Vol. 17(5), pp. 122-128, May, 2023
DOI: 10.5897/JMPR2022.7260
Article Number: 0B1D70B70679
ISSN 1996-0875

http://www.academicjournals.org/JMPR

ACADEMIC JOURNALS
expand your knowledge

Journal of Medicinal Plants Research

Full Length Research Paper

Sedative and hypnotic effects of the compatibility of *Schisandra chinensis* and *Polygala tenuifolia* on insomnia mice

Haifeng Sun*, Han Wang, Na Li and Hongyu Yang

College of Pharmacy, Heilongjiang University of Chinese Medicine, Harbin, Heilongjiang Province, 150040 China.

Received 26 July 2022; Accepted 18 April, 2023

The objective of this study is to study the sedative and hypnotic effects of the compatibility of *Schisandra chinensis* (SC) and *Polygala tenuifolia* (PT) on insomnia mice and its mechanisms. The 160 SPF KM mice were divided into 8 groups with 20 each group (10 female and 10 male mice), including control, model, estazolam, SC, PT, SC-PT 1:1, SC-PT 1:2, and SC-PT 2:1 group. Insomnia mice model was established with intraperitoneal injection of P-chlorophenylalanine (PCPA) for 3 days. The mice were investigated by the sleep time of mice induced by pentobarbital sodium, the levels of GABA, DA, 5-HT and 5-HIAA in hypothalamus by enzyme-linked immunosorbent assay, and the GABAα1 and DRD2 mRNA expression in hypothalamus by quantitative real-time polymerase chain reaction (RT-qPCR). SC-PT reduced the sleep latency of insomnia mice and prolonged the Pentobarbital-sodium induced sleep time than model group. SC-PT 2:1 group increased the levels of 5-hydroxytryptamine (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), γ-Aminobutyric acid (GABA) and the expression of GABAARα1 and decreased the levels of dopamine (DA) and the expression of DRD2 in the hypothalamus of insomnia mice. The compatibility of SC and PT can enhance the function of sedation and hypnosis than single group. This study provides a basis for the compatibility of SC and PT in the insomnia treatment.

Key words: *Schisandra chinensis*, *Polygala tenuifolia*, mice, sedative, hypnotic, γ-Aminobutyric acid, dopamine.

INTRODUCTION

Insomnia is defined as the disorder of sleep process, such as difficulty falling asleep, decline of sleep quality and sleep time, and even decline of memory function and attention function. It is a common disease all over the world. At present, the main focus on insomnia is behavioral therapies and drug therapies (Roth and Drake, 2004; Zammit, 2007), but the traditional hypnotic not only have tolerance, but also have serious rebound Insomnia

*Corresponding author. E-mail: 359896987@qq.com.

(Kripke, 2000). The development order of traditional hypnotics is roughly as pre-barbiturates, barbiturates and non-barbiturates, benzodiazepines, non-benzodiazepines, melatonin receptor agonists and orexin receptor blockers (Rui and Han, 2021). Traditional treatment of insomnia is mainly based on GABA-A Receptors (serotonin, histamine or melatonin); they are the main targets (Robinson et al., 2022). Z-drugs drive sleep mainly through extensive inhibition of central nervous system (CNS) activity. But they can cause many side effects such as the addiction, drug dependence and tolerance (Dolder et al., 2008; Frey et al., 2011). Benzodiazepines can cause adverse reactions such as drug resistance, dependence, withdrawal reaction and memory impairment. Daridorexant plays a role in promoting sleep by inhibiting OX1R and OX2R. Therefore, they can avoid regulating GABA-A receptor like traditional hypnotics, thus avoiding related side effects. (Roch et al., 2021) Therefore, traditional Chinese medicine has provided a new direction for the treatment of insomnia with the characteristics of more ingredients and less adverse reactions. Moreover, the compatibility of herbal medicine has been used in China for thousands of years, and it is still a hot spot in the treatment of diseases today. The compatibility of traditional Chinese medicine can increase the efficiency and reduce the toxicity by changing the content of chemical components, changing the pharmacokinetic parameters *in vivo*, and enhancing the protection of the body system (Wu et al., 2019).

Schisandra chinensis (SC) (Turcz.) Baill is mostly used to treat insomnia, asthma, night sweats, palpitations and other diseases. In recent years, SC extract has been studied to have antioxidant, antitumor, anti-virus, liver protection, platelet activating factor inhibition, central nervous system regulation and other activities (Huang et al., 2007). At present, SC is still widely used in nervous system diseases such as insomnia and Alzheimer's disease. The pharmacokinetic study of SC alcohol extract showed that its hypnotic activity may be related to the increase of the levels of serotonin and 5-hydroxyindoleacetic acid γ-Aminobutyric acid, norepinephrine, dopamine, dihydroxyphenylalanine and homovanillic acid are related to the decline of their levels (Yan et al., 2016; Wang et al., 2020; Zhang et al., 2014; Wei et al., 2014).

PT is the dry root of *Polygala tenuifolia* Willd. and *Polygala sibiria* L. which is commonly used for insomnia, dreaminess, forgetfulness, palpitation, trance, expectoration, sores, swelling and toxin, breast swelling and pain caused by heart kidney disharmony in China. As a commonly used traditional Chinese medicine for insomnia, it was found that PT ethanol extracts can significantly increase the content of 5-hydroxytryptamine (5-HT) and γ-Aminobutyric acid (GABA) levels and decrease Glu levels (Ren et al., 2020). A study in 2016 showed that the tenuifolin has hypnotic effect in male cancer research mice, which could prolong the total sleep time, reduce the ability of noradrenaline and increase the acetylcholine and γ-GABA. These results suggested that tenuifolin may enhance sleep by activating GABAAergic system or inhibiting noradrenergic system (Cao et al., 2016). Tenuifolin also showed strong sleep promoting activity in zebrafish and are mediated by 5-hydroxytryptaminergic and GABAergic systems (Zhao et al., 2020).

SC and PT are common traditional Chinese medicine and folk medicine since ancient time. Traditionally, *S. chinensis* is used to treat chronic cough and asthma, frequent enuresis, spontaneous sweating and night sweating, palpitations and insomnia (Dong et al., 2021). *P. tenuifolia* is often used to treat insomnia, dreaminess, amnesia, palpitations, expectoration discomfort, breast swelling and pain caused by heart kidney disjunction (Yao et al., 2022). At present, there were many studies on SC in the treatment of depression, anxiety, Alzheimer's disease and insomnia and PT in the treatment of insomnia and Alzheimer's disease. SC and PT, as Chinese traditional classical drugs, have a long history in the treatment of insomnia, and there are many prescriptions about their compatibility. SC and PT were the second most frequent compatibility with 51.61%. A study conducted in 2021 by Wang et al. (2021) investigated the medication patterns of SC through patented information mining technology, and found that among many herbs compatible with SC, PT is the second most frequently used, but there are few studies about the combined treatment of SC and PT for insomnia and its underlying mechanism. Therefore, the main purpose of this study is to explore the sedative and hypnotic mechanism of the compatibility of SC and PT.

MATERIALS AND METHODS

Drugs and reagents

Sodium chloride injection was purchased from Harbin Sanlian Pharmaceutical Co. Ltd; (210802D05); Pentobarbital sodium was purchased from Sinopharm Chemical Reagent Co., Ltd. (WS20170421); Estazolam tablets were purchased from Shandong Xinyi Pharmaceutical Co., Ltd. (H37023047, batch No. 211102); P-chlorophenylalanine (PCPA) was purchased from Shanghai Aladdin Biochemical Technology Co., Ltd (No. J2114483). Mouse γ-Aminobutyric acid (GABA) ELISA kit, Mouse dopamine (DA) ELISA kit, Mouse 5-hydroxytryptamine (5-HT) ELISA kit, and Mouse 5-hydroxyindoleacetic acid (5-HIAA) ELISA kit were purchased from Jiangsu Jingmei Biological Technology Go., Ltd. The Supersmart^{TM} 3rd generation ZAPA SYBR Green qPCR premix (ZS-M13002), Supersmart^{TM} 6 min heat resistant first strand cDNA synthesis Kit (ZS-M14003) and 6-min high purity RNA Extraction Kit (ZS-M11005) was from Zhongshi (Tianjin) inspection and Testing Co., Ltd. *S. chinensis* and *P. tenuifolia* were purchased in Sankeshu Medicinal Material market in Harbin, and identified by Professor Haifeng Sun (Department of Pharmacy, Heilongjiang University of Chinese Medicine) according to the guidelines of the Chinese Pharmacopoeia (2020).

Animals

SPF KM mice (6-8 weeks old and weighing 20 ± 2 g) were purchased from Jinan Pengyue Experimental Animal Breeding Co., Ltd. (production license: SCXK (LU) 2019 0003; Laboratory License: SYXK (Hei) 2018-007). The animals were housed in acrylic cages with water and food available *ad libitum* under an artificial 12-h light/dark cycle (light from 5:00 a.m. to 5:00 p.m.) in a sound-proof room (25 ± 1°C) with food and water available *ad libitum* for the duration of the study. Every effort was made to minimize the number of animals used and any pain and discomfort experienced by the subjects.

Preparation of SC and PT extracts

A proper amount of SC was weighed and crushed. According to the best extraction method selected by the research group, 75% ethanol (eight times the weight of SC) was refluxed and extracted twice at 85°C for 2 h each time. Combine the filtrate extracted twice for reduction and evaporation, so that the final crude drug content is 0.5 g/ml liquid drug. The extract of PT was the same as that of SC, except for adding 6 times the amount of 75% ethanol.

Establishment of insomnia mouse model, grouping and administration

The mice were randomly divided into 8 groups, including control, model, estazolam (positive control group), SC, PT and SC-PT 1:1, SC-PT 1:2, and SC-PT 2:1 group, with an average of 10 males and 10 females in each group. The latter 7 groups were injected intraperitoneally with PCPA (350 mg/kg) for 3 days to induce insomnia mouse model (Lan et al., 2022), and the control group was injected with the same amount of normal saline at the same time point.

On the fourth day, according to the Chinese Pharmacopoeia and the experimental results of the earlier research group, the classic Eszolam group was given Eszolam (1.02 mg/kg, equivalent to the adult clinical dose of 5 mg/kg), SC (1.635 g/kg, equivalent to the adult clinical dose of 8 g/kg), PT (1.635 g/kg) In SC-PT 1:1, 1:2 and 2:1 groups, the test drug (1.635:1.635, 1.635:3.270, 3.270:1.635 g/kg, equivalent to 8:8, 8:16, 16:8 g/kg of clinical dose for adults, respectively (Direct conversion algorithm based on body surface area) (Zhao and Sun, 2010) was administered orally for 10 days. The control group was given the same amount of saline.

Pentobarbital sodium induced sleep in mice

On the 11th day, mice were intraperitoneally injected with pentobarbital (40 mg/kg). The sleep latency and sleep time in each group was from injection to disappearance of righting, reflex was recorded. The sleep time is the correct time from the disappearance of the flip to the turning over of the mice and the inability to maintain the supine position for 30 s (Long et al., 2021).

Enzyme-linked immunosorbent assay

The mice were decapitated by removing their necks and the hypothalamus is separated immediately on ice. According to the manufacturer's protocol, the levels of GABA, DA, 5-HT and 5-HIAA in hypothalamus were detected by standard enzyme-linked immunosorbent assay (ELISA). All experiments were conducted at least 3 times.

Quantitative real-time polymerase chain reaction analysis

Quantitative real-time polymerase chain reaction (RT-qPCR) was carried out to detect the mRNA expression of GABAARα1 and DRD2. Supersmart^{TM} 6 min heat resistant first strand cDNA synthesis kit was used to synthesize cDNA. The Supersmart^{TM} 3rd generation ZAPA SYBR Green qPCR premix was used for PCR. The level of GABAARα1 and DRD2 were analyzed by the 2-△△Ct method.

Statistical analysis

All data were analyzed using the SPSS version 26 statistical software package (IBM, China) and expressed as the mean ± standard deviation ($\bar{x}$ ± s). One-way ANOVA was used for comparisons among groups followed by Student Newman Keuls for differences between two groups. $P < 0.05$ was considered significant and $P < 0.01$ was considered as extremely significant.

RESULTS

In the study, the differences in sleep latency and sleep time, the contents of GABA, DA, 5-HT and 5-HIAA in the hypothalamus and the expression of GABAAR α 1 and DRD2 of each group were statistically analyzed.

SC-PT enhanced pentobarbital sodium induced sleeping behaviors of insomnia mice

Compared with the control group, the sleep latency of the model group was significantly prolonged ($P<0.05$). Compared with the model group, estazolam, SC, PT, 1:1, 1:2 and 2:1 group could significantly reduce the sleep latency of mice induced by pentobarbital sodium ($P < 0.01$) (Figure 1, left), and the decrease trend of sleep latency in the 2:1 group was the largest except for the estazolam group.

Compared with the control group, the sleep time of model group mice was significantly shortened ($P<0.05$). Compared with the model group, estazolam, SC, PT, SC-PT 1:1, SC-PT 1:2 and SC-PT 2:1 group can significantly prolong the sleep time of mice induced by pentobarbital sodium ($P<0.01$) (Figure 1, right). Except for the estazolam group, the SC-PT 2:1 group had the largest trend of prolonging sleep time in each group.

Levels of GABA, DA, 5-HT and 5-HIAA in hypothalamus of mice

Compared with the control group, the levels of GABA, 5-HT and 5-HIAA of insomnia mice in model group decreased significantly ($P<0.01$), while the level of DA increased significantly in the model group ($P<0.05$). Compared with the model group, the levels of GABA, 5-HT and 5-HIAA of insomnia mice increased significantly

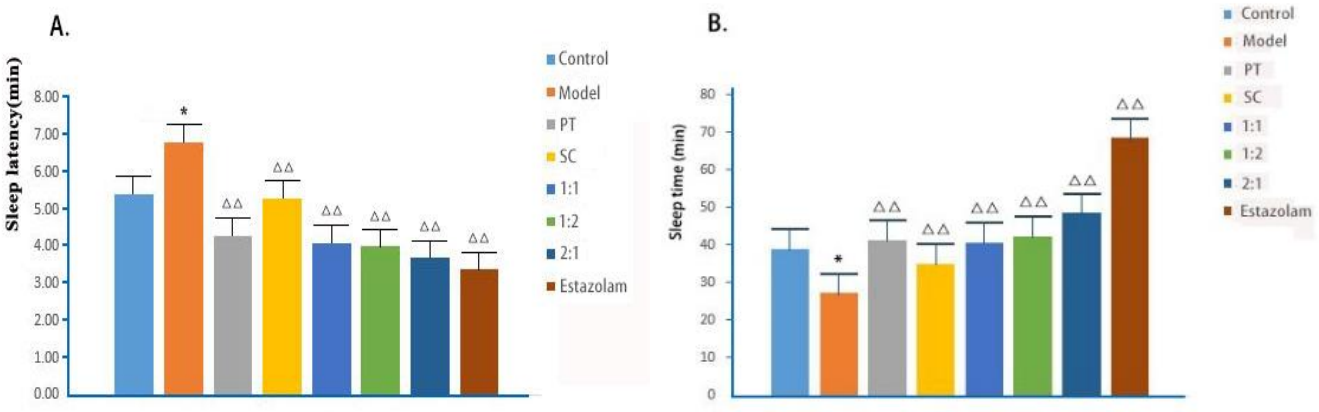

Figure 1. The compatibility of SC and PT decreased sleep latency (A) and increased sleep time (B) of insomnia mice in pentobarbital sodium test (±s, n=10). *P<0.05, **P<0.01 vs. control group; △P<0.05, △△P<0.01 vs. model group.
Source: Authors

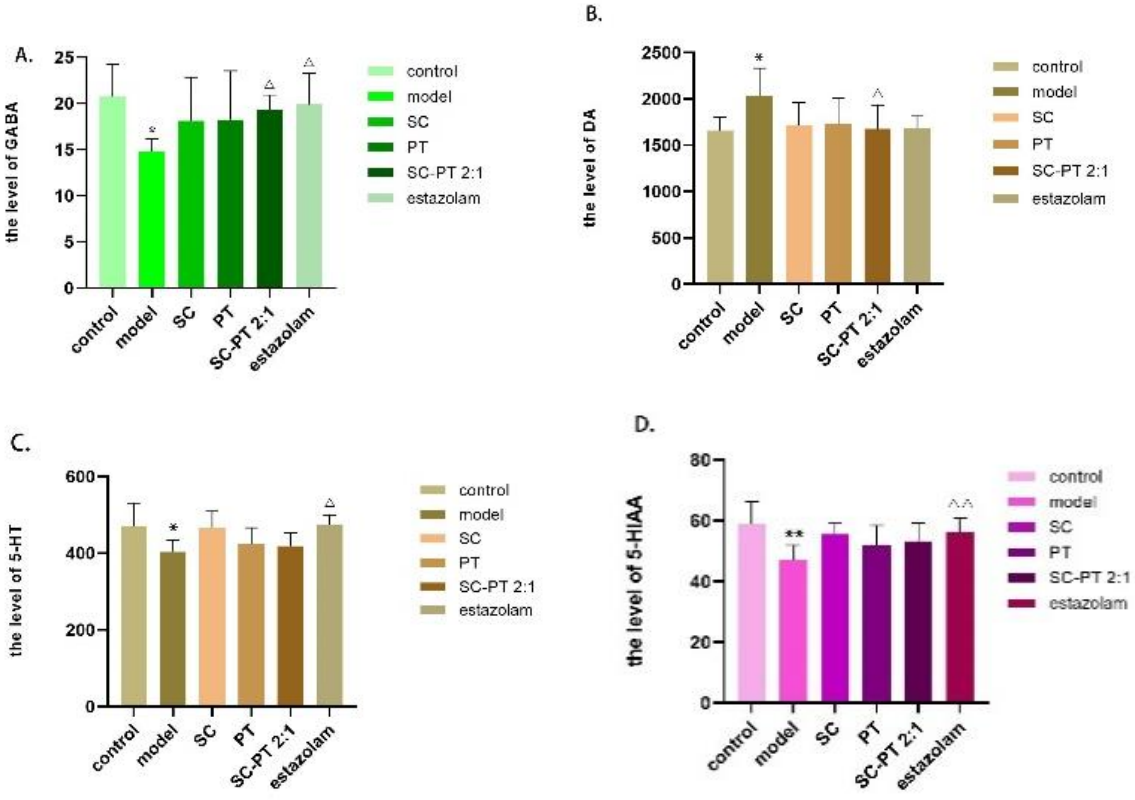

Figure 2. Neurotransmitters of each treatment group on insomnia mice (n=6). The levels of GABA (A), DA (B), 5-HT (C) and 5-HIAA (D) in hypothalamus of insomnia mice were detected by ELISA (*P<0.05, **P<0.01 vs. control group; △P<0.05, △△P<0.01 vs. model group).
Source: Authors

in the estazolam group (P<0.05 or P<0.01). The SC-PT 2:1 group augmented the levels of GABA and reduced the level of DA in hypothalamus of aged insomnia rats compared with the model group (P<0.05 or P<0.01, Figure 2), and the levels of GABA in SC-PT 2:1 group was higher than that of SC and PT groups, lower than

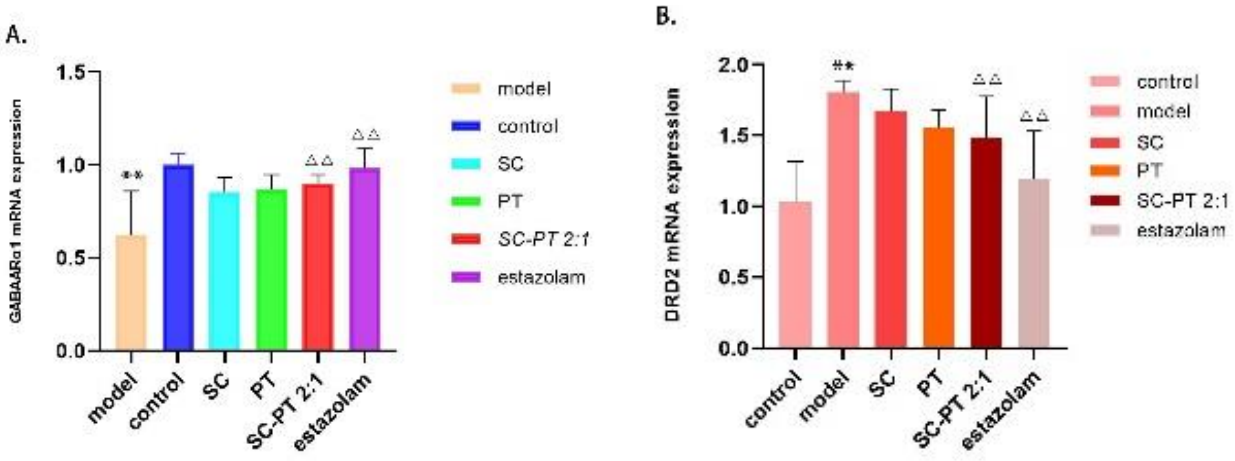

Figure 3. Expression of GABAARα1 and DRD1 in the hypothalamus of 2:1 group and estazolam group on insomnia mice (n=6). The expression of GABAARα1 (A) and DRD1 (B) in hypothalamus of insomnia mice (*P<0.05, **P<0.01 vs. control group; △P<0.05, △△P<0.01 vs. model group).
Source: Authors

that of SC and PT groups.

Expression of GABAARα1 and DRD2 in hypothalamus in 2:1 group on insomnia mice

Compared with the control group, the expression of GABAARα1 mRNA in model group was decreased, the expression of DRD2 mRNA was increased significantly (P＜0.01). The SC-PT 2:1 group and estazolam group were significantly augmented the expression of GABAARα1 mRNA, and reduced the expression of DRD2 mRNA in hypothalamus of insomnia mice compared with the model group (P<0.01), and the expression of GABAARα1 mRNA in 2:1 group was higher than that in SC group and Pt group, and the expression of DRD2 mRNA was lower than that in SC group and Pt group (Figure 3).

DISCUSSION

Among the drugs for insomnia, the first-line drugs include nonbenzodiazepines and antidepressants, but they have serious abnormal sleep and central nervous system inhibition; second line drugs include melatonin and suvorexant (Bragg et al., 2019). Insomnia drugs similar to suvorexant have some problems, melatonin is one of the safest drug substitutes at present. Although melatonin does not affect psychomotor function compared with other hypnotics, it increases subjective sleepiness and (Paul et al., 2003) has low oral bioavailability (De Muro et al., 2000; Di et al., 1997). Other drugs such as benzodiazepines, antihistamines and antipsychotics are rarely used because of their low effectiveness and high side effects (Bang et al., 2022). Herbal medicines play an important role in preventing and treating insomnia. As one of the important means of disease treatment, the compatibility of traditional Chinese medicine may produce effects other than those of two traditional Chinese medicine alone (Sun et al., 2023).

Modern medical research shows that insomnia is related to neurotransmitters such as dopamine, 5-hydroxytryptamine, neuropeptide, acetylcholine and γ-aminobutyric acid, hormones such as melatonin and prostaglandin D2, and cytokines such as interleukin-1, interleukin-6, and six necrosis factor regulate the occurrence of sleep wake alternation (Liu and Zhang, 2012). In this study, the compatibility of SC and PT can increase the content of GABA, 5-HT, 5-HIAA in the hypothalamus of mice and reduce the content of Glu.

Tenuigenin B in PT can improve learning and memory ability, and has the potential to treat cognitive dysfunction and Alzheimer's disease (Wang et al., 2019). Tenuifolin can significantly enhance the hypnotic effect on mice by activating GABAergic system and inhibiting noradrenergic system (Li et al., 2016). PT could increase the weight of senile insomnia rats, improve their memory ability, shorten the sleep latency induced by pentobarbital, prolong the sleep time, increase the level of 5-HT and GABA and reduce Glu level in hippocampus (Ren et al., 2020). SC can increase GABAARα1 and GABAARγ2 expression and down-regulation of NKCC1 expression in hippocampal to prolong sleep duration, reduce the number of autonomous activities and time of autonomous activities, shorten the time of platform piercing and

increase the number of platform piercing in insomnia rats, showing sedative and hypnotic effects (Wang, 2021). The sedative and hypnotic mechanisms of SC and PT studied are consistent with the results of these studies.

The results of this study show that the compatibility of SC and PT could shorten the sleep latency and prolong the sleep time of insomnia mice by increasing the levels of GABA and the expression of GABAARα1 mRNA and reducing the level of DA and the expression of DRD2 mRNA in hypothalamus. The sedative and hypnotic effect of SC combined with PT was better than that of SC group and PT group, and SC-PT 2:1 had the best effect. GABA is an inhibitory neurotransmitter with hypnotic, sedative and antianxiety functions (Jembrek et al., 2015). GABA regulates GABAergic system pathway through GABA receptors. GABA receptors are divided into GABAA, GABAB and GABAC, among which GABAARα1, GABAARγ2 are most closely related to sleep-wakefulness (Hu et al., 2017). GABA activates GABAA receptor to make Cl^- influx, excites immature neurons during early development, hyperpolarizes cell membrane, and thus produces central inhibitory effect (Ben-Ari et al., 2007). DA is an important endogenous catecholamine. DA neurons are mainly distributed in caudate nucleus and putamen, and their fibers project to the preoptic area, locus coeruleus, raphe and other neural structures related to sleep-wakefulness (Xiao, 2014). According to modern medical research, DA is an important neurotransmitter in the human body (Li et al., 2021). It can participate in the regulation of a variety of physiological activities in the body (such as self-cognition, emotional expression, etc). It is found that when animals are stimulated or awakened, the string discharge form of DA neurons can be obviously expressed in the dense area of substantia nigra and ventral tegmental area of midbrain, which can make DA release more effectively and promote the occurrence of awakened state (Peng et al., 2021). DA functions through its corresponding membrane receptors. DA receptors can be divided into dopamine D1 and D2. DA receptor can directly excite the cerebral cortex, and its metabolites can activate the β-Adrenaline produces awakening state (Han et al., 2013). DR2 is an important receptor that regulates the phase transition from sleep to wakefulness and participates in the maintenance of wakefulness. Diseases caused by abnormal DA system function will be accompanied by sleep disorders (Xei, 2020). It was found that blocking DR2 significantly shortened the sleep latency and prolonged the sleep time of mice.

In ancient, many medical practitioners realized that the pathogenesis of diseases was very complex, so the effect of single herb treatment may not be ideal. In pharmacodynamics, the compatibility of herbal medicine can enhance the efficacy and reduce the toxicity of drugs, and even lead to new pharmacological activities that a single herbal medicine does not have (Steven and Yi, 2021). Therefore, the compatibility of herbs may be more suitable for the treatment of more complex diseases.

Conclusion

The compatibility of SC and PT can enhance the sleep behavior of pentobarbital sodium induced insomnia mice. The combination of SC and PT can achieve sedative and hypnotic effects by up-regulating GABA neurotransmitter levels and down-regulating DA levels in hypothalamus. The sedative and hypnotic effect of SC and PT is better than that of SC and Pt, and the best compatibility ratio of SC and PT in the treatment of insomnia is determined to be 2:1. This study showed that the efficacy of two herbal medicines with calming effect was significantly better than that of single use, and the optimal ratio of the two drugs was determined. It has laid a scientific foundation for the in-depth study of the compatibility of the two drugs in the treatment of insomnia, as well as the development of efficient and safe drugs for the treatment of insomnia and their clinical application.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Bang YR, Jeon HJ, Yoon IY (2022). Effect of Long-Term Benzodiazepines for Chronic Insomnia on Cognitive Function and Waking Electroencephalography: A Case-Control Study. Psychiatry Investigation 19(4):259-267. https://doi.org/10.30773/pi.2021.0316

Ben-Ari Y, Gaiarsa JL, Tyzio R, Khazipov R (2007). (GABA): a pioneer transmitter that excites immature neurons and generates primitive oscillations. Physiological Reviews 87(4):1215-1284. https://doi.org/10.1152/physrev.00017.2006

Bragg S, Benich JJ, Christian N, Visserman J, Freedy J (2019). Updates in insomnia diagnosis and treatment. The International Journal of Psychiatry in Medicine 54(4-5):275-289. https://doi.org/10.1177/0091217419860716

Cao Q, Jiang Y, Cui SY, Tu PF, Chen YM, Ma XL, Cui XY, Huang YL, Ding H, Song JZ, Yu B, Sheng ZF, Wang ZJ, Xu YP, Yang G, Ye H, Hu X, Zhang YH (2016). Tenuifolin, a saponin derived from *Radix Polygalae*, exhibits sleep-enhancing effects in mice. Phytomedicine 23(14):1797-1805. https://doi.org/10.1016/j.phymed.2016.10.015

De Muro RL, Nafziger AN, Blask DE, Menhinick AM, Bertino JS J (2000). The absolute bioavailability of oral melatonin. The Journal of Clinical Pharmacology 40(7):781-784. https://doi.org/10.1177/00912700022009422

Di WL, Kadva A, Johnston A, Silman R (1997). Variable bioavailability of oral melatonin. New England Journal of Medicine 336(14):1028-1029. https://doi.org/10.1056/NEJM199704033361418

Dolder CR, Nelson MH (2008). Hypnosedative-induced complex behaviours: incidence, mechanisms and management. CNS drugs 22:1021-1036.. https://doi.org/10.2165/0023210-200822120-00005

Dong P, Liu J, Han H (2021). Research progress in *Schisandra chinensis* and its drug pairs. Information on Traditional Chinese Medicine 38(7):69-73.

Frey DJ, Ortega JD, Wiseman C, Farley CT, Wright KP J (2011).

Influence of zolpidem and sleep inertia on balance and cognition during nighttime awakening: A randomized placebo-controlled trial Journal of the American Geriatrics Society 59(1):73-81. https://doi.org/10.1111/j.1532-5415.2010.03229.x.

Han X, Li Q, Guo H, Gao J, Yu G, Jiang M, Ju W (2013). Effect of Zhenzao Capsule on the contents of 5-HT and DA in elderly insomnia patients with Yin deficiency and fire hyperactivity. Journal of Nanjing University of traditional Chinese Medicine 29(05):429-431.

Hu D, Yu ZL, Zhang Y, Han Y, Zhang W, Lu L, Shi J (2017). Bumetanide treatment during early development rescues maternal separation-induced susceptibility to stress. Scientific Reports 7(1):11878.. https://doi.org/10.1038/s41598-017-12183-z

Huang F, Xiong Y, Xu L, Ma S, Dou C (2007). Sedative and hypnotic activities of the ethanol fraction from Fructus Schisandrae in mice and rats. Journal of Ethnopharmacology 110(3):471-475.. https://doi.org/10.1016/j.jep.2006.10.008

Kripke DF (2000). Chronic hypnotic use: deadly risks, doubtful benefit. Sleep Medicine Reviews 4(1):5-20. https://doi.org/10.1053/smrv.1999.0076

Lan T, Wang L, Yu X, Yu C, Yao H, Xu H, Tan H, Nie S (2022). The sleep improvement effect of royal jelly and its extract on insomnia mice. Food Research and Development 43(20):83-90.

Li M, Zhou L, Sun X, Yang Y, Zhang C, Wang T, Fu F (2021). Dopamine, a co-regulatory component, bridges the central nervous system and the immune system. Biomedicine and Pharmacotherapy 145:112458. https://doi.org/10.1016/j.biopha.2021.112458

Li X, Cui J, Yu Y, Li W, Hou Y, Wang X, Qin D, Zhao C, Yao X, Zhao J, Pei G (2016). Traditional Chinese Nootropic Medicine Radix Polygalae and Its Active Constituent Onjisaponin B Reduce β-Amyloid Production and Improve Cognitive Impairments. PLoS One 11(3):e0151147. https://doi.org/10.1371/journal.pone.0151147

Liu Y, Zhang B (2012) Research progress of insomnia regulation mechanism in Chinese and Western medicine. Massage and Rehabilitation Medicine 3(9):55-56.

Long P, Hu X, Hu Q, Tao L, Zhou X, Shen X, Zhang M (2021). Study on the sedative and hypnotic effects of gastrodin on mice with insomnia induced by chlorophenylalanine (PCPA). Pharmacology and Clinic of Traditional Chinese Medicine 37(05):33-38.

Paul MA, Gray G, Kenny G, Pigeau RA (2003). Impact of melatonin, zaleplon, zopiclone, and temazepam on psychomotor performance. Aviation, Space, and Environmental Medicine 74:1263-70.

Peng Z, Zhang X, Bian Y, Chen J, Xiao C, Deng N, Liang Z (2021). Study on the characteristics of serum Da content and PSG sleep structure parameters of insomnia patients with five types of insomnia. Chinese Journal of Traditional Chinese Medicine 36(03):1637-1639.

Ren XJ, Wang GY, Zhang XP, Wang QQ, Peng ZP (2020). Sedative and hypnotic effects and transcriptome analysis of polygala tenuifolia in aged insomnia rats. Chinese Journal of Integrative Medicine 26(6):434-441. https://doi.org/10.1007/s11655-020-3087-6

Robinson CL, Supra R, Downs E, Kataria S, Parker K, Kaye A, Viswanath O, Urits I (2022). Daridorexant for the Treatment of Insomnia. Health Psychology Research 10(3):37400. https://doi.org/10.52965/001c.37400

Roch C, Bergamini G, Steiner MA, Clozel M (2021). Nonclinical pharmacology of daridorexant: a new dual orexin receptor antagonist for the treatment of insomnia. Psychopharmacology 238(10):2693-2708. https://doi.org/10.1007/s00213-021-05954-0

Roth T, Drake C (2004). Evolution of insomnia: current status and future direction. Sleep Medicine 5(Suppl1):S23-30. https://doi.org/10.1016/S1389-9457(04)90004-4

Rui M, Han N (2021). A brief history of research and development of modern sleeping pills. Chinese Journal of Pharmaceutical Chemistry 31(6):460-469.

Steven LCT, Yi GX (2021) Discussion on Relevance and Studies of Prescription Compatibility in Chinese Medicine. Chinese Journal of Integrative Medicine 27:788-793. https://doi.org/10.1007/s11655-020-3217-1

Sun N, Sun J, Zhong H, Pei S, Sun J (2023). Progress in the study of sedative and hypnotic mechanisms of traditional Chinese medicine. Chinese Herbal Medicine 54(04):1299-1310.

Wang L, Jin GF, Yu HH, Lu XH, Zou ZH, Liang JQ, Yang H (2019). Protective effects of tenuifolin isolated from *Polygala tenuifolia* Willd roots on neuronal apoptosis and learning and memory deficits in mice with Alzheimer's disease. Food and Function 10(11):7453-7460. https://doi.org/10.1039/C9FO00994A

Wang M (2021). Effects of *Schisandra chinensis* extract on GABAAR in hippocampus of insomnia rats α 1、GABAARγ2. Effect of NKCC1 expression. World Journal of Sleep Medicine 8(11):1916-1918.

Wang M, Li N, Jing S, Wang C, Sun J, Li H, Liu J, Chen J (2020). Schisandrin B exerts hypnotic effects in PCPA-treated rats by increasing hypothalamic 5-HT and γ-aminobutyric acid levels. Experimental and Therapeutic Medicine 20(6):1-1. https://doi.org/10.3892/etm.2020.9271

Wang Y, Xie W (2021). Study on the drug law of *Schisandra chinensis* for calming nerves based on patent information mining and verification. Chinese modern traditional medicine (11):1916-1920.

Wei B, Li Q, Fan R, Su D, Chen X, Jia Y, Bi K (2014). Determination of monoamine and amino acid neurotransmitters and their metabolites in rat brain samples by UFLC-MS/MS for the study of the sedative-hypnotic effects observed during treatment with *S. chinensis*. Journal of Pharmaceutical and Biomedical Analysis 88:416-422. https://doi.org/10.1016/j.jpba.2013.09.022

Wu XJ, Li MF, Gu XM (2019). Modern research progress on the compatibility of traditional Chinese medicine to reduce toxicity and increase efficiency. World's Latest Medical Information Abstract 19(99):77-79.

Xiao Y (2014). Effect of Jianpi Yangxin Anshen Recipe on dopamine in brain of insomnia mice. Clinical Research of Traditional Chinese Medicine 6(14):31-32.

Xei G (2020) Clinical observation on the effect of Du moxibustion combined with Chaihu Shugan Pill on constitution bias, 5-hydroxytryptamine (5-HT) and dopamine (DA) in patients with qi stagnation insomnia. Guangming traditional Chinese Medicine 35(17):2728-2731.

Yan T, Xu M, Wu B, Liao Z, Liu Z, Zhao X, Bi K, Jia Y (2016) The effect of *Schisandra chinensis* extracts on depression by noradrenergic, dopaminergic, GABAergic and glutamatergic systems in the forced swim test in mice. Food Function 7(6):2811-9. https://doi.org/10.1039/C6FO00328A

Zammit GK (2007). The prevalence, morbidities, and treatments of insomnia. CNS & Neurological Disorders-Drug Targets (Formerly Current Drug Targets-CNS and Neurological Disorders) 6(1):3-16. https://doi.org/10.2174/187152707779940754

Zhang C, Zhao X, Mao X, Liu A, Liu Z, Li X, Bi K, Jia Y (2014). Pharmacological evaluation of sedative and hypnotic effects of schizandrin through the modification of pentobarbital-induced sleep behaviors in mice. European Journal of Pharmacology 744:157-163. https://doi.org/10.1016/j.ejphar.2014.09.012

Zhao X, Cui Y, Wu P, Zhao P, Zhou Q, Zhang Z, Wang Y, Zhang X (2020). *Polygalae Radix*: A review of its traditional uses, phytochemistry, pharmacology, toxicology, and pharmacokinetics. Fitoterapia 147:104759. https://doi.org/10.1016/j.fitote.2020.104759

Zhao W, Sun G (2010). Conversion of dosage between different experimental animals. Animal Husbandry and Veterinary Science and Technology Information (5):52-53.