The Influence of Sucrose on Ultra-Micro Powder

Qiweibaizhusan to Reconcile Intestinal Microecological Balance

Xinxin Peng¹, Qiling Zhang², Zhifeng Peng³, Lan Shu¹, Huan Wang³, Rong Cao³, Kangxiao Guo³, Zhoujin Tan³*

¹The First Affiliated Hospital of Hunan University of Chinese Medicine, Changsha, Hunan Province, 410007, PRC
²Hunan Institute of Nuclear Agricultural Sciences and Space-Induced Breeding, Changsha, Hunan Province, 410125, PRC
³Hunan University of Chinese Medicine, Changsha, Hunan Province, 410208, PRC

*Correspondence Author: Zhoujin Tan, Hunan University of Chinese Medicine, Changsha, Hunan Province, 410208, PRC.

Xinxin Peng, Huan Wang, Rong Cao And Kangxiao Guo Are All Postgraduate Students.

Both Xinxin Peng and Qiling Zhang are the first authors.

ABSTRACT:

OBJECTIVE: To study the influence of sucrose on ultra-micro powder Qiweibaizhusan to regulate intestinal microorganism and enzyme activity.

METHODS: Mice diarrheal model with dysbacteriosis diarrhea were built by oral administration with gentamycin sulfate and cefradine mixed antibiotics. Then the mice were randomly divided into 4 groups: normal group, model group, group treated with 50% ultra-micro powder Qiweibaizhusan and group treated with 50% ultra-micro powder Qiweibaizhusan with 8% sucrose. The curative effect was judged by weight, feces, intestinal microorganisms amount and enzyme activity.

RESULTS: The antibiotics have no effect on the weight of mice. The level of bacteria number, xylanase activity, protease activity, amylase activity of model group were all significantly below the average level of normal group (P<0.01 or P<0.05). The cellulase activity was higher than the normal group (P<0.05). There was no influence on the weight for the 50% ultra-micro powder Qiweibaizhusan group and 50% ultra-micro powder of Qiweibaizhusan with 8% sucrose group. For 50% ultra-micro powder Qiweibaizhusan group, there was some recovery of xylanase activity, but there was a notable difference when compared with normal group (P<0.01). There was no significant difference in cellulase activity and amylase activity (P>0.05). There was a notable difference in protease activity compared with the normal group. The feces of 50% ultra-micro powder Qiweibaizhusan group was more like normal group. 50% ultra-micro powder Qiweibaizhusan had more approachable therapy effect than 50% ultra-micro powder of Qiweibaizhusan with 8% sucrose. 50% ultra-micro powder Qiweibaizhusan group had a better effect on improving the fungi and bacteria of mice with dysbacteriosis diarrhea. 50% ultra-micro powder Qiweibaizhusan group had a higher number of bacteria but a lower number of Escherichia coli (p<0.05), a lower protease activity (P<0.05), and a higher amylase activity (P<0.01).

CONCLUSIONS: Both 50% ultra-micro powder Qiweibaizhusan group and 50% ultra-micro powder Qiweibaizhusan group with sucrose could cure mice’s dysbacteriosis. And the former had a better effect than the latter. It was advised to adopt the ultra-micro powder Qiweibaizhusan without the additive of sucrose.

KEY WORDS: Qiweibaizhusan; Traditional Chinese Medicine; Sucrose; Intestinal microbiota; Intestinal enzyme
I. INTRODUCTION

It is believed that the cause of diarrhea comes from the spleen and stomach in Traditional Chinese Medicine (TCM) theory. Exogenous evil or internal damage is prone to cause stomach dysfunction. And wet turns into hygro. Grain turns into stagnate. Diarrhea originates from stagnants of aquefer and hygro. Generally speaking, the spleen preferring dryness to dampness. Dampness hurts the spleen easily, and the spleen is also easy to be a paterner with hygro. Therefore, doctors believe that the key of treating diarrhea is benefit to vital energy and invigorate the spleen. As long as to keep the spleen health and let the hygro go, then the diarrhea can be cured by itself [1]. The spleen and stomach are the hub of human body. They transport and transform nutrients from foodstuff, and transmit and distribute the fluids. So the treatment of antibiotic-associated diarrhea (AAD) need to process from the spleen and stomach, which is to invigorate the spleen and take care of the stomach, ascend the clear and descend the turbid.

Qiweibaizhusan is not only rigorous prescription but also accurate and appropriate compatibility. The holo-prescription integrated the processes of reinforce, transporting and hoisting. It is reinforce rather than stagnating. It proliferates the intestinal beneficial bacteria and inhibits the growth of harmful bacteria, and plays an important role in regulating the balance of the gastrointestinal microbial flora [2]. Qiweibaizhusan takes its meaning of replenishing qi and strengthening the spleen. Therefore ginseng is used to complement the spleen. Costustoot is used to promote the circulation of Qi. Largehead Atractylodes Rhizome is used to deprive the evil wetness and to invigorate the spleen and stomach. Poria cocos is used to invigorate the spleen and excrete dampness. Cablin patchouli Herb with aroma is used to dissipate dampness. Kudzuvine Root is to send up the lucid YANG. Radix Glycyrrhizae is to recuperate the splenogastric qi and invigorate spleen. The holo- prescription is committed to nourish qi by invigorating spleen and excreting dampness, and to check the diarrhea [1]. At present, the use of Qiweibaizhusan can help many children avoid the adverse effects resulting from abuse of antibiotics [3].

The sweetening agent is essential in the daily life. It provides the everyday needs of energy. It can also change the taste and adjust flavor [4]. There are three major categories of food additives sweetener. The first category covers the carbohydrate of sweetener (nutritive sweeteners) such as sucrose, glucose, and so on. The second category consists in the synthetic sweetener. It is pathogenic and contains so much insecure factors on human life so that it has been gradually phased out. The third category refers to the developing of non-sugar sweeteners (non-nutritive or low calorific sweetener) [5]. An ideal sweetener should possess characteristics such as high safety, good taste, a higher stability, better solubility in water and a lower price [6]. Sugar in preparation not only plays a role in shaping and flavoring agent from the TCM point of view but also has a therapeutic effect. It is sweet in property and flavor, meridian distribution belongs to the hepar and spleen. It possesses functions like moistening the heart and lung, anesis of liver-energy and aiding spleen [7]. Adding a certain amount of sucrose and its metabolites to some health food and medicine can improve the taste of the TCM preparation, reduce the difficulty of taking medication and improve medication compliance. When children take orally TCM preparation, adding sucrose for taste is inevitable. Thus the effect of sweeteners on efficacy of TCM is necessary by comparing the efficacy of TCM pros and cons. The results made a bedding to serve as a link between past and future for preferred sweetener clearly. Therefore, to study the influence of sucrose on ultra-micro powder Qiweibaizhusan to reconcile intestinal microecological balance and to explore into the relationship between the efficacy and the intestinal flora can offer help and guidance for the preparation and clinical medicine. In the long run, it helps selective advantage sweeteners.

II. MATERIAL AND METHODS

Medicine

Qiweibaizhusan were prepared according to the Chinese Pharmacopoeia 2010. Ginseng 6 g, Common Aucklandia Root 6 g, Poria cocos 10 g, Largehead Atractylodes Rhizome 10 g, Cablin Patchouli Herb 10 g, Kudzuvine
Root 10 g and Radix glycyrrhizae 3 g. The ultra-micro powder Qiweibaizhusan was made by Ultra-powder Chinese Medicine Engineering Research Center of Hunan Academy of Chinese Medicine. Superfine-comminuted the medicinal materials by following the above proportion, added suitable amount of boiling water, stirred, centrifuged at a low speed after cooling, and the resulting supernatant was divided into two: one-second ultra-micro powder and one-second ultra-micro powder with 8% sucrose, and stored at 4 °C. The sweetness of sweetener was evaluated mainly by specially trained personnel through the sense organs. The sweetness of the sucrose concentration between 10% to 25% is more preferable to general people [8]. The sweetness of sweetener was a natural sweetener, after the sweet taste test, 8% was the most suitable sweetness of sucrose in this experiment.

Agents

The gentamycin sulfate (Batch Lot :5120106) and cefradine (Batch Lot :110804). The concentration of the antibiotics mixture was 62.5 g.L⁻¹ with the stroke-physiological saline solution (SPSS) [9], and then stored at 4 °C.

Animals

32 Kunming mice (SPF grade) with half male and half female were provided by Hunan Sly Fox animal experiments Co., Ltd. Each mouse weighs about 18-22 g.

Feedstuff

Mouse food was provided by Experimental Animal Center of Hunan University of Chinese Medicine.

Media [10]

Beef extract-peptone medium for bacteria, EMB (eosin methylene blue agar) medium for *Escherichia coli*, Martin Rose Bengal Medium for fungi and BBL medium for bifidobacteria.

Animal groups

After adaptive feeding 4 days, then the mice were randomly divided into 4 groups (8 mice per group): control group, model group, 1/2 volume of ultra-micro powder Qiweibaizhusan group (1/2 volume group), 1/2 volume of ultra-micro powder Qiweibaizhusan group with sucrose group (1/2 volume group with sugar).

Modeling Method

The control group’s mice were treated with SPSS (0.35 mL/mouse) by gavage administration, and the other groups’ mice were treated with antibiotics mixture (0.35 mL/mouse) by gavage administration [9]. They were treated twice a day for 5 d in succession.

Medication and Dose

After the modeling, the control group and model group were treated with SPSS, and the rest were treated with the clinical equivalent dose of oral administration, twice a day for 4 d.

Extracting the intestinal contents

Mice intestinal (jejunum to the rectum) contents were collected in a sterile environment and transferred into clinical flask equipped with glass beads and sterile water.

Clinical observation of experimental animals

Weighed and recorded the mice before modeling, modeling successfully and after the end of treatment.
respectively. Observed and recorded the activities of the diet of mice, mental condition, fecal dilution, death and body mass growth rate. Calculated as follows [11]:

\[
\text{Rate of weight change after the model (\%) = \frac{(\text{body weight after the model} - \text{Initial body weight})}{\text{body weight after the model}} \times 100.}
\]

\[
\text{Rate of weight change after treatment (\%) = \frac{(\text{final weight} - \text{body weight after the model})}{\text{final weight}} \times 100.}
\]

**Determining intestinal microorganisms**

A certain amount of intestinal contents were weighed in a sterile environment and transferred into conical flask equipped with glass beads and sterile water. In order to release microorganisms from intestinal contents into sterile water completely, the conical flasks were put on an oscillators for 30 min shaken with 120 rpm. The number of intestinal microorganisms was determined with dilution plate counting method. Total numbers of bacteria and *Escherichia coli* were counted after being cultured for 24 hours at 37°C. Total numbers of fungi and bifidobacteria were determined after being cultured for 48 hours at 37°C.

**Analyzing intestinal enzyme activities**

Intestinal contents were dissolved completely in sterile water and incubated at 40 °C for 30 min. The crude enzyme extracts were centrifuged for 10 min at 2000 rpm and then the supernatants were collected for enzyme activity analysis. The activities of cellulase, xylanase and amylase were determined with DNS colorimeter as previous described. Protease activity was determined with Folinphend method [12]. The protease activity was defined which generates 1µg amino acid of 1 g contents at 37 °C for 30 min as an unit. The cellulase activity was defined which generates 1 µg reduced sugar of 1 g contents at 50°C for 30 min as an unit. The xylanase activity was defined which generates 1 µg reduced sugar of 1 g contents at 50°C for 30 min as an unit. The amylase activity was defined which generates 1 µg reduced sugar of 1 g contents at 40 °C for 30 min as an unit.

**Statistical analysis**

Measured the data of every group with mean-standard deviation (x±s) and analyzed by using DPS v7.05 statistical software. The two groups of data were compared by using T test, *P*<0.05 was statistically significant.

**III. RESULTS**

**Effect of sucrose on ultra-micro powder Qiweibaizhusan to weight change of dysbacteriosis diarrheal mice**

The weights of mice before treatment and after treatment were analyzed and shown in Figure 1. As shown in Figure 1, after modeling, weight growth rates of the administration groups and model group were slower than the control group. This might be the damage of intestinal mucosa by antibiotics to affect the intestinal absorption function. After intragastric, weight growth rates of administration groups and model group were higher than the normal control group, but were not statistically significant (*P*> 0.05) to normal control group. After treatment, changes in weight and weight change rate of the normal group were minimum. Because the weight of the mice reached the maximum physiological weight probably, weight increase no further or extremely slow. Therefore, sucrose does not have much effect on ultra-micro powder Qiweibaizhusan to the weight change of antibiotics-dysbacteriosis mice.
Figure 1 Effect of sucrose on ultra-micro powder Qiweibaizhusan to weight change of dysbacteriosis diarrheal mice (x±s, n=8)

Note: Compared to normal control group: A: P<0.05  a: P<0.01; Compared to model group: B: P<0.05  b: P<0.01; Compared to 1/2 volume group: C: P<0.05  c: P<0.01.

Effect of sucrose on ultra-micro powder Qiweibaizhusan to treat dysbacteriosis diarrheal mice

After diarrheal modeling 5 days with mix antibiotics, treatment was started with Traditional Chinese Medicine in the sixth day according to the above methods. When any group of mice with diarrhea recovered, the Traditional Chinese Medicine treatment would be ended and the treatment lasted for 4 days. Then the intestinal contents of mice were sampled and analyzed. The inherent moisture and color of fresh feces from mice were observed and all the information was shown in Table 1. Before modeling, the mice were active and normal, smooth coat, dry stool in dark brown. After modeling, the control group mice had a normal diet, active in action, smooth coat, grainy stool in khaki. When pressed hard, the feces were still sticking together, and the feces were squashed but not crushed. They were not sticky. The feces of model group mice and the medication administered mice were grainy but "thin wet" stool and the moisture content increased relatively. The feces were in deeper color, black, easily crushed and sticker after being broken. After being treated, feces of administration groups were dry and faded. The 1/2 volume group recovered closer to that of the control group. While the feces of model group mice were still dilute wet and dark. The 1/2 volume group with sucrose had a poorer curative effect than the 1/2 volume group in this respect.

Table 1 Effect of sucrose on ultra-micro powder Qiweibaizhusan to treat dysbacteriosis diarrheal mice

<table>
<thead>
<tr>
<th></th>
<th>The dilution of feces after molding</th>
<th>The dilution of feces after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>control group</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>model group</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>1/2 volume group</td>
<td>---</td>
<td>+++</td>
</tr>
<tr>
<td>1/2 volume group with sugar</td>
<td>---</td>
<td>++</td>
</tr>
</tbody>
</table>

Note: + constipation; - wet feces
Effect of sucrose on ultra-micro powder Qiweibaizhusan to intestinal microbiota in dysbacteriosis diarrheal mice

Intestinal contents of mice were collected in sterile environment, and the microorganisms in the intestinal contents were detected by diluting plate counting method. From Figure 2, the amount of both *Escherichia coli* and fungi in the model mice were higher than in the control group (*P*<0.05), and the mount of bacteria in the model mice were less than in the control group (*P*<0.01). There was no notable difference for bifidobacteria compared with the control group (*P*>0.05). After being treated with Traditional Chinese Medicine, the number of *Escherichia coli* in administration group mice (both 1/2 volume group and 1/2 volume group with sugar) was less than that in the model group mice (*P*<0.05). The number of bacteria in 1/2 volume group mice was more than in the normal group. The number of bacteria in 1/2 volume group mice was closer to the control group and had no significant difference (*P*>0.05). The number of fungi in 1/2 volume group with sugar was more than the control group (*P*<0.05). Compared with 1/2 volume group, the number of bacteria and *Escherichia coli* of the 1/2 volume group with sugar were significantly different (*P*<0.05). The intestinal flora in both 1/2 volume group and 1/2 volume group with sugar recovered validly. The recovery efficacy of the 1/2 volume group was more close to control group. At the same time, 1/2 volume group had an idea therapeutic efficacy on increasing intestinal fungi and bacteria in mice. It may be relative to osmotic pressure of sucrose on microorganisms [13]. It may also be that sucrose gets into the intestines and stomach as the carbon source of intestinal microorganisms. In addition, the unabsorbed disaccharides (sucrose) which gets into the colon can cause the high osmotic pressure and be fermented to produce short-chain fatty acids (such as formic), CO₂, CH₄, and other gases. Then it causes fermented diarrhea, along with abdominal pain, abdominal distention, diarrhea and a series of clinical symptoms [14]. The intestinal microbial number may also be changed.

Effect of sucrose on ultra-micro powder Qiweibaizhusan to intestinal enzyme activity in mice with dysbacteriosis diarrhea

As is shown in Table 2. The xylanase activity, protease activity, amylase activity of model group were all lower than that of normal group (*P*<0.01 or *P*<0.05). The cellulase activity is higher than that of the normal group (*P*<0.05). Cellulase activity of medication administration group and normal group reduced. There was a notable difference (*P*<0.05). There was no notable difference with control group (*P*>0.05). In 50% ultra-micro powder Qiweibaizhusan group,
xylanase activity recovered partly, but still had a extremely notable difference compared with control group\( (P<0.01) \). There was no notable difference for cellulase activity and amylase activity \( (P>0.05) \). There was a notable difference in protease activity compared with that of the control group. Xylanase activity and protease activity recovered partly, but still had extremely notable difference compared with model group \( (P<0.01) \), cellulase activity and amylase activity had a notable difference compared with model group \( (P<0.05) \), protease activity and xylanase activity had extremely notable difference compared with model group \( (P<0.01) \). In 50% ultra-micro powder Qiweibaizhusan with 8% sugar group, cellulase activity and protease activity had a notable difference with that of the model group \( (P<0.05 \mbox{ or } P<0.01) \), amylase activity had a extremely notable difference with that of the control group and 1/2 volume group \( (P<0.01) \). It shows that the 1/2 volume with 8% sugar group had a better effect than 1/2 volume group on protease activity and cellulase activity, while the effect on the xylanase activity and amylase activity were completely opposite. It indicates that ultra-micro powder Qiweibaizhusan combined with sucrose will not improve xylanase activity and amylase activity. Both the 1/2 ultra-micro powder Qiweibaizhusan group and the 1/2 ultra-micro powder Qiweibaizhusan group with sugar could recover the intestinal enzyme activity of mice with dysbacteriosis. But sucrose did harm to the curative effect of ultra-micro powder Qiweibaizhusan. The sugar was an important material to the body, for example, the glycoprotein is an integral part of the body of hormones, enzymes, antibodies and so on. Sugar could affect metabolic level, synthesis of secondary metabolites, cell morphology and development \[ 15 \].

Table 2 Effect of sucrose on ultra-micro powder Qiweibaizhusan to intestinal enzyme activity in mice with dysbacteriosis diarrhea \( (x±s, \ n=8) \)

<table>
<thead>
<tr>
<th></th>
<th>protease(U)</th>
<th>cellulase(U)</th>
<th>xylanase(U)</th>
<th>amylase(U)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control group</td>
<td>17.215±1.359</td>
<td>2.114±0.692</td>
<td>2.064±0.174</td>
<td>2.617±0.231</td>
</tr>
<tr>
<td>model group</td>
<td>4.721±0.238a</td>
<td>4.112±0.412A</td>
<td>0.267±0.175a</td>
<td>1.333±0.287A</td>
</tr>
<tr>
<td>1/2 volume group</td>
<td>9.765±0.708Ab</td>
<td>1.765±0.930B</td>
<td>1.177±0.270ab</td>
<td>2.029±0.404B</td>
</tr>
<tr>
<td>1/2 volume group with sugar</td>
<td>14.227±0.614AbC</td>
<td>2.602±0.254B</td>
<td>1.102±0.758</td>
<td>0.656±0.266ac</td>
</tr>
</tbody>
</table>

Note: Compared to normal control group: A: \( P<0.05 \) a: \( P<0.01 \); Compared to model group: B: \( P<0.05 \) b: \( <0.01 \); Compared to 1/2 volume group: C: \( P<0.05 \) c: \( P<0.01 \).

IV. DISCUSSION

According to the effect of microbes on human health, the intestinal microbiota were divided into three categories: harmful bacteria, beneficial bacteria and facultative bacteria. Harmful bacteria can degrade proteins and produce stench, and also be related to diarrhea, inflammation, even induce tumor and promote aging, do harm to human health \[ 16 \]. The main intestinal bacteria play an important role in the process of human's immune, nutrition, growth, development, anti-infective, anti-tumor and anti-aging. Escherichia coli is facultative bacteria but will lead disease when antibiotics destroyed the intestinal flora balance of the body.

Qiweibaizhusan may contain “Key to Therapeutics of children’s Diseases”. The prescription is not only rigorous but also compatibility accurate and appropriate. It is the classical prescription for infantile diarrhea\[ 17 \]. The research showed that many of these ingredients had an extremely important role in gut microbes\[ 18 \]. Qiweibaizhusan contains a variety of complex chemical composition of TCM and has a variety of functions. Sucrose can promote intestinal bacteria to proliferate specially, especially for bifidobacteria. At the same time, bifidobacteria could use plant polysaccharides to produce organic acid in the intestinal tract. Thereby the intestinal pH reduced to inhibit the propagation of pathogens including many Gram-negative bacteria. Bifidobacteria also can synthesis proteins, vitamins B and K in animal intestines. Sucrose is a disaccharide. It’s susceptible to acid hydrolysis, and has no reducibility. Sucrose consumption was converted
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into glucose and fructose by converting enzyme in the gastrointestinal and absorbed through the small intestine. This study showed that it was not synergistic effect but a certain antagonism effect of sucrose on ultra-micro powder Qiweibaizhusan to reconcile intestinal microecological balance of diarrheal mice. Symptoms of dysbacteriosis diarrhea by ultra-micro powder Qiweibaizhusan with 8% sucrose might be improved but intestinal enzyme activity had not returned to normal levels. Recovery of feces and intestinal microbiota by ultra-micro powder Qiweibaizhusan with 8% sucrose was not better than that by ultra-micro powder Qiweibaizhusan. It suggested that the effect of ultra-micro powder Qiweibaizhusan with 8% sucrose on dysbacteriosis diarrhea may be linked to some functional composition of Qiweibaizhusan to gut microbes[19]. We can learn from the test, ultra-micro powder Qiweibaizhusan associated with 8% sucrose could increase harmful intestinal bacteria in mouse. In particular, the growth of Escherichia coli increased the damage to intestine and thus affected the clinical efficacy. Studies had shown that a variety of problems, such as denitrification, dissolved oxygen limit vulnerable and easy to produce nitrite, might results from sucrose as a single external carbon source[20]. The supply of nutrients could affect the intestinal microbial metabolism. This example came from the suppression phenomenon of the carbon metabolites[21]. The substance having a high efficiency of energy utilization, such as sucrose, usually inhibits the intestinal microbiota to use other energy substance. This suppression phenomenon prevalents in nature [22]. This may be caused due to sucrose inhibiting the expression or activity of some key enzymes. Moreover, the carbon source and temperature factors have a significant interaction effect [23]. This may be the important reason that 1/2 volume group with sugar is less efficacious than the 1/2 volume group.

In addition, the sugar could improve children’s adrenaline levels and bring impaired concentration or become anxious. The Japanese scholars’ trial showed that the mice’s cancer rate was 8 fold more than normal feeding after long-term consumption of sucrose or having large amounts of sucrose food[24]. Most patients with diabetes can not take sucrose-containing granule. When diarrheal patient without clinical symptoms with diabetes would inevitably take some Traditional Chinese Medicine with sugar granules, it will be worse. And you should pay close attention [25]. The mechanism that sucrose affect the curative effect of TCM are not yet clear. This study failed to clear the antagonism function of sucrose to specific herbs. It needs to be further probed. Therefore, when ultra-micro powder Qiweibaizhusan was used for disease, it’s a good idea to use no sucrose. You had better consider the selection of the other sweeteners. The specific type needs be further studied.

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