Insomnia is associated with menopausal transition and is a major determinant affecting women’s Quality of Life (QOL) (Hsien-Chang et al., 2011). It is estimated that more than 27% people in the world suffer from insomnia with difficulty in initiating or maintaining sleep and this figure is expected to grow (Weyerer and Dilling, 1991; Freeman, 1996). However, it is well known that the most extensively used benzodiazepines showed many unpleasant reactions, such as drug dependence, tolerance, rebound insomnia and amnesia. The new type of hypnotics, such as zolpidem, zolpidem etc., also showed subjective residual effects (Griffiths et al., 1986). Therefore, many people have turned to traditional medicine to manage their own symptoms.

Ganoderma lucidum, which is called “Lingzhi” in China, is a widely used fungus in traditional Chinese medicine for preventing and treating a large number of diseases (Xianliang et al., 2012). The estimated global production of G. lucidum was about 4,700 tons in 2002, of which 3, 800 tons were produced in China (Lai et al., 2004). It has been widely used for the treatment of a variety of diseases such as cancer, hepatitis, neurasthenia, deficiency fatigue (Gao et al., 2004, 2002). Triterpenoids and polysaccharide are the main components of G. lucidum, which are reported to play an important role in the pharmacological effects mentioned above (Ko et al., 2008). It was also reported that the G. lucidum was a herbal medicine with not only hypnotic effects but also sleep quality enhancing effects (Wang et al., 2001). It has also been used as a tranquilizing agent to treat insomnia for thousands of years. Cui (1987) revealed that G. lucidum extract influenced the sleep of freely moving rats GLE and significantly increased total sleep time (Wang et al., 2001). Some literature reported on the hypnotic effects of G. lucidum extract in human beings.

Semen Ziziphi spinozae, a traditional tranquilizing medicine frequently used in China, has been used extensively for the treatment of a variety of syndromes and diseases, including insomnia, neurasthenia and climacteric period syndrome. Spinosin, also known as 2’-β-o-glucopyranosyl swertisin, is one of the major flavonoids of semen Ziziphi spinozae (Li-En et al., 2008). Z. spinozae has been in widespread use for thousands of years in traditional Chinese medicine for the treatment of a variety of syndromes and diseases, including insomnia, neurasthenia and climacteric period syndrome (Peng and Zhu, 2001). Animal studies indicated that both the decoction of semen Z. spinozae and its total flavonoids prolonged barbiturate induced sleep time.
For the first time the capsule of *G. lucidum* and *Z. spinosae* was made and its effect on the sleep improving function for the treatment of insomnia. The present study investigated groups of mice injected with pentobarbital and the criterion was if it was placed on its back and exhibited a loss-of-righting reflex.

**MATERIALS AND METHODS**

**Materials:** *G. lucidum* crude powder was the dry spore bodies of *G. lucidum* (Leyss. ex Fr.) Karst, which was purchased from the Health Food Co., Ltd. Yancheng Shen Nong (Province, China PR). The spore powder of *G. lucidum* was obtained through breaking the *G. lucidum* (Leyss. ex Fr.) Karst, which was purchased from Shen Nong's Health Food Co., Ltd. Yancheng (province, China PR).

*Z. Spinozae* was the dry mature seeds of buckthorn plants of jujube *Ziziphus* jujube mill.var.spinosa (Bunge) Hu ex H.F.Chou, which was purchased from Longquan Pharmaceutical Company (Province, P.R. China).

**Animals:** Female ICR mice (Grade CL, Shanghai Slac Laboratory Animal Co. Ltd., Shanghai, China), weighing 18-22 g were used. The mice were housed in the SPF grade animal facility and were fed with standard diet and water.

The Ginsenoside Re (purity of 88.8%) and Rutin (purity of 90%) standard were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China PR). Pentobarbital sodium and barbital sodium were analytically pure, produced from Shanghai Chemical Reagent Company.

**Methods:**

**Preparation of the capsule of Semen Z. spinosae and Gabiderma lucidum:** Powdered *G. lucidum* 2.5 kg and *Z. spinosae* 1.0 kg were extracted twice with 75% v/v ethanol, 80°C for 2 h (1:8) and then ethanol insoluble matter were filtered, combined and extracted by water. The extraction was done at 100°C for 1.5 h (1:10) (*Z. spinosae*, yield 12%, *G. lucidum*, yield 7%). The combined filter residue was extracted by water. Finally the filter (ρ = 1.03-1.04) was concentrated (ρ = 1.02-1.03) under reduced pressure (50°C, 0.09 MPa) and then spray-dried (inlet temperature 230°C, outlet temperature 90°C) to yield powder, the broken spore powder of *G. lucidum* (0.13 kg) was mixed with it.

**Determination of the content of polysaccharide, total flavone and total spinosin:** Content of polysaccharide was determined by the method of phenol-sulfuric acid (Gao et al., 2004). Determination of total flavone was in accordance with literature (Gao et al., 2004), polyamide column chromatography was used to make the standard curve with rutin as the standard. Determination of total spinosin was in accordance with literature by D-101 macroporous adsorbent resin (Gao et al., 2004), the calibration curve was made and the ginsenoside Re as a standard. The content of triterpenoids was determined by the column chromatography method with the calibration curve made using oleanolic as a standard.

**Drugs and drug administration:** The capsules of the drug were obtained from the Fang Ge Co., Ltd. in Zhejiang, China PR. The mice were divided into four groups, each group included 12 mice. The mice were treated with the drug capsules (225, 450 and 1350 mg/kg b.wt/day, respectively), or distilled water as a control. The freshly prepared drug capsules were dissolved in distilled water at 450, 800, 2700 mg in 40 mL distilled water. The mice were treated with this solution (20 mL/kg b.wt) by Stomach Lavaging for 30 days, distilled water was administered as the reference. One of the groups was used to directly evaluate the sleep induced. To evaluate the effect of the drug on the weight of the mice, each animal was weighed before and after administration.

**Evaluation of the direct sleep induction:** Following pentobarbital injection, each mouse was observed for the direct sleep induction. A mouse was considered asleep if it was placed on its back and exhibited a loss-of-righting reflex for 5 min. The mice that rolled over to right themselves in less than 5 min were considered to be awake.

**Prolonging sleeping time induced by pentobarbital sodium:** The animals in each group were injected with pentobarbital 50 mg/kg, the injection quantity was 0.2 mL/20 g. Decision was based whether on it being placed on its back it exhibited a loss-of-righting reflex for 1 min. Each mouse was observed to determine whether the drug could prolong the sleeping time induced by pentobarbital sodium.

**Affecting sleeping rate under the threshold hypnogenesis dosage of pentobarbital sodium:** The animal of each group were injected with pentobarbital 130 mg/kg, the injection quantity was 0.2 mL/20 g. Decision was based whether on being placed on its back it exhibited a loss-of-righting reflex for 1 min. Also, the number of mice which fell asleep within 30 min was recorded.

**Sleep delitescence induced to tobarbital sodium:** The animals in each group were injected with pentobarbital 205 mg/kg at 0.2 mL/20 g. Decisions were based on whether on being placed on its back, a mouse exhibited a loss-of-righting reflex. The sleep delitescence of all group animals was recorded. Sleep latency time was recorded from the time of pentobarbital injection until 1 min after mice exhibited a loss-of-righting reflex. Sleep time was recorded from 1 min after exhibiting a loss-of-righting reflex until regaining the righting reflex. In the test with a subhypnotic dose of pentobarbital, the percentage of
sleep onset was calculated as number of animals falling asleep/total number of animals ×100.

**Statistical analysis:** All values are expressed as mean±standard deviation (S.D.). For multiple comparisons, data were analyzed by one-way Analysis of Variance (ANOVA) followed by Student-Newman-Keuls test. For the test with a subhypnotic dose of pentobarbital, the x² test was used to compare the percentages of sleep onset between the group that received a subhypnotic dose of pentobarbital alone and each of the other groups with significance at p<0.05.

**RESULTS AND DISCUSSION**

Content of functional composition of *G. lucidum* and *Z. spinozae*: The active biochemical ingredient of the herbal formula is unknown. *G. lucidum* contains triterpenes, polysaccharides and sterols. *Z. spinozae* contains spinosin and flavonoid, which were reported the most important bioactive constituents (Guo and Fan, 1996, 1998). The present study showed that the content of polysaccharide was high, about 12.08%. The content of triterpenoid, total flavone and spinosin are 1.50, 1.35 and 0.70%, respectively (Table 1).

Effect of the capsules of the drug on body weight of mice: There was no significant difference (p=0.05) between the initial body weight and the final body weight (Table 2). The drug capsules showed no effect on mice body weight.

Evaluation of the sleep induced directly: None of the mice fell asleep, the drug capsules showed no effect on sleep induced directly on the mice (Table 3).

Prolonging sleeping time induced by pentobarbital sodium: Significant effect of prolonging sleeping time induced by pentobarbital sodium was observed according to the control group (p=0.01, p<0.05) and the effect is closely related with the dose (Table 4).

Affecting sleeping rate under the threshold hypnogenesis dosage of pentobarbital sodium: The number of mice falling asleep increased with the dose. The number of mice falling asleep in high-dose group was significantly different from the control group (p<0.005) (Table 5).

Sleep delitescence induced by tobarbital sodium: The drug capsules could shorten the sleep delitescence compared to the control group. The sleep delitescence in the high-dose group was significantly different from the control group (p=0.005) (Table 6).

Comparison of the effect on sleep of *G. lucidum* or *Z. spinozae* used alone or in combination: The effect on sleep of the capsules of combined *G. lucidum* and *Z. spinozae* was compared with the effect of *G. lucidum* and *Z. spinozae* used individually. All of the three drugs showed no effect on sleep induced directly on mice. The data of reference article Huang and Jin (2008) and Jiang and Pen (2008) were cited.
For the prolonging sleeping time induced by pentobarbital sodium, the effect of the G. lucidum was the best while the Z. spinozae was better than the effect of capsules of G. lucidum and Z. spinozae.

As for the effects on the sleeping rate under the threshold hypnogenesis dosage of pentobarbital sodium, the effect of the G. lucidum extract was better than the other two, the effect of the capsules of G. lucidum and Z. spinozae was better than the Z. spinozae.

As for the sleep delitescence induced by tobarbital sodium, the effect of the G. lucidum extract was the best, the effect of the capsules of G. lucidum and Z. spinozae was better than the Z. spinozae.

The present study showed that the content of polysaccharide, total flavone, spinosin and triterpenoid was high in the G. lucidum and Z. spinozae capsule. The capsule significantly improved the sleep induced by pentobarbital in mice by shortening the sleep delitescence and increasing the sleep time, but it had no effect on the sleep induced directly just like G. lucidum and Z. spinozae. The effects were dose-dependent.

It was reported that both the G. lucidum and Z. spinozae had the function of improving sleep and the polysaccharide extract of G. lucidum, improved the insomnia severity scores in patients with neurasthenia. The mechanism of the beneficial effect of G. lucidum on insomnia remains unknown. In our experiment, the content of polysaccharide was the highest (12.08%) in the functional composition. A recent animal study showed that aqueous extracts from G. lucidum exerted sedative effects by decreasing sleep latency and increased sleep time in pentobarbital-treated rats via a GABAergic mechanism (Chu et al., 2007). The hypnotic effect and possible mechanism of action of spinosin on pentobarbital-induced sleep was assessed by the loss-of-righting reflex in the reference (Li et al., 2008). Literature demonstrates the hypnotic effects of Z. spinozae (Cui, 1987; Li et al., 2002) and its total flavonoids (Wang et al., 2006, 2008). Animal studies indicated that both the decoction of Z. spinozae and its total flavonoids prolonged barbiturate-induced sleep time. The hypnotic effect of spinosin, one of the major flavonoids, also had been assessed (Sui et al., 2007; Yuan et al., 1987). In our experiment, the content of total flavonoids and saponin was high, being 1.25 and 0.67%, respectively in the functional composition. The G. lucidum material was very expensive, our study found out a way to reach the same function with the lower cost.

The search for novel pharmacotherapy for psychiatric illness from medicinal plants has progressed significantly in the past decade. A considerable number of herbal constituents whose behavioral effects and pharmacological actions have been well characterized may be good candidates for further investigations that may ultimately lead to clinical use. An increasing number of herbal products have been introduced into psychiatric practice in the past decade (Eun et al., 2006). The potential benefits of herbal remedies such as St. John’s wort and Kava-kava in psychiatric practice have been addressed (Zhang, 2004; Ma et al., 2009). The potential benefits of herbal remedies such as St. John’s wort and Kava-kava in psychiatric practice have been addressed. The capsule of G. lucidum and Z. spinozae might be another good candidate for use in psychiatric illnesses such as sleep disorders.

These results indicated that caution should be taken when the capsule is used at higher doses or combined with other drugs. Monitoring of adverse events should be systematically carried out and potential drug interactions should be identified. This would enable a safer use of the capsule. In conclusion, the capsule had the function of improving sleep.

CONCLUSION

In the present study, G. lucidum and Z. spinozae were combined and the active fractions were extracted to make the capsule. The functional compositions of the capsule were polysaccharide, total flavone, spinosin and triterpenoid, with the content being 12.08, 1.35, 0.67 and 1.50 g/100 g, respectively. The effect of the capsule on improving sleep in mice was studied. Results showed no effects on the sleep induced directly in mice assessed with the loss-of-righting reflex even at the high dose of 450, 1350 mg/kg/day. However, the capsule significantly decreased sleep latency and increased sleeping time and prolonged sleeping time induced by pentobarbital sodium at high doses. In conclusion, the capsule of G. lucidum and Z. spinozae combined had the function of improving sleep.

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