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 biotechnological methods for the study of antidepressants in Chinese medicine**

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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APPROVE
Head of Department of Biotechnology, Leather and Fur, Professor, Doctor of Technical Science
Olena MOKROUSOVA

_____
«___»_____2024

ASSIGNMENTS
FOR THE QUALIFICATION THESIS
Geng Ruhui

1. Thesis topic **Application of biotechnological methods for the study of antidepressants in Chinese medicine**
Scientific supervisor Olga Iungin, Ph.D., Assoc. Prof.

approved by the order of KNUTD "____"_____2024, №____

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

3. Content of the thesis (list of questions to be developed): literature review; object, purpose, and methods of the study; experimental part; conclusions

4. Date of issuance of the assignments_____

EXECUTION SCHEDULE

№	The name of the stages of the qualification thesis	Terms of performance of stage	Note on performance
1	Introduction	From 01 April 2024 to 11 April 2024	
2	Chapter 1. Literature review	From 06 April 2024 to 20 April 2024	
3	Chapter 2. Object, purpose, and methods of the study	From 21 April 2024 to 30 April 2024	
4	Chapter 3. Experimental part	From 01 May 2024 to 10 May 2024	
5	Conclusions	From 07 May 2024 to 12 May 2024	
6	Draw up a bachelor's thesis (final version)	From 20 May 2024 to 25 May 2024	
7	Submission of qualification work to the supervisor for feedback	From 25 May 2024 to 27 May 2024	
8	Submission of bachelor's thesis to the department for review (14 days before the defense)	27 May 2024	
9	Checking the bachelor's thesis for signs of plagiarism (10 days before the defense)	31 May 2024	
10	Submission of bachelor's thesis for approval by the head of the department (from 7 days before the defense)	03 June 2024	

I am familiar with the task:

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SUMMARY

Depression is a global frequent mental illness, which usually brings a great economic burden on the society. The main symptoms of depression are memory loss, difficulty concentrating, and various cognitive disorders. For the treatment of depression, common treatments include drugs such as sertraline, cognitive behavioral therapy, and corresponding neuromodulation techniques for depression. Traditional Chinese medicine has a long history of treating depression in China. The common therapies to relieve depression include Traditional Chinese medicine and acupuncture, among which this Chinese medicine has a variety of pharmacological effects and has significant effects in the treatment of depression and other diseases. Sini powder has the effects of regulating liver and spleen, penetrating evil and relieving depression, and can play antidepressant effects by regulating immune inflammation and synaptic plasticity.

Keywords: depression; sini powder; depression intervention; animal model.

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INTRODUCTION

Depression can bring a variety of cognitive disorders to people, its common treatment methods are medication and psychological treatment. Traditional Chinese medicine in China has a long history of treating depression. The common treatments include acupuncture and traditional Chinese medicine. Xiaoyao powder, ginsenoside xiaobuhu soup and Sini powder are commonly used prescriptions, and the clinical treatment of liver depression qi knot type depression has good results. The corresponding research methods include the preparation of mouse model, mouse model first, and behavioral detection to evaluate the antidepressant effect of Sini powder intervention on depression model mice. At the same time,learning inflammatory cytokine IL-1 β levels with the Elisa kit. It is hoped that through this study, we can have a deep understanding of the improvement effect of depression and depression on depressive symptoms, and provide new ideas and theoretical support for the study of depression in depression.

The relevance of the topic is Sini powder and depression.

The purpose of the study is the study of depression in depression provides new thinking and theoretical support.

The objectives of the study is in-depth understanding of depression and the improvement of depressive symptoms.

The object of the study is Sini powder with the mouse model.

The subject of the study is Sini powder and its role in the development of depression.

Research methods is mouse model.

The scientific novelty is Chinese traditional medicine Sini powder for the treatment of depression.

The practical significance of the results obtained is playing an antidepressant role in many ways.

Approbation. (Appendix).

CHAPTER 1
LITERATURE REVIEW

1.1 An overview of depression

The main characteristics of depression are reduced interest in life, decreased attention for more than two weeks, and a long period of low mood. It is a persistent and severe mental illness worldwide. Today, about 350 million people in the world suffer from depression.[1] Depression, which is expected to become the leading cause of functional disability after several decades, is often accompanied by high morbidity and mortality, with a huge economic burden on the world. According to the World Health Organization, major depression will be the leading cause of disease burden in many parts of the world by 2030.[2] The symptoms of depression are mainly concentration difficulty, visual impairment, memory loss, executive dysfunction, lack of interest, abnormal social function and hallucinations. At present, there is still a lack of understanding of cognitive impairment in depression and the corresponding prevention and treatment methods, so it is very important to conduct more in-depth research. The incidence of depression is increasing year by year, and research has shown that after the COVID-19 event, various mental diseases, especially depressive symptoms and anxiety symptoms, increasingly appear and worsen. Depression, a chronic disease, often occurs with other chronic diseases, which seriously affects people's normal life. People with depression with diseases such as cardiovascular disease and diabetes have severely impaired body function and increase the risk of death. Depression, which places a huge burden on people, is now an important public health problem.[3]

1.2 Progress in domestic research in depression

Depression, as a multidimensional mental disorder that affects human psychology and cognition, is particularly evident in terms of cognitive function, and therefore is also included in the International Classification of Diseases-10 and the

Diagnostic and Statistical Manual-5.[1] The treatment method for depression in China is mainly divided into western medicine therapy and traditional Chinese medicine therapy. Drugs such as sertraline and duroxetine in western medicine are effective in improving depressive symptoms. Common treatments include medication, such as sertraline hydrochloride, doloxetine, vortixetine, and bupropion. Sertraline improves psychomotor, and duroxetine improves memory and language function. Wartixetine, similar to doloxetine, can both improve depression and cognition. Bupropion is able to help improve memory. Common antidepressant drugs include quintiapine fumarate and escitalopram oxalate. In addition, there are psychotherapy methods such as cognitive behavioral therapy that can help people with depression.[2] The pathogenesis of depression is complex. Related studies show that the combination of genetic and environmental influences can lead to depression, and genetic factors account for about 30% to 40% of the incidence. Some people believe that depression is influenced by multiple mechanisms, but the exact mechanism is still unclear. For the study of the depression in China, some data show that the treatment method can adopt stepped strategies from low to high intensity: for patients with mild illness, psychological therapy and exercise intervention are preferred to improve the symptoms; for patients with severe symptoms, they generally tend to choose higher intensity psychotherapy or drug therapy; and for patients with severe depression, more intensive treatment like electroconvulsive therapy or repeated transcranial stimulation should be considered.[3]

1.3 Research progress in depression abroad

According to some foreign data, the duration of depression and the increase in the frequency of attacks will affect the function of the hippocampus and amygdala in the brain, and then affect some cognitive functions, including executive function, working memory and situational response. According to the study, we can learn that the reduction of hippocampal volume is closely associated with the number of major depressive episodes. Bilateral hippocampal volume reduction usually occurs in patients with major depression, and memory declines once these patients have episodes.[2]

Cognitive deficits in some depressed patients are associated with an impairment in their memory. Some negative information will be retained in long-term memory for a long time. A foreign experimental study showed that patients with major depression were given a coding working memory intervention in the first two weeks, after which they received about three weeks of treatment, with an average of about five weeks to complete. During this period, the participating patients took various self-report measures and objective neuropsychological tests, and they gave feedback to the tester every week. They were then tested to a series of standard tests, including working memory. The more mistakes the participants make, the lower the suppression ability. For planning ability as well as language long-term memory, participants also took the California Language Learning Test as well as the D-KEFS Tower test. In addition, these participants assessed their processing speed and intelligence by the D-KEFS color word interference test and the Wechsler Intelligence Scale, respectively. After that, they completed their self-report including self-perception and reflection. After statistics and analysis, the tester concluded that there were significant improvements in memory function in those involved in encoding working memory training compared with MDD patients without intervention.[4] Vision is also affected due to damage to the hippocampus and the medial temporal lobe. There is a test of scene recognition, where patients with medial temporal lobe (MTL) loss need to observe and find out the deformed scene to determine which scene is most similar to the end scene on the screen. The cognitive deficits in patients with impaired medial temporal lobe appear more commonly than in patients with focal hippocampal lesions. The hippocampus will also participate in the scene construction, and according to the relevant experiment, the participants will need to imagine a richer and more vivid scene at the prompt of a certain scene. The results showed that patients with hippocampal damage lacked an immersive perception of imagery. Through a popular study in vision science, we can learn that memories can also have some effects on eye movements under the influence of the hippocampus [7]. Testing patients for visual contrast inhibition and luminance

sensing for retinal processing, the researchers concluded that cortical processing of visual contrast was altered during disease onset, but retinal processing was normal [5].

Patients often develop abnormalities in other ways. Patients with MDD may have hallucinations, and in their eyes, the shape and size of the surrounding environment may change. Sometimes patients are unable to correctly distinguish between the right side and the left side. Sometimes the images they see can even move. In the eyes of these depressed people, the night becomes very long, or the surrounding environment can change quickly. They often lose confidence in themselves, have fear and even collapse.[6] People with MDD will also have executive dysfunction, such as difficulties in solving problems and performing tasks. Also, patients often have difficulty maintaining attention while watching TV or reading a book. In general, patients with first-episode depression have no attention deficits in processing information, while relapsed patients often have attention deficits in processing various information, and attention deficits are associated with depression both in the acute period and in remission. Usually, patients with first-episode depression process more normally during remission, while those with relapse generally slow. People with MDD need more time to process the information.[7]Although the majority of depressed patients respond well to medication and psychotherapy, some are resistant to this treatment approach. In addition, many people with depression have limited access to treatment. Relevant data show that the coverage rate of depression treatment in low- and middle-income countries is about 20%, and that in high-income countries is only about 50%. Studies have also found that exercise may be an effective supplement or alternative to this treatment. And exercise can also help improve a range of physical and cognitive outcomes in addition to mental health. Many sports can help to treat depression, especially when walking or jogging, etc. Studies have found that depression is more likely to reduce life satisfaction than diabetes, divorce or debt problems, and exacerbates disease symptoms, including anxiety or heart problems. Major depression can also trigger disability worldwide. The World Health Organization recommends that people, especially those with chronic diseases and

disabilities, such as jogging, a lower-cost intervention, are effective for patients with different levels of depression. Some competent people want to further reduce depressive symptoms and can also choose more intense exercise in a permissive environment. These treatments can be used as an adjunct or alternative to other established methods such as pharmacotherapy, while also reducing the risk of developing other depression-related diseases.[8]

1.4 The Chinese TCM of depression and the origin of Chinese medicine

Traditional Chinese medicine has a long history of treating depression. The ancient“Huangdi Neijing” has a record of “depression”, and it mentioned: “to the depression of emotion is always from the heart”.[9] “The essence of Chinese medicine” mentioned: “the spleen Yang is short, spleen Yin is insufficient memory more forget”. Modern clinical medicine shows that TCM therapies such as Chinese medicine and acupuncture have their unique advantages for relieving and treating depression.[10] Xiaoyao powder, ginsenoside and acupuncture and iguan point can improve depression symptoms, Xiaobuhu soup is a common prescription in the treatment of depression, clinical treatment of liver depression qi knot depression effect is good. The jujube kernel soup in traditional Chinese medicine compound can nourish the heart and spleen, nourish blood and calm the mind, and is often used in the treatment of heart and spleen deficiency depression.[11] Left-home lowering sugar and relieving depression has good anti-depressant effect. Zhao Hongqing et al. found the effect of relieving the liver and relieving depression and nourishing the liver and kidney. In Zhang Zhongjing's “Theory on Febrile Diseases”, the Sini powder has the functions of dredging liver and spleen, and penetrating evil and relieving depression. Modern studies have found that the Sini powder can exert antidepressant effects by regulating immune inflammation and synaptic plasticity.[12] The Sini powder is composed of peony, bitter fruit, licorice and bupleurum, which has the effect of regulating the liver and spleen. Chai Hu rise Yang, liver depression; zhi qi depression, heat break knot; white peony root soft liver nourishing blood, reconcile qi and blood; and licorice with

various medicine, spleen and medium. Four medicine combined, shu liver solution depression, evil to depression, qi and stomach, qi and blood adjustment smooth, clear Yang stretch.[13]

1.5 Current development in the treatment of depression at home and abroad

There are corresponding interventions for the adverse symptoms of depression. In terms of pharmacotherapy, drugs such as vortixetine and sertraline can help to improve depressive symptoms. As the only antidepressant approved by the FDA, voxicetine has a more widespread impact on memory function in patients. In addition, both intranasal insulin and brain insulin have important roles in the treatment of depressive symptoms in patients.[2] There are other treatments other than drug therapy. Studies have shown that some people with major depression can help them improve their depression and cognition through breathing relaxation training and cognitive behavioral therapy.[14] In addition, brain stimulation techniques such as cognitive remediation therapy can also help treat patients with depressive symptoms. Aerobic exercise plays an important role in the process of improving cognitive impairment in depressed patients.[2] Creative art therapy is an innovative treatment method, and this non-pharmacological treatment modality can effectively intervene in the cognitive impairment in depressed patients.[15]The full course of antidepressant treatment can effectively prevent and control the recurrence of depression. Its treatment is divided into three stages, including acute, consolidation, and maintenance periods, which can effectively improve the prognosis. Currently, first-line antidepressant drugs such as SNRIs, NASSAs, and SSRIs are effective in relieving patients. Generally, when starting treatment with these drugs, SSRIs or SNRIs are applied at low doses, and the usual effective time is approximately 2 to 4 weeks. When the first time this antidepressant method fails to achieve good results, the intake dose and use time of the drug can be appropriately increased. If the desired treatment effect is still not achieved, then other synergistic drugs can be considered, but it should be noted that this may

aggravate the risk of drug interaction, which will also increase the treatment cost to some extent. In addition, it can also be replaced with other drugs with attention to the occurrence of drug withdrawal syndrome. These antidepressants have fewer side effects than other drugs. However, long-term use of SSRIs and SNRIs can cause side effects such as weight gain, intestinal discomfort and osteoporosis. According to the data, the most common side effects of SSRIs and SNRIs are the gastrointestinal discomfort, whose symptoms include dyspepsia, abdominal pain, nausea and gastrointestinal bleeding. About half of the patients will experience the above discomfort symptoms for several days or more after using the medication, which may be related to 5-HT's main regulating role in sensory regulation and gastrointestinal motility. In addition to medication, psychotherapy is also an important treatment for depression. Common psychotherapy treatments include hypnotherapy and cognitive-behavioral therapy. Most patients prefer psychotherapy. Generally, for patients with multiple medication conditions or patients with multiple diseases, psychotherapy can also be considered as an efficient treatment method to prevent the possible interactive effect between drugs and disease. Using pharmacotherapy combined with psychotherapy has a more significant effect than using one method alone. Therefore, psychotherapy is one of the very important interventions in the mild and moderate depression stages of patients.

As the main type of psychotherapy recommended in the majority consensus and guidelines, cognitive behavioral therapy can help depression correct the dysregulated function. Studies have shown that the most effective psychotherapy treatment is CBT, which can be used as a separate therapy or in combination with other treatments. After the cessation of CBT treatment, it still had a long-term effect in patients, and during long-term follow-up, similar effects were found between patients with long-term antidepressant medication and CBT treatment without continued treatment. It is noteworthy that CBT cannot be used as a treatment for major depression alone, and that CBT combined with antidepressants should be considered in patients with major depression. In addition to psychotherapy, there are improved electroconvulsive therapy

MECT and other physical therapy methods. The fied electroconvulsive therapy is to make the depressed patient in a state of unconsciousness through short general anesthesia, and then stimulate the patient's brain through a certain amount of current. A British survey showed that more than 30 percent of patients receiving electroconvulsive therapy had improved significantly, and studies showed that most of them had depressive symptoms subsided in just a week. However, for this relatively rapid treatment method, its various side effects have been highly concerned. Some practical evidence suggests that the efficacy of electroconvulsive shock is related to the location and dose of electrode placement. The effect was more pronouAnother neuromodulation technique for depression is repetitive transcranial magnetic stimulation, which aims to regulate the network of local areas in the brain through weak currents or magnetic pulses. The rTMS is generally used in the treatment of major depression. During the rTMS treatment, coils with different intensity and different frequencies and shapes deliver magnetic pulses in order to produce excitability changes in specific areas of the brain. It has been shown that rTMS alone has similar efficacy to antidepressants and MECT. There are also related trials that combine rTMS with other therapies to greatly benefit patients, such as rTMS combined with psychotherapy to achieve remission and response rates of 56% and 66% in depressed patients, respectively. The follow-up after half a year showed that the continuous remission rate remained at about 60%. For example, rTMS combined with paroxetine or citalopram can also benefit patients widely from antidepressant treatment. However, it should be noted that there are still many unsolved problems with rTMS, although it has developed rapidly in the clinical field in recent years. Future research directions will also include identifying rTMS targets and mechanisms, evaluating their combination methods in combination with other treatment modalities, and developing effective personalized treatment plans to fully realize the therapeutic potential of rTMS.[3]nced with bilateral electrode placement and using high doses.

1.6 Research progress of traditional Chinese medicine prescription of Sini powder

Sini powder has a variety of pharmacological effects and has a wide range of treatment. For example, it can alleviate some diseases such as depression, gastrointestinal diseases and diabetes. Bupleurum in Sini powder is rich in buside side and polysaccharide, which has anti-inflammatory and analgesic effects. Studies have shown that firewood saponin is helpful in a variety of digestive diseases, including its ability to inhibit gastric fluid secretion, reduce pepsin activity, and reduce ulcer area. Moreover, firewood saponin also has a role in alleviating depressive symptoms, which can increase the expression of brain-derived neurotrophic factors in the hippocampus. In anti to cancer, firewood saponin also plays a role in blocking the G1 cell cycle and inducing p53 expression to inhibit the proliferation of cancer cells. The pharmacological effects of white peony root in the Sini powder prescriptions are also very extensive, with antibacterial, anti-inflammatory, anti-fibrosis and immune regulation effects. In addition, both bitter orange fruit and licorice have been proven to have similar effects.

It is found that compared with single drug, the metabolites of sersaponin are more widely distributed in vinegar and white peony root. There were some changes in the efficacy of vinegar, such as the decrease of volatile oil in the drug, the increase of nine components associated with anti-inflammation, and the increase of ten components and 16 components. Sini powder also has obvious anti-inflammatory effect, whether single agent or prescription can regulate inflammation to play a therapeutic effect. It has a good effect in regulating gastrointestinal function, It can regulate the movement of the gastrointestinal tract to a certain extent, improve the abnormalities of gastrointestinal neurotransmitters, gastric acid and gastric mucosa, and have a good prevention and treatment effect on a variety of gastrointestinal ulcers.[16]

Conclusions to Chapter 1

1. The treatment methods to treat depression in China mainly include psychotherapy and exercise intervention. Most patients with depression can improve their situation through these methods.
2. Depression is associated with the hippocampus and amygdala in the brain, and depression is often accompanied by memory loss and visual impairment.
3. Traditional Chinese medicine has a long history of treating depression. And traditional Chinese medicine Sini powder scattered can relieve depression.

CHAPTER 2
OBJECT, PURPOSE, AND METHODS OF THE STUDY

2.1 Object and purpose of the study

The object of the study is Sini powder with the mouse model.

The purpose of the study is the study of depression in depression provides new thinking and theoretical support.

2.2 Progress in mold modeling in depressed mice

At present, mice are the most common animal model in disease research. Its advantages include low price, small size, and convenience of genetic engineering technology to induce mouse models, which are inaccessible to other animal models. The outstanding strain in the mouse model of depression is C57BL / 6 mice, which is the preferred strain for the stress model, which is characterized by high stability and small individual differences. The selection of appropriate mold making methods needs to master the advantages and characteristics of different strains of mice, so as to ensure the scientific nature of experimental research. In addition, the mouse models commonly used at home and abroad include ICR mice, BALB / C mice, Kunming KM mouse model, and FST, TST, CUMS, CRS mouse models, etc.[17]

2.3 Progress in the remaining animal models of depression

In addition to several common mouse models, there are other animal models, such as the Flinders Sensitive Line (FSL) model found by Overstreet and Wegener, which has prolonged swimming test mobility duration and prolonged FEM sleep, characterized by high immobility and no significant anxiety symptoms. In addition, there is a new animal model of depression, the fecal bacteria transplantation (fecal microbiota transplantation, FMT) model, which has found that mood changes affect gut microbial ecology, which can be molded by using multiple antibiotics to destroy

the gut ecology or fill the gut flora of depressed patients. Studies have found that inflammation in the brain is also correlated to the gut. In order to regulate some functions of the brain and build a normal intestinal ecology, some people have considered transplanting fecal bacteria from healthy people into the intestines of patients as an adjunct to depression therapy. However, the stability of the current FMT model is not high, and usually needs to cooperate with some other modeling methods, the most common method is to combine with CUMS model.[18]

2.4 Progress in mold modeling in mouse models

Mice have the validity, sensitivity and reliability suitable for depression modeling, which can help to develop relevant experimental standards and provide feasible experimental reference to a great extent. The C57BL / 6 strain of mice has undergone early mother and infant separation, and thus is suitable for continuous chronic stress after weaning to construct a novel long-term depression LTD model. Compared with the traditional ELS and CUMS mice, this mouse model can show a more stable depressive state, which is conducive to behavioral studies. Li et al proposed that in the case of acute stress in C57BL / 6 mice, the results of the absence experiment found that mice of this strain were more likely to show depressive-like behavior than the remaining mice. Therefore, this strain of mice has the potential to become various forms of stress modeling.[17]

Conclusions to Chapter 2

1. Mice are the most common animal model, whose advantages include low price, small size, etc. The excellent strain is C57BL / 6 mice, which are characterized by high stability and small individual differences.
2. C57BL / 6 strain mice were separated by early maternal and infant separation, suitable for continuous chronic stress after weaning to construct a new long-term depression LTD model. Through absenteeism experiment, this mouse

model can show a more stable depression state, which is conducive to behavioral studies.

CHAPTER 3
EXPERIMENTAL PART

3.1 Laboratory animals and the subgroups

3.1.1 Experimental animals and groups

The model animals were molded in CSDS for ten days, and the mice were tested by sugar spraying experiments before and after molding. The mice with depression model that significantly reduced the number of hair management after mold making compared with before mold making.

3.1.2 Preparation of animal models

The depression model mice were treated with gardenia black bean soup, and the mice received the antidepressant effect of the depression.

3.1.3 Use of Elisa reagent test method (detection of inflammatory cytokine IL-1 β levels)

The detection of inflammatory cytokine IL-1 β levels was performed with the Elisa kit, through the detection of peripheral blood inflammatory cytokine IL-1 β to evaluate the intervention effect of the Sini powder intervention on pathological indicators in depression model mice.

3.2 Animal model preparation

Laboratory animals and the subgroups

SPF male healthy C57BL / 6J mice with body mass 18~22g, 36 mice; SPF male healthy CD-1 mice with body mass 30~35g, 8 mice. After entering the laboratory, the mice adapted to the environment for one week, had a free diet and drank water, kept the temperature at 21 ± 1℃, 12h / 12h light and dark cycle, day and night reversed (reverse the light at 20:00, and turn off the light at 8:00); grasped parallel operation every day to eliminate the influence of human operation. All animal experimental procedures were complied with the Guide for the Care and Use of Laboratory Animals.

All behavioral tests were performed in the mouse dark period with a fixed time of 09:00am – 17:00pm.

40 C57BL / 6J mice were left with 10 blank control mice, and the remaining mice were subjected to CSDS modeling. After the modeling, the successful depression model mice were divided into model group and Sini powder intervention group. Mice in the four intervention group were treated with 14 days of gavage (600 mg / kg) and the remaining groups were given the same volume of saline. The experimental flow chart is shown below.

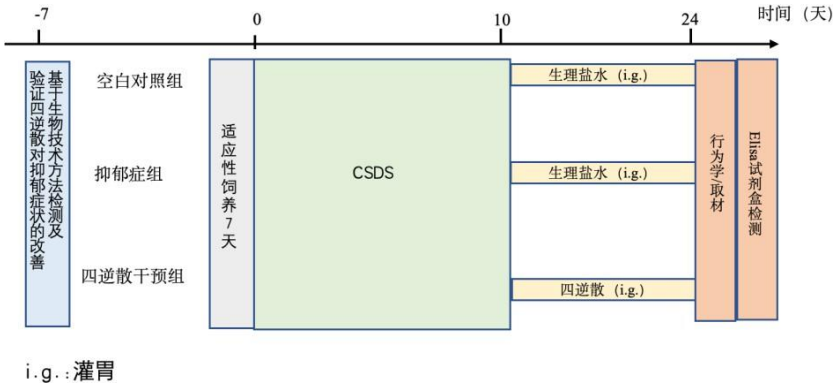

Figure 3.1 – Intragastric administration

Animal model preparation

C57BL / 6J mice (invading mouse) were placed in a CD1 mouse (resident mouse) cage for 5 minutes. To avoid individual differences in fight intensity, daily resident rats faced different invading rats designed by Latin squares. The CSDS was stressed once daily for 10 consecutive days. After the fight, the resident and invading rats were placed in the same perforated Plexiglas cage for 24 h, allowing visual, auditory, and olfactory interactions. The procedure was then repeated for 10 days and ensured that C57BL / 6J invaded a previously seen new CD1 cage. The control group (n=10) was paired-separated by perforated plexiglas and never exposed to CD1.Detection of behavioral indicators (behavior test)

Sugar spray experiment (Sucrose Splash Test, SUST)

A 10% sucrose solution was sprayed on the mouse dorsal hair. Sucrose solution could stain the back hair and cause grooming, and the camera synchronously recorded the activity of the mice for 6 minutes. The experimental indicators to be recorded during the experiment were the incubation duration of grooming, grooming duration, grooming times, and the proportion of grooming duration in the total duration.

Female urine sniff assay (Female Urine Sniffing Test, FUST)

Female urine sniffing experiments evaluated reward-seeking behavior. One hour before the test, a sterile cotton head nozzle was inserted into the mouse cage. The test was divided into three stages: a: submerged cotton tip into the mouse cage for 6min; b: 45min interval without inserting the mouse cage; c: fresh urine collected from the same strain female during estrus into the mouse cage (6min), during which the camera video video to measure the duration of sniffing.

Taiiling tail (Tail Suspension Test, TST)

The desensitization tape was attached to 1 / 3 of the distal tail tip and fixed in the hanging tail box to keep the mouse in an upright state and the head facing the lens, about 30cm above the ground. The Smart 3.0 animal behavior analysis system was used to record the behavior state in real time and calculate the stationary time. At the motionless time, the mice stopped struggling and were overhanging as the standard for determining their behavioral despair. The hanging time and incubation period were collected and analyzed.

Organization materials

Two hours after the last behavioral session, the mice were taken for eyeball blood extraction. After the materials were completed, centrifugation was performed quickly to prevent blood coagulation, and the upper serum was collected for later use.

Detection of the levels of the inflammatory cytokine IL-1 β

1) First, prepare the required standards and reagents;

2) Remove the microplate from the sealed bag that has been balanced to room temperature, and the unused strip should be put back into the aluminum foil bag and re-sealed;

3) Add 300μL of washing liquid to the microplate, stand and soak for 30 seconds, discard the washing liquid, and beat the microplate dry on absorbent paper;

4) Add different concentrations of experimental samples and standard or control products to the corresponding wells, 100μL of each well. Incubation for 2 hours at room temperature with the reaction wells on sealed paper;

5) Abup the liquid in the plate and use the bottle, automatic plate washing machine or multi-channel plate washer.300μL of wash solution was added to each well, after which the in-plate wash solution was aspirated. Repeat 3 operations. Try to suck up the residual liquid on each wash plate. After the last washing board, drain all the liquid in the board or upside the board, and dry all the residual liquid on the absorbent paper;

6) 100μL of the detection antibody was added to each microwell. The reaction wells were sealed with sealing plate adhesive paper and incubated at room temperature for 2 hours;

7) Repeat the step 5 plate washing operation;

8) SA-HRP100 μL was added to each microwell and incubated at room temperature for 20 minutes from light;

9) Repeat the step 5 plate washing operation;

10) 100μL of color development solution was added to each microwell, and incubated at room temperature for 5-30 minutes;

11) 50μL of termination solution is added to each microwell, and the color of the solution will change from blue to yellow. If the color change of the solution is not consistent, to tap the microplate to make the solution evenly mixed;

12) Within half an hour after the addition of the termination solution, the absorbance value of 450nm should be measured, and 540nm or 570nm should be set as the calibration wavelength;

13) Calculation result: Take the average value of the corrected absorbance value (OD 450-OD 540 / OD 570) for each sample and standard, and subtract the average zero standard OD value. You can create the standard curves by using the computer

software. Another way is to generate a curve by drawing the logarithm of the standard concentration and the corresponding logarithm of the OD value, and finally determine the best line of fit by regression analysis. If the sample is diluted, the concentration should be multiplied by the corresponding dilution.

Statistical method

The method of GraphPad Prism 9.0 can be used for data statistics. Before analysis, the homogeneity of variance test and normality test should be used for all data groups. Data need to meet the homogeneity of variance and normal distribution, including one-way ANOVA for inter-group comparisons, and Tukey's test for further pairwise comparisons. Body mass growth rate to be repeatedly measured was analyzed by two-way ANOVA, qualitative data expressed as percentage (%), quantitative data expressed as mean ± standard error, and the significance level was set at $P < 0.05$. When statistics, remove abnormal data with twice the standard deviation, and mice with abnormal behavior will also be removed.

3.3 Data handling

The analysis method to be used is the GraphPad Prism 8.02 software.

Results of the preparation data of the depression model mice

The model animals were molded in CSDS for ten days, and the mice were tested by sugar spraying experiments before and after molding. The mice with depression model that significantly reduced the number of hair management after mold making compared with before mold making.

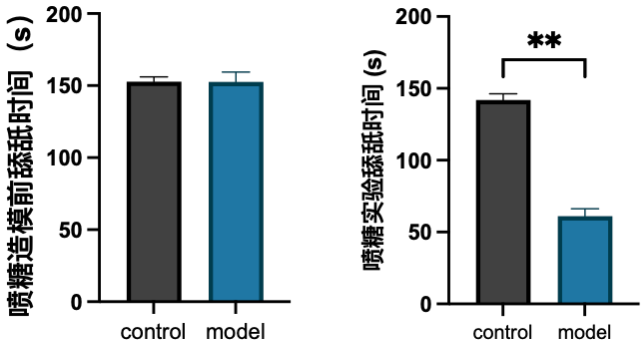

Figure 3.2 – Sugar spraying experiments before and after

Before the start of experimental modeling, licking time statistics did not differ in the two groups, indicating that the two groups were not compare. The mice were tested before and after the molding, and it was found that the number of mice in the model group was significantly less than that of the blank control group, indicating that the model mice of depression were successfully molded and basically in line with the symptoms of clinical depression.

Behavioral evaluation of mice with depression

Gavage was treated with Sini powder, and the antidepressant effect of Sini powder dispersion intervention on depression model mice was evaluated by behavioral detection with sugar spraying experiment, female urine sniffing experiment and hanging tail test.

Sugar spraying experiment:

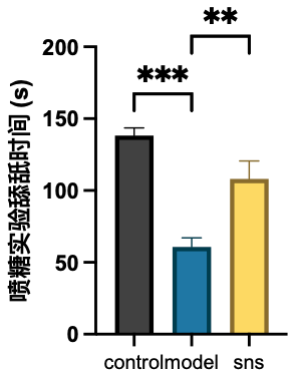

Figure 3.3 – Sugar spraying experiments

This group of data shows that the three groups of experimental data are significantly different. The first group is the blank control group, the second group is the depression model group, and the third group is the Sini powder intervention group. The results found that the licking time of the depression model mice was significantly lower than that of the blank control group, indicating that there were depressive symptoms, while the data of the mouse model of Sini powder intervention group were significantly higher than that of the depression model mice, and close to

the data of the blank control mouse model, indicating that the depressive symptoms were in remission and close to normal.

Female urine sniff experiment:

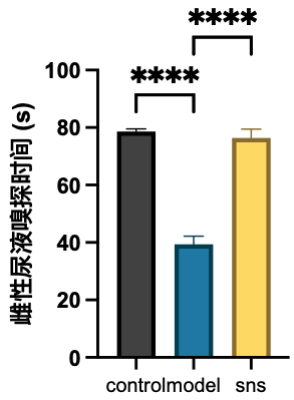

Figure3.4 – Female urine sniff experiment

This group of data shows that the three groups of experimental data are significantly different. The first group is the blank control group, the second group is the depression model group, and the third group is the Sini powder intervention group. Results found: female urine sniff experiment data, depression model of female urine sniff time significantly lower than the blank control group, the model group mice appear depression symptoms, and Sini powder intervention model of female urine sniff time data is significantly higher than the model of depression mouse data, and close to the blank control mouse model data, shows that intervention after Sini powder depression symptoms in relief, close to normal.

Suspension tail experiment:

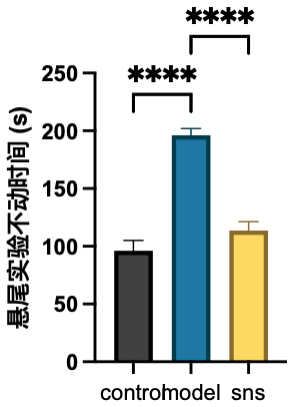

Figure 3.5 – Suspension tail experiment

This group of data shows that the three groups of experimental data are significantly different. The first group is the blank control group, the second group is the depression model group, and the third group is the Sini powder intervention group. The results found that the hyperactivity time of the suspended tail experiment of the depression model mice was significantly higher than that of the blank control group, indicating that the depression symptoms appeared in the model group, while the hyperactivity time of the suspended tail experiment in the Sini powder intervention group was significantly lower than that of the depression model group, indicating that the depression symptoms were relieved after the intervention and close to normal.

Elisa kit:

The detection of inflammatory cytokine IL-1 β levels was performed with the Elisa kit, through the detection of peripheral blood inflammatory cytokine IL-1 β to evaluate the intervention effect of the Sini powder intervention on pathological indicators in depression model mice.

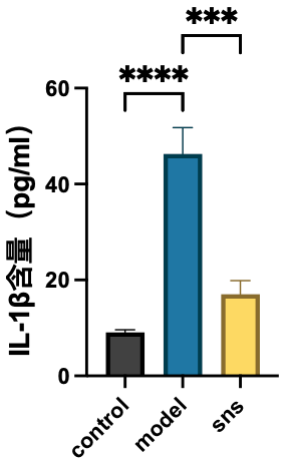

Figure 3.6 – IL-1 β levels

Results of Elisa kit monitoring peripheral blood inflammatory factor IL-1 β: the content of IL-1 β was basically normal, while the content of the depression model group was significantly higher than that of the blank control group, indicating that the peripheral blood inflammatory factor IL-1 β was increased in the peripheral blood of

depressed mice, which was an abnormal indicator. However, after the intervention, the content of inflammatory factor IL-1 β in the peripheral blood was reduced, which was lower than that of mice in the depression model group and close to normal, indicating that it can reduce depressive symptoms from the perspective of microscopic indicators.

3.4 Animal model preparation and discussion of behavioral indicators

First, the animal model preparation of this experiment was divided into blank control group, depression model group, and three groups of TCM intervention group. At the same time, the depression model mice were treated with gavage, and behavioral detection was verified by sugar spraying, female urine sniffing and tail hanging. The purpose of this step is two: one is to learn the preparation of disease animal model; the other is to verify the success of animal model preparation through the results of the behavioral indicators of the animal model, and whether the improvement of depression.

The results show: 1) the preparation of the depression model mice successful analysis: licking model before and after the data found that the model group of mice hair significantly less than blank control mice, data have differences, model of depression model success, see from the mouse behavior, the characteristics of depression mice less, basic accord with clinical depression don't want to move symptoms, anyway from the symptoms of success.

Behavioral evaluation and analysis of the depression mice of the Sini powder intervention: Analysis of sugar spraying experiment results: The licking time of the depression model mice was significantly lower than that of the blank control group, indicating that the model mice showed symptoms of depression and the behavior were not interested in sweet food. The data of the mouse models of the Sini powder intervention group were significantly higher than the data of the depression model mice, and that indicates that the depression symptoms are reduced and close to normal, indicating that the improvement of depression can be reflected by the behavior indicators of mice. Female urine sniff experimental data analysis: depression model mice female urine sniff time significantly lower than the blank control group, model

group mice appear not interested in urine sniff, response is symptoms of depression, and Sini powder intervention model of female urine sniff time data is significantly higher than the model of depression mouse data, and close to the blank control mouse model data, shows that intervention after Sini powder, improve interest and sensitivity, surrounding depression symptoms in relief, Sini powder has certain curative effect. Analysis of the mobility time of the suspended tail experiment: the hyperactivity time of the suspended tail model mice was significantly higher than that of the blank control group, indicating that the movement of the model group mice were slow or unwilling to move, and showed depressive symptoms. However, the motionless time of the suspended tail experiment in the Sini powder intervention group was significantly lower than that of the depression model group, indicating that the movements of the mice after the Sini powder intervention increased and gradually returned to normal suspended tail movements.

The behavioral detection indexes of the above sugar spraying experiment, female urine sniffing experiment and suspended tail test showed that the compound Sini powder intervention improved the depression movements of the depression model mice, indicating a certain effect.

3.5 Discussion of Elisa kit for Sini powder intervention depression mice

After the detection of behavioral indicators, Elisa kit was used to monitor peripheral blood inflammatory factor IL-1 β, which could reflect the effect of depression mice from the perspective of microscopic indicators. Results analysis: The content of peripheral blood inflammatory factor IL-1 β in the depression model group was significantly higher than that in the blank control group, indicating that the content of peripheral blood inflammatory factor IL-1 β in the peripheral blood of depressed mice was increased, which is an abnormal indicator, so as to analyze and speculate that the inflammatory factor IL-1 β is closely related to depression. The inflammatory cytokine IL-1 β has proved to be closely related to inflammation, and is an important factor in the inflammatory regulation process, and is present in various

tissues and organs. It has been shown to be associated with Alzheimer's disease (AD), atherosclerosis (AS) and other diseases. Currently our data could demonstrate the association of the inflammatory cytokine IL-1 β with depression. However, after the intervention of Sini powder, the content of inflammatory-factor IL-1 β in the peripheral blood was reduced, which was lower than that of mice in the depression model group and close to normal, indicating that Sini powder can reduce the content of inflammatory factor IL-1 β, indicating that Sini powder can reduce depressive symptoms from the perspective of microscopic indicators.

Sini powder from the Han Zhang Zhongjing "on typhoid Diseases", it is mainly used to relieve the liver depression, very consistent with the pathogenesis of liver depression. The original prescription records licorice (zhi), bitter fruit (broken, water stains, dry), bupleurum, peony 6g, which is mainly for qi medicine, which can play a role in relieving liver and relieving depression.

Conclusions to this experiment

(1) The purpose of this experiment is mainly as follows: one is to learn the animal model of disease and learn from the mice with depression; the other is to learn the detection method of Elisa kit through the detection of microindex inflammatory factor IL-1 β. At the same time, the use and comparison of compound Sini powder in the experiment, so as to lay a foundation for the next research of TCM compound.

At present, the purpose of this experiment is basically completed. First, the animal model of depression is basically prepared successfully, and the indicators are different. At the same time, the animal modeling of disease is learned. Secondly, through the improvement of behavioral detection indicators of mouse model in sugar spray experiment, female urine sniffing experiment and suspended tail experiment, the efficacy of compound Sini powder intervention on depression was also verified from the perspective of animal behavior. As a student of my major, I learned the operation and use of Elisa kit. Meanwhile, the content of inflammatory factor IL-1 β in peripheral

blood verified the improvement and effect of compound Sini powder intervention on depressive symptoms from the perspective of microscopic indicators.

(2) Meanwhile, there are still some deficiencies in this experiment. For example, the relevant part of the experiment was superficial. It only studied the relationship between peripheral blood inflammatory factor IL-1 β content and depression and the role of Sini powder, not the specific mechanism of IL-1 β, nor how the monomer played its role.

Conclusions to Chapter 3

1. Successful preparation of depression model mice; Analysis of the immovable time results of sugar spray experiment and female urine sniffing experiment and hanging tail experiment showed that Sini powder can improve depressive symptoms.

2. The test results of Elisa kit discuss that Sini powder can reduce the inflammatory factor IL-1 β content, indicating that Sini powder can reduce depressive symptoms from the perspective of microscopic indicators.

CONCLUSIONS

1. Depression, as a common mental disorder, brings a huge burden on people. Symptoms of depression in memory loss, difficult to focus and various cognitive impairment, etc., its treatment mainly has medication, including sertraline hydrochloride, doxoxetine, voetixetine and bupropion drugs, and psychological treatment methods such as cognitive behavior therapy are helpful to patients with depression.

2. Chinese medicine has a significant effect in the treatment of depression. The Sini powder is composed of licorice, bitter, bupleurum and peony, which has the effect of penetrating evil and relieving depression and regulating liver and spleen.

3. Model-making studies in depressed mice are more common than those in other animals, with many advantages that cannot be reached by other animal models. Among them, mice of the C57BL / 6 strain have the potential to become various forms of stress modeling.

4. Animal models of depression also have various shortcomings that can not be ignored, and there is still a certain gap between animal depression models and reality. This Chinese medicine has a variety of pharmacological effects in the treatment of depression and other diseases. It can play antidepressant effects by regulating immune inflammation and synaptic plasticity.

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