MINISTRY OF EDUCATION AND SCIENCE OF UKRAINE
KYIV NATIONAL UNIVERSITY OF TECHNOLOGIES AND DESIGN
Faculty of Chemical and Biopharmaceutical Technologies
Department of Biotechnology, Leather and Fur

QUALIFICATION THESIS

on the topic **Application of protein immunoblotting method in the study of the efficacy of Chaihu Shugang San**

First (Bachelor's) level of higher education
Specialty 162 "Biotechnology and Bioengineering"
Educational and professional program "Biotechnology"

Completed: student of group BEBT-20
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ASSIGNMENTS
FOR THE QUALIFICATION THESIS
Li Tiantian

1. Thesis topic **Application of protein immunoblotting method in the study of the efficacy of Chaihu Shugang San**

Scientific supervisor Olga Andreyeva, Dr. Sc., Prof.

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2. Initial data for work: assignments for qualification thesis, scientific literature on the topic of qualification thesis, materials of Pre-graduation practice

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2	Section 1 Literature review	From 06 April 2024 to 20 April 2024	
3	Section 2 Object, purpose, and methods of the research	From 21 April 2024 to 30 April 2024	
4	Section 3 Experimental part	From 01 May 2024 to 10 May 2024	
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I am familiar with the task:

Student _____Li TIANTIAN

Scientific supervisor _____Olga ANDREYEVA

SUMMARY

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Anxiety is a common mental illness. It can seriously affect patients' quality of life. Traditional Chinese medicine believes that the main pathogenesis of anxiety is liver qi stagnation. Chaihu Shugan San is a commonly used Chinese herbal medicine, the main ingredients include bupleurum, peony, tuckahoe and so on. These herbs have a good effect of clearing heat and detoxifying and soothing liver and gallbladder.

In this study, biotechnological methods were used to detect and validate bupleurum Shugan powder. In this study, normal C57BL/6J mice were selected and the gradient dose of Chaihu Shugan San was administered by intragastric administration. After the intragastric administration, behavioral evaluation methods were used: The Open Field Test (OFT) and Elevated Plus Maze (EPM) tests were conducted to determine the effect of the drug on anxiety-like behavior improvement in mice and to identify the optimal dose. Chronic restraint stress (CRS) was used to construct an anxiety model of mice, and Chaihu Shugan San was administered. Behavioral evaluation methods OFT and EPM were used to evaluate the efficacy of Chaihu Shugan San in the treatment of the anxiety model mice constructed by CRS. To explore whether changes in behavioral phenotype and the anti-anxiety effects of Chaihu Shugan San are related to Brain-derived neurotrophic factor (BDNF) in the hippocampus, In this study, the content of BDNF in the hippocampus of mice was detected by Western blot to reflect the pathological effect of Chaihu Shugan San on anxiety model mice.

The experimental results showed that the anxiety symptoms of the treatment group were significantly alleviated, and the results of biochemical indicators supported the theory of traditional Chinese medicine. The effect of the treatment of anxiety was possible through clearing heat and detoxifying, soothing the liver and opening the gallbladder, and improving the stagnation of liver qi. However, the sample size of this study is small, and it is necessary to expand the sample in the later stage to further verify the conclusion. In general, this study adopts modern biotechnology methods to preliminarily detect and verify that Chaihu Shugan San may play a role in the treatment of anxiety through various ways.

Keywords: Anxiety disorder; Chaihu Shugan San; Brain-derived neuroinfluence factor; Western blot

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INTRODUCION

Relevance of the topic. Depression is a severe, common mood disorder with a high suicide rate and one of the most painful medical diseases. The mechanism of depression is quite complex and unclear, and many currently available synthetic chemical antidepressants have low response and remission rates and even serious side effects. Therefore, it is necessary to research and develop a more effective antidepressant without side effects for patients. Natural plants are an important source of new antidepressants, and the safety of natural plants may be higher than that of synthetic antidepressants. Chaihu Shugan San, a traditional Chinese medicinal herbal formula consisting of several Chinese herbs and recorded in the Chinese medical classics, has been used as a remedy for relieving symptoms of depression caused by stagnation of Liver Qi, which according to Traditional Chinese Medicine is the cause of stress and anger. There are reports of the effectiveness of the drug in the treatment of depression. However, its mechanism has not been studied.

The purpose of the study is to identify and study the effect of the herbal preparation Chaihu Shugan San on anxiety using the method of biotechnological immunoblotting of proteins.

To achieve this goal, a **task** was set and solved:

- to study the effect of Chaihu Shugan San powder on anxiety;

- to master a modern method of experimental work in biotechnology - the method of protein immunoblotting (Western blotting), which can be used to identify the microscopic index of BDNF to establish the connection between the anti-anxiety effect of Chaihu Shugan San and brain-derived neurotrophic factor.

Object of study: assessment of the efficiency of the herbal preparation Chaihu Shugan San on anxiety disorder.

Subject of research: behavioral indices of mice; the efficiency of the anti-anxiety effect of Chaihu Shugan San.

Research methods: To study the effect of Chaihu Shugan San on improving the anxiety-like behavior of mice and to determine the optimal dose of the drug, methods of behavioral assessment of animals were used: the Open Field Test (OFT) and the Elevated Plus Maze (EPM). The BDNF content in the hippocampus of mice was determined using Western blotting.

Scientific novelty: the results of Western blotting of proteins showed that the effectiveness of the anti-anxiety action of Chaihu Shugan San is due to brain-derived neurotrophic factor, as evidenced by changes in BDNF content in the hippocampus of model mice.

The practical significance of the results obtained is that the effect of Chaihu Shugan San on anxiety has been confirmed: it can reduce anxiety in animals. The optimal dose of the drug has been established, providing a result that is not inferior to the result of using diazepam, a modern first - line drug in Western medicine.

The structure and scope of the dissertation. A Qualification thesis consists of Introduction, three Chapters, Conclusion, and List of references.

CHAPTER 1
LITERATURE REVIEW

1.1 Overview of anxiety disorders

Anxiety disorder is a common psychological disorder, manifested as persistent and excessive anxiety and worry, patients will appear in daily life obvious tension, restlessness and fear seriously interfere with the patient's daily life, work and social functions. The symptoms of anxiety disorders can be divided into physical symptoms, emotional symptoms and cognitive symptoms. Physical symptoms include palpitation, chest tightness, dyspnea, muscle tension, etc. Emotional symptoms include nervousness, restlessness, fear, irritability, irritability, etc. Cognitive symptoms include excessive worry, uncontrollable thinking, and decreased attention and concentration. These symptoms can have a negative impact on the patient's life, leading to social avoidance, increased stress at work, sleep problems and physical discomfort, among others. The causes of anxiety disorders include the interaction of genetic factors, biochemical factors, environmental stress, and psychological factors. An individual's physical and psychological characteristics, personal experiences, and environmental factors may all influence the development of the disorder 0.

Symptoms of anxiety disorders include a variety of physical and emotional manifestations, such as rapid heartbeat, difficulty breathing, muscle tension, insomnia, fatigue, nausea, and so on. In addition to these physical symptoms, anxiety disorders can affect an individual's cognitive and emotional states, such as difficulty concentrating, irritability, irritability, fear, and depression.

The causes of anxiety disorders are complex and varied, and may be influenced by genetic, biological, and psychosocial factors. Stress in life, traumatic experiences, family environment, personal characteristics, etc., may be related to the occurrence and development of anxiety disorders. The diagnosis of anxiety disorder usually requires a doctor to confirm it through clinical evaluation and symptom

questioning, while ruling out other health problems that may be causing similar symptoms.

There are many ways to treat anxiety, including medication and psychotherapy. Medications are often used to relieve symptoms, such as anti-anxiety medications and antidepressants. Psychotherapy, including cognitive behavioral therapy, psychodynamic therapy, and relaxation therapy, is designed to help patients understand and manage the causes of anxiety and learn effective techniques to cope with anxiety. Family and social support also play an important role in the treatment process.

Overall, anxiety is a common but treatable mental illness, and with a comprehensive treatment approach and comprehensive support, patients can effectively manage their symptoms, improve their quality of life, and regain confidence and a joyful life.

1.2 Domestic research progress of anxiety disorder

In China, anxiety disorder, as a common mental disorder, has received extensive attention and research. Domestic research on the treatment of anxiety disorders involves many fields, including psychotherapy, drug therapy, neurobiological mechanism, social psychological factors and so on. The following are some important research advances in the treatment of anxiety disorders in China:

Optimization and personalization of psychotherapy: Psychotherapy plays an important role in the treatment of anxiety disorders. Domestic research is focused on optimizing treatment protocols to better adapt to Chinese culture and patient characteristics. Cognitive behavioral therapy CBT is one of the main methods for the treatment of anxiety disorders, and domestic researchers have continuously improved the implementation of CBT and the effectiveness evaluation methods through clinical practice and experiments. At the same time, personalized treatment is gradually receiving attention, and targeted treatment plans are formulated

according to individual differences and disease characteristics of patients to improve treatment effect and sustainability.

Innovation and progress in drug therapy: Drug therapy is one of the important means of anxiety management. Domestic researchers continue to explore new drug treatment options, including selective serotonin reuptake inhibitors (SSRIs), selective serotonin and norepinephrine reuptake inhibitors (SNRIs) and so on. The combination of traditional Chinese and western medicine in the treatment of patients with cardiovascular diseases accompanied by anxiety has obvious effects, improving patients' bad mood and heart function, increasing satisfaction, and playing a role in soothing the liver and spleen, nourishing the blood and calming the mind, which is worthy of application 0.

In-depth study of neurobiological mechanisms: The pathogenesis of anxiety disorders involves a complex neurobiological regulatory network. Through neuroimaging techniques, animal models and genomics methods, researchers in China have explored changes in brain structure and function in patients with anxiety disorders, as well as related changes at the molecular and cellular levels.

China has made important progress in the field of anxiety treatment, involving many aspects such as psychotherapy, drug therapy, neurobiological mechanisms and social psychological factors. These studies provide important theoretical and practical support for improving the quality of life of patients with anxiety disorders. With the continuous improvement of science and technology and medical level, it is believed that China will make more remarkable achievements in the field of anxiety treatment.

1.3 Foreign research progress of anxiety disorders

Recent foreign research on anxiety disorders involves many fields, including psychotherapy, neuroscience, drug therapy and interdisciplinary cooperation. In recent years, foreign researchers have made continuous efforts to understand the

pathogenesis and treatment of anxiety disorders, and have made a series of important progress.

In the field of psychotherapy, foreign research on anxiety disorders focuses on the development and optimization of innovative treatment methods. In addition to traditional cognitive behavioral therapy, new psychotherapy methods such as Mindfulness-based stress reduction therapy (MBSR) and mindfulness-based cognitive therapy (MBCT) have attracted much attention. These methods help patients reduce anxiety symptoms and prevent relapses by cultivating mindfulness and self-awareness, and have also shown good results in coping with stress and regulating emotions.

In the field of neuroscience, foreign researchers have used neuroimaging techniques and molecular biological methods to reveal the brain mechanism and genetic basis of anxiety disorders. Most psychotherapy research evidence on cognitive behavior therapy (cognitive behavioral therapy, CBT) in the treatment of anxiety disorders. A key component of CBT is exposing patients to internal or imagined fear stimuli. This exposure can break the vicious cycle of avoidance behavior and enable new safety learning so that the expected adverse outcomes do not occur or are manageable. Internet-based CBT has grown tremendously in the past decade and provides a convenient way to treat patients in situations with limited services or remote locations. Internet-based CBT also had a similar anxiety-reducing effect when compared to face-to-face CBT . There is less evidence for the use of CBT in children and adolescents than in adults, and given the risks of using medication in this age group, CBT deserves more research. Studies have found that patients with anxiety disorders have abnormal activity in brain regions such as the amygdala and the cingulate cortex, which is closely related to the onset of anxiety disorders.

In terms of drug therapy, foreign researchers continue to explore new drugs and treatment strategies. In addition to traditional anti-anxiety drugs, some novel drugs such as erythropoietin receptor antagonist (CRF-R1) antagonist have been

studied for the treatment of anxiety disorders0. Medication is also the first line of treatment for anxiety disorders. When there is no response to psychotherapy, chronic disease course or complexity, and comorbid depressive disorder, medication may be prioritized. The efficacy of SSRIs and SNRIs has been demonstrated in children and adolescents. Although drug approval status varied by country, overall, all SSRIs, SNRIs, MAO inhibitors, and the serotonergic tricyclic compound Clomipramine were superior to placebo in reducing anxiety symptoms in adults. However, SSRIs (and, to a lesser extent, SNRIs) are currently preferred because they have a good risk-benefit ratio across all age groups, especially in children and adolescents. A mesh meta-analysis of generalized anxiety disorder showed that duloxetine, Venlafaxine, Etapram, pregabalin, and quetiapine were more effective, although quetiapine had a relatively high discontinuation rate. At the same time, new drugs targeting neurotransmitters and receptors are also in the development stage, offering new possibilities for future treatments.

In terms of combination therapy, meta-analysis evidence suggests that psychotherapy combined with medication is superior to medication alone. In general, patients can only be considered resistant to treatment if both medication and psychotherapy have failed. Resistance to first-line therapy remains a clinical challenge, and although studies have shown that intensive drug therapy has a slight effect on reducing symptoms, it does not increase response rates. Attention bias training, drug-mediated memory reconsolidation, and transcranial brain stimulation techniques are currently experimental treatment methods, and more effective combination treatment strategies are expected. The recent research on anxiety disorders in foreign countries presents the characteristics of diversification, innovation and interdisciplinary, which provides important theoretical and practical support for the treatment and management of anxiety disorders worldwide. With the continuous progress of science and technology and the deepening of research, it is believed that there will be more breakthrough progress in the future.

1.4 Current research methods of anti-anxiety Chinese medicine and Chinese medicine

At present, the research methods of anti-anxiety Chinese medicine and Chinese medicine mainly include clinical observation, experimental research and the application of modern scientific and technological means. TCM treatment of anxiety stress syndrome differentiation treatment, according to the patient's specific condition and physical characteristics, the use of acupuncture, traditional Chinese medicine, massage, Qigong and other methods for treatment. There are several methods to study the anti-anxiety effect of traditional Chinese medicine:

Clinical observation: By observing the changes of patients' clinical symptoms and treatment effects, summarize the experience of TCM treatment of anxiety. Clinical observation can help doctors understand the efficacy and indications of Chinese medicine for anxiety disorders.

Experimental studies: including animal experiments and in vitro experiments, Chinese medicine extracts or Chinese medicine compounds are added to experimental animals or cell culture media to observe their effects on anxiety-related indicators, such as behavioral, biochemical and neurotransmitter levels, so as to evaluate the anti-anxiety effect of Chinese medicine and its possible mechanism.

The application of modern scientific and technological means: such as functional magnetic resonance imaging (fMRI), single cell sequencing technology, etc., through these technologies, we can study the influence of Chinese medicine on the brain function of anxiety disorders, and explore the mechanism of action of Chinese medicine in the treatment of anxiety disorders.

Application of modern scientific and technological means: For example, functional magnetic resonance imaging (fMRI), single cell sequencing technology, etc., through these technologies, we can study the influence of Chinese medicine on the brain function of anxiety disorders, and explore the mechanism of action of Chinese medicine in the treatment of anxiety disorders. For patients with depression and anxiety disorders, drugs are usually used for treatment. This drug is a highly

selective serotonin reuptake inhibitor, which has an inhibitory effect on human 5-HT transporters and can inhibit 5-HT reuptake in the presynaptic membrane of nerve endings in the central nervous system0.

To sum up, there are various methods of TCM and TCM research on anti-anxiety, which combine traditional medical theories and modern scientific and technological means, aiming to comprehensively and deeply study the mechanism of action and clinical application of TCM in the treatment of anxiety, and provide scientific basis for the application of TCM in the treatment of anxiety.

1.5 The study on the biological detection methods of traditional Chinese medicine

1.5.1 Research progress of Chaihu Shugan Powder

Chaihu Shugan powder is a commonly used Chinese herbal medicine prescription, the main components of bupleurum, peony, podia and so on. These herbs have a good effect of clearing heat and detoxifying and soothing liver and gallbladder. In recent years, domestic and foreign scholars have conducted in-depth research on this prescription, explored its therapeutic mechanism and indications, and made certain progress.

Regarding the therapeutic mechanism, some scholars have adopted high throughput sequencing technology to study the effect of Bupleurum Shugan Powder on liver cells. The results show that this formula can regulate important metabolic pathways and signaling pathways in liver cells, such as heparin metabolic pathway and cell cycle regulation, etc., explaining its role in clearing heat and detoxifying and soothing liver and gallbladder from the genetic level0.

In general, in recent years, the in-depth research on the mechanism and indications of bupleurum Shugan powder has provided more theoretical and empirical basis for the rational clinical application of this important Chinese medicine, and its research prospects are broad. However, the mutual confirmation with western medicine needs to be further developed.

1. Pharmacological effects of Bupleurum Shugan Powder: Pharmacological effects of Bupleurum Shugan powder mainly include cell and animal experiments. Several studies have investigated the effects of this formula on liver cells using in vitro cell culture models. The results showed that Chaihu Shugan powder could promote the proliferation and repair of hepatocytes and inhibit the apoptosis of hepatocytes caused by cytokines. Another study used animal models of liver injury to observe the effects of bupleurum Shugan Powder on liver pathological changes 0.

2. Clinical study of Bupleurum Shugan Powder: Clinical study mainly focuses on the efficacy of bupleurum Shugan powder in treating chronic liver disease. A multicenter, randomized, double-blind clinical trial included 120 patients with mild chronic liver injury. The results showed that the liver function indexes such as ALT and AST decreased after bupleurum Shugan powder, and the difference was statistically significant compared with the control group.

3. Research on biological active ingredients of Bupleurum Shugan Powder: Current research shows that the main active ingredients of bupleurum Shugan powder include bupleurum, berberis saponin and so on. By means of separation staining and high-performance liquid chromatography, the researchers isolated and identified a variety of chemical components from bupleurum, and evaluated their anti-liver toxicity and pro-liver repair effects. For example, bupleurum can inhibit the oxidative stress response of hepatocytes, and berberine can promote the energy production of hepatocytes. This has laid a foundation for revealing the therapeutic mechanism of Bupleur Shugan powder from the component level 0.

1.5.2 Research progress of animal models of anxiety disorder

The study of the animal model of anxiety disorder is of great significance in exploring its pathological mechanism and screening new drugs. At present, the commonly used animal models mainly include the following categories:

Behavioral model:

Open field testing: This is one of the most commonly used animal models for anxiety disorders, and evaluates anxiety levels by placing a mouse or rat in an open area and observing its exploration and avoidance behavior in a new environment.

Exploratory behavior and fear memory in mice were evaluated by the light shelter chamber test. By setting up light and dark areas in the box, the mice were observed to enter the dark area and other indicators. This model simulates human fear and avoidance behavior.

The other was the forced swim test, which looked at the restlessness and swimming time of mice in a helpless state in the water. This model is suitable for evaluating anxiety levels. There was also a conditioned fear model, which created fear memories in mice by pairing harmful stimuli with the environment.

In recent years, researchers have also developed genetically based models of anxiety in mice, such as 5-HT transporter deficient mice, which can help explore the biological mechanisms of anxiety. With the development of neuroimaging and molecular biology technologies, animal models have broad application prospects in anxiety research0.

Maze Crossing tests, including Elevated Plus Maze (EPM) and Light-Dark Box (LDB), assess anxiety levels by measuring how animals transition between open and closed Spaces.

Neurobiological model:

In addition to behavioral models, neurobiological models also play an important role in revealing the mechanisms of anxiety disorders.

Neurotransmitter regulation: Anxiety disorders are associated with abnormalities in a variety of neurotransmitters, such as GABA, glutamate, and serotonin. By modulating these neurotransmitter systems, researchers mimic anxiety symptoms.

Monoamine neurotransmitters such as 5-hydroxytryptamine and norepinephrine play an important role in the generation and regulation of anxiety,

and the relevant animal experiments have been well verified. For example, an anxiety-like state can be rapidly induced or suppressed in mice by microinjections of 5-HTagonist or antagonist, suggesting a critical role for 5-HT in regulating anxiety.

In addition, people have also studied the neural pathways related to anxiety, such as the amygdaloid-olfactory bulb pathway, and found that its activation can cause anxiety, and its inhibition can reduce anxiety. In recent years, changes in the activation of brain regions associated with human anxiety have also been observed in mice and non-human primates using neuroimaging techniques such as fMRI 0.

Genetic model:

Genetic model is another important model to reveal the mechanism of anxiety disorder. By studying genetically engineered mice, it has been found that some genetic variants may be associated with anxiety disorders in humans. For example, mice lacking the 5-HT transporter show increased anxiety and fear behaviors. At the same time, the study also found that the knockout or overexpression of BDNF and CRF genes also affected their anxiety levels. These gene models provide an important way to study the pathogenesis of anxiety at the molecular and genetic level.

Environmental model:

Environmental factors also play an important role in the onset of anxiety disorders. The researchers designed some environmental models to simulate human psychological stress in the living and working environment, and explored the influence of environmental factors such as early trauma on anxiety. The loneliness model, for example, isolated mice when they were young and observed an increase in anxious behavior as adults. The social stress model also induces sustained physiological and behavioral changes in mice by establishing competitive relationships within the domain. In addition, the study uses a conditioned stress model, which associates stressful stimuli such as electric shocks with the environment to form fear memories. These environmental models can simulate the common psychosocial stress in human life, providing an effective way to study the

impact of environmental factors such as early trauma on anxiety, and also providing references for the prevention and intervention of mental diseases0.

Neuroimaging model:

Brain imaging techniques, such as functional magnetic resonance imaging (fMRI) and electroencephalography (EEG), were used to observe the changes of brain nerve activity in animal models of anxiety disorder under different conditions and explore its neural mechanism.

Drug therapy model:

Animal models of anxiety disorders were treated with anxiety medications (e.g., benzodiazepines, SSris, etc.) or natural medications (e.g., plant extracts) to assess their effects on anxious behavior and therapeutic mechanisms.

The research progress of animal models of anxiety disorders is constantly enriched, through these models can better understand the pathogenesis of anxiety disorders, evaluate the effectiveness of treatment methods, and provide theoretical and experimental basis for clinical treatment. The research results of these neurobiological models are helpful to reveal the neurobiological mechanism of anxiety pathogenesis from the cellular and molecular levels, and lay the foundation for the discovery of new targets and drug development 0.

1.5.3 Research progress of biological detection methods of traditional Chinese medicine

The research on unilateral and compound biological detection methods of traditional Chinese medicine has always been a hot spot in the field of traditional Chinese medicine, and great progress has been made in the following aspects in recent years:

Establishment of quality control standards:

The researchers used high performance liquid chromatograph-mass spectrometry (HPLC-MS) to efficiently separate and accurately identify the main components. At the same time, the standard material comparison method was used

to establish the quantitative detection method and quality evaluation standard for the main active ingredients, which provided the basis for the follow-up study.

Fingerprint analysis:

A fingerprint database containing many kinds of traditional Chinese medicine was established. Multi-dimensional statistical methods such as principal component analysis and cluster analysis were used to systematically evaluate the traditional Chinese medicine from different origin and processing technology, and the stability and consistency of fingerprint were studied.

Biological activity was evaluated by cell and animal experiments:

For example, adipose tissue cells were used to study the mechanism of regulation of fat metabolism by traditional Chinese medicine, and mouse liver injury models were used to evaluate the protective effect of traditional Chinese medicine on liver function. This helps to reveal the specific therapeutic effects of traditional Chinese medicine 0.

Multi-omics techniques reveal the mechanism of action:

For example, RNA seq and proteomics technologies systematically study the molecular mechanisms of Chinese medicine regulation of cell signaling pathways and gene expression, laying the foundation for in-depth exploration of new targets of Chinese medicine.

Safety evaluation:

Long-term toxicity tests were carried out in mice and rats to determine the maximum safe dose and potential toxic side effects of traditional Chinese medicine and ensure its safe application.

Through the comprehensive application of multi-disciplinary technology, the research of biological detection methods of traditional Chinese medicine has made great progress, which provides a solid basis for the modernization of traditional Chinese medicine. The research level will be further improved in the future.

1.5.4 Overview of Western blot

Protein immunoblotting (Western blot) is a commonly used technique for protein analysis. It can quantitatively or qualitatively detect the expression level of a specific protein in a sample. The Western blot operation process consists of the following steps:

First, the proteins in the sample are separated by molecular weight by SDS-PAGE electrophoresis. The protein is then transferred from the gel to the PVDF membrane, leaving it in its original separated state. After that, western blotting was performed using specific primary and secondary antibodies. The primary antibody recognizes specific proteins, while the secondary antibody binds to the primary antibody and produces a visible color response. The expression level of target proteins is determined by color depth and standardized by internal reference proteins 0.

In addition, Western blot can also be used to detect the modification level of proteins. For example, phosphorylation levels are detected by phospho-specific antibodies. In recent years, using near-infrared fluorescence to label antibodies, Western blot technology has been improved in terms of sensitivity and quantitative ability. It has been widely used to study the mechanism of regulating cell signaling pathway of traditional Chinese medicine, and to provide a basis for revealing the effect of traditional Chinese medicine. In general, Western blot technique is simple to operate and has strong specificity, which plays an important role in proteomics research. Here is a detailed overview of protein immunoblotting:

Principles and basic steps:

The basic principle of Western blot technology is to use antibodies to recognize and detect specific proteins. The main steps are as follows:

1. Protein extraction and quantification: Total protein is extracted from cells or tissues and quantified to determine protein concentration.

2. Protein separation: The protein in the sample is separated according to molecular weight by SDS-PAGE electrophoresis, so that the protein of different molecular weight forms obvious bands on the gel.

3. Protein transfer: The separated protein is transferred from the gel to the PVDF membrane or cellulose nitrate membrane, retaining its separated state in the gel.

4. Western blot: The specific primary antibody is added and incubated so that the primary antibody can bind to the target protein. Then, the second antibody combined with the primary antibody was added for color development to detect the expression of the target protein.

5. Image analysis: The imaging system is used to scan and analyze the results, and the expression level of the target protein is quantified semi-quantitatively by the concentration depth of the target protein band.

6. Standardized treatment: The internal reference protein is standardized to eliminate systematic errors in sample processing and obtain quantitative results.

The above is the basic principle and main steps of Western blot technology, which identifies specific proteins through antibodies and can detect changes in protein levels qualitatively and quantitatively.

Advantages and applications

The main advantages and applications of Western blot technology are as follows:

1. Strong specificity. By using specific primary antibodies, a single protein molecule can be clearly identified without being affected by other proteins.

2. Good sensitivity. Proteins as low as a few hundred femtogram levels can be detected using chemiluminescence.

3. Strong quantitative ability. The relative expression level of proteins in different samples was detected semi-quantitatively or quantitatively by the depth and shallow concentration of target protein bands 0.

4. The modified form of protein can be detected. The changes of modification levels such as phosphorylation and acetylation were detected by specific antibodies.

5. Combine with other technologies. If combined with immunocytochemistry or proteomics, both quantitative level and structural localization are considered to improve the depth of research.

In general, Western blot technology has strong specificity, simple operation and wide application, which provides important support for proteomic research.

Improvement and development

Western blot technology is also constantly improving and developing, mainly in the following aspects:

1. Increased sensitivity. Using near-infrared fluorescence to label antibodies, lower concentrations of proteins can be detected, improving the sensitivity of the technique.

2. Enhanced quantitative ability. A variety of internal reference proteins were standardized, and gray scanning software was used for quantitative analysis, which improved the accuracy of quantitative results.

3. The level of automation is improved. Some experimental steps, such as electrophoresis and film transfer, are carried out by automatic instruments to reduce manual error and improve repeatability.

4. Multiple detection methods. In addition to conventional chemiluminescence detection, radiolabels and enzyme-linked immunosorbent assay are also used to detect and expand the scope of application.

5. Analyze software updates. Use deep learning and other technologies to develop more efficient image recognition and quantitative analysis software to improve detection efficiency and accuracy.

Overall, Western blot technology will continue to integrate deeply with other technologies to improve quantitative accuracy and high-throughput detection capabilities, and play a broader role in life science research.

Conclusions to chapter 1

Anxiety disorder, a common psychological disorder, is characterized by persistent and excessive worry and worry. In the patient's daily life, obvious tension, anxiety and fear appear, which significantly interfere with the person's daily life, work and social functions. The causes of anxiety disorders include the interaction of

genetic factors, biochemical factors, environmental stress, and psychological factors. However, anxiety is a treatable mental illness, and with a comprehensive approach to treatment and comprehensive support, patients can manage their symptoms, improve their quality of life, gain confidence and normalcy.

China has made important progress in the field of anxiety treatment, including many aspects such as psychotherapy, drug therapy, neurobiological mechanisms and socio-psychological factors. These studies provide important theoretical and practical support for improving the quality of life of patients with anxiety disorders. It is believed that with continuous improvement in science, technology and medical level, China will make even greater strides in the field of anxiety treatment.

There are various ways to treat anxiety, including medication and psychotherapy. Medications, such as anti-anxiety medications and antidepressants, are often used to relieve symptoms. Regarding the therapeutic mechanism, some scientists have applied high-throughput sequencing technology to study the effect of Bupleurum Shugan powder on liver cells. The results show that this formula can regulate important metabolic pathways and signaling pathways in liver cells, such as heparin metabolic pathway, cell cycle regulation, etc., which explains its role in heat removal, detoxification and calming of the liver and gallbladder on genetic level.

Overall, in recent years, in-depth research on the mechanism and indications of Buplerum Shugang powder has provided more theoretical and empirical basis for the rational clinical application of this important Chinese medicine, and the prospects for its research are broad. However, mutual confirmation with Western medicine needs further development. In this regard, Western blotting is attracting increasing interest from researchers, which is widely used in molecular biology, biochemistry, genetics and other natural science disciplines. Western blotting technology can be integrated with other technologies to improve quantitative accuracy and high-throughput detection capabilities, and will also play a broader role in life sciences research.

Studying an animal model of anxiety disorder is of great importance for studying its pathological mechanism and screening for new drugs. The above served as the basis for shaping the direction of this study, its goals and objectives.

CHAPTER 2
OBJECT, PURPOSE AND METHODS OF THE STUDY

2.1 Object and purpose of the study

The purpose of the study is to identify and study the effect of the herbal preparation Chaihu Shugan San on anxiety using the method of biotechnological immunoblotting of proteins.

To achieve this goal, a task was set and solved: *a)* to study the effect of Chaihu Shugan San powder on anxiety; *b)* to master a modern method of experimental work in biotechnology – the method of protein immunoblotting (Western blotting), which can be used to identify the microscopic index of BDNF to establish the connection between the anti - anxiety effect of Chaihu Shugan San and brain-derived neurotrophic factor.

Object of study: assessment of the efficiency of the herbal preparation Chaihu Shugan San on anxiety disorder. Subject of research: behavioral indices of mice; the efficiency of the anti-anxiety effect of Chaihu Shugan San.

2.2 The proposed research program and the analysis of modeling result

2.2.1 Research Methods

1. Evaluation of anti-anxiety efficacy of Chaihu Shugan Powder

In this study, normal C57BL/6J mice were selected and the gradient dose of Chaihu shugan Powder was administered by intragastric administration. After the intragastric administration, behavioral evaluation methods were used: Open Field Test (OFT) and Elevated Cross Maze (EPM) tests were conducted to detect the effect of the drug on the improvement of anxiety-like behavior in mice and to screen out the optimal dose.

2. Preparation of anxiety model mice and efficacy evaluation of Chaihu Shugan Powder for treating anxiety mice

In this study, the anxiety model mice were constructed by Chronic restraint stress (CRS) and given Chaihu Shugan Powder. Behavioral evaluation methods OFT and EPM were used to evaluate the efficacy of Chaihu Shugan Powder in the treatment of the anxiety model mice constructed by CRS.

3. Biotechnological method detection of Bupleurum Shugan Powder in the intervention of anxiety mice

For change in the phenotype of exploratory behavior and radix bupleurum scattered role of anti-anxiety whether liver and Brain hippocampus Brain derived neurotrophic factor (Brian – derived neurotrophic factor, BDNF), In this study, the content of BDNF in the hippocampus of mice was detected by Western blot to reflect the pathological effect of Chaihu Shugan Powder on anxiety model mice.

2.2.2 Experimental animals and grouping

Efficacy evaluation of Chaihu Shugan Powder for anti-anxiety: SPF grade male healthy C57BL/6J mice, body weight 18 ~ 22g, 60 mice; Efficacy evaluation of Chaihu Shugan Powder on anxiety model mice: SPF grade male healthy C57BL/6J mice, body weight of 18 ~ 22g, 48 mice; After entering the laboratory, the mice adapted to the environment for 1 week, kept the temperature 22±1℃, 12h/12h light and dark cycle, day and night were reversed (the light was on at 20:00 and the light was off at 8:00); Daily line grip parallel operation, eliminate the influence of human operation. All animal experiments are conducted in accordance with the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals. All behavioral tests were performed during the dark phase of the mice, and the time was fixed from 09:00 to 17:00.

Efficacy evaluation of Chaihu shugan Powder for anti-anxiety: open field experiment was conducted after 7 days of adaptive feeding, and mice were randomly divided into the following 5 groups with 12 mice per group according to body weight and total distance of open field exercise: normal control group, Chaihu shugan

Powder low-dose group, Chaihu shugan powder medium-dose group, Chaihu shugan powder high-dose group, and diazepam treatment group.

Preparation of anxious mouse model and efficacy evaluation of Chaihu Shugan Powder for treatment: 12 experiments were carried out on after 7 days of adaptive feeding of mice. After screening according to body mass and total distance of open field movement, they were randomly divided into the following 4 groups with 10 participants in each group: normal control group, CRS model group, CRS+ Chaihu shugan powder treatment group, and CRS+ diazepam group.

2.2.3 Animal model preparation

CRS model group, Chaihu Shugan powder group and positive drug group were used to construct anxiety animal models. Restraint stress was performed with a transparent plastic tube (inner diameter 3.5cm, length 13cm), the length of which could be adjusted according to the weight of mice. In the restrained state, the mice were guaranteed to breathe freely, and none of the mice had physical inhibition or pain. After the end of intragastric administration at 9:00 every day, the mice were placed in the restraint tube and bound for 4h for 10 days. Mice in the control group were transferred to the confinement stress room at the same time every day, and the rest were not treated.

2.2.4 Western blot method was used to detect BDNF content in the hippocampus of mice

The hippocampus of 3 mice in each group was lysed with RIPA lysate, and the protein concentration was measured with BCA protein quantitative assay kit after lysate was obtained. The electrophoretic product was transferred to polyvinylidene fluoride (PVV) film, and then closed with 5% skim milk powder at room temperature for 1h after 120min. The primary antibody of BDNF (1:2000) was added overnight at 4℃. Wash the film, add the diluent of the second antibody (1:10000) and incubate for 1h; Wash the film and finally expose it with ECL luminescent solution. Protein

expression levels were analyzed by ImageJ software. GAPDH was used as an internal parameter to represent the relative expression of BDNF in each group.

2.2.5 Data Processing

GraphPadPrism9.0 was used for data statistics, and normality test and variance homogeneity test were required for each group of data before analysis. The data were normally distributed and homogeneity of variance was satisfied. One-way analysis of variance was used for comparison among multiple groups, and Tukey test was used for further pound-wise comparison. Qualitative data was expressed as percentage (%), quantitative data was expressed as mean ± standard error ($x \pm s$), and data with a significance level set at $P > 0.05$ that deviated from the mean by two or more standard deviations were regarded as outliers and were excluded during statistical analysis. Mice with abnormal behavior were also excluded.

Conclusions to chapter 2

To achieve this goal, a number of traditional and modern research methods were used in the work. CRS model group, Chaihu Shugan powder group, and positive drug group were used to establish animal models of anxiety.

To evaluate the anti-anxiety efficacy of Chaihu Shugang powder, this study selected normal C57BL/6J mice, and a gradient dose of Chaihu Shugang powder was administered intragastrically. Following intragastric administration, behavioral assessment methods such as the open field test (OFT) and the elevated transverse maze test (EPM) were used to determine the effect of the drug on improving anxiety-like behavior in mice and to determine the optimal dose.

To determine the content of BDNF in the hippocampus of mice, we used Western blotting method. The hippocampus of three mice in each group was lysed with RIPA lysate, and after obtaining the lysate, protein concentration was measured using a BCA protein quantitation kit. Protein expression levels were

analyzed using ImageJ software. GAPDH was used as an intrinsic parameter to represent the relative expression of BDNF in each group.

For statistical processing of the data, the GraphPadPrism9.0 package was used and a test of normality and a test of homogeneity of variance were required for each group of data before analysis. Data were normally distributed and homogeneity of variance was ensured. One-way analysis of variance was used to compare multiple groups, and Tukey's test was used for further comparisons by pound.

CHAPTER 3
EXPERIMENTAL PART

3.1 Experiment results

3.1.1 Screening of anti-anxiety efficacy evaluation results of Chaihu Shugan Powder

In this study, normal C57BL/6J mice were selected and the gradient dose of Chaihu shugan Powder was administered by intragastric administration. After the intragastric administration, behavioral evaluation methods were used: Open Field Test (OFT) and Elevated Cross Maze (EPM) tests were conducted to detect the effect of the drug on the improvement of anxiety-like behavior in mice and to screen out the optimal dose.

Open field experiment:

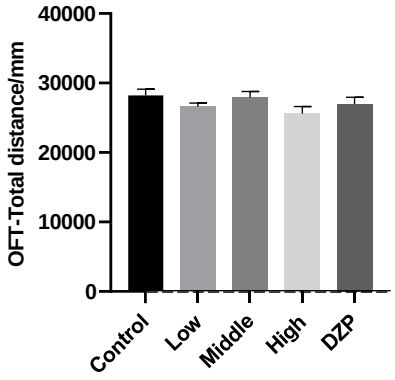


Figure 3.1 Results of open field experiment 1

As shown in Figure 3.1, the results of total journey showed that compared with the blank control group, there was no significant difference in the comparison of total journey between different doses of Chaihu Shugan Powder and diazepam, indicating that the motor function of mice was not affected.

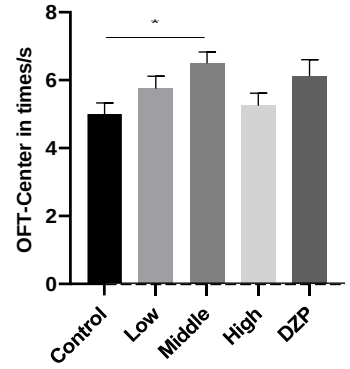


Figure 3.2 Open field experiment results II

As shown in Figure 3.2, this result reflects the comparison of the number of mice entering the center, mainly reflecting the anxiety state of the mice. The results showed that: First of all, different doses of Chaihu Shugan Powder and diazepam increased the number of visits to the center compared with the blank control group, indicating that these drugs can improve the active movement and increased exercise of mice. At the same time, the comparison between these drugs found that the most significant improvement was in the medium-dose group, which was significantly better than the other doses, and better than the diazepam group. These results indicated that the optimal dose was the medium dose group from the point of view of the number of mice entering the center.

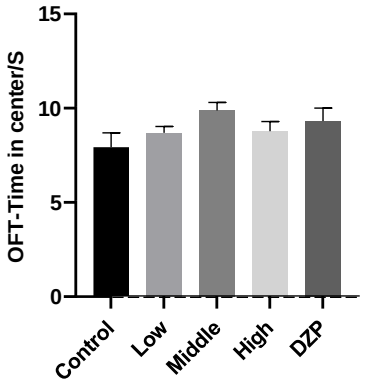


Figure 3.3 Open field experiment results III

As shown in Figure 3.3, this result reflects the comparison of the residence time of the mice in the central region, indicating the mice's interest in exploring the new environment. The results showed that different doses of Chaihu shugan powder and diazepam increased the residence time in the central region compared with the blank control group, indicating that different doses of medicine and diazepam can improve the desire to explore the new environment of mice. At the same time, the comparison of different doses of Chaihu Shugan powder and diazepam showed that the longest improvement time was in the medium-dose group, which was significantly longer than the other groups. The results indicated that the optimal dose of mice in the central region was the medium dose group.

Elevated Cross Maze:

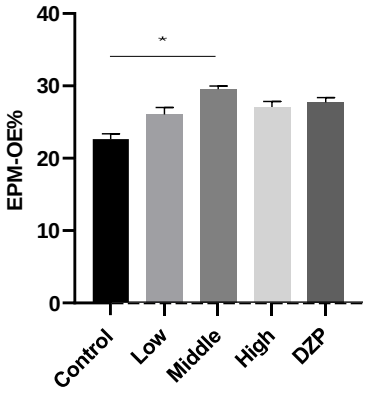


Figure 3.4 Results of the elevated Cross maze experiment

As shown in Figure 3.4, this result reflects the ratio of the number of times the mice entered the open arm, which is proportional to the anxiety of the mice. The results showed that different doses of Chaihu shugan powder and diazepam increased the proportion of times of entering the open arm compared with the blank control group, indicating that different doses of medicine and diazepam can improve the behavioral state of mice. At the same time, the comparison of different doses of Chaihu Shugan Powder and diazepam showed that the medium-dose group had the highest proportion of times entering the open arm, which was significantly longer

than the other groups. The results showed that the optimal dose of mice entering the open arm was the medium dose group.

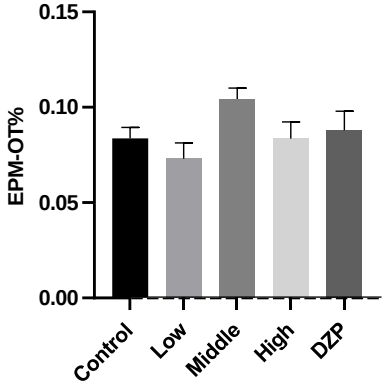


Figure 3.5 Experiment results of elevated cross maze II

As shown in Figure 3.5, this result reflects the residence time of mice entering the open arm, which reflects the exploratory behavior of mice to the new environment. The results showed that compared with the blank control group, the drug results of the medium dose of Chaihu shugan powder and diazepam at different doses were significantly higher than that of the blank group, indicating that the optimal dose for mice entering the open arm was the medium dose group.

Results: According to the above indexes, it was found that the medium dose was the best for improving the anxiety-like behavior in mice. Therefore, the results of the second step choose the best medium dose to continue the next step of the experiment.

3.1.2 Preparation of anxiety model mice and efficacy evaluation of Chaihu Shugan Powder for treating anxiety mice

In this study, Chronic restraint stress (CRS) was used to construct an anxiety model for mice, and Chaihu Shugan Powder was administered. Behavioral evaluation methods OFT and EPM were used to evaluate the efficacy of Chaihu Shugan Powder in the treatment of the anxiety model mice constructed with CRS.

Open field experiment:

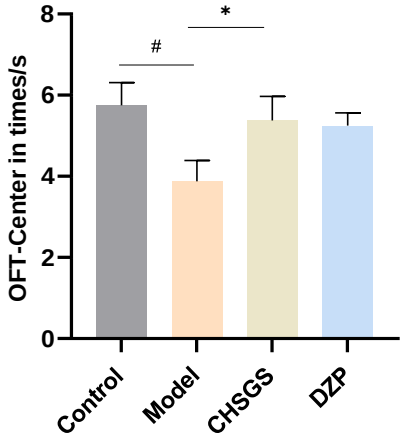


Figure 3.6 Results of open field experiment 1

As shown in Figure 3.6, this result reflects the comparison of the number of mice entering the center, mainly reflecting the anxiety state of the mice. The results showed as follows: First, compared with the blank control group, the anxiety model group reduced the number of visits to the center, while the medium dose of Chaihu Shugan Powder and diazepam both increased the number of visits to the center, indicating that the mice in the anxiety model group showed anxiety state, and the use of Chaihu Shugan powder and diazepam could improve the active movement and increased exercise of the mice. It shows that both drugs can treat anxiety.

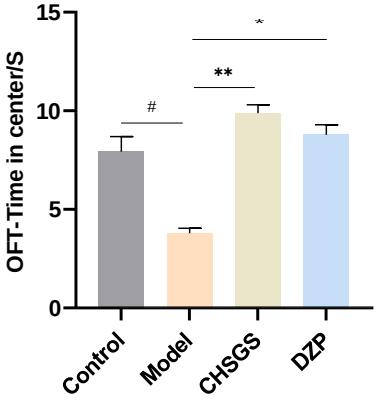


Figure 3.7 Open field experiment results II

As shown in Figure 3.7, this result reflects the comparison of the residence time of mice in the central region, indicating the mice's interest in exploring the new

environment. The results showed that: compared with the blank control group, the residence time in the central area of the anxiety model group was reduced, while the residence time of the medium dose of Chaihu Shugan Powder and diazepam was increased, indicating that both Chaihu Shugan powder and diazepam could improve the desire to explore the new environment of mice.

Elevated Cross Maze:

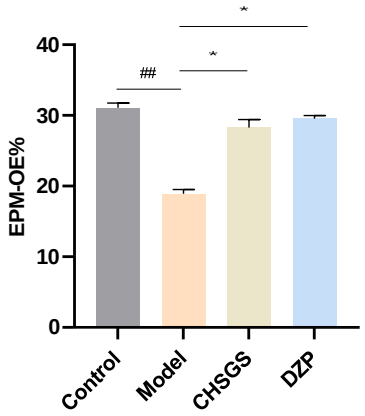


Figure 3.8 Results of the elevated Cross maze experiment

As shown in Figure 3.8, this result reflects the ratio of the number of times the mice entered the open arm, which is proportional to the anxiety of the mice. The results showed that compared with the blank control group, the proportion of mice entering the open arm decreased in the anxiety model group, while the proportion of mice entering the open arm increased in the medium dose of Chaihu Shugan Powder and diazepam, indicating that the medium dose of Chaihu Shugan powder and diazepam can improve the behavior state of mice.

As shown in Figure 3.9, this result reflects the residence time of mice entering the open arm, which reflects the exploratory behavior of mice to the new environment. The results showed that: compared with the blank control group, the indexes of the three groups were lower than that of the blank control group, but the administration of the medium dose of Chaihu Shugan powder and diazepam was significantly higher than that of the model group, indicating that the mice took longer time to enter the open arm after medication.

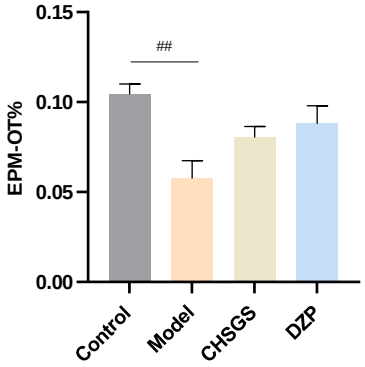
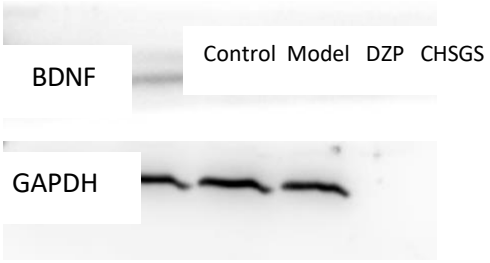


Figure 3.9 Experimental results of elevated cross maze II

3.1.3 Biotechnological method detection of Chaihu Shugan Powder in the intervention of anxiety mice

To explore whether changes in behavioral phenotype and the anti-anxiety effects of Buplehushugan Powder are related to Brain-derived neurotrophic factor (BDNF) in the hippocampus, In this study, the content of BDNF in the hippocampus of mice was detected by Western blot to reflect the pathological effect of Chaihu Shugan Powder on anxiety model mice.



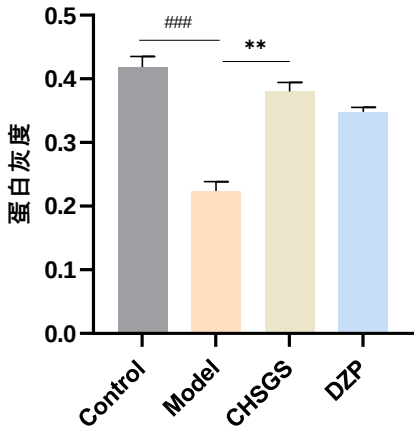


Figure 3.10. Brain-derived neurotrophic factor (BDNF) levels

As shown in Figure 3.10, compared with the control group, the level of brain-derived neurotrophic factor (BDNF) in the hippocampus of the anxiety model group was significantly reduced, indicating that the level of BDNF in the Brain of the anxiety mice is closely related to anxiety. At the same time, the medium-dose Chaihu shugan powder and diazepam were basically the same as the blank control group, but significantly higher than the model group, indicating that the BDNF content in both groups could be increased, indicating that the two drugs could improve the state of anxiety, and the effects of the two groups of drugs were reflected through the perspective of micro-index BDNF.

3.2 Discussion of the experiment results

3.2.1 Screening results of anti-anxiety efficacy evaluation of Chaihu Shugan Powder and analysis of optimal dosage results

This step is divided into two parts, the first part is to first screen the different doses of Chaihu Shugan powder, screen out the dose with the best effect on improving team anxiety, and then conduct the second experiment. The second experiment is to compare the best use of Chaihu Shugan powder, the use of diazepam, and the efficacy of compound Chinese medicine and Western medicine.

The results showed that: The first experiment selected low dose, medium dose and high dose of Chaihu Shugan powder. Through the comparison of the behavioral indexes of mice in Open Field Test (OFT) and Elevated Plus Maze (EPM) experiment, it was found that the three doses could improve the behavioral indexes of mice. In the open field experiment, the number of entering the center increased and the stay time in the central area increased, indicating that the three doses can improve the active movement and increase the movement of mice. It can improve mice's desire to explore the new environment. In the elevated cross maze experiment, the proportion of mice entering the open arm and the time of entering the open arm were both increased, reflecting the improvement of anxiety symptoms by the drug.

At the same time, the comparison between the three doses found that the best dose was the middle dose, and the middle dose had the most obvious effect on improving the data, thus laying the foundation for the second step.

In the second step, the main purpose of the experiment was to compare the improvement of medium dose of Chaihu Shugan Powder and diazepam on anxiety, in order to prove the effectiveness of compound Chinese medicine Chaihu Shugan Powder on anxiety. Mice with Open Field Test (OFT) and Elevated Plus Maze (EPM) were also used for comparison of behavioral indicators. The results showed that the indicators of the anxiety model group were significantly lower than those of the blank group, indicating that the behavioral indicators were used to verify the success of the anxiety model, which was also consistent with the symptoms of clinical anxiety. Then, the two groups were compared, and the medium dose of Chaihu Shugan Powder and diazepam could both improve the behavioral indexes of mice. In the open field experiment, the number of entering the center increased and the stay time in the central area became longer, indicating that both the compound Chinese medicine and Western medicine could improve the active movement and increased exercise of mice. It can improve mice's desire to explore the new environment. In the elevated cross maze experiment, the proportion of times and time of entering the open arm of mice were increased, which reflected the improvement of anxiety symptoms of the compound Chinese medicine Chaihu Shugan powder and diazepam.

Chaihu Shugan powder composition: orange peel (vinegar stir-fried), bupleurum 6g, Chuanxiong, Xiangfu, Fructus aurantii (bran stir-fried), Peony 4.5g each, licorice (grilled)1.5g. The main function is to ease the liver depression, the clinical effect of anxiety is very obvious improvement. This step verified that Chaihu Shugan Powder can first improve the symptoms of anxiety disorder through mouse behavioral indicators, and also detected the best dose to improve symptoms, mainly medium dose.

3.2.2 Discussion on the results of detection of BDNF content in the hippocampus of mice by Western-blot

This step has two purposes, the first is to master the basic experimental operation methods in the biotechnology profession, and choose the Western-blot method. Secondly, through the change of BDNF content in the hippocampal region of mice, the improvement of Chaihu shugan powder and diazepam on anxiety was quantitatively compared.

The results showed that the level of brain-derived neurotrophic factor (BDNF) in the hippocampus of the anxiety model group was significantly reduced, indicating that the level of BDNF in the Brain of the anxiety mice was reduced, and the two have a certain correlation. At the same time, the medium dose of Chaihu shugan powder and diazepam group both increased the content of BDNF, indicating that the two drugs could improve the state of anxiety, and the micro-index BDNF reflected the improvement.

BDNF, which means brain-derived neurotrophic factor in medicine, is a protein synthesized in the brain, which is generally widely distributed in the central nervous system and plays a very close role in the survival and differentiation of neurons. In the course of the experiment, the change of this index also verified the close relationship between anxiety and BDNF.

Conclusions to chapter 3

Screening results evaluating the anti-anxiety efficacy of Chaihu Shugan powder showed that a medium dose best improved anxiety behavior in mice. Therefore, she was chosen to continue the next stage of the experiment.

When studying the behavior of mice with anxiety model and evaluating the effectiveness of Chaihu Shugan powder for treating mice with anxiety, it was found that compared with the control group, the performance of the three groups was lower than that of the control group, but the administration of the average dose of Chaihu Shugan

powder and diazepam was significantly higher than the model group, indicating that the mice took longer to enter the open arm after taking the drug.

By applying the biotechnological detection method of Chaihu Shugang powder to treat anxious mice, it was found that the level of brain-derived neurotrophic factor (BDNF) in the hippocampus of the anxiety model group was significantly reduced, indicating that the level of BDNF in the brain of anxious mice was reduced, and these two indicators have a certain correlation. At the same time, the average dose of Chaihu Shugan powder and the diazepam group increased BDNF content, indicating that these two drugs can improve anxiety and the BDNF micro-index reflects this improvement.

CONCLUSIONS

The work is devoted to solving an important scientific and practical problem – studying the effect of the herbal medicine Chaihu Shugan Strength on anxiety using the method of biotechnological immunoblotting of proteins.

To solve this problem, two tasks were set and solved:

a) to study the effect of Chaihu Shugan San powder on anxiety;

b) to master a modern method of experimental work in biotechnology – the method of protein immunoblotting (Western blotting), which can be used to identify the microscopic index of BDNF to establish the connection between the anti-anxiety effect of Chaihu Shugan San and brain-derived neurotrophic factor.

Taking into account the intended goal, the optimal dose of Chaihu Shugan powder was first determined in comparison with diazepam, a first-line Western medicine drug. It was found that the best effect is achieved when a medium dose is administered. The improvement in anxiety in the two groups of mice was further compared, and the behavioral indices of the mice in the Open Field Test (OFT) and Elevated Plus Maze (EPM) were used as response indices.

The results of the experiment showed that:

1. The herbal medicine Chaihu Shugan San affects the anxious state of experienced mice towards its improvement;

2. The positive effect of Chaihu Shugan San powder on anxiety was basically the same as that of the famous Western medicine drug diazepam; in this case, the best effect is achieved with an average dose of Chaihu Shugan San;

3. The effectiveness of Chaihu Shugan San on anxiety is associated with brain-derived neurotrophic factor, as indicated by the content of BDNF in the hippocampus of mice.

There are still some shortcomings in this experiment, such as ambiguity in studying the relevant mechanism, as well as some unfamiliar operations and

uncontrolled factors in the mouse experiment, which are expected to be improved in the future experiment.

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