Effect of Confucius' Pillow Elixir on Cognitive Ability of Agrypnia Mice and Its Mechanism

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Abstract. Aim: To investigate the influence of Confucius' Pillow Elixir on cognitive ability of agrypnia mice and its mechanism. Methods: Twenty-four mice were randomly divided into normal group, model group, Confucius' Pillow Elixir group and Diazepam group. Mice were suffered from continuous sleep deprivation with improved multi-platform water environment sleep deprivation boxes. Normal group and model group were given the same amount of normal saline, Confucius' Pillow Elixir group was given Confucius' Pillow Elixir decoction and Diazepam group were given Diazepam. During the experiment, we need to observe and record the general state and body weight of each group of mice. The open-field experiment and Morris water maze were conducted. Gamma-aminobutyric acid (GABA) concentration in serum of mice was determined by Elisa. Results: In the open-field experiment, compared with the model group, the number of horizontal motion grids in the Confucius' Pillow Elixir group increased significantly, with statistical difference \((P<0.05)\). In the Morris water maze positioning navigation test, compared with the model group, the escape latency of Confucius' Pillow Elixir group was significantly reduced, with statistical difference \((P<0.01)\). Compared with the model group, the GABA concentration in Confucius' Pillow Elixir group was significantly decreased, with statistical difference \((P<0.01)\).

1. Introduction

With the accelerated pace of life and increased work pressure, the number of agrypnia people is increasing year by year [1]. Agrypnia is a kind of clinical multiple disease, which is characterized by frequent abnormal sleep. And it has a great impact on the physical and mental health of patients.
Common clinical symptoms include difficulty falling asleep, decreased sleep quality, inattention, memory loss and decreased autonomous behavior [2].

At present, Western medicine mainly treats sedative and hypnotic drugs such as benzodiazepines and barbiturates, which can effectively reduce the excitability of the cerebral cortex. It can inhibit the central nervous system and relieve abnormal brain excitation and will produce similarly physiological sleep state. However, the side effects of these drugs are relatively large, and they are addictive and dependent. Through the research on the literature, it is found that there is a certain research foundation for the application of Confucius' Pillow Elixir in both clinical application and basic experimental research. Confucius' Pillow Elixir was first published in Sun Sizhen’s *A Handbook of Prescriptions for Emergencies*. Tortoiseshell, raw keel nourish kidney and replenish yin. And calamus, Yuanzhi sputum and nourish the heart, with the raw keel, tortoise coordinate heart-kidney. The whole prescription of medicine to treat agrypnia. Clinical application shows that Confucius' Pillow Elixir achieved satisfactory results in the treatment of yin deficiency of liver and kidney, floating disturbance of virtual fire, and menopausal agrypnia [3].

2. Materials

2.1. Animal
Animals were housed at a specific environment that room temperature was 20-24°C, with a 12:12h light-dark cycle and fed with a standard rodent diet and water.

2.2. Drugs and reagents
Confucius' Pillow Elixir (granules) consisting of Polygala, Acorus calamus, Tortoise plastron, raw keel purchased from Affiliated Hospital of Henan University of Traditional Chinese Medicine. Elisa kit for γ-aminobutyric acid purchased from Shanghai Langton Biotechnology Co., Ltd.

2.3. Experimental equipment
Constant temperature water bath, bench top centrifuge, microplate reader.

3. Methods

3.1. Grouping and administration
Twenty-four mice were randomly divided into the normal group, model group, the Confucius' Pillow Elixir group and the Diazepam group. The mice were subjected to continuous sleep deprivation in modified multi-platform water environment sleep deprivation boxes. The normal group and the model group were intragastrically administered with the same amount of normal saline, the Confucius' Pillow Elixir group was intragastrically administered Confucius' Pillow Elixir decoction and the Diazepam group was intragastrically administered Diazepam.

3.2. Model preparation
Molding mice were manually intervened by touching lightly during the day, when the mice were snoring, leaning against or nodding, then gently patted the cage to wake up the mice and keep the mice awake. Avoid direct contact with mice during tapping. Use sleep deprivation box for sleep deprivation at night. In the evening, the sleep deprivation boxes were used for sleep deprivation. The mice were stood on some platforms which were placed in the deprivation box and around the platform was filled water. When the mouse goes to sleep, the animals will rhythmically drops the water or falls into the water to wake up due to the decrease of the whole body muscle tension, so that the animal can never enter sleep. The mice were continuously deprived of sleep for 72 hours to establish a mouse model of insomnia [4].
4. Observation and detection indicators

4.1. Mouse weight change
The weight of the mice was measured once a day and recorded.

4.2. The open-field experiment
The open-field experiments can detect spontaneous activity and exploration behavior in mice. The enclosed arena (50 cm L × 50 cm W × 40 cm H) was divided into center, and the four squares in the middle were defined as the central zone [5]. Mice were placed individually in an open-field permitted to move freely for 3 min. Each mouse was measured for 3 minutes, then the environment was cleaned with a low concentration of alcohol, and the alcohol was evaporated and tasteless before proceeding to the next one. The locomotor activity in the central and peripheral part of open-field was observed. The number of horizontal motion grids of the mice was recorded manually for 3 minutes.

4.3. Morris water maze test
Mice performed four trials per day. In each trial, the animal was allowed to swim with a maximum length of 60s to find the platform and then remained on it for 10s. The animal that failed to find the platform within 60s was manually guided to the platform by the experimenter and allowed to stay on it for 20s. The interval time of each trial lasted for 10–15 min. The mice were continuously trained for 3 days, and the escape latency was recorded every day (if the platform was not found within 60s, the escape latency was recorded as 60 s), and the trajectory was recorded [6].

4.4. \(\gamma\)-Aminobutyric acid
Serum GABA concentration was measured according to the kit instructions [7].

4.5. Statistical Analysis
Data are presented as the mean ± SD. Differences were evaluated using Statistical Package for Social Science 21.0. Statistical analysis was performed using One-way ANOVA followed by least-significant difference (LSD). \(P < 0.05\) was considered to be statistically significant.

5. Results

5.1. Effect of Confucius' Pillow Elixir on the body weight of agrypnia mice
In the experiment 2-5 days, compared with the model group, the body weight of the Confucius' Pillow Elixir group increased slightly, and there was no statistically significant difference.

| Group            | Second day(g) | Fifth day(g) |
|------------------|---------------|--------------|
| Normal           | 24.50±3.20    | 23.63±4.17   |
| Model            | 24.20±2.65    | 22.30±2.49   |
| Chinese medicine | 24.19±2.79    | 22.89±0.93   |
| Western medicine | 22.17±4.60    | 19.99±4.69   |

5.2. The influence of Confucius' Pillow Elixir on the behavior of agrypnia mice
Compared with the model group, the number of horizontal motion grids in the Confucius' Pillow Elixir group increased significantly, with statistical difference \((P<0.05)\). The number of horizontal motion grids in the Diazepam group decreased slightly, without statistically difference. \(*P<0.05, **P<0.01\) vs. normal group; \(\dagger P<0.05, \ddagger P<0.01\) vs. model group).
Table 2. The influence of Confucius’ Pillow Elixir on the behavior of agrypnia mice

| Group               | Number of horizontal motion grids |
|---------------------|-----------------------------------|
| Normal              | 80.67±15.82                       |
| Model               | 79.50±2.50                        |
| Chinese medicine    | 107.22±2.41 #                     |
| Western medicine    | 72.67±10.83                       |

5.3. Influence of Confucius’ Pillow Elixir on Morris water maze test

Compared with the model group, the escape latency of the Confucius’ Pillow Elixir group was significantly lower, with statistical differences ($P<0.01$) and the escape latency of the Diazepam group was also significantly lower, with statistical differences ($P<0.05$). (*$P<0.05$, **$P<0.01$ vs. normal group; #*$P<0.05$, ##*$P<0.01$ vs. model group).

Table 3. Influence of Confucius’ Pillow Elixir on Morris water maze test

| Group           | Escape latency(s) |
|-----------------|-------------------|
| Normal          | 14.70±4.50        |
| Model           | 30.58±4.63**      |
| Chinese medicine| 15.85±3.76##      |
| Western medicine| 20.95±2.39#       |

5.4. Effect of Confucius’ Pillow Elixir on serum GABA concentration in agrypnia mice

Compared with the model group, the serum GABA concentration in the Confucius’ Pillow Elixir group was significantly lower, with statistical differences ($P<0.01$), and the Diazepam group was slightly different, without statistically difference. (*$P<0.05$, **$P<0.01$ vs. normal group; #*$P<0.05$, ##*$P<0.01$ vs. model group).

Table 4. Effect of Confucius’ Pillow Elixir on serum GABA concentration in agrypnia mice

| Group           | GABA concentration(Umol/l) |
|-----------------|---------------------------|
| Normal          | 0.82±0.02                 |
| Model           | 0.83±0.02                 |
| Chinese medicine| 0.76±0.03##               |
| Western medicine| 0.82±0.01                 |

6. Discussion

This experiment mainly explored the influence of Confucius’ Pillow Elixir on the cognitive ability of agrypnia mice. The open-field experiment is to detect the spontaneous activity and exploration behavior of mice [8]. And Morris water maze experiment is to analyze the spatial learning of mouse animals and memory behavior [9]. And finally through the detection of the serum GABA content of mice to explore the changes in learning and memory in mice. In the Morris water maze experiment, the escape latency of the Confucius’ Pillow Elixir group was significantly reduced compared with the model group. In the open-field experiment, the number of horizontal motion grids of the Confucius’ Pillow Elixir group was significantly increased compared with the model group. The results showed that Confucius’ Pillow Elixir had a certain effect on improving the cognitive ability of agrypnia mice. GABA is an important inhibitory neurotransmitter that has a broad inhibitory effect on neuronal electrical activity. The effects of learning and memory can also be achieved through inhibitory regulation, which plays an important role in the formation of learning and memory [10]. Studies have shown that Confucius' Pillow Elixir can significantly reduce serum GABA concentration in agrypnia mice, suggesting that the improvement mechanism of Confucius’ Pillow Elixir on learning and memory in agrypnia mice may be related to the decrease of serum GABA concentration.
In the experiment, a multi-platform water environment method was used to prepare the insomnia model. Common methods for preparing insomnia models include multi-platform water environment method and chemical reagent stimulation method. By comparison, the multi-platform water environment method is more suitable for short-term sleep deprivation, which reduces the impact of isolation of a single mouse from a population and effectively reduces the stress population instability in mice while selectively depriving REM sleep. Although the chemical reagent stimulation method can change the neurotransmitters in brain or other chemical substances to effect the sleep of animals, this method has many limitations. It has a high demanding of the selection of the site of administration, the control of the dose administered, and the operation of the investigators. Therefore, the multi-platform water environment method is more in line with the modeling needs of this experiment [11]. At the end of the modeling, the model mice became darker, slower, and listless and weight loss, which was successful modeling [12].

Sleep is a high-level function of the brain, which has the function of maintaining the survival of the individual, and can promote growth and development, facilitate learning, and form memory. Adequate sleep is a basic condition for maintaining health. It is well known that sleep rhythms can be divided into rapid eye movement sleep (REM) and non-rapid eye movement sleep (NREM). Experiments have confirmed that NREM can restore the body's physical strength, while REM can restore the body's energy and intelligence by regulating brain cell function and its protein synthesis. Learning and memory are achieved through the function of the nervous system, and the completion of this process requires not only adequate blood and nutrient supply, but also adequate and efficient sleep. Sleep is indispensable for short-term memory. Animals and people are coding and processing information during this period. Insomnia can cause electrophysiological and biochemical changes in the central nervous system, leading to disturbances in the neurotransmitters in the central nervous system. Therefore, it is believed that the decline in learning and memory ability may be related to changes in neurotransmitters in the brain after insomnia.

7. Conclusion
Confucius' Pillow Elixir can improve the cognitive ability of agrypnia model mice, and the mechanism may be related to reducing the GABA content in the serum of agrypnia mice.

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