# Based on the ' gut-brain axis ' theory, the intervention effect and related mechanism of wall-removed Ganoderma lucidum spore powder on depressive symptoms in patients with thyroid cancer were investigated

**SETTING : Zhejiang Cancer Hospital**

**Ethical approval number：IRB-2023-307**

**Scheme revision time : 2023.3.28**

**Version number : 3.0**

# Scheme signature page

I have read this program, I understand and agree with the content of this program, agree to follow this program for clinical research. I agree to keep this scheme and related content confidential.

Research Center：Zhejiang Cancer Hospital

Date ： 2023.3.28

## Ⅰ.Test background

Depression is an important cause of global disability and mortality, and its prevalence continues to rise. Compared with the general population, the incidence of depressive symptoms in patients with thyroid cancer is high. The psychological survey of thyroid cancer patients after thyroid cancer surgery found that the number of positive symptom self-rating scale, depression, anxiety and terror factors, and pessimism factors in the thyroid cancer postoperative group were higher than those in the control group. In modern society, the acceptance of mental diseases such as depression is generally low. Patients with depression often do not choose to seek medical treatment because of their stigma and lack of understanding of mental illness. As a result, many patients miss the best treatment opportunity because they do not get timely and accurate diagnosis.

Due to the characteristics of multi-component, multi-target, dynamic and holistic in the research of traditional Chinese medicine, it is necessary to continuously develop new strategies to study the therapeutic mechanism of active components of traditional Chinese medicine. Metabonomics combined with intestinal metagenomics, metagenomics and other multi-omics platforms can jointly identify new functional metabolites and functional bacteria, laying a good foundation for the study of the mechanism of active ingredients related to traditional Chinese medicine. It has been found that the composition and function of intestinal microorganisms in patients with depression are imbalanced. Imbalanced microbiota may induce depression and anxiety through the microbial-gut-brain axis ( biosynthesis and metabolic pathways of tryptophan ). It may also be involved in the occurrence of depression through other flora metabolites ( amino acids, fatty acids, short-chain fatty acids, etc. ). Therefore, this study used the wall-removed Ganoderma lucidum spore powder to intervene in patients with thyroid papillary carcinoma accompanied by depressive symptoms, and explored the antidepressant mechanism of the wall-removed Ganoderma lucidum spore powder through microbial 16 s diversity analysis and metabolomics methods.

Ganoderma lucidum spores are rich in polysaccharide peptides, adenine

nucleosides, proteins, enzymes, trace elements and other special components. The key effective components known now are Ganoderma lucidum polysaccharides and Ganoderma lucidum triterpenes. Ganoderma lucidum is widely used as a medicinal fungus to prevent and treat many diseases. Studies have shown that Ganoderma lucidum spore powder has antidepressant effect.

This study was carried out only in this research institution. A total of 300 patients with papillary thyroid carcinoma accompanied by depressive symptoms were recruited to participate in this clinical study. This study was divided into study group and control group, ratio ( 2 : 1 ). From the first day, the experimental group was given oral administration of 4g of Ganoderma lucidum spore powder ( 1 bag at a time, 2 times a day, 2g / bag ) for 90 days, and the control group was given oral administration of 4g ' placebo ' ( 1 bag at a time, 2 times a day, 2g / bag ) for 90 days. Ganoderma powder product instructions : daily care dose is 2g / d, 4g / d can achieve the therapeutic effect. In the previous study, ' wall-removing Ganoderma lucidum spore powder ' was used in clinical practice, prevention and reduction of cancer-related fatigue symptoms in cancer patients, and improvement of the quality of life of cancer patients in the Second Zhejiang Hospital.Ganoderma lucidum medication method 2g / time, once in the morning and once in the evening, a total of 4g. The same dose was used.

## Ⅱ.test purpose

1.In this study, the antidepressant mechanism of ganoderma lucidum spore powder was studied by microbial 16 s diversity analysis and metabolomics method, and the clinical efficacy of ganoderma lucidum spore powder intervention on postoperative depressive symptoms of papillary thyroid carcinoma was determined.

2.To investigate the effect of Ganoderma lucidum spore powder on the depression of patients through the communication pathway of microbial-intestinal-brain axis ( biosynthesis and metabolic pathway of tryptophan ), or through other microbial metabolites ( amino acids, fatty acids, short-chain fatty acids, etc. ) to inhibit the occurrence of depression in patients. To clarify the antidepressant mechanism of

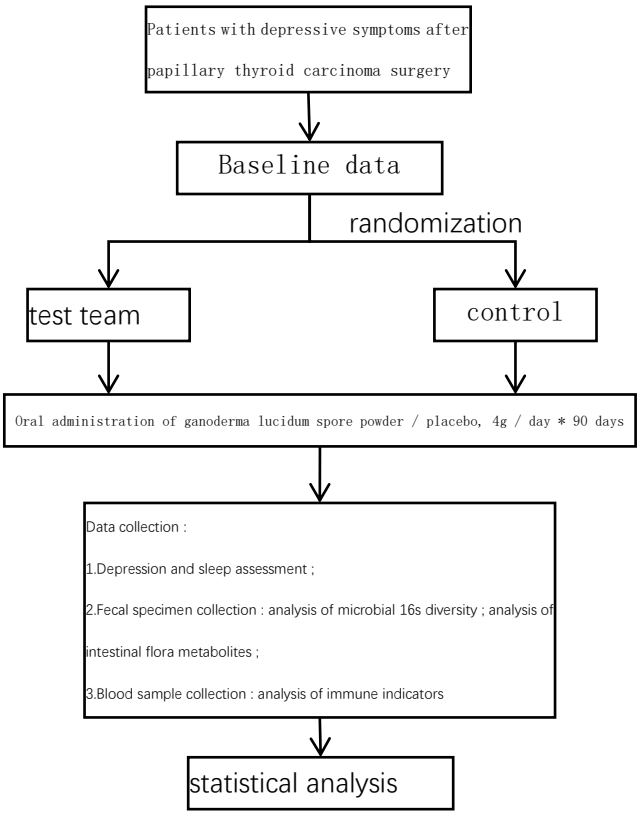
wall-removed Ganoderma lucidum spore powder and provide objective basis for its application.

3.To construct a clinical database of patients with depressive symptoms after papillary thyroid carcinoma surgery, and to explore the optimal mode of intervention of exfoliated ganoderma lucidum spore powder in patients with depressive symptoms. The multi-omics platforms such as metabolomics and intestinal flora were used to directly find the functional components related to the intervention effect of wall-removed Ganoderma lucidum spore powder ; combined with clinical data, mining potential clinical indicators or more sensitive biomarkers ; on this basis, the in vivo function and mechanism of the target flora ( potential metabolic beneficial bacteria ) or metabolites ( potential epigenetic or parabiotic ) alone or in combination will be verified.

## Ⅲ.experimental design

1.design description: A randomized, double-blind, parallel, placebo-controlled study.

2.technical route



## Ⅳ.case selection

4.1 inclusion criteria

( 1 ) Patients with depressive symptoms after papillary thyroid carcinoma surgery in the outpatient department of Zhejiang Cancer Hospital ( patients with depressive symptoms by clinical psychiatrist and Hamilton Depression Scale score of 8-20 points ) ;

( 2 ) Han nationality ;

( 3 ) no previous depression and other mental diseases ;

( 5 ) 18-80 years old ;

( 6 ) Women ;

( 8 ) BMI : 19 ~ 24. ( BMI calculation method : BMI = weight ( kg ) / height 2 ( m ) )

4.2 excluded criteria

( 1 ) suffering from other diseases of the intestinal system ;

( 2 ) Have received gastrointestinal surgery before intervention ;

( 3 ) Including patients with other malignant tumors, who need chemotherapy, radiotherapy, biological therapy or traditional Chinese medicine treatment ;

( 4 ) received antibiotics or microecological modulators within 3 months before the intervention ;

( 5 ) Acute intestinal obstruction ;

( 6 ) Patients with severe depressive symptoms who must receive antidepressant treatment ;

( 7 ) organic diseases such as heart and brain diseases, brain trauma ;

( 8 ) history of mental illness, use of psychoactive drugs such as drugs ;

( 9 ) Severe liver and kidney dysfunction ;

( 10 ) Pregnancy, lactation

4.3rejecting standard

( 1 ) After enrollment, it was found that the included objects did not meet the inclusion criteria ;

( 2 ) Subjects who did not take oral ganoderma lucidum spore powder or intervene in the course of dissatisfaction after enrollment according to the study protocol ;

( 3 ) poor compliance, midway active withdrawal or lack of main indicators, incomplete data, etc., can not determine the efficacy or safety.

4.4 The subjects withdrew from the test standard

( 1 ) It is necessary for researchers to stop the trial from the perspective of medical ethics ;

( 2 ) patients with severe adverse reactions ( SAE ), complications or special physiological changes should not continue to accept the test ;

( 3 ) Pregnancy during treatment.

## Ⅴ.test scheme

5.1 Number of cases and distribution of cases

According to the ' Drug Registration Management Method ', the study sample should be no less than 100 pairs. It is assumed that the case shedding rate is 15 %, and the sample size is not less than 117 pairs. Finally, the sample size was determined to be 300 cases. In order to reduce the use of placebo in the control group, so that more patients benefit from treatment, the experimental group : the control group was assigned to 2 : 1, that is, 200 cases in the experimental group and 100 cases in the control group.

5.2 Research Drug Information

（1）testing medicine

The main component is the wall-removed Ganoderma lucidum spore powder, specification : 2g / bag, batch number : the same batch number ;

（2）control drug

Placebo : the main component is the wall shell and accessories of the wall-removed Ganoderma lucidum spore powder, specification : 2g / bag, batch number : same batch number

（3）Drug source

The above drugs were produced and provided by Jinhua Shouxiangu Pharmaceutical Co., Ltd., and all research drug tests were qualified. If the drug expiration test is not completed, the drug needs to be re-prepared and blinded.

5.3 medication regimen

（1）From the first day after enrollment, the subjects were given oral research drugs ( wall-removing spore powder / placebo ), once in the morning and once in the evening, 1 packet ( 2g ) each time, for 90 days. Taking method : 50ml warm boiled water into suspension.

（2）dosage adjustment：If this scheme has not been revised in writing, the dose of the test drug shall not be adjusted.

5.4 drug packaging

One patient in the trial was treated with 6 medium packs of medicine, each containing 30 small bags. The drugs in the pouch are ganoderma lucidum spore powder ( 2g ) or placebo ( 2g ).

5.5 Drug storage and recovery

The storage conditions of the drugs studied were : closed, placed in a clean, ventilated, shady place, away from children 's contact.

All test drugs must be stored safely and sealed, kept and distributed by a special person, and supervised the subjects to take them on time as required. The researcher should register and sign the medication record form after each administration to the patient, and be responsible for recycling the used test drug packaging bags. Researchers and sponsor supervisors must count all drugs at the end of the trial and check with the number of drugs issued, the number of missing drugs and the cause of CRF. At the end of the trial, all unused drugs should be returned to the sponsor, and the relevant handover procedures and records should be completed according to GCP requirements.

## Ⅵ.drug combination

It is forbidden to use any interfering drugs, such as biological therapy, traditional Chinese medicine treatment, antibiotic treatment, microecological modulators, antidepressants, other health care products with immune regulation function,

psychotropic drugs and so on.

**Ⅶ.research process**

7.1 Screening / Baseline ( Week 0 )

( 1 ) Sign informed consent ;

( 2 ) Complete the verification of inclusion and exclusion criteria ;

( 3 ) to obtain demographic data and medical history data ;

( 4 ) Psychiatric examination and HAMD-24 scale evaluation ;

( 5 ) PSQI scale evaluation ;

( 6 ) Stool specimen collection ;

( 7 ) Thyroid function test residual blood collection ;

( 8 ) Women of childbearing age to do urine pregnancy test ;

( 9 ) Record the combined medication ;

( 10 ) Drug distribution.

7.2 Visit 1（D28±3d）

( 1 ) Evaluation of HAMD-24 scale ;

( 2 ) PSQI scale assessment ;

( 3 ) Record drug combination and adverse events ;

( 4 ) Drug recovery and distribution.

7.3 Visit 2（D90±3d）

( 1 ) Evaluation of HAMD-24 scale ;

( 2 ) PSQI scale assessment ;

( 3 ) Fecal specimen collection ;

( 4 ) Thyroid function test residual blood collection ;

( 5 ) Record the combined medication and adverse events ;

( 6 ) Drug recovery.

7.4 Principles of index collection

( 1 ) Hamilton Depression Scale ( HAMD-24 ) score and Pittsburgh Sleep Quality Index score :

The HAMD-24 was evaluated by two senior clinical psychologists, who were responsible for the assessment of HAMD-24 by Dr. Qiu Yaju. PSQI was evaluated by

the subjects themselves and guided by psychologists.

（2）Fecal sample collection method :

①The stool collector was placed in the toilet, the feces were discharged into the collector, and the fresh fecal samples were fully mixed with a slender handle spoon ( Note : to avoid urine contaminated feces ).

②The mixed fecal samples were divided into two 5 ~ 10 mL sterile tubes marked with good names or pre-sterilized collection tubes, and about 2 ~ 3 mL of each tube was collected.

③About 1 ml fecal samples were sub-packed into 1.5 ml centrifuge tubes from 5-10 ml sample collection tubes with long-stem wooden rods. Or take about 1 ml to 1.5 ml centrifuge tube directly from the mixed fecal samples in 1.

④The centrifuge tube was covered well, and the fit was checked. According to the numbering order, it was placed in the sample box and placed in a refrigerator at －80 ° C for cryopreservation.

If the feces are collected at home, the collection tube is packed with a self-sealing bag and sent to the hospital sample receiving place as soon as possible ( within two hours ).

Notes for sample collection and preservation :

Samples should not be exposed to room temperature for more than 2 hours.

Sample collection should be carried out at the same time of the day to avoid the influence of rhythm on metabolism.

The sample should be mixed immediately after collection, and frozen after separation.

Prepare two samples at the same time to avoid repeated freezing and thawing.

（3）Blood sample collection：

①The remaining venous blood after thyroid function test in the laboratory was injected into the serum / plasma separation tube, and mixed upside down for 5-8 times.

②The whole blood was placed at room temperature ( 20 ° C ~ 25 ° C ), standing for about 30min ( no more than 2h ), and the blood was completely solidified,

and the anticoagulant was added as plasma.

③After the serum / plasma was fully separated, centrifuged at 1000 g for 10 min. About 500 μl-1mL of the supernatant was taken to a 1.5mL centrifuge tube ( recommended multi-tube subpackage ) and placed in a cryopreservation box.

④. The cryopreservation box was placed in a refrigerator at － 80 ° C and sent by dry ice.

（4）Stool and blood samples preservation and transportation：①Liquid nitrogen rapid cryopreservation for 15 min and then transferred to -80 ° C refrigerator cryopreservation.②Ensure that the refrigerator continues to work constantly power, power will cause the sample freeze-thaw③Shunfeng and other dry ice transport ( in transit 5kg / day ) or cold chain transport.

**VIII.response evaluation criteria**

8.1 leading indicator

( 1 ) Hamilton Depression Scale ( HAMD-24 ) score classification

No obvious depressive symptoms : total score 0-7 points ; may have depression : total score 8-20 points ; mild or moderate depression : total score 21-35 ; severe depression : total score 36-76 points.

( 2 ) Validity standard

Depression relief : After 90 days of treatment, HAMD score decreased.

8.2 secondary index

( 1 ) Changes of intestinal microbial diversity and metabolites in patients

( 2 ) Remission of sleep status : After 90 days of treatment, the Pittsburgh Sleep Quality Index score decreased.

( 3 ) Immune enhancement

**IX.Evaluation of adverse events**

1.Definition of adverse events : Any disease that occurs during a drug clinical trial, new symptoms, signs or abnormal laboratory tests, or deterioration of the original symptoms and signs, whether or not related to the test drug, is an adverse event.

2.Relationship between adverse reactions and experimental drugs : Searching through the CNKI database, no side effects and related adverse reactions of Ganoderma lucidum were found in the current study. If complained of discomfort, through laboratory tests such as blood routine, liver and kidney function, platelets, hemoglobin, determine or determine whether it is related to the test drug.

3.Adverse event recording and reporting channels :

In the event of adverse events, the occurrence time, clinical manifestations, treatment process and duration, outcome and the relationship between the test drugs should be recorded in detail on the case report form page. Serious adverse events should fill in the serious adverse events form and report to SFDA Registry Drug Research and Supervision Office within 24 hours, notify the team leader unit and the bidder, and report to the center ethics committee, and submit details within 7 days thereafter.

Safety evaluation at the end of the trial should include all patients who have used the test drug, regardless of whether they meet the evaluable criteria.

## X.Statistical analysis of test data

1. Data Selection for Statistical Analysis

( 1 ) Full Analysis Set ( FAS ) : Constituted of all cases with depressive symptoms after papillary thyroid carcinoma surgery that were randomized into groups and at least 1 month after surgery, that is, Intention-to-treat. The FAS set was used for efficacy analysis, and the missing data was filled with the last observed data filling ( LOCF ) criterion.

( 2 ) Per-Protocol Set ( PPS ) : PPS is composed of all cases that meet the test plan, have good compliance, do not use prohibited drugs during the test, and complete the content of CRF requirements. The PPS set is used for efficacy analysis.

( 3 ) Safety Analysis Set ( SAS ) : It is composed of all cases that have been randomly grouped, used at least one drug and have safety evaluation. The SAS set is used for safety analysis.

2. statistical analysis plan

Statistical analysis was performed using SPSS 9.1.3 statistical software.

( 1 ) Distribution of two groups of cases : the comparison of the shedding and elimination rates of the two groups was performed by $\chi^2$ test or exact probability method.

( 2 ) Comparability analysis : Demographic data and other basic indicators were compared to measure the comparability of the two groups. According to the type and distribution of data, $\chi^2$ test or exact probability method, CMH test, t test, variance analysis or non-parametric statistical method were used.

( 3 ) Compliance analysis : To compare whether the two groups of patients use the test drugs on time and in quantity. The drugs that were not banned in the regimen were mainly tested by $\chi^2$ test or exact probability method.

( 4 ) Validity analysis : Repeated measures were used to compare the differences in measurement data such as depression scores and sleep scores between the experimental group and the control group, or linear regression models were used to control baseline depression scores or sleep scores. Differences in depression scores and sleep scores between the two groups. Compare the score after the end of the experiment with the baseline score, calculate the difference before and after the change, and use the t test to compare the difference between the score changes of the experimental group and the control group.

( 5 ) Safety analysis : adverse reactions were statistically described, and the incidence of adverse reactions in the two groups was compared by $\chi^2$ test or exact probability method.

( 6 ) All statistical tests should give test statistics and corresponding p values. When using the exact probability method, the p value is given directly. $P \leqslant 0.05$ indicated that the difference was statistically significant.

## XI.Setting up Blindness, Uncovering Blindness and Emergency Uncovering Blindness

1.Blinding

( 1 ) Subjects were randomly numbered and grouped : The cases were numbered

according to the time sequence of enrollment, and divided into three groups : A, B, and C. The patients were divided into three groups according to the time sequence of enrollment.

( 2 ) Randomly determined grouping : Randomly determined by drawing lots, group A, group B and group C correspond to ' experimental group ' or ' control group ', respectively.

2.Provisions on Blinding

Uncover the blind bottom envelope : The three groups of A, B, and C are provided in the letter as test drugs or reference drugs. The groups corresponding to each number are assigned to statistical analysts for statistical analysis and statistical conclusions are made. According to the statistical conclusions, combined with professional knowledge, make professional conclusions.

3. Emergency Blinding

When the patient has serious adverse reactions or emergency events need to determine the group for treatment, when the patient has serious complications or symptoms of deterioration must take emergency measures, emergency cases do need to break the blind, by the researchers in the center of the person in charge, the person in charge after consultation with the bidding unit can read the emergency letter to break the blind. Once the corresponding numbered emergency letter is opened, the case is treated as a shedding case.

**XII.Test schedule arrangement**

2023.3-2024.5 Time for clinical trials

2024.9 Complete the summary repor