures, especially the overwrought working and study, the morbidity of the disease has been on the rise, which seriously affects the living, working and study qualities of the patients and has caused adverse effects on the working, study and the social communication. 45% of traffic accidents are related to sleep insufficiency, 50% of work injuries are related to sleep insufficiency, and the risk of accident for a chronic insomniac is 4.5 times as that of a normal person (Da JING, China Medical Tribune, March 20, 2003, Issue 853).

[04] With the accelerated pace of life and increased working pressure, there are more and more patients with insomnia, and there is a more and more urgent requirement for effectively preventing and treating this disease. Studies in recent years show that the efficacy of sedative alone is poor, especially when the toxic and side effects of chemically synthesized drugs have become increasingly evident, and the dependence of human body, withdrawal symptom and rebound phenomenon have also caused widespread concern of medical practitioners and patients.

[05] There is a long history for traditional Chinese medicine to treat insomnia. Based on holistic conception and syndrome differentiation and treatment, the efficacy of traditional Chinese medicine in treatment of insomnia, is definite and lasting with favorable safety, as well as satisfactory patient compliance. Thus, people seek to prevent and treat insomnia under the guidance of the theory of traditional Chinese medicine, and the demand for treating insomnia with a medicament prepared from natural traditional Chinese materia medica is also rising.

Summary of the invention

[06] In one aspect, the present invention provides a pharmaceutical composition, comprising Radix Polygoni Multiflori and/or extracts thereof, Semen Ziziphi Spinosae and/or extracts thereof, and Fructus Mori and/or extracts thereof.

[07] In some embodiments, the pharmaceutical composition of the present invention further comprises Ganoderma and/or extracts thereof, Bulbus Lilii and/or extracts thereof, Rhizoma Anemarrhenae and/or extracts thereof, Radix Salviae Miltiorrhizae and/or extracts thereof, Flos Chrysanthemi and/or extracts thereof, and Poria and/or extracts thereof.

[08] In some embodiments, the pharmaceutical composition of the present invention further comprises Flos Albiziae and/or extracts thereof.

[09] In some embodiments, said extract is each independently solvent extract. Said solvent may be any suitable solvent, e.g. water, ethanol, aqueous ethanol solution, supercritical carbon dioxide, or any mixture thereof.

[10] In some embodiments, said pharmaceutical composition is prepared from crude medicines of the following ratios by weight parts: Radix Polygoni Multiflori 150-270, Semen Ziziphi Spinosae 145-275, and Fructus Mori 160-255.

[11] In some embodiments, said pharmaceutical composition is prepared from crude medicines of the following ratios by weight parts: Radix Polygoni Multiflori 150-270, Semen Ziziphi Spinosae 145-275, Fructus Mori 160-255, Ganoderma 80-135, Bulbus Lilii 75-160, Rhizoma Anemarrhenae 50-110, Radix Salviae Miltiorrhizae 120-200, Flos Chrysanthemi 45-120, and Poria 75-145.

[12] In some embodiments, said pharmaceutical composition is prepared from crude medicines of the following ratios by weight parts: Radix Polygoni Multiflori 150-270, Semen Ziziphi Spinosae 145-275, Fructus Mori 160-255, Ganoderma 80-135, Bulbus Lilii 75-160, Rhizoma Anemarrhenae 50-110, Radix Salviae Miltiorrhizae 120-200, Flos Chrysanthemi 45-120, Poria 75-145, and Flos Albiziae 165-288.

[13] In some embodiments, the ratios by weight parts of the crude medicines are: Radix Polygoni Multiflori 158, Semen Ziziphi Spinosae 270, Fructus Mori 165, Ganoderma 135, Bulbus Lilii 80, Rhizoma Anemarrhenae 110, Radix Salviae Miltiorrhizae 125, Flos Chrysanthemi 118, Poria 80, and Flos Albiziae 280.

[14] In some embodiments, the ratios by weight parts of the crude medicines are: Radix

Polygoni Multiflori 265, Semen Ziziphi Spinosae 147, Fructus Mori 250, Ganoderma 81, Bulbus Lilii 158, Rhizoma Anemarrhenae 55, Radix Salviae Miltiorrhizae 188, Flos Chrysanthemi 50, Poria 145, and Flos Albiziae 170.

[15] In some embodiments, the ratios by weight parts of the crude medicines are: Radix Polygoni Multiflori 225, Semen Ziziphi Spinosae 163, Fructus Mori 220, Ganoderma 83, Bulbus Lilii 113, Rhizoma Anemarrhenae 50, Radix Salviae Miltiorrhizae 190, Flos Chrysanthemi 45, Poria 113, and Flos Albiziae 178.

[16] In some embodiments, the ratios by weight parts of the crude medicines are: Radix Polygoni Multiflori 165, Semen Ziziphi Spinosae 228, Fructus Mori 173, Ganoderma 110, Bulbus Lilii 75, Rhizoma Anemarrhenae 75, Radix Salviae Miltiorrhizae 150, Flos Chrysanthemi 78, Poria 78, and Flos Albiziae 218.

[17] In some embodiments, the ratios by weight parts of the crude medicines are: Radix Polygoni Multiflori 200, Semen Ziziphi Spinosae 200, Fructus Mori 200, Ganoderma 100, Bulbus Lilii 100, Rhizoma Anemarrhenae 66.7, Radix Salviae Miltiorrhizae 166.7, Flos Chrysanthemi 66.7, Poria 100, and Flos Albiziae 200.

[18] In some embodiments, each gram of the pharmaceutical composition contains not less than 0.5 mg, preferably not less than 1.0 mg, more preferably not less than 1.5 mg 2,3,5,4'-tetrahydroxydiphenylethene-2-O- β -D-glucoside (mainly derived from Radix Polygoni Multiflori or extracts thereof), based on the dry weight of the pharmaceutical composition.

[19] In some embodiments, the dosage form of the pharmaceutical composition is capsule, tablet, powder, oral solution, soft capsule, pill, tincture, syrup, suppository, gel, spray or injection.

[20] In some embodiments, the method for preparing the pharmaceutical composition comprises preparing one or more of the crude medicines separately or collectively into extract.

[21] In some embodiments, the method for preparing the pharmaceutical composition comprises: crude medicines are weighed according to the ratios by weight parts, soaked for 20-40 minutes by addition of water, decocted for 30-40 minutes after boiling, filtered, and again decocted for 25-30 minutes after addition of water and boiling, filtered, and the two filtrates are combined.

[22] In some embodiments, the method for preparing the pharmaceutical composition comprises the following steps:

- a) preparing alcohol extract with Radix Polygoni Multiflori, Rhizoma Anemarrhenae and Semen Ziziphi Spinosae;
- b) preparing aqueous extract with Fructus Mori, Poria, Ganoderma, Radix Salviae Miltiorrhizae, Flos Albiziae and Flos Chrysanthemi;
- c) preparing powder with Bulbus Lilii;
- d) using the alcohol extract of step a), the aqueous extract of step b) and the powder of Bulbus Lilii of step c) as the active ingredients of the pharmaceutical

composition.

[23] In some embodiments, the method for preparing the pharmaceutical composition comprises the following steps:

- a) Radix Polygoni Multiflori, Rhizoma Anemarrhenae and Semen Ziziphi Spinosae are weighed according to the ratios by weight parts, 6-12 times amount of 30%-70% ethanol is added; heat under reflux is performed for 1-3 times, with 1-3 hours each time; the extract is filtered, combined with ethanol recovered under reduced pressure, and concentrated to a relative density of 1.10-1.15 determined at 60°C, to obtain alcohol extract;
- b) Fructus Mori, Poria, Ganoderma, Radix Salviae Miltiorrhizae, Flos Albiziae, and Flos Chrysanthemi are weighed according to the ratios by weight parts, 10-15 times amount of water is added to extract for 1-3 times, with 1-3 hours each time; the extract is filtered, combined, and concentrated to a relative density of about 1.10-1.15 determined at 60°C, to obtain aqueous extract;
- c) Bulbus Lilii is weighed according to the ratio by weight parts, and crushed into 100 mesh powder of Bulbus Lilii;
- d) the alcohol extract of step a), the aqueous extract of step b) and the powder of Bulbus Lilii of step c) are used as the active ingredients of the pharmaceutical composition.

[24] The active ingredients can be prepared into desired dosage forms together with optional pharmaceutical adjuvants.

[25] The dosage form of the pharmaceutical composition according to the present invention can be any suitable dosage form, e.g. capsule, tablet, powder, oral solution, soft capsule, pill, tincture, syrup, suppository, gel, spray or injection.

[26] To achieve the above-mentioned dosage forms, pharmaceutically acceptable adjuvants can be added during the preparation of these dosage forms, for example: filler, disintegrant, lubricant, suspending agent, adhesive, sweetener, flavoring agent, preservative and the like. Fillers include starch, pregelatinised starch, lactose, mannitol, chitin, microcrystalline cellulose, sucrose and the like. Disintegrants include starch, pregelatinised starch, microcrystalline cellulose, sodium carboxymethyl starch, crospolyvinylpyrrolidone, low substituted hydroxypropyl cellulose, cross-linked sodium carboxymethyl cellulose and the like. Lubricants include magnesium stearate, sodium dodecyl sulfate, talc powder, silicon dioxide and the like. Suspending agents include polyvinylpyrrolidone, microcrystalline cellulose, sucrose, agar, hydroxypropyl methyl cellulose and the like. Adhesives include starch slurry, polyvinylpyrrolidone, hydroxypropyl methyl cellulose and the like. Sweeteners include saccharin sodium, aspartame, sucrose, sodium cyclamate, glycyrrhethinic acid and the like. Flavoring agents include sweetener and various essences. Preservatives include parabens, benzoic acid, sodium benzoate, sorbic acid and salts thereof, benzalkonium bromide, chlorhexidine acetate, eucalyptus oil and the like.

[27] The pharmaceutical composition according to the present invention can be prepared by various methods. Such preparation methods are well known to those ordinary skilled in

the art, and are taught in various technical documents, Remington's Pharmaceutical Manual etc., for example, can be read for reference. These methods include regular technologies for preparations, e.g. mixing, dissolving, emulsifying, suspending, etc.

[28] The preparation method of the tablet of the pharmaceutical composition according to the present invention comprises the following steps:

- a) selected and cleaned Radix Polygoni Multiflori, Rhizoma Anemarrhenae and coarsely crushed Semen Ziziphi Spinosae are weighed according to the ratios, 6-12 times amount of 30%-70% ethanol is added; heat under reflux is performed for 1-3 times for extraction, with 1-3 hours each time; the extract is filtered, combined with ethanol recovered under reduced pressure, and concentrated to a relative density of 1.10-1.15 determined at 60°C, to obtain alcohol extract, ready for use;
- b) selected and cleaned Fructus Mori, Poria, Ganoderma, Radix Salviae Miltiorrhizae, Flos Albiziae and Flos Chrysanthemi are weighed according to the ratios, 10-15 times amount of water is added to extract for 1-3 times, with 1-3 hours each time; the extract is filtered, combined, and concentrated to a relative density of about 1.10-1.15 determined at 60°C, to obtain aqueous extract, ready for use;
- c) selected and cleaned Bulbus Lili is weighed according to the ratios, and crushed into powder of 100 mesh, ready for use;
- d) the alcohol extract obtained in step a), the aqueous extract of step b) and the fine powder of Bulbus Lili obtained in step c) are subjected to dry granulation; appropriate adjuvants are added after granule sizing; mixing, tableting and coating are performed.

[29] The preparation method of the tablet of the pharmaceutical composition according to the present invention preferably comprises the following steps:

- a) selected and cleaned Radix Polygoni Multiflori, Rhizoma Anemarrhenae and coarsely crushed Semen Ziziphi Spinosae are weighed according to the ratios, and 60% ethanol is added; heat under reflux is performed twice for extraction, with the first time adding 10 times amount and extracting for 2 hours and the second time adding 8 times amount and extracting for 1 hour; the extract is filtered, combined with ethanol recovered under reduced pressure, and concentrated to a relative density of 1.10-1.15 determined at 60°C, to obtain alcohol extract, ready for use;
- b) selected and cleaned Fructus Mori, Poria, Ganoderma, Radix Salviae Miltiorrhizae, Flos Albiziae and Flos Chrysanthemi are weighed according the ratios, 11 times amount of water is added to extract twice, with 1.5 hours each time; the extract is filtered, combined, and concentrated to a relative density of about 1.10-1.15 determined at 60°C, to obtain aqueous extract, ready for use;
- c) selected and cleaned Bulbus Lili is weighed according to the ratios, and crushed into powder of 100 mesh, ready for use;
- d) the fine powder of Bulbus Lili obtained in step c) is added into the container for raw materials of the spraying drier granulator, the alcohol extract obtained in step a) and the aqueous extract of step b) are sprayed in for spraying granulation; 0.5% magnesium stearate is added after granule sizing; mixing, tableting and coating are performed.

[30] The preparation method of the decoction of the pharmaceutical composition according to the present invention comprises: Radix Polygoni Multiflori, Semen Ziziphi Spinosae, Fructus Mori, Ganoderma, Bulbus Lilii, Rhizoma Anemarrhenae, Radix Salviae Miltiorrhizae, Flos Chrysanthemi, Poria, and Flos Albiziae are weighed according to the ratios, and placed in a boiler; water is added for the first time till 3-5 cm over the drug top surface; the crude medicines are soaked for 20-40 minutes, decocted for 30-40 minutes after boiling, and filtered; water is added again till 1-3 cm over the drug top surface; the crude medicines are decocted for 25-30 minutes after boiling, filtered, and the two filtrates are combined.

[31] The preparation method of the watered pill of the pharmaceutical composition according to the present invention comprises:

- a) selected and cleaned Radix Polygoni Multiflori, Rhizoma Anemarrhenae and coarsely crushed Semen Ziziphi Spinosae are weighed according to the ratios, 6-12 times amount of 30%-70% ethanol is added; heat under reflux is performed for 1-3 times for exaction, with 1-3 hours each time; the extract is filtered, combined with ethanol recovered under reduced pressure, concentrated, dried at 60-80°C, and crushed, to obtain dry alcohol extract, ready for use;
 - b) selected and cleaned Fructus Mori, Poria, Ganoderma, Radix Salviae Miltiorrhizae, Flos Albiziae and Flos Chrysanthemi are weighed according to the ratios, 10-15 times amount of water is added to extract for 1-3 times, with 1-3 hours each time; the extract is filtered, combined, concentrated, and dried at 60-80°C, and crushed, to obtain dry aqueous extract, ready for use;
 - c) selected and cleaned Bulbus Lilii is weighed according to the ratios, and crushed into powder of 100 mesh, ready for use;
- the dry alcohol extract obtained in step a), the dry aqueous extract of step b) and the fine powder of Bulbus Lilii obtained in step c) are collectively made into pills.

[32] All of the capsule, powder, oral solution, soft capsule, pill, tincture, syrup, suppository, gel, spray and injection of the pharmaceutical composition according to the present invention can be prepared from the crude medicines for the pharmaceutical composition according to the present invention or the active ingredients of the pharmaceutical composition according to the present invention in accordance with conventional pharmaceutical methods.

[33] The pharmaceutical composition according to the present invention can be administered to various animals, including mammals, especially human. For animal or human subjects in need of administration of the pharmaceutical composition according to the present invention, the dosage thereof can be determined by medical practitioners according to the conditions of the subject, including e.g. severity of the disease, general health condition, body weight, age and the like of the patient. The pharmaceutical composition according to the present invention can be administered in various appropriate routes, including e.g. oral, injection, percutaneous, transdermal, and local administration, etc. The administration frequency of the pharmaceutical composition according to the present invention can be determined by various factors, including for example the specific

disease to be treated, general health condition of the subject, etc. Generally, for human subjects, the dosage of the pharmaceutical composition according to the present invention is 14-20 g/day based on the total weight of the crude medicines, and can be taken once per day or be divided into 2-4 doses per day, preferably 16.8 g/day divided into 3 doses per day.

[34] The terms as used in the present invention have the common meanings in the field of traditional Chinese medicine.

[35] Radix Polygoni Multiflori: *POLYGONI MULTIFLORI RADIX*. It is the dry root tuber of *Polygonum multiflorum* Thunb.. It is excavated in autumn and winter when the leaves have withered; both of its ends are chipped off; it is cleaned, and cut into pieces for big ones; and then is dried.

[36] Prepared Radix Polygoni Multiflori (*POLYGONI MULTIFLORI RADIX PRAEPARATA*) is the processed product of Radix Polygoni Multiflori. Slices or pieces of Radix Polygoni Multiflori are taken and mixed evenly with black bean juice and carry out stewing method (Appendix II D of Chinese Pharmacopoeia, 2010 edition) in an appropriate nonferrous container, until the juice is exhausted or carry out steaming method (Appendix II D of Chinese Pharmacopoeia, 2010 edition) and steam it alone or steam it after being mixed evenly with black bean juice to a brown color on all sides, dry in the sun to partial dryness, then cut into slices, and dried. 10 kg black bean is used for every 100 kg slices (pieces) of Radix Polygoni Multiflori. Preparation method for black bean juice: 10 kg black beans are taken, and an appropriate amount of water is added; boiled for 4 hours to obtain about 15 kg juice, and additional water is added to the bean residue and boiled for 3 hours to obtain about 10 kg juice; the juice is combined and about 25 kg black bean juice is obtained.

[37] Semen Ziziphi Spinosae: *ZIZIPHI SPINOSAE SEMEN*. It is the dried ripe seed of *Ziziphus jujuba* Mill. var. *spinosa* (Bunge) Hu ex H. F. Chou, which is a plant of Rhamnaceae. The ripe fruits are collected in the late autumn and early winter, the pulps and the shells (endocarp) are removed and dried in the sun.

[38] Stir-baked Semen Ziziphi Spinosae: the clean Semen Ziziphi Spinosae is taken and baked according to the simple stir-baking method (Appendix II D of Chinese Pharmacopoeia, 2010 edition) to inflate and the color slight darken. Break to pieces before use.

[39] Fructus Mori: *MORI FRUCTUS*. It is the dried fruit-spike of *Morus alba* L., which is a plant of Moraceae. The raw material is collected from April to June when the fruits turn red, and dried in the sun, or dried after steaming briefly.

[40] Ganoderma: *GANODERMA*. It is the dried sporophore of *Ganoderma lucidum* (Leyss. ex Fr.) Karst. or *Ganoderma sinense* Zhao, Xu et Zhang, (Fam. Polyporaceae). The raw material is collected all the year round; removed from foreign matter, attached rotten wood, sand or lower stripe of the culture matrix; dried in the shade or stove at 40-50°C.

[41] Bulbus Lilii: *LILII BULBUS*. It is the dried fleshy scale leaf of *Lilium lancifolium*

Thunb., *Lilium brownii* F. E. Brown var. *viridulum* Baker or *Lilium pumilum* DC., which are plants of Liliaceae. The raw material is collected in autumn and washed, and the scale leaves are stripped off, treated with boiling water for a moment, and dried.

[42] Rhizoma Anemarrhenae: *ANEMARRHENAE RHIZOMA*. It is the dried rhizome of *Anemarrhena asphodeloides* Bge., which is a plant of Liliaceae. The raw material is collected in autumn or winter, removed from fibrous roots and soil, dried in the sun, known as “Maozhimu”; or removed from outer tissue, and dried in the sun.

[43] Radix Salviae Miltiorrhizae: *SALVIAE MILTIORRHIZAE RADIX ET RHIZOMA*. It is the dried root and rhizome of *Salvia miltiorrhiza* Bge., which is a plant of Labiatae. The raw material is collected in spring or autumn, removed from soil, and dried.

[44] Flos Chrysanthemi: *CHRYSANTHEMI FLOS*. It is the dry capitulum of *Chrysanthemum morifolium* Ramat., which is a plant of Compositae. The raw material is collected in batches from September to November on flowering, dried in shade or by baking or dried in the sun after being fuming and steaming. It is classified into “Boju”, “Chuju”, “Gongju” and “Hangju” according to different localities of production and variations in processing methods.

[45] Poria: *PORIA*. It is the dried sclerotium of fungus, *Poria cocos* (Schw.) Wolf, (Fam. Polyporaceae). The raw material is collected mostly in July to September, removed from soil, piled up to the surface wetted, then spread to the surface dried. Repeat for several times until wrinkles appear and inside water evaporated, then dried in the shade, it is known as “Fulingge”; or the fresh sclerotium is cut and dried in the shade, according to cut portions, known as “Fulingpian” and “Fulingkuai”.

[46] Flos Albiziae: *ALBIZIAE FLOS*. It is the dried inflorescence of *Albizia julibrissin* Durazz, which is a plant of Leguminosae. They are collected on sunny days in summer when the flower is in blossom, and dried in the sun without delay.

[47] The traditional Chinese material medica of the present invention can be replaced by traditional Chinese material medica with the same or similar efficacy, and all these material medica can be processed in accordance with “National processing standards for traditional Chinese material medica” or “Dictionary of Traditional Chinese Medicine”. For example, Radix Polygoni Multiflori can be replaced by prepared Radix Polygoni Multiflori, and Semen Ziziphi Spinosae can be replaced by stir-baked Semen Ziziphi Spinosae; although the efficacies after the replacement may be better than that of the original crude drugs, they all belong to the content of the present invention.

[48] In another aspect, the present invention provides use of the pharmaceutical composition for the treatment of the symptoms of insomnia, amnesia, dizziness, lassitude, and/or soreness and weakness of waist and knees, etc.

[49] In another aspect, the present invention further provides use of the pharmaceutical composition for sedation, hypnosis, promoting memory, anti-fatigue, and/or pain relief.

[50] In another aspect, the present invention further provides a method for the treatment of insomnia, which comprises administering the pharmaceutical composition to a patient

with insomnia.

[51] The functions and indications of the pharmaceutical composition according to the present invention are supplementing kidney and invigorating brain, and nourishing heart to calm the mind, and is used for insomnia which is caused by insufficiency of heart and kidney and the symptoms thereof include sleep disorder, e.g. sleep onset insomnia, non-deep sleep, nocturnal awakening, excessive dreaming, etc, accompanied with amnesia, dizziness, palpitation, lassitude, soreness and weakness of waist and knees.

[52] The present invention is obtained by Professor WU Yiling, through repeat practice based on the theoretic exploration of the traditional Chinese medicine in treating insomnia, in combination with the clinical results. It is observed clinically that the symptoms of insomnia, such as amnesia, dizziness, palpitation, lassitude, soreness and weakness of waist and knees, etc. caused by insufficiency of heart and kidney can be improved effectively.

Detailed embodiments of the invention

[53] The following examples are provided only for the illustration of preparing the pharmaceutical composition according to the present invention, but they do not limit the scope of the present invention in any way.

Example 1: Preparation of the tablet of the pharmaceutical composition according to the present invention (also known as Jiannao Anshen Tablet)

[54] Crude Medicines: Radix Polygoni Multiflori 200 g; Semen Ziziphi Spinosae 200 g; Fructus Mori 200 g; Ganoderma 100 g; Bulbus Lilii 100 g; Rhizoma Anemarrhenae 66.7 g; Radix Salviae Miltiorrhizae 166.7 g; Flos Chrysanthemi 66.7 g; Poria 100 g; and Flos Albiziae 200 g.

Preparation method:

[55] a) selected and cleaned Radix Polygoni Multiflori, Rhizoma Anemarrhenae, and coarsely crushed Semen Ziziphi Spinosae were weighed according to the ratios, 60% ethanol was added; heated under reflux to extract twice, 10 times amount of ethanol was added for the first time and extracted for 2 hours, and 8 times amount of ethanol was added for the second time and extracted for 1 hour; the extract was filtered, combined with ethanol recovered under reduced pressure, and concentrated to a relative density of 1.10-1.15 determined at 60°C, to obtain alcohol extract, ready for use;

[56] b) selected and cleaned Fructus Mori, Poria, Ganoderma, Radix Salviae Miltiorrhizae, Flos Albiziae, and Flos Chrysanthemi were weighed according to the ratios, 11 times amount of water was added to extract twice, with 1.5 hours each time; the extract was filtered, combined, and concentrated to a relative density of about 1.10-1.15 determined at 60°C, to obtain water extract, ready for use;

[57] c) selected and cleaned Bulbus Lilii was weighed according to the ratios, and crushed into powder of 100 mesh, ready for use;

[58] d) the fine powder of Bulbus Lilii obtained in step c) was added into the container

for raw materials of the spraying drier granulator, the alcohol extract obtained in step a) and the aqueous extract of step b) were sprayed in for spraying granulation; 0.5% magnesium stearate was added after granule sizing; mixing, tableting and coating were performed to obtain 975 tablets of said tablet (0.4g per tablet).

Example 2: Preparation of the capsule of the pharmaceutical composition according to the present invention

[59] Crude medicines: Radix Polygoni Multiflori 158 g; Semen Ziziphi Spinosae 270 g; Fructus Mori 165 g; Ganoderma 135 g; Bulbus Lilii 80 g; Rhizoma Anemarrhenae 110 g; Radix Salviae Miltiorrhizae 125 g; Flos Chrysanthemi 118 g; Poria 80 g; and Flos Albiziae 280 g.

Preparation method:

[60] a) selected and cleaned Radix Polygoni Multiflori, Rhizoma Anemarrhenae, and coarsely crushed Semen Ziziphi Spinosae were weighed according to the ratios, 6 times amount of 30% ethanol was added; heated under reflux to extract for 3 times, 3 hours each time; the extract was filtered, combined with ethanol recovered under reduced pressure, and concentrated to a relative density of 1.10-1.15 determined at 60°C, to obtain alcohol extract, ready for use;

[61] b) selected and cleaned Fructus Mori, Poria, Ganoderma, Radix Salviae Miltiorrhizae, Flos Albiziae and Flos Chrysanthemi were weighed according to the ratios, 10 times amount of water was added to extract for 3 times, 3 hours each time; the extract was filtered and combined, with ethanol recovered under reduced pressure, and concentrated to a relative density of about 1.10-1.15 determined at 60°C, to obtain aqueous extract, ready for use;

[62] c) selected and cleaned Bulbus Lilii was weighed according to the ratios, and crushed into powder of 100 mesh, ready for use;

[63] d) the alcohol extract obtained in step a), the aqueous extract of step b) and the fine powder of Bulbus Lilii obtained in step c) were subjected to dry granulation; appropriate adjuvants were added after granule sizing; mixed evenly and filled into the capsules to obtain 983 capsules (0.42 g per capsule).

Example 3: Preparation of the powder of the pharmaceutical composition according to the present invention

[64] Crude medicines: Radix Polygoni Multiflori 265 g; Semen Ziziphi Spinosae 147 g; Fructus Mori 250 g; Ganoderma 81 g; Bulbus Lilii 158 g; Rhizoma Anemarrhenae 55 g; Radix Salviae Miltiorrhizae 188 g; Flos Chrysanthemi 50 g; Poria 145 g and Flos Albiziae 170 g.

Preparation method:

[65] a) selected and cleaned Radix Polygoni Multiflori, Rhizoma Anemarrhenae, and coarsely crushed Semen Ziziphi Spinosae were weighed according to the ratios, 12 times amount of 70% ethanol was added; heated under reflux to extract for 2 times, 2 hours each

time; the extract was filtered and combined, with ethanol recovered under reduced pressure, and concentrated to a relative density of 1.10-1.15 determined at 60°C, to obtain alcohol extract, ready for use;

[66] b) selected and cleaned Fructus Mori, Poria, Ganoderma, Radix Salviae Miltiorrhizae, Flos Albiziae and Flos Chrysanthemi were weighed according to the ratios, 15 times amount of water was added to extract for 2 times, 2 hours each time; the extract was filtered, combined, and concentrated to a relative density of about 1.10-1.15 determined at 60°C, to obtain aqueous extract, ready for use;

[67] c) selected and cleaned Bulbus Lili was weighed according to the ratios, and crushed into powder of 100 mesh, ready for use;

[68] d) the alcohol extract obtained in step a), the aqueous extract of step b) and the fine powder of Bulbus Lili obtained in step c) were subjected to dry grinding to obtain 400 g powder, which was dispensed by 2g per bag.

Example 4: Preparation of the oral solution of the pharmaceutical composition according to the present invention

[69] Crude medicines: Radix Polygoni Multiflori 225 g; Semen Ziziphi Spinosae 163 g; Fructus Mori 220 g; Ganoderma 83 g; Bulbus Lili 113 g; Rhizoma Anemarrhenae 50 g; Radix Salviae Miltiorrhizae 190 g; Flos Chrysanthemi 45 g; Poria 113 g and Flos Albiziae 178 g.

Preparation method:

[70] a) selected and cleaned Radix Polygoni Multiflori, Rhizoma Anemarrhenae, and coarsely crushed Semen Ziziphi Spinosae were weighed according to the ratios, 10 times amount of 50% ethanol was added; heated under reflux to extract for 3 hours; the extract was filtered with ethanol recovered under reduced pressure, and concentrated to a relative density of 1.10-1.15 determined at 60°C, to obtain alcohol extract, ready for use;

[71] b) selected and cleaned Fructus Mori, Poria, Ganoderma, Radix Salviae Miltiorrhizae, Flos Albiziae and Flos Chrysanthemi were weighed according to the ratios, 12 times amount of water was added to extract for 3 hours; the extract was filtered, and concentrated to a relative density of about 1.10-1.15 determined at 60°C, to obtain aqueous extract, ready for use;

[72] c) selected and cleaned Bulbus Lili was weighed according to the ratios, and crushed into powder of 100 mesh, ready for use;

[73] d) the alcohol extract obtained in step a), the aqueous extract of step b) and the fine powder of Bulbus Lili obtained in step c) were prepared into oral solution according to conventional methods (Chunlin CAO, *Pharmaceutics of Traditional Chinese Medicine*, Shanghai Science & Technology Press, 1st Edition in November 1986, 8th printing in October 1993: the preparation method for oral solution, pages 477-488), to obtain 10000 ml oral solution, which was dispensed by 10 ml per container.

Example 5: Preparation of the soft capsule of the pharmaceutical composition according to

the present invention

[74] Crude medicines: Radix Polygoni Multiflori 225 g; Semen Ziziphi Spinosae 163 g; Fructus Mori 220 g; Ganoderma 83 g; Bulbus Lilii 113 g; Rhizoma Anemarrhenae 50 g; Radix Salviae Miltiorrhizae 190 g; Flos Chrysanthemi 45 g; Poria 113 g and Flos Albiziae 178 g.

Preparation method:

[75] a) selected and cleaned Radix Polygoni Multiflori, Rhizoma Anemarrhenae, and coarsely crushed Semen Ziziphi Spinosae were weighed according to the ratios, 12 times amount of 70% ethanol was added; heated under reflux to extract for 1.5 hours; the extract was filtered, combined with ethanol recovered under reduced pressure, and concentrated to a relative density of 1.10-1.15 determined at 60°C, to obtain alcohol extract, ready for use;

[76] b) selected and cleaned Fructus Mori, Poria, Ganoderma, Radix Salviae Miltiorrhizae, Flos Albiziae and Flos Chrysanthemi were weighed according to the ratios, 15 times amount of water was added to extract for 3 times, 1 hour each time; the extract was filtered, and concentrated to a relative density of about 1.10-1.15 determined at 60°C, to obtain aqueous extract, ready for use;

[77] c) selected and cleaned Bulbus Lilii was weighed according to the ratios, and crushed into powder of 100 mesh, ready for use;

[78] d) the alcohol extract obtained in step a), the aqueous extract of step b) and the fine powder of Bulbus Lilii obtained in step c) were prepared into soft capsules according to conventional methods (Chunlin CAO, Pharmaceutics of Traditional Chinese Medicine, Shanghai Science & Technology Press, 1st Edition in November 1986, 8th printing in October 1993: the preparation method for soft capsule, pages 232-235), to obtain 980 soft capsules (0.6 g per capsule).

Example 6: Preparation of the pill of the pharmaceutical composition according to the present invention

[79] Crude medicines: Radix Polygoni Multiflori 165 g; Semen Ziziphi Spinosae 228 g; Fructus Mori 173 g; Ganoderma 110 g; Bulbus Lilii 75 g; Rhizoma Anemarrhenae 75 g; Radix Salviae Miltiorrhizae 150 g; Flos Chrysanthemi 78 g; Poria 78 g and Flos Albiziae 218 g.

Preparation method:

[80] a) selected and cleaned Radix Polygoni Multiflori, Rhizoma Anemarrhenae, and coarsely crushed Semen Ziziphi Spinosae were weighed according to the ratios, 8 times amount of 40% ethanol was added; heated under reflux to extract twice, 2 hours for each time; the extract was filtered, combined with ethanol recovered under reduced pressure, and concentrated to a relative density of 1.10-1.15 determined at 60°C, to obtain alcohol extract, ready for use;

[81] b) selected and cleaned Fructus Mori, Poria, Ganoderma, Radix Salviae Miltiorrhizae, Flos Albiziae and Flos Chrysanthemi were weighed according to the ratios,

12 times amount of water was added to extract twice, with 1.5 hours each time; the extract was filtered, and concentrated to a relative density of about 1.10-1.15 determined at 60°C, to obtain aqueous extract, ready for use;

[82] c) selected and cleaned *Bulbus Lili* was weighed according to the ratios, and crushed into powder of 100 mesh, ready for use;

[83] d) the alcohol extract obtained in step a), the aqueous extract of step b) and the fine powder of *Bulbus Lili* obtained in step c) were prepared into pills according to conventional methods (Chunlin CAO, *Pharmaceutics of Traditional Chinese Medicine*, Shanghai Science & Technology Press, 1st Edition in November 1986, 8th printing in October 1993: the preparation method for pill, pages 248-280), to obtain 900 pills (0.5 g per pill).

Example 7: Preparation of the tincture of the pharmaceutical composition according to the present invention

[84] Crude medicines: *Radix Polygoni Multiflori* 158 g; *Semen Ziziphi Spinosae* 270 g; *Fructus Mori* 165 g; *Ganoderma* 135 g; *Bulbus Lili* 80 g; *Rhizoma Anemarrhenae* 110 g; *Radix Salviae Miltiorrhizae* 125 g; *Flos Chrysanthemi* 118 g; *Poria* 80 g and *Flos Albiziae* 280 g.

Preparation method:

[85] a) selected and cleaned *Radix Polygoni Multiflori*, *Rhizoma Anemarrhenae*, and coarsely crushed *Semen Ziziphi Spinosae* were weighed according to the ratios, 11 times amount of 60% ethanol was added; heated under reflux to extract for 3 times, 1 hour for each time; the extract was filtered, combined with ethanol recovered under reduced pressure, and concentrated to a relative density of 1.10-1.15 determined at 60°C, to obtain alcohol extract, ready for use;

[86] b) selected and cleaned *Fructus Mori*, *Poria*, *Ganoderma*, *Radix Salviae Miltiorrhizae*, *Flos Albiziae* and *Flos Chrysanthemi* were weighed according to the ratios, 14 times amount of water was added to extract for 3 times, with 1.5 hours each time; the extract was filtered, and concentrated to a relative density of about 1.10-1.15 determined at 60°C, to obtain aqueous extract, ready for use;

[87] c) selected and cleaned *Bulbus Lili* was weighed according to the ratios, and crushed into powder of 100 mesh, ready for use;

[88] d) the alcohol extract obtained in step a), the aqueous extract of step b) and the fine powder of *Bulbus Lili* obtained in step c) were prepared into tincture according to conventional methods (Chunlin CAO, *Pharmaceutics of Traditional Chinese Medicine*, Shanghai Science & Technology Press, 1st Edition in November 1986, 8th printing in October 1993: the preparation method for tincture, pages 163-165), to obtain 10000 ml tincture, which was dispensed by 10 ml per container.

Example 8: Preparation of the syrup of the pharmaceutical composition according to the present invention

[89] Crude medicines: Radix Polygoni Multiflori 265 g; Semen Ziziphi Spinosae 147 g; Fructus Mori 250 g; Ganoderma 81 g; Bulbus Lilii 158 g; Rhizoma Anemarrhenae 55 g; Radix Salviae Miltiorrhizae 188 g; Flos Chrysanthemi 50 g; Poria 145 g and Flos Albiziae 170 g.

[90] Preparation method: 10000 ml syrup was prepared according to conventional methods (Chunlin CAO, Pharmaceutics of Traditional Chinese Medicine, Shanghai Science & Technology Press, 1st Edition in November 1986, 8th printing in October 1993: the preparation method for syrup, pages 166-170), and dispensed by 10 ml per container.

Example 9: Preparation of the suppository of the pharmaceutical composition according to the present invention

[91] Crude medicines: Radix Polygoni Multiflori 158 g; Semen Ziziphi Spinosae 270 g; Fructus Mori 165 g; Ganoderma 135 g; Bulbus Lilii 80 g; Rhizoma Anemarrhenae 110 g; Radix Salviae Miltiorrhizae 125 g; Flos Chrysanthemi 118 g; Poria 80 g; Flos Albiziae 280 g.

[92] Preparation method: 500 suppositories were prepared according to conventional methods (Chunlin CAO, Pharmaceutics of Traditional Chinese Medicine, Shanghai Science & Technology Press, 1st Edition in November 1986, 8th printing in October 1993: the preparation method for suppository, pages 352-361), 0.5 g per suppository.

Example 10: Preparation of the gel of the pharmaceutical composition according to the present invention

[93] Crude medicines: Radix Polygoni Multiflori 225 g; Semen Ziziphi Spinosae 163 g; Fructus Mori 220 g; Ganoderma 83 g; Bulbus Lilii 113 g; Rhizoma Anemarrhenae 50 g; Radix Salviae Miltiorrhizae 190 g; Flos Chrysanthemi 45 g; Poria 113 g and Flos Albiziae 178 g.

[94] Preparation method: 3000 g gel was prepared according to conventional methods (Chunlin CAO, Pharmaceutics of Traditional Chinese Medicine, Shanghai Science & Technology Press, 1st Edition in November 1986, 8th printing in October 1993: the preparation method for gel, pages 349-350), and dispensed by 10 g per container.

Example 11: Preparation of the spray of the pharmaceutical composition according to the present invention

[95] Crude medicines: Radix Polygoni Multiflori 225 g; Semen Ziziphi Spinosae 163 g; Fructus Mori 220 g; Ganoderma 83 g; Bulbus Lilii 113 g; Rhizoma Anemarrhenae 50 g; Radix Salviae Miltiorrhizae 190 g; Flos Chrysanthemi 45 g; Poria 113 g and Flos Albiziae 178 g.

[96] Preparation method: 10000 ml spray was prepared according to conventional methods (Chunlin CAO, Pharmaceutics of Traditional Chinese Medicine, Shanghai Science & Technology Press, 1st Edition in November 1986, 8th printing in October 1993: the preparation method for spray, pages 454-466), and dispensed by 100 ml per container.

Example 12: Preparation of the injection of the pharmaceutical composition according to

the present invention

[97] Crude medicines: Radix Polygoni Multiflori 165 g; Semen Ziziphi Spinosae 228 g; Fructus Mori 173 g; Ganoderma 110 g; Bulbus Lilii 75 g; Rhizoma Anemarrhenae 75 g; Radix Salviae Miltiorrhizae 150 g; Flos Chrysanthemi 78 g; Poria 78 g and Flos Albiziae 218 g.

[98] Preparation method: 1000 injections were prepared according to conventional methods (Chunlin CAO, *Pharmaceutics of Traditional Chinese Medicine*, Shanghai Science & Technology Press, 1st Edition in November 1986, 8th printing in October 1993: the preparation method for injection, pages 364-4193), 5ml per container.

Example 13: Preparation of the decoction of the pharmaceutical composition according to the present invention

Crude medicines: the same as Example 1.

Preparation method:

[99] Radix Polygoni Multiflori, Semen Ziziphi Spinosae, Fructus Mori, Ganoderma, Bulbus Lilii, Rhizoma Anemarrhenae, Radix Salviae Miltiorrhizae, Flos Chrysanthemi, Poria and Flos Albiziae were weighed according to the ratios, and placed in a boiler; water was added for the first time to 3-5 cm above the top surface of the crude medicines; soaked for 30 minutes, and decocted for 35 minutes after boiling, and filtered; water was added again to 1-3 cm above the top surface of the crude medicines; decocted for 30 min after boiling, filtered, and the two filtrates were combined to obtain 200 doses, 500 ml per dose.

Example 14: Preparation of the watered pill of the pharmaceutical composition according to the present invention

Crude medicines: the same as Example 1.

Preparation method:

[100] a) selected and cleaned Radix Polygoni Multiflori, Rhizoma Anemarrhenae, and coarsely crushed Semen Ziziphi Spinosae were weighed according to the ratios, 8 times amount of 60% ethanol was added; heated under reflux to extract twice, 2 hours each time; the extract was filtered, combined with ethanol recovered under reduced pressure, concentrated and dried at 75°C, and crushed to obtain dry alcohol extract, ready for use;

[101] b) selected and cleaned Fructus Mori, Poria, Ganoderma, Radix Salviae Miltiorrhizae, Flos Albiziae and Flos Chrysanthemi were weighed according to the ratios, 12 times amount of water was added to extract twice, 1 hour each time; the extract was filtered, combined, concentrated, and dried at 75°C, and crushed, to obtain dried aqueous extract (the yield for the dry extract was 20%), ready for use;

[102] c) selected and cleaned Bulbus Lilii was weighed according to the ratios, and crushed into powder of 100 mesh, ready for use;

[103] d) the dry alcohol extract obtained in step a), the dried aqueous extract of step b) and the fine powder of Bulbus Lilii obtained in step c) were collectively prepared into pills and

1 kg watered pills were obtained, which were dispensed by 100 g per bag.

Pharmacological testing

[104] In order to confirm the activities of the pharmaceutical composition according to the present invention for the treatment of insomnia, the tablets prepared according to the method of Example 1 (hereinafter referred to as the pharmaceutical according to the present invention) were used to perform the following pharmacological testing.

1. The sedative effect of the pharmaceutical according to the present invention

1.1 Experimental materials

1.1.1 Experimental animals

[105] 90 male healthy Kunming mice of clean grade (body weight 18-22 g) were purchased from Laboratory Animal Center of Hebei Province (License No.: SCXK (JI) 2003-1-003, Certificate No.: DK0409-0040), and were fed in Laboratory of Pharmacology of Yiling Research Institute, under 12 hours/day illumination, 20-23°C temperature, and 40-60% relative humidity. The mice were caged, with 6 mice/cage. The feedstuff for mice was the complete pellet feed provided by the Laboratory Animal Center of Hebei Province, and the mice were provided with sufficient drinking water. The mice were acclimatized for 3 days.

1.1.2 Experimental drug

[106] The pharmaceutical according to the present invention (the tablet of Example 1) is equivalent to 3.47 g crude drug/g, and the human dosage is 17.0 g crude drug/day, i.e. 0.28 g crude drug/kg body weight (standard body weight 60 kg). It is prepared into a suspension with 0.5 % CMC-Na, and stored at 4°C. In pharmacological experiments, the term “crude drug” refers to the above-mentioned “crude medicine” or “materia medica”, and the amount of the “crude drug” refers to the converted amount of the “material medica”.

[107] Anshenbunao Ye: It is prepared from Cornu Cervi Pantotrichum, Radix Polygoni Multiflori Preparata, Herba Epimedii, Rhizoma Zingiberis (dried ginger), Fructus Jujubae, and Vitamin B1; functions and indications: invigorating brain and calm the mind, generating essence and reinforcing the marrow, replenishing Qi and nourishing blood; and it is commonly used for neurasthenia, insomnia, amnesia, dizziness, lassitude; it is manufactured by JILIN AODONG YANBIAN PHARMACEUTICAL CO., LTD, Approval No.: Z22022453 (by SFDA); clinical dosage for adults: 20ml/d; batch No.: 040269.

[108] Estazolam Tablet, an anxiolytic of benzodiazepines, can cause inhibitions in different sites of the central nervous system; the clinical manifestation can range from mild sedation to hypnosis and even coma, with the increasing of dosage used. It is provided by Shijiazhuang Pharmaceutical Group Co., LTD; Approval No.: H13020974(by SFDA); strength: 1 mg; Clinical dosage for adults: 2-4 mg each time for hypnosis, and 1-2 mg each time for sedation, 3 times/day; batch No.: 040101.

1.1.3 Experimental instruments

[109] Instrument for locomotor activities of mice (locomotor-activity box, software for locomotor activities), purchased from Beijing Shuolin Xueyuan Science and Technology Co., LTD.

[110] AB204-N type electronic analytical balance, purchased from Mettler-Toledo Instruments (Shanghai) Co., Ltd.

1.2. Experimental method

1.2.1 The principles for dosage setting

[111] In the experiment, the pharmaceutical according to the present invention was set into groups of 5.6 g crude drug/kg, 2.8 g crude drug/kg and 1.4 g crude drug/kg, which are equivalent to 20, 10 and 5 times of the clinical human dosage (0.28g crude drug/kg) respectively. Group of Anshenbunao Ye 13ml/kg and group of estazolam 2.67 mg/kg were set, according to the results of preliminary experiment. Mice were divided into 6 groups according to the above dosage setting, as is shown in Table 1.

Table 1: the effect of the pharmaceutical according to the present invention on the locomotor activities of mice

Groups	Dosages	Animal number	equivalent to the times of clinical dosage (ratio)
Group of Blank Control	—	12	—
Group of Anshenbunao Ye	13ml/kg	12	39
Group of Estazolam	2.67mg/kg	12	27
The pharmaceutical according to the present invention	5.6g Crude drug/kg	12	20
	2.8g Crude drug/kg	12	10
	1.4g Crude drug/kg	12	5

1.2.2 Method of administration

[112] The administering concentrations of the pharmaceutical according to the present invention were 0.08 g dry powder/ml, 0.04g dry powder/ml and 0.02g dry powder/ml respectively; the administering concentration of Anshenbunao Ye group was 0.65 ml concentrated liquid/ml, and the administering concentration of estazolam was 0.13mg/ml. 0.2ml/10g was administered by gavage daily, which is in accordance with the clinically recommended oral route, for 7 consecutive days, and estazolam was administered only once on the seventh day.

1.2.3 Experimental period: 7 days.

1.2.4 Detection indicators and detection methods

[113] The mice were trained once in the morning, the afternoon and the evening on the 1st day, for every time, the mice were acclimatized for 5 min after each training, and the number of locomotor activities within 10 minutes was recorded. 72 mice with an average number of 150-250 times/10 minutes were chosen to participate in the experiment. The

number of locomotor activities was tested 1 hour after the last administration, the mice were acclimatized for 5 min, and the number of locomotor activities within 10 minutes was then recorded.

1.2.5 Statistical method: One-way ANOVA was performed by using spss11.5 statistical software.

1.3 Experimental results

[114] The numbers of locomotor activities of mice in the groups of Anshenbunao Ye and Estazolam were both decreased in comparison with that of the group of blank control ($P<0.01$ or $P<0.05$). Compared to the group of blank control, the numbers of locomotor activities of mice in the groups of 5.6 g Crude drug/kg and 2.8 g Crude drug/kg of the pharmaceutical according to the present invention were significantly decreased ($P<0.05$), and the inhibitory rates were 27.5% and 23.4%, respectively. For the group of 1.4 g Crude drug/kg, the number of locomotor activities of mice tended to decrease, and the inhibitory rate was 9.7%. The results are shown in Table 2.

Table 2: The effect of the pharmaceutical according to the present invention on the number of locomotor activities of mice ($\bar{x} \pm s$, $n=12$)

Groups	Dosages	Number of Locomotor Activities (times/10min)	Inhibitory Rate (%)
Group of Blank Control		240.3±30.1	
Group of Anshenbunao Ye	13ml/kg	159.7±34.2**	33.5
Group of Estazolam	2.76mg/kg	174.0±58.9*	27.5
Group of the pharmaceutical according to the present invention	5.6g Crude drug/kg	173.3±50.1*	27.8
	2.8g Crude drug/kg	184.0±55.2*	23.4
	1.4g Crude drug/kg	216.8±72.6	9.7

Note: * $P<0.05$, ** $P<0.01$, compared with the group of blank control

1.4 Conclusion

[115] The pharmaceutical according to the present invention can decrease the number of locomotor activities of mice, which confirms that the pharmaceutical according to the present invention has a sedative effect.

2. The hypnotic effect of the pharmaceutical according to the present invention

2.1 Experimental materials

2.1.1 Experimental animals

[116] 72 male healthy Kunming mice of clean grade (body weight 18-22 g) were purchased from Laboratory Animal Center of Hebei Province (License No.: SCXK (JL)

2003-1-003, Certificate No.: DK0409-0016). They were fed in Laboratory of Pharmacology of Yiling Research Institute, under 12 hours/day illumination, 20-23°C temperature, and 40-60% relative humidity. The mice were caged, 6 mice per cage. The complete granular mice feedstuff was provided by the Laboratory Animal Center of Hebei Province, and the mice were provided with sufficient drinking water. The mice were acclimatized for 3 days.

2.1.2 Experimental drug

[117] The pharmaceutical according to the present invention is equivalent to 3.47 g crude drug/g dry powder, and the human dosage is 17.0 g crude drug/day, i.e. 0.28 g crude drug/kg body weight (standard body weight 60 kg). It is provided by Shijiazhuang Yiling Pharmaceutical co., LTD (batch No. 20040201). It is prepared into a suspension with 0.5 % CMC-Na, and stored at 4°C.

[118] Anshenbunao Ye: It is prepared from Cornu Cervi Pantotrichum, Radix Polygoni Multiflori Preparata, Herba Epimedii, Rhizoma Zingiberis (dried ginger), Fructus Jujubae, and Vitamin B1; functions and indications: invigorating brain and calm the mind, generating essence and reinforcing the marrow, replenishing Qi and nourishing blood; and it is commonly used for neurasthenia, insomnia, amnesia, dizziness, lassitude; it is manufactured by JILIN AODONG YANBIAN PHARMACEUTICAL CO., LTD, Approval No.: Z22022453 (by SFDA); clinical dosage for adults: 20ml/d; batch No.: 040269.

[119] Estazolam Tablet, an anxiolytic of benzodiazepines, can cause inhibitions in different sites of the central nervous system; the clinical manifestation can range from mild sedation to hypnosis and even coma, with the increasing of dosage used. It is provided by Shijiazhuang Pharmaceutical Group Co., LTD; Approval No.: H13020974(by SFDA); strength: 1 mg; Clinical dosage for adults: 2-4 mg each time for hypnosis, and 1-2 mg each time for sedation, 3 times/day; batch No.: 040101.

2.1.3 Experimental reagent

[120] Pentobarbital sodium, provided by Sinopharm (Group) Shanghai Chemical Reagent Co., Ltd, batch No.: F20030816.

2.2 Experimental method

2.2.1 The principles for dosage setting

[121] In the experiment, the pharmaceutical according to the present invention was set into groups of 5.6 g crude drug/kg, 2.8 g crude drug/kg and 1.4 g crude drug/kg, which are equivalent to 20, 10 and 5 times of the clinical human dosage (0.28g crude drug/kg) respectively. Group of Anshenbunao Ye 13ml/kg and group of estazolam 2.67 mg/kg were set, according to the results of preliminary experiment. Mice were divided into 6 groups according to the above dosage setting, as is shown in Table 3.

Table 3: the effect of the pharmaceutical according to the present invention on the sleeping time of mice induced by pentobarbital sodium

Groups	Dosage	Animal number	equivalent to the times of clinical dosage (ratio)
Group of Blank Control	—	12	—
Group of Anshenbunao Syrup	13ml/kg	12	39
Group of Estazolam	2.67mg/kg	12	27
Group of the pharmaceutical according to the present invention	5.6g Crude drug/kg	12	20
	2.8g Crude drug /kg	12	10
	1.4g Crude drug /kg	12	5

2.2.2 Method of administration

[122] The administering concentrations of the pharmaceutical according to the present invention were 0.08 g dry powder/ml, 0.04g dry powder/ml and 0.02g dry powder/ml respectively; the administering concentration of Anshenbunao Ye group was 0.65 ml concentrated liquid/ml, and the administering concentration of estazolam was 0.13mg/ml. 0.2ml/10g was administered by gavage daily, which is in accordance with the clinically recommended oral route, for 7 consecutive days, and estazolam was administered only once on the seventh day.

2.2.3 Animal model establishment method

[123] 1 hour after the last administration of each group, 45mg/kg pentobarbital sodium was administered by intraperitoneal injection.

2.2.4 Experimental period: 7 days.

2.2.5 Detection indicators and detection methods

[124] Reference is made to “Methodology on Pharmacology of Experiment” (3rd Edition) for the experiments. The i.p. dosage of pentobarbital sodium, which induced 100% mice to sleep and did not incur too long sleeping time, was determined to be 45mg/kg by preliminary experiments. 1 hour after the last administration of each group, 45mg/kg pentobarbital sodium was administered by intraperitoneal injection, and the sleeping time of mice was recorded (the time period from the disappearance of the righting reflex to the recovery of the righting reflex).

2.2.6 Statistical method: t-test was performed by using spss11.5 statistical software.

2.3 Experimental results

[125] The sleeping time of mice in the group of estazolam was extended in comparison with that of the group of model control ($P < 0.01$). The sleeping time of mice in the groups of 5.6 g Crude drug/kg and 2.8 g Crude drug/kg of the pharmaceutical according to the present invention was extended compared with that of the group of model control ($P < 0.05$).

or 0.01).The results are shown in Table 4.

Table 4: The effect of the pharmaceutical according to the present invention on the sleeping time of mice induced by pentobarbital sodium ($\bar{x} \pm s$, n=12)

Groups	Dosage	Sleep Time (min)
Group of Model Control		26.64±18.50
Group of Anshenbunao Ye	13ml/kg	21.60±11.01
Group of Estazolam	2.76mg/kg	72.89±18.01*
Groups of the pharmaceutical according to the present invention	5.6g Crude drug/kg	41.55±14.28*
	2.8g Crude drug /kg	57.90±17.04*
	1.4g Crude drug /kg	25.75±16.69

Note: *P<0.05, **P<0.01, compared with the group of model control

2.4 Conclusion

[126] The pharmaceutical according to the present invention has the effect of extending the sleeping time of mice induced by pentobarbital sodium.

3. The memory-promoting effect of the pharmaceutical according to the present invention

3.1 Experimental materials

3.1.1 Experimental animals

[127] 72 male healthy Kunming mice of clean grade (body weight 18-22 g) were purchased from Laboratory Animal Center of Hebei Province (License No.: SCXK (JH) 2003-1-003, Certificate No.: DK0504-0019). They were feed in Laboratory of Pharmacology of Yiling Research Institute, under 12 hours/day illumination, 20-23℃ temperature, and 40-60% relative humidity. The mice were caged, 6 mice per cage. The granular mice feedstuff was provided by the Laboratory Animal Center of Hebei Province, and the mice were provided sufficient drinking water. The mice were acclimatized for 3 days.

3.1.2 Experimental drug

[128] The pharmaceutical according to the present invention was provided by Shijiazhuang Yiling Pharmaceutical co., LTD, batch No. 20040201. It is prepared into a suspension with 0.5 % CMC-Na, and stored at 4℃.

[129] HABOYIN (Huperzine A tablet). Huperzine A is a reversible, high selective acetylcholinesterase inhibitor. It is manufactured by Henan Zhulinzhongsheng Pharmaceutical Co., Ltd. Approval No.: H10940156 (by SFDA); strength: 0.05 mg/tablet; batch No.: 050201.

[130] Scopolamine Hydrobromide Injection, Approval No.: H31021519 (by SFDA),

Shanghai Hefeng Pharmaceutical Co., Ltd.; batch No.: 4A18003. Strength: 0.3 mg.

3.1.3 Experimental instruments: SMG-2 programmed control water maze, manufactured by Institute of Materia Medica, Chinese Academy of Medical Sciences.

3.2 Experimental method

3.2.1 The principles for dosage setting

[131] In the experiment, the pharmaceutical according to the present invention was set into groups of 5.6 g crude drug/kg, 2.8 g crude drug/kg and 1.4 g crude drug/kg, which are equivalent to 20, 10 and 5 times of the clinical human dosage (0.28g crude drug/kg) respectively. Groups of blank control, model control and huperzine A 0.4mg/kg were set additionally. Mice were divided into 6 groups according to the above dosage setting, as is shown in Table 5.

Table 5: the effect of the pharmaceutical according to the present invention on the memory-reappearance disorder of mice induced by scopolamine

Groups	Dosage	Animal number	equivalent to the times of clinical dosage (ratio)
Group of Blank Control	—	12	—
Group of Model Control	—	12	—
Group of Huperzine A	0.4mg/kg	12	—
Group of the pharmaceutical according to the present invention	5.6g Crude drug/kg	12	20
	2.8g Crude drug/kg	12	10
	1.4g Crude drug/kg	12	5

3.2.2 Method of administration

[132] The administering concentrations of the pharmaceutical according to the present invention were 0.08 g dry powder/ml, 0.04g dry powder/ml, and 0.02g dry powder/ml respectively, and the administering concentration of Huperzine A group was 0.02mg/ml. 0.2ml/10g was administered by gavage daily, which is in accordance with the clinically recommended oral route, for 14 consecutive days.

3.2.3 Animal model establishment method

[133] 30 minutes after the administration on the 14th day, 2mg/kg scopolamine hydrobromide was administered by intraperitoneal injection except in the group of blank control to make a memory-reappearance disorder model.

3.2.4 Experimental period: 14 days.

3.2.5 Detection indicators and detection methods

[134] The mice were trained (water temperature 24-26°C, water depth 10 cm). When being trained, a mouse was first placed on the platform for 10 seconds so that it knew the existence of this safety zone, then the mouse was placed near the platform to allow it climbing the stairs by itself to be acquainted with path of escape. The whole training was divided into 5 days: Point A on the 9th day, Point B on the 10th day, Point B on the 11th day, Point S on the 12th day, and Point S on the 13th day, the mice were trained once every day, each training was set to 2 minutes, and the animal was guided to the platform by a glass rod if timeout. When starting to swim, the mouse's head was facing the wall of the starting point. After the training was performed to Point S, the mice were required to be able to swim to the platform within 2 minutes, otherwise they would be rejected. 30 min after the administration on the 14th day, 2mg/kg scopolamine hydrobromide was administered by intraperitoneal injection, except for the group of blank control, to make a memory-reappearance disorder model. The test was performed 30 min after the modeling. The mice started to swim from Point S, and the number of errors (the number of entering a blind end) and the latent period (the time period to swim from Point S to the platform) were recorded.

3.2.6 Statistical method: non-parametric test was performed by using spss11.5 statistical software.

3.3 Experimental results

[135] The swimming latent period of mice in the model group was extended in comparison with that of the group of blank control ($P<0.01$). The swimming latent period of mice in the groups of huperzine A was shortened in comparison with that of the model group ($P<0.01$). The latent period of in the group of 5.6 g Crude drug/kg of the pharmaceutical according to the present invention was shortened ($P<0.05$). The results are shown in Table 6.

Table 6: the effect of the pharmaceutical according to the present invention on the memory-reappearance disorder of mice induced by scopine ($\bar{x} \pm s$, $n=12$)

Groups	Dosage	Number of Errors (times)	Latent Period (sec)
Group of Blank Control		11.58±7.94	54.50±37.61
Group of Model Control		21.08±13.13	109.3±19.7 ^{△△}
Group of Huperzine A	0.4mg/kg	16.83±14.21	63.33±44.30**
Groups of the pharmaceutical according to the present invention	5.6g Crude drug/kg	18.83±11.68	79.67±40.96*
	2.8g Crude drug /kg	17.83±13.56	67.75±50.67
	1.4g Crude drug /kg	23.67±13.49	92.92±34.29

Note: ^{△△} $P<0.01$, compared with the group of blank control;

* $P<0.05$, ** $P<0.01$, compared with the group of model control

3.4 Conclusion

[136] The pharmaceutical according to the present invention has the effect of improving memory disorder of mice induced by scopine.

[137] In addition, in the mouse model of memory-reappearance disorder induced by scopine, the swimming latent period in the group of 5.6 g Crude drug/kg of the pharmaceutical according to the present invention was shortened. Furthermore, in the mouse model of acquired-dysmnnesia induced by scopine, the swimming latent period in the groups of 5.6 g Crude drug/kg and 2.8 g Crude drug/kg of the pharmaceutical according to the present invention was shortened. These results suggest that the pharmaceutical according to the present invention has the effect of enhancing memory.

[138] In the mice burden swimming experiment, the swimming time in the group of 5.6 g Crude drug/kg of the pharmaceutical according to the present invention was extended, and the swimming time in the groups of 2.8 g Crude drug/kg and 1.4 g Crude drug/kg of the pharmaceutical according to the present invention tended to be extended by 25.5% and 11.3%, respectively. In the Rota Rod experiments, the Rota Rod time in the groups of 5.6 g, 2.8 g and 1.4 g Crude drug/kg of the pharmaceutical according to the present invention tended to be extended by 93.6%, 55.9% and 3.0%, respectively. These results suggest that the pharmaceutical according to the present invention has anti-fatigue effect to a certain degree.

[139] In the twist experiment, the twisting latent period of mice in the group of 5.6 g Crude drug/kg of the pharmaceutical according to the present invention was extended, and the times of twisting reduced. In the hot-plate experiment, the pain threshold of mice in the group of 2.8 g Crude drug/kg of the pharmaceutical according to the present invention was extended. These results suggest that the pharmaceutical according to the present invention has pain relief effect.

[140] In conclusion, the pharmaceutical according to the present invention has the effects of sedation, hypnosis, promoting memory, anti-fatigue and pain relief. The clinically recommended dosage of the pharmaceutical composition according to the present invention is 17.0g crude drug/day (standard weight 60 kg), and the toxicity test of the pharmaceutical composition according to the present invention confirmed that no acute toxic reaction was observed in mice with maximum dosage 166.80g crude drug/kg, and no obvious toxic reaction was observed in rats when administered by gavage with 5.6, 11.2 and 22.4 g crude drug/kg for 3 consecutive months, thereby such composition can be recommended for clinical use.

CLAIMS

1. A pharmaceutical composition, comprising *Radix Polygoni Multiflori* and/or extracts thereof, *Semen Ziziphi Spinosae* and/or extracts thereof, and *Fructus Mori* and/or extracts thereof,
wherein the pharmaceutical composition further comprises *Ganoderma* and/or extracts thereof, *Bulbus Lilii* and/or extracts thereof, *Rhizoma Anemarrhenae* and/or extracts thereof, *Radix Salviae Miltiorrhizae* and/or extracts thereof, *Flos Chrysanthemi* and/or extracts thereof, and *Poria* and/or extracts thereof.
2. The pharmaceutical composition according to claim 1, further comprises *Flos Albiziae* and/or extracts thereof.
3. The pharmaceutical composition according to claim 1 or claim 2, wherein the extracts is independently solvent extract, wherein said solvent is water, ethanol, aqueous ethanol solution, supercritical carbon dioxide, or any mixture thereof.
4. The pharmaceutical composition according to claim 1, wherein the pharmaceutical composition is prepared from crude medicines of the following ratios by weight parts: *Radix Polygoni Multiflori* 150-270, *Semen Ziziphi Spinosae* 145-275, and *Fructus Mori* 160-255.
5. The pharmaceutical composition according to claim 1, wherein the pharmaceutical composition is prepared from crude medicines of the following ratios by weight parts: *Radix Polygoni Multiflori* 150-270, *Semen Ziziphi Spinosae* 145-275, *Fructus Mori* 160-255, *Ganoderma* 80-135, *Bulbus Lilii* 75-160, *Rhizoma Anemarrhenae* 50-110, *Radix Salviae Miltiorrhizae* 120-200, *Flos Chrysanthemi* 45-120, *Poria* 75-145.
6. The pharmaceutical composition according to claim 2, wherein the pharmaceutical composition is prepared from crude medicines of the following ratios by weight parts: *Radix Polygoni Multiflori* 150-270, *Semen Ziziphi Spinosae* 145-275, *Fructus Mori* 160-255, *Ganoderma* 80-135, *Bulbus Lilii* 75-160, *Rhizoma Anemarrhenae* 50-110, *Radix Salviae Miltiorrhizae* 120-200, *Flos Chrysanthemi* 45-120, *Poria* 75-145, and *Flos Albiziae* 165-288.

7. The pharmaceutical composition according to claim 5, wherein the ratios by weight parts of the crude medicines are: Radix Polygoni Multiflori 158, Semen Ziziphi Spinosae 270, Fructus Mori 165, Ganoderma 135, Bulbus Lilii 80, Rhizoma Anemarrhenae 110, Radix Salviae Miltiorrhizae 125, Flos Chrysanthemi 118, Poria 80, and Flos Albiziae 280.
8. The pharmaceutical composition according to claim 5, wherein the ratios by weight parts of the crude medicines are: Radix Polygoni Multiflori 265, Semen Ziziphi Spinosae 147, Fructus Mori 250, Ganoderma 81, Bulbus Lilii 158, Rhizoma Anemarrhenae 55, Radix Salviae Miltiorrhizae 188, Flos Chrysanthemi 50, Poria 145, and Flos Albiziae 170.
9. The pharmaceutical composition according to claim 5, wherein the ratios by weight parts of the crude medicines are: Radix Polygoni Multiflori 225, Semen Ziziphi Spinosae 163, Fructus Mori 220, Ganoderma 83, Bulbus Lilii 113, Rhizoma Anemarrhenae 50, Radix Salviae Miltiorrhizae 190, Flos Chrysanthemi 45, Poria 113, and Flos Albiziae 178.
10. The pharmaceutical composition according to claim 5, wherein the ratios by weight parts of the crude medicines are: Radix Polygoni Multiflori 165, Semen Ziziphi Spinosae 228, Fructus Mori 173, Ganoderma 110, Bulbus Lilii 75, Rhizoma Anemarrhenae 75, Radix Salviae Miltiorrhizae 150, Flos Chrysanthemi 78, Poria 78, and Flos Albiziae 218.
11. The pharmaceutical composition according to claim 5, wherein the ratios by weight parts of the crude medicines are: Radix Polygoni Multiflori 200, Semen Ziziphi Spinosae 200, Fructus Mori 200, Ganoderma 100, Bulbus Lilii 100, Rhizoma Anemarrhenae 66.7, Radix Salviae Miltiorrhizae 166.7, Flos Chrysanthemi 66.7, Poria 100, and Flos Albiziae 200.
12. The pharmaceutical composition according to any of claims 1 to 10, wherein each gram of the pharmaceutical composition contains not less than 0.5 mg, preferably not less than 1.0 mg, more preferably not less than 1.5 mg 2,3,5,4'-tetrahydroxydiphenylethene-2-O- β -D-glucoside based on the dry weight of the pharmaceutical composition.
13. The pharmaceutical composition according to any of claims 1 to 12, wherein the dosage form of the pharmaceutical composition is capsule, tablet, powder, oral solution, soft capsule, pill, tincture, syrup, suppository, gel, spray or injection.

14. The pharmaceutical composition according to any of claims 3 to 12, wherein the method for preparing the pharmaceutical composition comprises preparing one or more of the crude medicines separately or collectively into an extract.

15. The pharmaceutical composition according to claim 14, wherein the method for preparing the pharmaceutical composition comprises: materia medica are weighed according to the ratios by weight parts, soaked for 20-40 minutes by adding water, decocted for 30-40 minutes after boiling, filtered, and again decocted for 25-30 minutes after addition of water and boiling, filtered, and the filtrates are combined.

16. The pharmaceutical composition according to any of claims 5 to 12, wherein the method for preparing the pharmaceutical composition comprises the following steps:

a) preparing Radix Polygoni Multiflori, Rhizoma Anemarrhenae and Semen Ziziphi Spinosae into alcohol extract;

b) preparing Fructus Mori, Poria, Ganoderma, Radix Salviae Miltiorrhizae, Flos Albiziae and Flos Chrysanthemi into aqueous extract;

c) preparing Bulbus Lilii into its powder; and

d) using the alcohol extract of step a), the aqueous extract of step b) and the powder of Bulbus Lilii of step c) as the active ingredients of the pharmaceutical composition.

17. The pharmaceutical composition according to any of claims 5 to 12, wherein the method for preparing the pharmaceutical composition comprises the following steps:

a) Radix Polygoni Multiflori, Rhizoma Anemarrhenae and Semen Ziziphi Spinosae are weighed according to the ratios by weight parts, 6-12 times amount of 30%-70% ethanol is added; heat under reflux is performed to extract for 1-3 times, with 1-3 hours each time; the extract is filtered, and combined to recover ethanol under reduced pressure, and concentrated to a relative density of 1.10-1.15 determined at 60°C, to obtain alcohol extract;

b) Fructus Mori, Poria, Ganoderma, Radix Salviae Miltiorrhizae, Flos Albiziae and Flos Chrysanthemi are weighed according to the ratios by weight parts, 10-15 times amount of water is added to extract for 1-3 times, with 1-3 hours each time; the extract is filtered,

combined, and concentrated to a relative density of about 1.10-1.15 determined at 60°, to obtain aqueous extract;

c) Bulbus Lili is weighed according to the ratios by weight parts, and crushed into 100 mesh powder of Bulbus Lili; and

d) using the alcohol extract of step a), the aqueous extract of step b) and the powder of Bulbus Lili of step c) as the active ingredients of the pharmaceutical composition.