Effect of *Glycyrrhiza uralensis* Fisch polysaccharide on growth performance and immunologic function in mice in Ural City, Xinjiang

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ABSTRACT

**Objective:** To discuss the effect of *Glycyrrhiza uralensis* (G. uralensis) Fisch polysaccharide on growth performance and immunologic function in mice in Ural City, Xinjiang and to provide important data supporting the application of Glycyrrhiza polysaccharide.

**Methods:** A total of 100 Kunming mice aged 3 weeks old were randomly divided into 5 groups with 20 mice in each group (10 were females and 10 were males). About 0.5 mL normal saline was given to the mice of control group every day and 0.5 mL *G. uralensis* Fisch polysaccharide was given to the mice of other groups at the concentration of 1, 20, 50 and 100 mg/mL, respectively. The growth performance (average body weight, average daily feed intake and feed efficiency), immune organ indexes (spleen index and thymus index) and immunologic function (serum IL-2, CD4+/CD8+ and the activity of NK cells) of mice in each group were detected continuously.

**Results:** The average body weight, feed efficiency, serum IL-2, CD4+/CD8+ and the activity of NK cells of mice were increased with the increase of administrated time after administrating *G. uralensis* Fisch polysaccharide and were reached up the largest level on Day 28. At the same time, each index was proportional to the given dose and was significantly higher than those of control group and reached up the largest level at the administrated dose of 100 mg/mL. After administrating *G. uralensis* Fisch polysaccharide, the spleen index and thymus index of mice were increased with the increase of administrated dose and the spleen index and thymus index of mice administrated with the dose of 100 mg/mL were maximum which was more than 1.51 times and 1.43 times of that in control group, respectively and the comparative differences showed statistical significance ($P < 0.05$). The average daily feed intake of mice in each group was increased with the passage of time and at the same time, the comparison of average daily feed intake of mice in each group was not significantly different ($P > 0.05$).

**Conclusions:** *G. uralensis* Fisch polysaccharide can significantly improve the growth performance and immunologic function of mice and laid a research basis for the clinical application of *G. uralensis* Fisch polysaccharide.

1. Introduction

Polysaccharide is a biopolymer connected by ketose or aldose through glycosidic bond which widely exists in various kinds of plant tissue and possesses significant and important biological activity and pharmacological effects [1]. In recent years, a large number of studies have shown that polysaccharide can widely regulate various biological phenomena and influence signal transduction and feelings among cells and also has an important regulating role on immune function and physical function [2-3]. *Glycyrrhiza uralensis* Fisch (*G. uralensis*) is a characteristic Chinese medicine in Xinjiang region which possesses some efficacies like fortifying the spleen and supplementing Qi, and clearing heat and removing toxicity and is widely used in various Chinese medicine formulas [4]. Glycyrrhiza polysaccharide is one of the main active ingredients of licorice and its biological activity is closely connected with the spatial structure and spatial structure given priority to with alpha-D-pyran polysaccharide [5],

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Glycyrrhiza polysaccharide possesses strong immune activity and antioxidant activity [6], meanwhile it has some functions such as enhancing body function, reduce the production of cancer cells and antivirus which is widely used in some aspects of anti-tumor, immunoregulation and anti-aging etc. and suitable for people of all ages and it is been recognized [7,8]. However, it is still lack of relevant data about the effect of G. uralensis Fisch polysaccharide on growth performance and immunologic function so far which seriously impact the application of G. uralensis Fisch polysaccharide [9].

In this study, we extract the polysaccharide from G. uralensis Fisch and study the effect of G. uralensis Fisch polysaccharide on growth performance (average body weight, average daily feed intake and feed efficiency), immune organ indexes (spleen index and thymus index) and immunologic function (serum IL-2, CD4+/CD8− and the activity of NK cells) in Kunming mice and also discuss the effect of glycyrrhiza polysaccharide on growth performance and immunologic function in mice, Xinjiang and provide important data supporting the application of G. uralensis.

2. Materials and methods

2.1. Research methods

About 5 kg medicinal powder of G. uralensis Fisch was extracted and soaked at 90% ethyl alcohol for 48 h to remove impurities and lipophilic small molecule compounds. Hot reflux extraction was performed 3 times and each extracting time was 3 h, 2 h, 1.5 h in order. The material was centrifuged at 5000 g for 10 min and combined with supernatant extract and then was concentrated. The right amount of 90% ethyl alcohol was added into the solution to make the concentration of ethyl alcohol to be 85% and was precipitated at 4 °C for 24 h. The sediment was extracted after centrifuging at 5000 g for 10 min and frozen and dried. The dried sample and crude polysaccharide were obtained and distilled water was dissolved the configured into 1% crude polysaccharide solution. The protein was removed by using Sevag method, namely, Sevag reagent (chloroform: N-butyl alcohol = 4:1) and polysaccharide samples (1:1) were shaken well for 20 min and kept still for 30 min and the denatured layer of lower protein was removed by centrifuging. The samples were concentrated and operation was conducted repeatedly for several times to remove the proteins. The samples which were concentrated to remove the proteins were washed with 95% ethyl alcohol and were concentrated again and freeze-dried to obtain crude polysaccharide samples.

2.2. Anthrone-sulfuric acid method detecting the content of polysaccharide

The right amount of glucose standard sample was made into 0.1 mg/mL glucose standard solution after precise weighing and adding water. About 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 mL glucose standard solutions were obtained respectively and placed into 10 mL test tube with cork. The water was added up to 2.0 mL and anthrone-sulfuric acid was added precisely up to 6.0 mL and then all were mixed well and heated in water bath for 15 min and put into ice bath immediately for 15 min. The absorbance was detected at the wavelength of 625 nm of ultraviolet spectrophotometer and the absorbance was regarded as ordinate and concentration was regarded as ordinate to draw a standard curve. The content of polysaccharide was measured.

2.3. Mice groups

SPF Kunming mice aged 3 weeks old were purchased from Xinjiang Medical University (half male and half female) and the body weight was between 20 and 25 g [Certificate of Animal Quality No.: SCXK(Xin)2013-0001]. Breeding mice and related experiments were carried out strictly in accordance with the related requirements of the Laboratory Animal Administration Rules. The experimental operations were all accomplished in Animal Nutrition and Feed Science Laboratory and Chinese Veterinarian Laboratory of College of Animal Science and Technology, Shihezi University. All experiments related with animals were approved by the Ethics Committee of Shihezi University. All Kunming mice were randomly divided into 5 groups with 20 mice in each group (10 were females and 10 were males), namely, 1 mg/mL group, 20 mg/mL group, 50 mg/mL group, 100 mg/mL group and control group. About 0.5 mL normal saline was given into the mice of control group every day and 0.5 mL G. uralensis Fisch polysaccharide was given into the mice of other groups at the concentration of 1, 20, 50 and 100 mg/mL, respectively. Experimental period was persisted for 28 days.

2.4. Detection of growth performance in mice

Each mouse in every experimental group was marked before experimental treatment. The body weight and daily feed intake were detected continuously using electronic scales under the situation of empty stomach before and after feeding to calculate average body weight, average daily feed intake and feed efficiency, feed efficiency = (average body weight of mice–initial body weight of mice)/average daily feed intake.

2.5. Detection of immune function in mice

2.5.1. Detection of immune organ index in mice

The body weight of mice with empty stomach in each group was weighted and sterile spleen and thymus were weighted and recorded after death with cervical dislocation. Spleen index = spleen mass of mice/empty body weight of mice; thymus index = thymus mass of mice/empty body weight of mice.

2.5.2. Determination of immune related factors in mice

The peripheral blood was collected before and after feeding and kept at −80 °C and then was used to detect subsequently. IL-2 and lactic dehydrogenase were detected by ELISA Kit (Enzyme-linked biological technology co., LTD., Shanghai, Wuhan Hua Mei Biological Engineering co., LTD) and related operation was performed strictly according to the specification of the kit. CD4+/CD8− value was detected by flow cytometry (CytoFLEX, USA) and related antibody was provided by Genscript Biotechnology Co., LTD and experimental operation was performed strictly according to the specification.

2.6. Statistical methods

Statistical analysis was performed by using statistical software SPSS20.0. The relevant data were expressed by mean ± SD. The comparison of two groups was performed by using t-test. The comparison among groups was performed using ANOVA. Pairwise comparison was conducted by SNK-q test. P < 0.05 indicated significantly difference.
3. Results

3.1. The effect of *G. uralensis* Fisch polysaccharide on growth performance in mice

The average body weight, average daily feed intake and feed efficiency of mice in each group were detected and the results showed that the comparison of average body weight and average daily feed intake of mice in each group before feeding was not significantly different (*P* > 0.05). The body weight and feed efficiency of mice were increased with the increase of administrated time after administrating *G. uralensis* Fisch polysaccharide and were reached up the largest level on Day 28. At the same time, the body weight and feed efficiency of mice was proportional to the given dose and was significantly higher than those of control group and reached up the largest level at the administrated dose of 100 mg/mL. The comparative differences showed statistical significance (*P* < 0.05). The average daily feed intake of mice in each group was increased with the passage of time and at the same time, the comparison of average daily feed intake of mice in each group was not significantly different (*P* > 0.05) (Table 1).

3.2. The effect of *G. uralensis* Fisch polysaccharide on immune organ index in mice

The mice with empty stomach were weighted and recorded and the spleen index and thymus index of mice were detected after death with cervical dislocation and the results showed that the spleen index and thymus index of mice in control group were minimum, (35.48 ± 3.21) mg/10 g, (17.02 ± 1.85) mg/10 g, respectively. After administrating *G. uralensis* Fisch polysaccharide, the spleen index and thymus index of mice were increased with the increase of administrated dose and the spleen index and thymus index of mice administrated with the dose of 100 mg/mL were maximum, 53.57 ± 5.01, 24.35 ± 2.34 (mg/10 g), respectively which was more than 1.51 times and 1.43 times of that in control group, respectively and the comparative differences showed statistical significance (*P* < 0.05) (Table 2).

3.3. The effect of *G. uralensis* Fisch polysaccharide on immunologic function in mice

The serum IL-2, CD4+/CD8+ and the activity of NK cells of mice in each group were detected and the results showed that the comparison of serum IL-2, CD4+/CD8+ and the activity of NK cells of mice in each group before feeding was not significantly different (*P* > 0.05) (Table 1).

### Table 1

The effect of *G. uralensis* Fisch polysaccharide on growth performance in mice (X ± S).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight (g)</th>
<th>Body weight (g)</th>
<th>Feed intake (g)</th>
<th>Feed efficiency</th>
<th>Feed intake (g)</th>
<th>Feed efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>17.65 ± 1.78</td>
<td>3.15 ± 0.54</td>
<td>0</td>
<td>20.78 ± 2.03</td>
<td>3.44 ± 0.76</td>
<td>0.91 ± 0.11</td>
</tr>
<tr>
<td>1 mg/mL group</td>
<td>17.51 ± 1.97</td>
<td>3.16 ± 0.50</td>
<td>0</td>
<td>22.58 ± 2.01</td>
<td>3.48 ± 0.75</td>
<td>1.45 ± 0.16</td>
</tr>
<tr>
<td>50 mg/mL group</td>
<td>17.71 ± 1.80</td>
<td>3.17 ± 0.52</td>
<td>0</td>
<td>23.95 ± 2.31</td>
<td>3.60 ± 1.14</td>
<td>1.73 ± 0.24</td>
</tr>
</tbody>
</table>

### Table 2

The effect of *G. uralensis* Fisch polysaccharide on immune organ index in mice (X ± S).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Spleen index (mg/10 g)</th>
<th>Thymus index (mg/10 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>35.48 ± 3.21</td>
<td>17.02 ± 1.85</td>
</tr>
<tr>
<td>1 mg/mL group</td>
<td>37.55 ± 4.16a</td>
<td>17.62 ± 1.98a</td>
</tr>
<tr>
<td>20 mg/mL group</td>
<td>43.74 ± 4.23abc</td>
<td>19.65 ± 2.01abc</td>
</tr>
<tr>
<td>50 mg/mL group</td>
<td>48.25 ± 4.64abcd</td>
<td>22.43 ± 2.25abcd</td>
</tr>
<tr>
<td>100 mg/mL group</td>
<td>53.57 ± 5.01abcd</td>
<td>24.35 ± 2.34abcd</td>
</tr>
</tbody>
</table>

*a* *P* < 0.05, compared with control group; *b* *P* < 0.05, compared with 1 mg/mL group; *c* *P* < 0.05, compared with 20 mg/mL group; *d* *P* < 0.05, compared with 50 mg/mL group.
Table 3

<table>
<thead>
<tr>
<th>Groups</th>
<th>IL-2 (ng/mL)</th>
<th>CD4+/CD8+ activity of NK cells (%)</th>
<th>Comparisons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>8.57 ± 0.10</td>
<td>8.55 ± 1.34</td>
<td></td>
</tr>
<tr>
<td>1 mg/mL group</td>
<td>8.55 ± 1.34</td>
<td>8.41 ± 1.45</td>
<td></td>
</tr>
<tr>
<td>20 mg/mL group</td>
<td>8.53 ± 1.27</td>
<td>8.32 ± 1.54</td>
<td></td>
</tr>
<tr>
<td>100 mg/mL group</td>
<td>8.56 ± 1.21</td>
<td>8.40 ± 1.44</td>
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</table>

**Comparisons:**
- *P* < 0.05 compared with Day 0 in this group;
- *P* < 0.05 compared with Day 7 in this group;
- *P* < 0.05 compared with Day 14 in this group;
- *P* < 0.05 compared with Day 21 in this group;
- *P* < 0.05 compared with the same time of control group;
- *P* < 0.05 compared with the same time of 20 mg/mL group;
- *P* < 0.05 compared with the same time of 50 mg/mL group.

4. Discussion

Except protein and nucleic acid, polysaccharide is an important substance in life movement, which is abundant among organisms in nature and widely participates in various biological functions such as structure support, energy storage and immune defense, and so on. More importantly, polysaccharide is a bridge to transfer energy and substance within cells and biotic environment leading to a mutual effect on biomacromolecule and cells [10,11]. In recent years, various polysaccharides have been extracted from different kinds of plants whose biological function has also been widely studied, which plays an important role in many aspects such as antineoplastic, decreased hyperglycemic, cura and immune function. Researches on polysaccharide have also become more and more active. Biological activity of polysaccharide is closely related to its structure, chemical group, formation and purity. Therefore, the focus and emphasis of the present biological research has still aimed at looking for polysaccharides of high activity [12,13]. Uralensis is a common Chinese herbal medicine. *G. uralensis* is the characteristic traditional Chinese medicine in Xinjiang region. Its quality has been recognized [14]. Uralensis has the effect of clearing heat and removing toxicity, invigorating spleen and replenishing qi, expelling phlegm to arrest coughing and concocting with multiple drugs, which is widely used in the treatment of cough, weakness of the spleen and the stomach, gastric ulcer and food poisoning [15,16]. In recent years, multiple researches have shown that the main active substances of uralensis are *G. uralensis* Fisch polysaccharide, triterpenoid saponin and flavonoids compounds [17]. Since glycyrrhiza polysaccharide was extracted from *G. uralensis* Fisch polysaccharide in 1965, clinical and pharmacologic researches have been found that the immunocompetence, antioxidant activity and antineoplastic activity were importantly related to *G. uralensis* Fisch polysaccharide [18]. *G. uralensis* Fisch polysaccharide can significantly improve the mitosis, anticomplement and enhance the ability of immune elimination [19]. Multiple researches have shown that *G. uralensis* Fisch polysaccharide has an effect of improving specific immunity and non-specific immunity of rats and plays an important role in maintaining a healthy body [20]. *G. uralensis* Fisch polysaccharide has a significant effect of elimination on hydroxyl radical, DPPH free radical and superoxide anion free radical and can slow down aging and maintain the normality of cells [21,22]. More importantly, *G. uralensis* Fisch polysaccharide will affect the expression of gene such as BCL-2 and Bax leading to an effect of antineoplastic [23]. At present, polysaccharide has been widely used in various prescriptions and Chinese patent medicine. *G. uralensis* Fisch polysaccharide also plays an irreplaceable role in the treatment of many diseases [24]. The widely use of different (*P* > 0.05). The serum IL-2, CD4+/CD8⁺ and the activity of NK cells of mice were increased with the increase of administrated time after administrating *G. uralensis* Fisch polysaccharide and were reached up the largest level on Day 28. At the same time, the serum IL-2, CD4+/CD8⁺ and the activity of NK cells of mice was proportional to the given dose and was significantly higher than those of control group and reached up the largest level at the administrated dose of 100 mg/mL. The comparative differences showed statistical significance (*P* < 0.05) (Table 3).
polysaccharide in different age and area has earned the focus from people on its toxic and side effect. Effect of *G. uralensis* Fisch polysaccharide on growth performance and immune function will severely affect the use of polysaccharide [25,26].

The study found that *G. uralensis* Fisch polysaccharide can significantly improve the growth performance of mice (average weight, average daily feed intake and feed efficiency), immune organ index (spleen and thymus index) and immune function (serum IL-2, CD4+/CD8+ and the activity of NK cells). The average daily intake of mice in every group was significantly increased with time, but at the same time, the daily intake of mice was not significantly different. The possible reason was that the growth of mice and the increase of gastric volume and energy consumption in mice would result in the increase of food intake. After Kunming mice were fed with glycyrrhiza polysaccharide, the average weight and the feed efficiency of mice were significantly higher than those of control group, but the food intake did not increased, which indicated that glycyrrhiza polysaccharide can promote the feed utilization rate or reduce energy consumption, thus speeding up the growth in mice. Spleen and thymus are important immune organs of the body. The immune organ index is a manifestation of the function of immune cells and the development of immune organs, which can reflect the immune function of the organism from the side [27]. The spleen index and thymus index of mice were significantly higher after administrating glycyrrhiza polysaccharide which indicates that glycyrrhiza polysaccharide has auxo-action on immune organs of body and can enhance the immune function of body. Immune cells and immune factor are the indispensable part of the body’s immune function and the levels of immune cells and immune factor can reflect well the level of body’s immune function [28]. Interleukin-2 (IL-2) is an important immune factor secreted by CD4+ T lymphocyte which widely participates in anti-virus infection and body’s immune response and can improve the activation and proliferation of T cell, B cell and NK cell and secretion of correlation factor. It plays an important role in the treatment of tumor and antivirus [29,30]. *G. uralensis* Fisch polysaccharide increases the secretion of IL-2 by improving the activation and proliferation of related immunity cells, which can simultaneously increase the secretion of Interleukin-2 dose-dependently. The secretion of IL-2 further improves the activation of NK cells. It has been found by ELISA that LDH level in rats irrigated by *G. uralensis* Fisch polysaccharide was significantly higher than that of control group, which by analyzing may result from the proliferation and activation of NK cells improved by *G. uralensis* Fisch polysaccharide. And it further improves the damage on tumor cell and virus-infection cells and the release of LDH from cells. T-lymphocyte subsets is a important cell participating in the function of body’s immune defenses, which mainly includes two classes, inhibitory CD8 T lymphocytes and auxiliary CD4+ T lymphocytes. CD4+ T lymphocytes contain secrete-related factors which improve the production of antibody and regulate the function of immunocompetence. While, CD8 T lymphocytes possess the effects of immunosuppression and cytotoxicity. Therefore, the value of CD4+/CD8+ can adequately indicate the situation of immune mechanism [31,32]. Researchers have found that the *G. uralensis* Fisch polysaccharide improve the increase of the value of CD4+/CD8+, which indicates that *G. uralensis* Fisch polysaccharide can regulate the body immune function.

In conclusion, in this study, we extract the polysaccharide from *G. uralensis* Fisch and study the effect of *G. uralensis* Fisch polysaccharide on growth performance (average body weight, average daily feed intake and feed efficiency), immune organ indexes (spleen index and thymus index) and immunologic function (serum IL-2, CD4+/CD8+ and the activity of NK cells) in Kunming mice and also discuss the effect of glycyrrhiza polysaccharide on growth performance and immunologic function in mice, Xinjiang and provide important help for the development and application of *G. uralensis*.

**Conflict of interest statement**

We declare that we have no conflict of interest.

**References**


