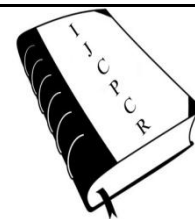




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BLOOD ROUTINE ASSOCIATED WITH QIWEIBAIZHUSAN COUPLED WITH YEAST ON DYSBACTERIOSIS DIARRHEAL MICE

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ABSTRACT

To explore the effect of Qiweibaizhusan coupled with yeast on blood routine in dysbacteriosis diarrheal mice. The mice model of dysbacteriosis diarrhea were established with antibiotics, and then they were treated with 1/2 dose of ultra-micro Qiweibaizhusan and 1/4 dose of ultra-micro Qiweibaizhusan combined with 1/4 dose of yeast. The blood gained from eyeball was analysed. The results showed that the amount of white blood cells of model group was lower than the normal group. The amount of white blood cells of 1/2 dose of ultra-micro Qiweibaizhusan group was twice more than the normal group. The white blood cells of the 1/4 dose of ultra-micro Qiweibaizhusan combined with 1/4 dose of yeast group was the same as that in the normal group. The average volume of red blood cells of the model group was lower than that in the normal group. The average red blood cells volume of the 1/4 dose of ultra-micro Qiweibaizhusan combined with 1/4 dose of yeast group was close to that in the normal group. The model group platelets number was minimum, normal group platelets number was maximum, and the platelets number of 1/4 dose of ultra-micro Qiweibaizhusan combined with 1/4 dose of yeast group was close to the normal group. The 1/4 dose of ultra-micro Qiweibaizhusan combined with 1/4 dose of yeast combination has more significant effect on the blood routine of dysbacteriosis diarrheal mice than 1/2 dose of ultra-micro Qiweibaizhusan.

Key words: Qiweibaizhusan, Dysbacteriosis, Ultra-micro powder, Chinese medicine, Blood routine.

INTRODUCTION

Qiweibaizhusan consists of ginseng, tuckahoe, fried atractylodes, radix glycyrrhizae, herba agastachis leaves, radix aucklandiae and radix puerariae, treating the asthenia of spleen and stomach, deficiency of body fluid and inner-heat[1]. Qiweibaizhusan integrates mending, transporting, rising and falling together, supplying without delay, specific to the characteristics of the diarrheal spleen deficiency of infant and children who are consume Yin fluid easily, having effect on both symptoms and cause. Professor Chen Baoyi use Qiweibaizhusan for the treatment of infantile diarrhea[2]. Qiweibaizhusan does not only play an important role in sterilization as it kills intestinal bacteria ,but can also factionalize the beneficial bacteria in biological antagonism, thus treating the autumn

diarrhea caused by rotavirus infection[3,4].

With the wider range of applications of probiotics, studies have shown that, the application of intestinal micro-ecological instead of traditional preoperative oral bowel antimicrobials during the radical resection for colorectal cancer surgery period, can not only effectively cure the dysbacteriosis, but also improves the immune function[5]. The use of intestinal micrological instead during the period of colorectal cancer surgery can cure the dysbacteriosis and reduce the inflammatory reaction and the postoperative Infectious complication of patients[6]. Application of probiotics can prevent and treat diarrhea flora [7,8], and this treatment is simple, safe and effective [9-12]. At present, a single bacteria preparations and

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compound bacteria preparations composed of lactic acid bacteria class [13], *Bacillus* class, *Aspergillus* class and yeast class are commonly used domestically and overseas. Yeast and yeast culture have been used for many years, which can improve health status [14,15].

There is a strong correlation between traditional Chinese medicine and emerging micro-ecology, with the further study of the micro-ecology of traditional Chinese medicine, the increasingly clear relationship between the two has shown. Researches have investigated that [16,17] probiotics and herbal medicine are complementary in the aspect of preventing growth-promoting. Chinese herbal medicine can contribute to the breeding of probiotics, probiotics can promote the absorption and utilization of Chinese herbal medicine. Probiotics and preparation of Chinese herbal medicine are natural, non-toxic, no side effect, no residual, safe to use and other advantages. Probiotics and traditional Chinese medicine combination is helpful to the treatment and prevention of diseases [18].

Blood index refers to the number of blood plasma and blood cells, the indicators of blood, shows us how well we human health varies from time to time. Some studies indicate that, traditional Chinese medicine can improve the number of white blood cells, red blood cells, hemoglobins, platelets and reticulocytes, which provides certain scientific basis for the application in clinic [19,20]. Data shows that traditional Chinese medicine compound can stimulate the generation of red blood cells and strengthen the body's metabolism and various life activities to improve the body immunity [21,22]. It's important to observe the effects of Qiweibaizhusan and yeast joint for the treatment of dysbacteriosis diarrhea in mice.

MATERIALS

Animal

48 SPF level Kunming mice, evenly composed of male and female, weight 20 ± 2 g, provided by Hunan silaikesi experiment Limited.

Fodder

Provided by the Hunan university of Chinese medicine experimental animal center of development .

Shielding environment and experimental facilities

Hunan university of Chinese medicine experimental animal center.

Medicine

Qiweibaizhusan: ginseng(Jilin province)6g, elecampane(yunnan province)6g, white poria (yunnan province)10g, roasted rhizoma atractylodis macrocephalae (anhui province)10 g, agastache rugosa kuntze (guangdong province)10g, Radix Puerariae (guangxi province)10 g, liquorice (neimenggu province)3 g. The same batch purchased from First Affiliated Hospital of Hunan University of Chinese Medicine.

Ultra-micro decoction: The single traditional Chinese medicine was grinded ultra-finely. Appropriate amount of boiling water was added in the proportion stated above, stir, after being cooled, the supernatant was extracted from low-speed centrifugation, superfine medicinal broth 1/2, 1/4 volume was obtained and preserved at 4 °C refrigerator.

Yeast: Took the intestinal yeast *Debaryomyces hansenii* which has been isolated and identified from the experimental animal intestine in the research group, Qiweibaizhusan has an obvious role in promoting the growth of the yeast [23], activated it and vaccinated to potato sucrose liquid medium, about 28 °C. The shaker was used to shake after 36h. After centrifugation the supernatant was discarded, precipitated and repeated washing 1-2 times. A certain amount of saline solution was added, tapped the centrifugal tube to scatter yeas. After hemocytometer counting, the number of bacteria diluted to 10^{10} /mL, which is the total amount of yeast [24], the total amount of yeast was diluted to 25% volume(0.25×10^{10} /mL), preserved at 4 °C refrigerator.

Reagent

Gentamycin sulfate (Yichang renfu liquor limited liability company, batch number: 5120106), cefradine (Shanxi xianyuan Pharmaceutical Co., Ltd., batch number:110804). The cephradine capsules and gentamicin sulfate injection paired with sterile saline concentration of 62.5 g / L of antibiotic mixture [25], (6 gentamicin (2ml) +3 cephalosporins (0.25g) formulated into a mixture), preserved at 4 °C refrigerator.

Methods

Animal groups

Randomly divided mice into four groups after adaptive breeding for 4 days, the normal group, model group, 1/2 ultra-micro Qiweibaizhusan group, 1/4 ultra-micro Qiweibaizhusan combined with 1/4 yeast group, each group of 12, with even number of male and female, were housed separately.

Modeling approach

Normal group was given sterile saline 0.35 mL/(only once) to fill the stomach, the rest of the group using antibiotics, the mixture of 0.35 mL/(only once) to fill the stomach, twice a day for 5days, once the stool becomes softer squishy, the model was established successfully.

Administration and dosage

After the success of modeling, administered orally, twice a day for 4 days. The normal group and model group was given sterile saline, the others according to the equivalent dosage for clinical medicine, namely: 50% amount of ultra-micro Qiweibaizhusan group:0.08 g/(kg·d) ; 25% amount of ultra-micro Qiweibaizhusan and

25% of yeast: 0.04 g ultra-micro Qiweibaizhusan + 0.25 x 10¹⁰ yeast/ (kg · d).

Blood sampling methods

Before experiment, experiment subjects were fasted for 12 hours, water deprivation for 1 hour, 1-1.5mL blood gained from eyeball, injected into EDTA-K2 anticoagulant tubes, after mixed evenly, submitted the sample in 2 hours , and detection need to be completed in 4 hours.

Instruments and measurement methods

CA-500 automatic blood analyzer, shandong lanqiao medical technology limited production, impedance method.

Statistical analysis

Data was processed with DPS v7.05 software, each resulting measurement data packet using the mean ± standard deviation (x ± s) between the two groups were compared using t test, P <0.05 was statistically significant.

RESULTS

Effects of Qiweibaizhusan coupled with yeast on white blood cells

Table 1 shows, the number of white blood cells in the model group were lower than the normal group, the white blood cell count compared with the normal group had statistical significance (P<0.01); 1/2 dose of ultra-micro Qiweibaizhusan group data were higher than those in the normal group 2 times, and compared with the other four groups had statistical significance (P<0.05 or P<0.01), with its antibacterial effect related to the number of white blood cells by enhancing the role played sterilization.1/4 dose of Qiweibaizhusan group combined with 1/4 dose of yeast combination group compared with normal group was not statistically significant, and the data is mostly flat with the normal group, may be due to the ultra-micro Qiweibaizhu scattered dose reduction, yeast beneficial bifidobacteria and lactobacillus added promote the role, which functioning as bacteriostatic, bactericidal was not needed to increase the

number of white blood cells to sterilize [26].

Effects of Qiweibaizhusan coupled with yeast on red blood cells

Hemoglobin: compare with the model group and the normal group: P <0.05, which is statistically significant, the value of model group is higher than the control group from the data, it might be caused by diarrhea loss of body fluid, blood and body fluids have the function of supporting and generating the blood, this is the body's compensatory increase in hemoglobin, thus generate blood. 1/2 dose of micro-ultra Qiweibaizhusan, 1/4 dose of Qiweibaizhusan and 1/4 dose of yeast combination group had increased in value, but the set of values of 1/2 dose of ultra-micro Qiweibaizhu group higher than the normal group, and 1/4 dose of Qiweibaizhusan and 1/4 dose of yeast combination group. Mean corpuscular volume: the other four groups and the model group were statistically significant, but the analysis from the data, 1/2 dose of ultra-micro Qiwei Baizhu Scattered group is less than 1/4 dose of Qiweibaizhusan and 1/4 dose of yeast combination group.

Effects of Qiweibaizhusan coupled with yeast on platelets

From the data of platelet count, the comparison between the model group and the normal group: P <0.01, the difference was highly statistically significant, therefore, Dysbacteriosis diarrhea cause the decrease in the number of platelets in mice;1/4 dose of Qiweibaizhusan and 1/4 dose of yeast combination group compared with the model group:P <0.05,the difference was statistically significant, platelet count increased as yeast was added, but did not reach normal levels, it might because of the long time required for the number of platelets to increase, and the time of collecting specimen was too short; 1/2 dose of QiweiBaizhu scattered groups in the data increased less than 1/4 dose of Qiweibaizhusan and 1/4 dose of yeast combination group, it most likely because the yeast has a high nutritional value, which can promote the body to produce platelets.

Table 1. Effects of Qiweibaizhusan coupled with yeast on white blood cells

Group	White blood cell count (×10 ⁹ /L)	The lymphocyte count (×10 ⁹ /L)	Mononuclear cell count (×10 ⁹ /L)	Neutrophil count (×10 ⁹ /L)
Normal group	7.800±1.387	6.833±1.134	0.317±0.133	0.467±0.350
Model group	7.217±0.431a	6.783±0.387	0.183±0.075	0.417±0.147
50% Ultra-micro Qiweibaizhusan group	16.983±4.988ab	11.433±2.690ab	2.267±1.397AB	2.117±1.283
25% Ultra-micro Qiweibaizhusan and 25% yeast group	6.150±1.242c	5.600±1.346c	0.117±0.075aC	0.317±0.264C

Note: compared with normal group:A:P<0.05,a:P<0.01.Compared with model group:B:P< 0.05,b:P<0.01. Compared with 1/2 dose of ultra-micro Qiweibaizhusan group:C:P<0.05,c:P< 0.01.Compared with 1/4 dose of ultra-micro Qiweibaizhusan and 1/4 dose of yeast group:D:P < 0.05, d:P < 0.01.

Table 2. Effects of Qiweibaizhusan coupled with yeast on red blood cells

Group	Red blood cell count ($\times 10^{12}/L$)	Hemoglobin (g/L)	Mean red blood cell volume(fL)
Normal group	17.688 \pm 1.697	326.167 \pm 29.431	52.317 \pm 1.504
Model group	18.372 \pm 1.211	349.333 \pm 35.115A	49.167 \pm 1.563a
50% Ultra-micro Qiweibaizhusan group	17.333 \pm 1.610	362.833 \pm 40.770	49.917 \pm 2.581B
25% Ultra-micro Qiweibaizhusan and 25% yeast group	18.008 \pm 2.422	350.500 \pm 25.336	53.917 \pm 2.041b

Note: Compared with normal group:A:P < 0.05, a:P<0.01.Compared with model group:B:P < 0.05,b:P<0.01.Compared with 1/2 dose of ultra-micro Qiweibaizhusan group:C:P<0.05,c:P< 0.01.Compared with 1/4 dose of ultra-micro Qiweibaizhusan and 1/4 dose of yeast group:D:P <0.05, d:P < 0.01.

Table 3. Effects of Qiweibaizhusan coupled with yeast on platelets

Group	The platelet count($\times 10^9/L$)	Thrombocytocrit (mL/L)	Mean platelet volume (fL)	Platelet volume distribution width (%)
Normal group	1167.833 \pm 234.628	6.415 \pm 2.818	8.233 \pm 0.446	13.326 \pm 6.469
Model group	697.500 \pm 53.459a	5.505 \pm 0.520	7.983 \pm 0.523	16.017 \pm 0.402
50% Ultra-micro Qiweibaizhusan group	808.333 \pm 348.595	6.590 \pm 2.801	8.317 \pm 0.458	16.017 \pm 0.705
25% Ultra-micro Qiweibaizhusan and 25% yeast group	906.000 \pm 200.849B	6.732 \pm 1.343	7.933 \pm 0.175	17.250 \pm 1.133

Note: Compared with normal group:A:P < 0.05,a:P<0.01.Compared with model group:B:P < 0.05,b:P<0.01.Compared with 1/2 dose of ultra-micro Qiweibaizhusan group:C:P<0.05,c:P< 0.01.Compared with 1/4 dose of ultra-micro Qiweibaizhusan and 1/4 dose of yeast group: D:P < 0.05, d:P < 0.01.

DISCUSSION

White blood cells are the defense system of the body that against microbial infection and foreign substances attack. In order to effectively defend the body, there must be a sufficient number of white blood cells to produce an appropriate stress response, reach the site where they are needed, kill and digest harmful micro-organisms and other substances. Like other blood cells, white blood cells are produced by the bone marrow, which are gradually differentiated from the precursor cells (stem cells) after a certain period of time, mature into five main types: neutrophils, lymphocytes, monocytes, eosinophils and basophils. Nowadays, most of the anti-inflammatory effect of the drug is according to the research of leukocytes, thus drawing the conclusion [27,28].

Hemoglobin contains red blood cells, when the level of red blood cells and hemoglobin decrease to a certain extent, is thought to be anemia. Some studies have indicated [29], in addition to the use of this indicator to determine hemoglobin anemia, further references to the mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and red blood cell volume distribution width are needed. The mean corpuscular reduces volume thalassemia minor, as well as iron deficiency anemia.

Platelets (blood platelet) is one of the formed elements in the mammals blood. Platelets have specific morphological structure and biochemical composition, in normal blood has a constant number (such as the number of platelets for 10 to 300000 per cubic millimeter), in the bleeding, wound healing, inflammation, thrombosis, and organ transplant rejection play an important role in the physiological and pathological process. The blood platelet is only found in mammals. Researches have shown that heavy use of antibiotics can cause the significant decrease in the number of platelets [30], so the reduction of the model group’s numbers of platelets has two reasons: firstly, caused by a antibiotics; Secondly, fluid loss, resulting in the decrease in the number of platelets.

Qiweibaizhusan by the sijunzitan with rugosa, woody and pueraria components. Sijunzitan can restore the rat model of intestinal bacteria spleen disorder to normal levels [31] and raise the number of white blood cells, enhancing specific immunity and nonspecific immunity level [32], there is also specific improvement in the red blood cells and hemoglobin for the patient with spleen deficiency [33]. Agastache has antibacterial effects [34, 35], arrowroot with immune function [36,37], woody way effects on intestinal [38].

Traditional Chinese medicine is based on Confucian Taoist thought of "neutral"(ZHONG HE) balanced thought as the main thinking method. Namely, "zhong" is unbiased, without too much, and balance; "he", is to coordinate everything with internal relations. Traditional Chinese medicine is also based on five elements, the climate change of nature, the modern activity of people, all cannot be too much, nor too less. Only keep this status of not "too much" and not "too less", then all can be attributed to calm, can make the person's life activity, natural phenomena, and all things of the world in orderly under the status of "governance", the existence and development variation can appear, otherwise "inverse" of disease will emergence or abnormal status of presence, production and development will appear [39].

Therefore, the number of white blood cells of 1/2 dose of ultra-micro Qiwei Baizhu Scattered groups increase by a lot, and the 1/4 dose of ultra-micro Qiweibaizhusan and 1/4 dose of yeast combined with dose reduction and

processing group in the case of yeast, because of the yeast bacteriostatic and antagonism, the number of white blood cell is consistent with the normal group. In the value of hemoglobin, mean corpuscular volume, platelet number, 1/4 dose of ultra-micro Qiweibaizhusan and 1/4 dose of yeast combination is closer to normal group. Can also be obtained, when compare the 1/2 dose of ultra-micro Qiweibaizhusan scattered group with the normal group, the phenomenon of "too much" and "too less" has appeared, which is contradictory to traditional Chinese medicine, "Zhong He" thinking. Therefore: 1/4 dose of ultra-micro Qiweibaizhusan and 1/4 dose of yeast flora associated diarrhea in mice with blood has more significant effect than 1/2 Ultrafine Powder Qiweibaizhusan.

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