HOSTED BY

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine



journal homepage: http://ees.elsevier.com/apjtm

Original Research http://dx.doi.org/10.1016/j.apjtm.2016.08.004

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

Jie Chen, Xiao-Qing Zhu, Li Yang, Yan Luo, Meng-Yuan Wang, Xiao-Ting Liu, Ke-Xun Liang, Xin-Li Gu<sup>⊠</sup>

College of Animal Science and Technology, Shihezi University, Shihezi 830002, Xinjiang, China

## ARTICLE INFO

ABSTRACT

Article history: Received 30 Jun 2016 Received in revised form 7 Jul 2016 Accepted 10 Aug 2016 Available online 22 Oct 2016

Keywords: Glycyrrhiza uralensis Fisch polysaccharide Immune organ index Growth performance

Immunologic function

## 2016 grov \_\_\_\_\_ nor \_\_\_\_\_ Fiso

**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<sup>+</sup>/CD8<sup>+</sup> and the activity of NK cells) of mice in each group were detected continuously.

**Objective:** To discuss the effect of *Glycyrrhiza uralensis* (G. uralensis) Fisch poly-

saccharide on growth performance and immunologic function in mice in Ural City, Xinjiang and to provide important data supporting the application of Glycyrrhiza polysaccharide.

**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].

1995-7645/Copyright © 2016 Hainan Medical University. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http:// creativecommons.org/licenses/by-nc-nd/4.0/).

First author: Jie Chen, College of Animal Science and Technology, Shihezi University, Shihezi 830002, Xinjiang, China.

Tel: +86 18699145825

E-mail: chenjie4984@163.com

<sup>&</sup>lt;sup>16</sup>Corresponding author: Xin-Li Gu, College of Animal Science and Technology, Shihezi University, Shihezi 830002, Xinjiang, China.

Tel: +86 993 2038582

E-mail: xlgu@shzu.edu.cn

Peer review under responsibility of Hainan Medical University.

Foundation Project: This study was supported by Scientific Research Innovation Project of Graduate Education Innovation Fund from Xinjiang (Grant No. XJGRI2014057).

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<sup>+</sup>/CD8<sup>+</sup> 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 freezed 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 shaked 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 removed 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<sup>+</sup>/CD8<sup>+</sup> 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  $\pm$  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.

	· .				/n			D14			171			D28	
Body Food Feed Body weight intake weight efficiency			Body weight		Food intake	Feed efficiency	Body weight	Food intake	Feed efficiency	Body weight	Food intake	Feed efficiency	Body weight	Food intake	Feed efficiency
$ \begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	$\begin{array}{l} 20.78 \pm 2.03^{a} \\ 21.25 \pm 1.97^{ac} \\ 2.58 \pm 2.01^{ac} \\ 3.95 \pm 2.01^{ac} \\ 3.14 \pm 2.44^{ac} \end{array}$	igh g	$\begin{array}{cccccccccccccccccccccccccccccccccccc$		$\begin{array}{l} 24.14\pm2.15^{ab}\\ 25.07\pm2.26^{abc}\\ 26.37\pm2.14^{abcf}\\ 27.67\pm2.79^{abcfg}\\ 27.67\pm2.79^{abcfg}\end{array}$	$\begin{array}{l} 4.25 \pm 0.94^{ab} \\ 4.17 \pm 1.25^{ab} \\ 4.20 \pm 0.93^{ab} \\ 4.23 \pm 1.31^{ab} \\ 4.21 \pm 1.22^{ab} \end{array}$	$\begin{array}{rrrr} 4.25 \pm 0.94^{\rm ab} & 1.53 \pm 0.16^{\rm ab} \\ 4.17 \pm 1.25^{\rm ab} & 1.79 \pm 0.18^{\rm abc} \\ 4.20 \pm 0.93^{\rm ab} & 2.11 \pm 0.24^{\rm abc} \\ 4.23 \pm 1.31^{\rm ab} & 2.35 \pm 0.31^{\rm abc} g^{\rm abc} \\ 4.21 \pm 1.22^{\rm ab} & 2.57 \pm 0.32^{\rm abc} g^{\rm abc} \end{array}$	$ 24.14 \pm 2.15^{\rm ab} - 4.25 \pm 0.94^{\rm ab} - 1.53 \pm 0.16^{\rm ab} - 27.78 \pm 2.24^{\rm abc} - 4.92 \pm 1.11^{\rm abc} - 2.06 \pm 0.26^{\rm abc} - 31.55 \pm 2.63^{\rm abcd} - 25.507 \pm 2.52^{\rm abcd} - 2.25^{\rm abcd} - 2.25^{\rm$	$\begin{array}{rrrr} 4.92 \pm 1.11^{\rm abc} & 2.06 \pm 0.26^{\rm abc} \\ 4.87 \pm 1.42^{\rm abc} & 2.33 \pm 0.25^{\rm abc} \\ 4.90 \pm 1.17^{\rm abc} & 2.60 \pm 0.30^{\rm abcet} \\ 4.89 \pm 1.38^{\rm abc} & 2.89 \pm 0.37^{\rm abcetg} \\ 4.81 \pm 1.39^{\rm abc} & 3.18 \pm 0.34^{\rm abcetg} \end{array}$	$\begin{array}{l} 2.06 \pm 0.26^{\rm abc}\\ 2.33 \pm 0.25^{\rm abce}\\ 2.60 \pm 0.30^{\rm abcef}\\ 2.89 \pm 0.37^{\rm abcefg}\\ 3.18 \pm 0.34^{\rm abcefgh} \end{array}$	31.55 ± 2.63 abcd 33.00 ± 2.57 abcde 34.21 ± 2.84 abcdef 35.55 ± 3.21 abcdefg 37.12 ± 3.48 abcdefgh	$5.44 \pm 1.45^{abcd} 2.56 \pm 0.38^{abcd}$ $5.42 \pm 1.62^{abcd} 2.84 \pm 0.33^{abcdc}$ $5.39 \pm 1.40^{abcd} 3.10 \pm 0.37^{abcdc}$ $5.38 \pm 1.52^{abcd} 3.32 \pm 0.41^{abcd}$ $5.38 \pm 1.49^{abcd} 3.33 \pm 0.44^{abcd}$	5.44 ± 1.45 <sup>abcd</sup> 2.56 ± 0.38 <sup>abcd</sup> 5.42 ± 1.62 <sup>bacd</sup> 2.84 ± 0.33 <sup>abcd</sup> 5.39 ± 1.40 <sup>abcd</sup> 3.10 ± 0.37 <sup>abcdef</sup> 5.38 ± 1.52 <sup>bacd</sup> 3.32 ± 0.44 <sup>abcdef</sup> 5.41 ± 1.49 <sup>abcd</sup> 3.63 ± 0.44 <sup>abcdefb</sup>
$^{a}P < 0.05$ compared with Day 0 in this group; $^{b}P < 0.05$ compared with Day 7 in this group; $^{c}P < 0.05$ compared with Day 14 in this group; $^{d}P < 0.05$ compared with Day 21 in this group; $^{e}P < 0.05$ compared with Day 0 in this group; $^{b}P < 0.05$ compared with Day 14 in this group; $^{d}P < 0.05$ compared with Day 21 in this group; $^{e}P < 0.05$ compared with Day 21 in this group; $^{e}P < 0.05$ compared with Day 0 in this group; $^{b}P < 0.05$ compared with Day 21 in this group; $^{e}P < 0.05$ compared with Day 21 in this group; $^{e}P < 0.05$ compared with Day 21 in this group; $^{e}P < 0.05$ compared with Day 21 in this group; $^{e}P < 0.05$ compared with Day 21 in this group; $^{e}P < 0.05$ compared with Day 21 in this group; $^{e}P < 0.05$ compared with Day 21 in this group; $^{e}P < 0.05$ compared with Day 21 in this group; $^{e}P < 0.05$ compared with Day 21 in this group; $^{e}P < 0.05$ compared with Day 21 in this group; $^{e}P < 0.05$ compared with Day 21 in this group; $^{e}P < 0.05$ compared with Day 21 in this group; $^{e}P < 0.05$ compared with Day 21 in this group; $^{e}P < 0.05$ compared with Day 21 in this group; $^{e}P < 0.05$ compared with Day 21 in this group; $^{e}P < 0.05$ compared with Day 21 in this group; $^{e}P < 0.05$ compared with Day 21 in this group; $^{e}P < 0.05$ compared with Day 21 in this group; $^{e}P < 0.05$ compared with Day 21 in this group; $^{e}P < 0.05$ compared with Day 21 in this group; $^{e}P < 0.05$ compared with Day 21 in this group; $^{e}P < 0.05$ compared with Day 21 in this group; $^{e}P < 0.05$ compared with Day 21 in this group; $^{e}P < 0.05$ compared with Day 21 in this group; $^{e}P < 0.05$ compared with Day 21 in this group; $^{e}P < 0.05$ compared with Day 21 in this group; $^{e}P < 0.05$ compared with Day 21 in this group; $^{e}P < 0.05$ compared with Day 21 in this group; $^{e}P < 0.05$ compared with Day 21 in this group; $^{e}P < 0.05$ compared with Day 21 in this group; $^{e}P < 0.05$ compared with Day 21 in this group; $^{e}P < $	y 0 in this group; $^{b}P < 0.05$	roup; $^{\rm b}P < 0.05$ .	P < 0.05	I S	npared with	Day 7 in this	s group; $^{c}P < 0$	).05 compare	d with Day 1	4 in this group;	$^{d}P < 0.05 \text{ con}$	npared with D	ay 21 in this gr	oup; <sup>e</sup> <i>P</i> < 0.0;	compared with

the same time of control group;  $^{f}P < 0.05$  compared with the same time of 1 mg/mL group;  $^{g}P < 0.05$  compared with the same time of 20 mg/mL group;  $^{h}P < 0.05$  compared with the same time of 50 mg/mL group.

## 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,  $(35.48 \pm 3.21) \text{ mg/10 g}$ ,  $(17.02 \pm 1.85) \text{ mg/10 g}$ , 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,  $53.57 \pm 5.01$ ,  $24.35 \pm 2.34$  (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<sup>+</sup>/CD8<sup>+</sup> 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<sup>+</sup>/CD8<sup>+</sup> and the activity of NK cells of mice in each group before feeding was not significantly

#### Table 2

The effect of *G. uralensis* Fisch polysaccharide on immune organ index in mice  $(\overline{x} \pm s)$ .

Groups	Spleen index (mg/10 g)	Thymus index (mg/10 g)
Control group 1 mg/mL group 20 mg/mL group 50 mg/mL group 100 mg/mL group	$\begin{array}{l} 35.48 \pm 3.21 \\ 37.55 \pm 4.16^{a} \\ 43.74 \pm 4.23^{ab} \\ 48.25 \pm 4.64^{abc} \\ 53.57 \pm 5.01^{abcd} \end{array}$	$17.02 \pm 1.85$ $17.82 \pm 1.98^{a}$ $19.65 \pm 2.01^{ab}$ $22.43 \pm 2.25^{abc}$ $24.35 \pm 2.34^{abcd}$

 ${}^{a}P < 0.05$ , 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.

The effect of G. uralensis Fisch polysaccharide on growth performance in mice  $(\overline{x} \pm s)$ .

**Fable 1** 

	activity of NK cells (%)	$\begin{array}{l} 2 \pm 1.55  \mathrm{albcd} \\ 1 \pm 1.88  \mathrm{albcde} \\ 5 \pm 2.14  \mathrm{albcde} \\ 11 \pm 2.08  \mathrm{albcdefg} \\ 11 \pm 2.43  \mathrm{albcdefg} \\ 1 \pm 2.43  \mathrm{albcdefg} \end{array}$
D20	CD4+/CD8+ ac	88 ± 0.37 <sup>abcd</sup> 8.5' 55 ± 0.41 <sup>abcde</sup> 9.6' 73 ± 0.47 <sup>abcdef</sup> 12.5' 9 ± 0.52 <sup>abcdefg</sup> 14.2 0 ± 0.77 <sup>abcdefgh</sup> 16.1
	IL-2 (ng/mL)	38 ± 0.18 abcd 1.5 .17 ± 1.39 abcde 1.6 t.26 ± 1.20 abcde 1.7 t.36 ± 1.22 abcdef 1.7 t.38 ± 1.22 abcdefg 1.7 t.48 ± 1.25 abcdefg 1.9
	activity of NK cells (%)	$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$
D21	CD4 <sup>+</sup> /CD8 <sup>+</sup> activity of NK cells (%)	$\begin{array}{l} 1.52 \pm 0.29^{\rm abc} \\ 1.57 \pm 0.33^{\rm abce} \\ 1.62 \pm 0.45^{\rm abcef} \\ 1.70 \pm 0.40^{\rm abcefg} \\ 1.80 \pm 0.68^{\rm abcefgh} \end{array}$
	IL-2 (ng/mL)	8.92 ± 0.15 <sup>abc</sup> 10.13 ± 1.24 <sup>abce</sup> 11.67 ± 1.08 <sup>abcef</sup> 13.22 ± 1.20 <sup>abcefg</sup>
	CD4 <sup>+</sup> /CD8 <sup>+</sup> activity of NK cells (%)	$\begin{array}{l} 8.27 \pm 1.34^{ab} \\ 8.77 \pm 1.45^{abc} \\ 10.15 \pm 1.87^{abcf} \\ 11.00 \pm 1.65^{abcfg} \\ 12.15 \pm 2.00^{abcfg} \end{array}$
D14	CD4 <sup>+</sup> /CD8 <sup>+</sup>	$\begin{array}{c} 1.46 \pm 0.31^{ab} \\ 1.52 \pm 0.35^{abe} \\ 1.51 \pm 0.41^{abef} \\ 1.51 \pm 0.47^{abefg} \\ 1.60 \pm 0.47^{abefg} \\ 1.68 \pm 0.57^{abefgh} \end{array}$
	IL-2 (ng/mL)	$\begin{array}{c} 8.79 \pm 0.14^{ab} \\ 8.89 \pm 1.33^{abe} \\ 8.81.7 \pm 1.17^{abef} \\ 10.17 \pm 1.17^{abef} \\ 11.94 \pm 0.96^{abef_1} \\ 11.94 \pm 0.14^{abef_2} \end{array}$
	activity of NK cells (%)	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$
<i>'</i> 1	IL-2         CD4 <sup>+</sup> /CD8 <sup>+</sup> activity of NK         IL-2         CD4 <sup>+</sup> /CD8 <sup>+</sup> activity of NG           ng/mL         cells (%)         (ng/mL)         cells         cells	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$
	tivity of NK IL-2 cells (%) (ng/mL)	$\begin{array}{l} 8.68 \pm 0.13^{\rm a} \\ 8.77 \pm 1.16^{\rm be} \\ 9.85 \pm 0.94^{\rm bef} \\ 10.33 \pm 1.11^{\rm aef} \\ 11.47 \pm 0.95^{\rm aef} \end{array}$
	<ul> <li>activity of NI cells (%)</li> </ul>	$\begin{array}{c} 8.05 \pm 1.21 \\ 8.06 \pm 1.06^{\circ} \\ f \\ 8.04 \pm 1.37^{\circ f} \\ f_{12} \\ 8.06 \pm 1.00^{\circ f_{1}} \\ 8.07 \pm 1.38^{\circ f_{2}} \end{array}$
M	CD4 <sup>+</sup> /CD8	$\begin{array}{c} 1.35 \pm 0.24 \\ 1.36 \pm 0.37^{\circ} \\ 1.34 \pm 0.33^{\circ} \\ 1.31 \pm 0.33^{\circ} \\ 10 \\ 1.37 \pm 0.21^{\circ} \\ 1.37 \pm 0.31^{\circ} \end{array}$
	IL-2 (ng/mL)	8.57 $\pm$ 0.10 8.55 $\pm$ 1.23° 9.8.58 $\pm$ 0.78° 9.8.54 $\pm$ 1.05° 10.8.86 $\pm$ 1.01° <sup>cl</sup>
Groups		Control group 1 mg/mL group 20 mg/mL group 50 mg/mL grou 100 mg/mL grou

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

**Fable 3** 

Jie Chen et al./Asian Pacific Journal of Tropical Medicine 2016: 9(11): 1078-1083  $^{a}P < 0.05$  compared with Day 0 in this group;  $^{b}P < 0.05$  compared with Day 7 in this group;  $^{c}P < 0.05$  compared with Day 14 in this group;  $^{d}P < 0.05$  compared with Day 21 in this group;  $^{c}P < 0.05$  compared with Day 14 in this group;  $^{d}P < 0.05$  compared with Day 21 in this group;  $^{c}P < 0.05$  compared with Day 14 in this group;  $^{d}P < 0.05$  compared with Day 21 in this group;  $^{c}P < 0.05$  compared with Day 14 in this group;  $^{d}P < 0.05$  compared with Day 21 in this group;  $^{c}P < 0.05$  compared with Day 21 in this group;  $^{c}P < 0.05$  compared with Day 21 in this group;  $^{c}P < 0.05$  compared with Day 21 in this group;  $^{c}P < 0.05$  compared with Day 21 in this group;  $^{c}P < 0.05$  compared with Day 21 in this group;  $^{c}P < 0.05$  compared with Day 21 in this group;  $^{c}P < 0.05$  compared with Day 21 in this group;  $^{c}P < 0.05$  compared with Day 21 in this group;  $^{c}P < 0.05$  compared with Day 21 in this group;  $^{c}P < 0.05$  compared with Day 21 in this group;  $^{c}P < 0.05$  compared with Day 21 in this group;  $^{c}P < 0.05$  compared with Day 21 in this group;  $^{c}P < 0.05$  compared with Day 21 in this group;  $^{c}P < 0.05$  compared with Day 21 in this group;  $^{c}P < 0.05$  compared with Day 21 in this group;  $^{c}P < 0.05$  compared with Day 21 in this group;  $^{c}P < 0.05$  compared with Day 21 in this group;  $^{c}P < 0.05$  compared with Day 21 in this group;  $^{c}P < 0.05$  compared with Day 21 in this group;  $^{c}P < 0.05$  compared with Day 21 in this group;  $^{c}P < 0.05$  compared with Day 21 in this group;  $^{c}P < 0.05$  compared with Day 21 in this group;  $^{c}P < 0.05$  compared with Day 21 in this group;  $^{c}P < 0.05$  compared with Day 21 in this group;  $^{c}P < 0.05$  compared with Day 21 in this group;  $^{c}P < 0.05$  compared with Day 21 in this group;  $^{c}P < 0.05$  compared with Day 21 in this group;  $^{c}P < 0.05$  compared with Day 21 in this group;  $^{c}P < 0.05$  compared with Day 21 in this group;  $^{c}P < 0.05$  compared with Day 21 in this group;  $^{c}P$ same time of control group;  $^{f}P < 0.05$  compared with the same time of 1 mg/mL group;  $^{g}P < 0.05$  compared with the same time of 20 mg/mL group;  $^{h}P < 0.05$  compared with the same time of 50 mg/mL group.

the

different (P > 0.05). The serum IL-2, CD4<sup>+</sup>/CD8<sup>+</sup> 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<sup>+</sup>/CD8<sup>+</sup> 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).

## 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 kind of plants whose biological function has also been widely studied, which plays an important role in many aspects such as antineoplastic, decreased hyperglycemic, cruor 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 on 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 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<sup>+</sup>/CD8<sup>+</sup> 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 glycyrrhiza significantly higher after administrating 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<sup>+</sup> 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. Tlymphocyte subsets is a important cell participating in the function of body's immune defenses, which mainly includes two classes, inhibitory CD8<sup>+</sup> T lymphocytes and auxiliary CD4<sup>+</sup> T lymphocytes. CD4<sup>+</sup> T lymphocytes contain secrete-related factors which improve the production of antibody and regulate the function of immunocompetence. While, CD8<sup>+</sup> T lymphocytes possess the effects of immunosuppression and cytotoxicity. Therefore, the value of CD4<sup>+</sup>/CD8<sup>+</sup> can adequately indicate the situation of immune mechanism [31,32]. Researchers have been found that the G. uralensis Fisch polysaccharide improve the increase of the value of CD4<sup>+</sup>/CD8<sup>+</sup>, 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<sup>+</sup>/CD8<sup>+</sup> 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

- [1] Ayeka PA, Bian Y, Mwitari PG, Chu X, Zhang Y, Uzayisenga R, et al. Immunomodulatory and anticancer potential of Gan cao (*Glycyrrhiza uralensis* Fisch.) polysaccharides by CT-26 colon carcinoma cell growth inhibition and cytokine IL-7 upregulation in vitro. *BMC Complement Altern Med* 2016; 16(1): 1-8.
- [2] Span EA, Marletta MA. The framework of polysaccharide monooxygenase structure and chemistry. *Curr Opin Struct Biol* 2015; 35(25): 93-99.
- [3] McCabe Orla, Spinelli Silvia, Farenc Carine, Labbé Myriam, Tremblay Denise, Blangy Stéphanie, et al. The targeted recognition of *Lactococcus lactis* phages to their polysaccharide receptors. *Mol Microbiol* 2015; 96(4): 875-886.
- [4] Bonten Marc JM, Huijts Susanne M, Bolkenbaas Marieke, Webber Chris, Patterson Scott, Gault Samantha, et al. Polysaccharide conjugate vaccine against pneumococcal pneumonia in adults. *New Engl J Med* 2015; **372**(12): 1114-1125.
- [5] Shen H, Zeng G, Sun B, Cai X, Bi L, Tang G, et al. A polysaccharide from Glycyrrhiza inflata Licorice inhibits proliferation of human oral cancer cells by inducing apoptosis via mitochondrial pathway. *J Int Soc Oncodev Biol Med* 2015; 36(6): 4825-4831.
- [6] Jassal PS, Kaur G, Kaur L. Synergistic effect of curcuma longa and glycyrrhiza glabra extracts with copper ions on food spoilage bacteria. *Int J Clin Pharm Net* 2015; 7(10): 371-375.
- [7] Hosseinzadeh H, Nassiri-Asl M. Pharmacological effects of *Gly-cyrrhiza* spp. and its bioactive constituents: update and review. *Phytother Res Ptr* 2015; 29(12): 1868-1886.
- [8] Barros L. Characterization of phenolic compounds and antioxidant properties of Glycyrrhiza glabra L. rhizomes and roots. *Rsc Adv* 2015; 5(34): 29-39.
- [9] Norazah B, Ayotunde OO, Ritchie KJ, Sarker S. Comparative cytotoxicity of *Glycyrrhiza glabra* roots from different geographical origins against immortal human keratinocyte (HaCaT), lung adenocarcinoma (A549) and liver carcinoma (HepG2) cells. *Phytother Res* 2015; **29**(6): 944-948.
- [10] Crouch LI, Labourel A, Walton PH, Davies GJ, Gilbert HJ. The contribution of non-catalytic carbohydrate binding modules to the activity of lytic polysaccharide monooxygenases. *J Biol Chem* 2016; **291**(14): 7439-7449.
- [11] Wietz M, Wemheuer B, Simon H, Giebel HA, Seibt MA, Daniel R, et al. Bacterial community dynamics during polysaccharide degradation at contrasting sites in the Southern and Atlantic Oceans. *Environ Microbiol* 2015; 17(10): 3822-3831.
- [12] Bryant KA, Frenck R, Gurtman A, Rubino J, Treanor J, Thompson A, et al. Immunogenicity and safety of a 13-valent pneumococcal conjugate vaccine in adults 18-49 years of age, naive to 23-valent pneumococcal polysaccharide vaccine. *Vaccine* 2015; **33**(43): 5854-5860.
- [13] Hosny Alaa El-Din Shawky, Diab Mohamed Reda, Khattab Rania Abdelmonem, Awad Heba Osama. Immune response to Vi

polysaccharide, heat-killed whole cells, and outer membrane protein of *Salmonella typhi. J Infect Dev Ctries* 2015; **9**(6): 642-649.

- [14] Link P, Wetterauer B, Fu Y, Wink M. Extracts of *Glycyrrhiza uralensis* and isoliquiritigenin counteract amyloid- $\beta$  toxicity in Caenorhabditis elegans. *Planta Med* 2015; **81**(5): 357-362.
- [15] Mousavi SA, Li L, Wei G, Lindström K. Evolution and taxonomy of native mesorhizobia nodulating medicinal *Glycyrrhiza* species in China. *Syst Appl Microbiol* 2016; **39**(4): 260-265.
- [16] Bhargava N, Singh SP, Sharma A, Sharma P, Capalash N. Attenuation of quorum sensing-mediated virulence of *Acinetobacter baumannii* by *Glycyrrhiza glabra* flavonoids. *Future Microbiol* 2015; **10**(12): 1953-1968.
- [17] Zeng G, Shen H, Tang G, Cai X, Bi L, Sun B, et al. A polysaccharide from the alkaline extract of Glycyrrhiza inflata induces apoptosis of human oral cancer SCC-25 cells via mitochondrial pathway. *Tumor Biol* 2015; 36(9): 6781-6788.
- [18] Li Jing, Wang Juan, Li Jinxin, Li Jianli, Liu Shujie, Gao Wenyuan. Salicylic acid induces the change in the adventitious root of *Gly-cyrrhiza uralensis* Fisch.: bioactive compounds and antioxidant enzymes. *Res Chem Intermed* 2016; **42**(2): 1503-1519.
- [19] Gao Haiyang, Sun Zhe, Xiao Chaoni, Zhenga Xiaohui, Zhang Yajun. The metabonomic study of Shaoyao-Gancao decoction in a rat model of acute bronchial asthma by 1H NMR. *Anal Methods* 2016; 8(3): 570-581.
- [20] Tu Y, Zhang GF, Deng KD, Diao Qiyu. Effects of supplementary bee pollen and its polysaccharides on nutrient digestibility and serum biochemical parameters in Holstein calves. *Anim Prod Sci* 2015; 55(10): 1318-1323.
- [21] Menchicchi B, Hensel A, Goycoolea FM. Polysaccharides as bacterial antiadhesive agents and smart constituents for improved drug delivery systems against *Helicobacter pylori* infection. *Curr Pharm Des* 2015; **21**(33): 4888-4906.
- [22] Hajirahimkhan A, Simmler C, Dong H, Lantvit DD, Li G, Chen SN, et al. Induction of NAD(P)H: quinone oxidoreductase 1 (NQO1) by glycyrrhiza species used for women's health: differential effects of the michael acceptors isoliquiritigenin and licochalcone A. Chem Res Toxicol 2015; 28(11): 2130-2141.
- [23] Kim DS, Hurh BS, Shin KS. Chemical characteristics and immunostimulatory activity of polysaccharides from fermented vinegars

manufactured with different raw materials. J Korean Phys Soc 2015; 44(2): 191-199.

- [24] Jeong SJ, Lim HS, Seo CS, Kim JH, Jin SE, Yoo SR, et al. Traditional herbal formula Jakyakgamcho-tang (*Paeonia lactiflora* and *Glycyrrhiza uralensis*) impairs inflammatory chemokine production by inhibiting activation of STAT1 and NF-κB in HaCaT cells. *Phytomedicine* 2015; **22**(2): 326-332.
- [25] Ma C, Wei F, Zhang J, Feng NC. Study of Pb(2<sup>+</sup>), Cd(2<sup>+</sup>) and Ni(2<sup>+</sup>)adsorption onto activated carbons prepared from glycyrrhiza residue by KOH or H3PO4 activation. *Water Sci Technol* 2015; 72(3): 451-462.
- [26] Dunlap TL, Wang S, Simmler C, Chen SN, Pauli GF, Dietz BM, et al. Differential effects of *Glycyrrhiza* species on genotoxic estrogen metabolism: licochalcone A downregulates P450 1B1, whereas isoliquiritigenin stimulates it. *Chem Res Toxicol* 2015; 28(8): 1584-1594.
- [27] Tang J, Chen Z. The protective effect of γ-aminobutyric acid on the development of immune function in chickens under heat stress. *J Anim Physiol Anim N* 2015; **103**(5): 99-102.
- [28] Deretic V, Kimura T, Timmins G, Pope M, Santosh C, Michael M. Immunologic manifestations of autophagy. J Clin Invest 2015; 125(1): 75-84.
- [29] Zhu E, Gai S, Opel C, Kwan BH, Surana R, Mihm MC, et al. Synergistic innate and adaptive immune response to combination immunotherapy with anti-tumor antigen antibodies and extended serum half-life IL-2. *Cancer Cell* 2015; 27(4): 489-501.
- [30] Weist BM, Kurd N, Boussier J, Chan SW, robev EA. Thymic regulatory T cell niche size is dictated by limiting IL-2 from antigen-bearing dendritic cells and feedback competition. *Nat Immunol* 2015; 16(6): 635-641.
- [31] Takeuchi A, Badr ME, Miyauchi K, Ishihara C, Onishi R, Guo Z, et al. CRTAM determines the CD4<sup>+</sup> cytotoxic T lymphocyte lineage. *J Exp Med* 2016; 213(1): 123-138.
- [32] Marshall MR, Varsha P, Mahantappa H, Maier-Peuschel M, Müller ML, Becherer U, et al. VAMP8-dependent fusion of recycling endosomes with the plasma membrane facilitates T lymphocyte cytotoxicity. *J Cell Biol* 2015; 210(1): 135-151.