

# *Effect of Qinghuo Anshen Decoction on Insomnia Rats with Qi-Stagnation Type*

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**Abstract: Purpose** Observe the effect of Qinghuo Anshen Decoction on the expression of 5-HT, Glu and GABA in qi-stagnation insomnia rats. **Methods** Sixty rats were divided into: peace quality control group, peace quality Qinghuo Anshen decoction group, qi depression quality control group, Qiyu quality Qinghuo Anshen decoction group. After treatment, the serum 5-HT, Glu and GABA contents of rats were detected. **Results** After the intervention of Qinghuo Anshen Decoction: 5-HT in the qi-stagnation insomnia treatment group was significantly higher than that in the qi-stagnation insomnia model group,  $P < 0.01$ , and the 5-HT in the peaceful insomnia treatment group was significantly higher than that in the peaceful insomnia model group,  $P < 0.01$ , indicating that Qinghuo Anshen Decoction is effective for qi-stagnation constitutional insomnia and calming and qualitative insomnia; comparison of 5-HT between the qi-stagnation insomnia treatment group and the peaceful insomnia treatment group was found to be  $P > 0.05$ , but due to the qi-stagnation insomnia rat serum before treatment 5-HT is lower than normal insomnia, which proves that Qinghuo Anshen Decoction is better than peaceful insomnia. Glu and GABA were compared between the two groups. There was no significant difference in serum GLU and GABA before and after the insomnia model ( $P > 0.05$ ), indicating that the physique model had little effect on the content of GLU and GABA in the rat serum. Glu and GABA Comparing the two groups, the serum GLU content of rats in each group was significantly higher than that before insomnia modeling ( $P < 0.01$ ); the GABA content in serum of rats in each group was significantly lower than that before insomnia modeling ( $P < 0.01$ ); It shows that Qinghuo Anshen Decoction is effective in improving insomnia in Qi-stagnation insomnia rats and peaceful rats. **Conclusion** Qinghuo Anshen Decoction can improve insomnia by reducing serum Glu and increasing GABA and 5-HT content. 5-HT can also improve qi stagnation physique. Qinghuo Anshen Tang is effective in treating qi stagnation insomnia rat model.

## 1. Introduction

Healthy sleep helps people restore energy and physical strength, relieve fatigue, strengthen immunity, and improve study and work efficiency. However, as the pace of modern social and economic development accelerates, various stresses have led to abnormal mood swings, irregular diet and daily life, neurotransmitter secretion disorders, etc., plus certain physiological characteristics,

resulting in different ages, different education levels, and different socioeconomics. People of status are deeply troubled by sleep problems. In addition, the length of a person's sleep time, the difference in sleep patterns, and the degree of insomnia vary from person to person, and there are individual differences. This difference is determined by physical factors, so physical factors often affect the pathogenesis and determine the symptoms of the disease. The occurrence of its syndromes depends on the stimulation intensity of the pathogenic factors on the human body on the one hand, and on the other hand the different degrees of the human body's response to the pathogenic factors. Studies have found that insomnia may cause mental illness (especially depression), cognitive dysfunction, and negatively affect patients' heart function, immune function, and endocrine function [1-3], thereby increasing early mortality [4]. Therefore, the experimental research of this subject is closely related to the clinical part and selects calmness and Qi-stagnation to study the effect of Qinghuo Anshen Decoction on the expression levels of 5-HT, Glu and GABA in rats with insomnia.

## 2. Materials and methods

**2.1 Research objects** 60 Sprague-Dawley (SD) adult male rats, weighing between 180-220g.

**2.2 Experimental methods:** (1) Establishment of physique model Sixty SD rats were randomly divided into 2 groups, each with 30 rats, and they were labeled as the calm constitution group and the qi depression group. After grouping, they were raised for one week with reference to the living environment of peace and depression. The feeding conditions are as follows: 1) Peace and quality group: Feed with ordinary feed, ensure adequate diet, suitable environment, suitable light and temperature, good ventilation, and timely cleaning. 2) Qi depression group: refer to the method of Zhu Qingjing [5] Fei Tang Yiting et al. [6], at the beginning of the experiment: give the restraint method, put the rat into the restraint box, adjust the front moving part to a suitable position, So that the rats do not produce a strong degree of tension, 6 hours a day (9:00-15:00), for 7 days. (2) Establishment of SD rat insomnia model Reference [7] data, combined with the previous experience and results obtained from our pre-experiment; using modern recognized PCPA-induced insomnia model. The specific operation was carried out from 8:00 to 8:30 in the morning every day. The rats were injected intraperitoneally with PCPA once a day for 2 consecutive days. That is, 24 to 28 hours after the first intraperitoneal injection of the drug into the rat, the rat lacked circadian rhythm and kept moving during the day and night. The overall situation was significantly different from that of the blank group. Before using PCPA, use the rat 0.430mg/g dosage,

*Table 1*

Physique group	Group again	Dosing
Peace quality group	Peace control group	Beginning on the 5th day after the successful model building, the patients were given normal saline intragastrically 0.2ml/day, once a day.
	Peaceful Quality Qinghuo Anshen Soup Group	Beginning on the 5th day after the successful model building, the Qinghuo Anshen Decoction was given by gavage 0.2ml/day, once a day.
Qi Depression Group	Qi depression control group	Beginning on the 5th day after the successful model building, the patients were given normal saline intragastrically 0.2ml/day, once a day.
	Peaceful Quality Qinghuo Anshen Soup Group	Beginning on the 5th day after the successful model building, the Qinghuo Anshen Decoction was given by gavage 0.2ml/day, once a day.

Weakly alkaline normal saline was used to prepare a suspension for later use; except for the blank group without any treatment, the rest were intraperitoneally injected with p-chlorophenylalanine (PCPA). 3) Regrouping and administration of insomnia. After successful insomnia modeling, 30 SD rats were selected from the peace and quality group, and they were randomly divided into a peace and quality control group, a peace and quality Qinghuo Anshen decoction group, each with 15; group. Groups and methods of administration Table 1

**2.3 Observation indicators and measurement methods** (1) Physique model establishment stage: 1) General situation: Every day of the experiment, observe and record the rat's dietary life and daily life, whether there is fatigue, diet, and water consumption. 2) Weight change: Weigh the rats before and after the experiment. The measurement time is between 7:00 and 8:00 in the morning, and then compare them in pairs to observe the difference. 3) Behavior observation: Weigh daily food intake; record body weight once a week; observe the spirit and activity of model animals. The 1% sucrose water intake was determined with reference to the method of Jin Guangliang [8]. Before the experiment, all rats were given 1% sucrose water intake training, that is, they were fed with sucrose water for 48 hours, then water was cut off for 24 hours, and then at 8:00 Measure the amount of sucrose water taken by each rat within 1 h from 9:00 to 9:00, and use this as the baseline of the sucrose water intake of each rat. At the end of the experiment, measure the sucrose water within 1 h from 8:00 to 9:00. Intake. (2) Testing indicators: After one week of feeding under the conditions of calm and qi-stagnation, the serum 5-HT, Glu and GABA contents of the two groups of rats were measured. (3) Establishment stage of insomnia model: Record the rats' daily routines during the test for abnormalities, general conditions, weight gains and losses, and serum 5-HT, Glu and GABA levels.

**2.4 Statistical methods** Use Excel to establish a database to input all the data into the patient data one by one, and use SPSS19.0 software to perform statistics on the relevant data. The main statistical methods are chi-square test and t-test.  $P < 0.05$  is statistically significant.

**2.5 Basic experimental research objectives:** This study intends to observe the effects of Qinghuo Anshen Decoction on the content of 5-HT, Glu and GABA in the serum of rats with calm insomnia and insomnia model rats with different constitutions, and to explore the treatment of insomnia by Qinghuo Anshen Decoction The curative effect and possible mechanism.

### 3. Result

3.1 Changes of 5-HT content in serum of rats in each group during the establishment of physique model. After the completion of the physique modeling, comparison within the group, the constitution of the Pinghe constitution group was compared with that before the modeling ( $P > 0.05$ ), and the constitution of the Qi-stagnation group was compared with before the modeling ( $P < 0.01$ ); The comparison between groups, compared with the peace constitution group, before the physique modeling ( $P > 0.05$ ), and after the physique modeling ( $P < 0.01$ ). The results are shown in Table 2.

*Table 2: Changes in 5-HT content of rats in calm and qi-stagnation groups after physique modeling*

Peace quality group Qi Depression Group	120.37±4.87	121.18±2.09*
Peace quality group Qi Depression Group	121.78±4.74☆	109.14±8.16☆☆**

Note: Intra-group comparison, compared with before physique modeling, \* $P > 0.05$ , ☆ $P < 0.01$ ; comparison between groups, compared with peaceful constitution group, \*\* $P < 0.01$ , ☆ $P > 0.05$

3.2 Changes in serum GLU and GABA contents of rats in each group during insomnia model establishment stage

After the insomnia model was established, the GLU content in the serum of rats in each group was

significantly higher than that before the insomnia model was established ( $P < 0.01$ ); the serum GABA content of the rats in each group was significantly lower than that after the insomnia model was established. Before modeling ( $P < 0.01$ ); comparison between groups, comparing the Qi-stagnation type and the peaceful type group, the levels of GLU and GABA in serum of rats before and after the insomnia modeling were both ( $P > 0.05$ ). The results are shown in Table 3.

Table 3: Serum GLU and GABA concentrations of rats in each group ( $\mu\text{mol/L}$ )

Group	GLU		GABA	
	Insomnia before modeling	Insomnia after modeling	Insomnia before modeling	Insomnia after modeling
Calm insomnia group	102.27±20.33	165.20±26.31★	5.67±1.10	4.06±1.01★
Qi depression insomnia group	103.78±24.00	173.01±18.98★	6.01±0.93	4.73±1.04★

Note: Compared with insomnia before modeling ★ $P < 0.01$ ;

### 3.3 Changes in serum 5-HT content after qi stagnation model and qi stagnation insomnia model

The results of the model of Qi-stagnation type showed that the serum 5-HT content of rats in each group was changed: the 5-HT content in the serum of rats in the Qi-stagnation type group was significantly lower than that of the Ping-type group ( $P < 0.01$ ), and the Qi-stagnation type insomnia model was made. The results showed that the serum 5-HT content of rats in each group changed: the 5-HT content in the serum of rats in the Qi-stagnation insomnia group was lower than that of the peaceful insomnia group ( $P < 0.05$ ); comparison between groups, the Qi-stagnation insomnia group and the Heping-type group ( $P < 0.01$ ); the serum 5-HT content of the rats in the Ping-he-type insomnia group was significantly lower than that of the Qi-stagnation group and the Pinghe-type group ( $P < 0.01$ ); The results are shown in Table 4.

Table 4: Changes of 5-HT content after each stage of modeling

Group	5-HT content (ng/L)
Peaceful Quality Group (Before Modeling of Qi Depression)	121.18±2.09
Qi depression group (after modeling of Qi depression)	109.14±8.16★
Calm insomnia group	101.89±3.40
Qi depression insomnia group	92.07±5.63 # *

Compared with the peace and quality group ★ $P < 0.01$ , compared with the Qi-stagnation group # $P < 0.01$ , compared with the peace and quality insomnia group \* $P < 0.05$ . 2.4 Changes in 5-HT content of Qinghuo Anshen Decoction on calm insomnia model and qi-stagnation insomnia model. In comparison within the group, the serum 5-HT content of rats in the peaceful insomnia treatment group was significantly higher than that in the peaceful insomnia model group ( $P < 0.01$ ). The serum 5-HT content of rats in the qi-stagnation insomnia treatment group was significantly higher than that in the qi-stagnation insomnia Model group ( $P < 0.01$ ); comparison between groups, the level of serum 5-HT in the calm insomnia model group was compared with that in the qi-stagnation insomnia model group ( $P < 0.01$ ), the serum 5-HT in the qi-stagnation insomnia treatment group. There was no significant difference between the content and the peaceful insomnia treatment group ( $P > 0.05$ ). The results are shown in Table 5.

Table 5: Serum 5-HT content of rats in each group after the intervention of Qinghuo Anshen Decoction

Group	5-HT(ng/L)
Peaceful Insomnia Model Group (Before Peaceful Insomnia Intervention)	101.89±3.40
Peaceful insomnia treatment group (after the intervention of peaceful insomnia)	121.53±4.81★
Qi-stagnation insomnia model group (before qi-stagnation insomnia intervention)	92.07±5.63*
Qi depression insomnia treatment group (after Qi depression insomnia intervention)	124.39±2.67☆ #

Note: Compared with the peaceful insomnia model group ★P<0.01; compared with the Qi-stagnation insomnia model group☆P<0.01; compared with the peaceful insomnia group treatment #P>0.05; compared with the peaceful insomnia model group \*P<0.05

3.4 Qinghuo Anshen Decoction changes the contents of GLU and GABA in calm insomnia models and qi-stagnation insomnia models

Comparing within the group, the serum GLU content in the moderate insomnia treatment group was significantly lower than that in the moderate insomnia model group P<0.01; the serum GLU content in the qi-stagnation insomnia treatment group was significantly lower than that in the qi-stagnation insomnia model group (P <0.01). The GABA content in the serum of rats in the peaceful insomnia treatment group was significantly higher than that in the peaceful insomnia model group (P<0.01); the GABA content in the serum of rats in the qi-stagnation insomnia treatment group was significantly higher than that in the qi-stagnation insomnia model group (P<0.01) ); comparison between groups, there is no significant difference between the Qi-stagnation insomnia treatment group and the peaceful insomnia treatment group (p>0.05); the Qi-stagnation insomnia model group has no significant difference compared with the peaceful insomnia model group (p>0.05) ); The results are shown in Table 6.

Table 6: Serum GLU and GABA contents of rats in each group after the intervention of Qinghuo Anshen Decoction

Group	GLU(μmol/L)	GABA(μmol/L)
Peaceful insomnia model group	160.66±19.04	4.05±0.88
(Before the peaceful insomnia intervention)	103.27±27.00★	5.69±1.03★
Peaceful Insomnia Treatment Group	165.96±22.81	4.19±0.84
(After the intervention of calm insomnia)	106.88±23.21☆ #	5.85±1.06☆ #

Note: Compared with the peaceful insomnia model group★P<0.01, compared with the Qi-stagnation insomnia model group☆P<0.01; compared with the peaceful insomnia treatment group#P>0.05.

#### 4. Discussion

This research is based on the support of TCM theory, after years of clinical practice and experimental research, combined with TCM symptoms, Pittsburgh Sleep Quality Scale, a large number of clinical and disassembled research to develop Qinghuo Anshen Decoction. It is composed of more than ten traditional Chinese medicines such as wild jujube seed, Shouwu vine, Bupleurum, Hypericum perforatum, Albizia julibrissin, Angelica, Radix Paeoniae Rubra, etc. Bupleurum is bitter, pungent and slightly cold in nature, enters the pericardium, liver, triple burnt, and gall bladder meridian. , It has the effects of clearing the surface and releasing heat, soothing the liver and relieving depression; Hypericum perforatum has a flat, bitter taste, non-toxic, returning to the liver meridian,

and has the effects of clearing heat and detoxification; Jujube seed nourishes the heart and soothes the nerves, Bupleurum, Hypericum perforatum, Wild jujube seed, A total medicine, not only relieves liver depression, but also clears the heart and calms the mind. The three medicines enter the liver at the same time, and the chaihu enters the pericardium meridian together, and treat the liver and heart simultaneously to treat anger, depression, unrest, forgetfulness and insomnia caused by the seven emotions. Turmeric and Albizia bark can enter the heart, liver meridian, clear fire and calm the heart, relieve depression and soothe the nerves, together with white peony root and angelica, nourish blood and soften the liver, serve as a minister medicine, Poria and Atractylodes spleen for nourishing qi and invigorating spleen, Shenglongmu, Shichangpu for sedation Soothing the nerves, dispelling dampness and relieving depression, it is a total adjuvant. Licorice and jujube are used as medicines to reconcile various medicines, nourish qi and nourish blood. All medicines are used at the same time to achieve the effects of soothing the liver and purging fire, nourishing the heart and calming the nerves. The compatibility of all prescriptions is rigorous, and the specimens are taken into consideration. After long-term clinical observation, the curative effect is positive, which can effectively relieve the symptoms of insomnia and improve the quality of life of patients.

Serotonin is an important part of the monoamine neurotransmitter receptor hypothesis. Serotonin is widely involved in human emotions, sleep and wakefulness, and cognition. Studies have confirmed that depression and sleep disorders are related to abnormal 5-HT expression [10]. Glu is the main excitatory neurotransmitter in the brain. It has a universal excitatory effect on neurons of the nervous system and plays an important role in sleep and wakefulness. GABA is the most important inhibitory neurotransmitter in the brain. It has a general inhibitory effect on neurons in the nervous system and promotes sleep. Studies have shown that the content of Glu and GABA in the brain and the changes in their receptor functions play an important role in sleep activities [11]. Studies have also shown that insomnia not only changes in neurotransmitters such as Glu, GABA, 5-HT, etc. in brain tissue, but also a series of changes in transmitters such as Glu, GABA, 5-HT in peripheral tissues, indicating that insomnia is a transmitter. Qualitative system disorder [12],

According to the above experimental data, after the intervention of Qinghuo Anshen Decoction, the serum 5-HT content of rats in the Qi depression insomnia treatment group was significantly higher than that in the Qi depression insomnia model group ( $P < 0.01$ ), indicating that Qinghuo Anshen Decoction treats Qi depression constitution Insomnia is effective, and its mechanism of action may be that Qinghuo Anshen Decoction can increase the secretion of 5-HT in rats in the Qi-stagnation insomnia group, thereby inhibiting arousal and excitement, promoting sleep and improving the qi-stagnation physique. The level of 5-HT in the serum of rats in the moderate insomnia treatment group was significantly higher than that in the moderate insomnia model group ( $P < 0.01$ ), indicating that Qinghuo Anshen Decoction is also effective in treating moderate insomnia. It is speculated that Qinghuo Anshen Decoction can improve moderate insomnia rats the secretion of 5-HT, thereby inhibiting arousal and excitement, has a certain effect on promoting sleep. Further comparison of the serum 5-HT content of rats in the Qi-stagnation insomnia treatment group and the Pinghe-insomnia treatment group showed that there was no significant difference ( $P > 0.05$ ), but due to the qi-stagnation insomnia rat serum 5-HT before treatment The content of Qinghuo Anshen Decoction is lower than that of peaceful quality insomnia, so it does not mean that Qinghuo Anshen Decoction has the same effect in treating Qi-stagnation insomnia and peaceful quality insomnia. It further shows that Qinghuo Anshen Decoction is better than peaceful quality insomnia in treating Qi-stagnation insomnia. After the intervention of Qinghuo Anshen Decoction, the serum GLU content of rats in the qi-stagnation insomnia treatment group and the peace-quality insomnia treatment group was significantly lower than that in the model group ( $P < 0.01$ ), and the qi-stagnation insomnia treatment group and the peace-quality insomnia treatment group were larger The content of GABA in rat serum was significantly higher than that in the model group ( $P < 0.01$ ), indicating that Qinghuo Anshen Decoction can improve

the insomnia of Qi-stagnation insomnia rats and peaceful rats, which may be achieved by reducing the secretion of Glu in serum and increasing the secretion of GABA. , Thereby inhibiting arousal and excitement, and promoting sleep. The results of this study are also consistent with the results of Huang Yunfang [13] and Gan Yu [14].

In summary, Qinghuo Anshen Decoction can improve insomnia by reducing serum Glu and increasing GABA and 5-HT content. 5-HT can also improve qi stagnation physique. Qinghuo Anshen Tang is effective for qi stagnation insomnia rat models. The treatment is effective.

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## References

- [1] Schwartz S, McDowell AW, Cole SR, et al. *Insomnia and heart disease: a review of epidemiologic studies [J]. J Psychosom Res, 1999, 47(4): 313-333.*
- [2] Suka M, Yoshida K, Sugimori H. *Persistent insomnia is a predictor of hypertension in Japanese male workers [J]. J Occup Health, 2003, 45(6):344-350.*
- [3] Burgos I, Richter L, Klein T, et al. *Increased nocturnal interleukin-6 excretion in patients with primary insomnia: a pilot study [J]. Brain Behav Immun, 2006, 20(3): 246-253.*
- [4] Janson C, Lindberg E, Gislason T, et al. *Insomnia in men—a 10-year prospective population based study [J]. Sleep, 2001, 24(4): 425-430.*
- [5] Zhu Qingjing, Luo Xinla, Xiong Zhenfang. *Effect of Chaihu Shugan Powder on Rats with Chronic Restrained Stress Liver-Stagnation Syndrome [J]. Chinese Journal of Integrated Traditional Chinese and Western Medicine on Digestion, 2004, 12(4): 220- 221.*
- [6] Tang Yiting, Chen Jiayu. *Three Chinese herbal compound prescriptions regulate the hypothalamus-pituitary-adrenal axis in rats with chronic restraint stress [J]. Journal of Beijing University of Traditional Chinese Medicine. 2002, 25(3): 23- 26.*
- [7] Xiao Chengrong, Ma Zengchun, Li Haijing, et al. *Preparation and mechanism of PCPA insomnia rat model [J]. Journal of Pharmacology and Toxicology, 2007, 21(4): 326.*
- [8] Jin Guangliang, Nan Rui, Guo Xiazhen. *Establishment of a rat model of chronic stress with liver depression [J]. Journal of Beijing University of Traditional Chinese Medicine, 2003, 26(2): 18-21.*
- [9] Long Qinghua. *Theoretical discussion on depression and insomnia and the intervention effect of Sancao Anshen Recipe on depression and insomnia rats [D]. Hubei University of Traditional Chinese Medicine, 2017, (05): 58.*
- [10] Regulation of central and peripheral neurotransmitters in insomnia rats [J/OL]. *Chinese Journal of Chinese Materia Medica: 1-9 [2020-03-23]. 20191230.402.*
- [11] Tononi G, Pano M, Cirelli C. *The locus coeruleus and and immediate-early genes in spontaneous Brain Res Bull, 1994, 5 — 6:589 — 596.*
- [12] Manfredi A, Brainoila D, Mancina M. *Sleep is differently modulated by basal forebrain GABA (A) and GABA (B) receptors [J]. AM J Physical Pegal Bore Geep Physite, 2001, 281R: 120-125.*
- [13] Huang Yunfang, Bi Xinning, Zheng Wei, Zhang Ting, Wei Guijie, Zhang Yujie, Wang Qi. *Regulation of Jiaotai Pills on Central and Peripheral Neurotransmitters in Heart-kidney Insomnia Rats [J/OL]. Chinese Journal of Chinese Materia Medica: 1-9 [2020-03-24] 20191230.402.*

[14] Gan Yu, Ma Jin, Qiao Min, Zhang Hong, Bao Yulong, Wu Xiaolin, Liu Xiaohu, Jiao Fuying. The effect of Anshen umbilical paste on autonomous activities and serum GABA and NE levels in experimental insomnia model rats [J]. *Experimental Animal Science*, 2018, 35(04): 45-48+55.