

# Effect of gynostemma pentaphyllum on Spleen Qi deficiency mice

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**Abstract.** Objective: To explore the therapeutic effect and mechanism of Gynostemma Pentaphyllum (GSM) on spleen-qi deficiency mice. Method: Mice were randomly divided into 4 groups: normal group, model group, Sijunzi decoction, Gynostemma Pentaphyllum group. The mice were given rhubarb decoction at the concentration of 1.56 g/kg by gavage for 5 days to establish spleen-qi deficiency mice model. The basic characteristics of weight, rectal temperature, stool, appetite and other characteristics of mice were observed. Swimming time, Water Maze Experiment, Open Field Test and serum immunoglobulin were detected. Result: Compared with model group, Gynostemma Pentaphyllum can significantly improve body weight of mice. Compared with model group, the swimming time of Gynostemma Pentaphyllum group was significantly increased on the fourth day, the vertical movement time and the number of swimming cycles, as well as the concentration of immunoglobulin IgG in the Gynostemma Pentaphyllum group was increased. Conclusion: Gynostemma Pentaphyllum has a certain effect on the overall treatment of spleen-qi deficiency mice. The therapeutic effect of Gynostemma Pentaphyllum on spleen-qi deficiency mice may be related to increase the immunoglobulin levels.

## 1. Introduction

According to the theory of traditional Chinese medicine, the spleen dominates transportation and transformation, which is considered to be the "acquired foundation", "the source of qi and blood". The spleen and stomach injury will cause many diseases. Due to the rapid pace of modern life and enormous social pressure, more and more people are eating disorders and excessive work, the incidence of modern spleen deficiency is increasing. Therefore, development of the functional products of traditional Chinese medicine has become an urgent problem to be solved. Gynostemma pentaphyllum, also known as the seven-leaf gall, Modern studies have shown that Gynostemma pentaphyllum has anti-fatigue, anti-aging, and anti-hypoxia effects, and long-term drinking can enhance human immunity. At the same time, it has significant sedative, hypnotic, hypolipidemic, hepatoprotective and treatment of migraine effects. Moreover, Gynostemma pentaphyllum drinking is very convenient and delicious, which is more easily accepted by the public. Based on this, this study was to investigate the therapeutic effect and mechanism of Gynostemma pentaphyllum for spleen-qi deficiency mice.

## 2. Materials and Methods

### 2.1. Animal

Kunming mice of SPF grade, 4 weeks old, male, weight 18~22 g, were provided by the experimental



animal center of Henan University of Chinese Medicine.

## 2.2. *Drugs and Reagents*

Sijunzi Decoction (main drugs are ginseng, *Atractylodes macrocephala*, peony, licorice, provided by Henan University of Traditional Chinese Medicine), *Gynostemma pentaphyllum*, rhubarb (provided by Henan University of Traditional Chinese Medicine), mouse immunoglobulin kit (Shanghai Jining Biotechnology Co., Ltd.).

## 2.3. *Instrument*

Water maze (Rongying Instrument Reagent Co., Ltd.) Mine box (Hebi Kate Instrument Co., Ltd.)

## 2.4. *Model Preparation*

The mice was put into the water bath with depth of 20 cm and the water temperature from 25 to 27°C and let the mice swim for 15 minutes. After swimming, the mice were given rhubarb decoction at the concentration of 1.56 g/kg by gavage for 5 days.

We believe the model of spleen qi deficiency is successful if Meet the following conditions: (1)Body weight increased slowly or decreased(2)Burnout, lazy movement, getting together(3)Soft or diluted stools (4)No significant changes in rectal temperature (5)educed food intake(6)dull backs and lusterless of backs (7)Swimming endurance decline. The animals met the above conditions and judged the success of the spleen-qi deficiency model.

## 2.5. *Grouping and Intervention Methods*

The mice were randomly divided into the normal group, the model group, the sijunzi decoction group and the gynostemma pentaphyllum group. The spleen deficiency model was established by using the rhubarb decoction. Sijunzi soup group was given Sijunzi decoction with 4.29g/kg and gynostemma pentaphyllum group was given gynostemma pentaphyllum decoction with 1.95g/kg.

## 2.6. *Observation Index*

**2.6.1. General Condition Observation** Observe the changes of anus temperature, stool, the mount of uptake material object and body mass in mice.

**2.6.2. Evaluation the Mental State of Spleen-qi Deficiency mice** Observe the basic signs of skin gloss, skin tone, activity level and sensitivity.

**2.6.3. Determination the Swimming Time** [7] The mice were put into a glass tank with water depth of 50 cm and the water temperature kept at  $20 \pm 0.5$  °C. We record the swimming time of mice when the mice head was sank in the water for 10 seconds

**2.6.4. The Serum Immunoglobulin IgG of Spleen-qi Deficiency Mice**[8] Levels of serum immunoglobulin IgG were analyzed using a commercially available ELISA kit according to the manufacturers' instructions.

**2.6.5. Water Maze Experiment** [9] The experimental device is a circular pool with diameter of 1m and height of 30cm. The inner wall of the pool is black and the depth of water in the pool is 15cm. The water temperature is room temperature. The light in the room is constant and no light is directly irradiated in the pool. The inside of the pool wall is marked with different shapes. A circular platform with a diameter of 10 cm and a height of 13 cm is fixed between one of the markers and the platform, and the platform is hidden 2 cm below the water surface.

**2.6.6. Open Field Test**[10] The testing box of mouse behaviour on open field is 100cm x 100cm x 80cm with bottom and peripheral wall in black and the bottom is divided into 25 small 20cm x 20cm cells by white lines. The operator holds the mouse which is put at the centre of the open field at 1/3 of

tail and the behaviour of it in 3 minutes is recorded by camera system. The operator record Vertical score. Vertical score is times the mouse standing (Include situations in which the mouse rears or climbs the cage). After model is established, it is measured 3 minutes each time. After experiment, the faces of each mouse are removed and the bottom of the field is sprayed by 70% ethanol and wiped by a clean duster cloth to avoid the influence of residual odor of the previous mouse on the experiment.

2.7. *Statistical Method* Data are presented as the mean  $\pm$  SD. Differences were evaluated using Statistical Package for Social Science 19.0 (SPSS11.0, Chicago, IL, USA). Statistical analysis was performed using One-way ANOVA followed by least-significant difference (LSD).  $p < 0.05$  was considered to be statistically significant[5]

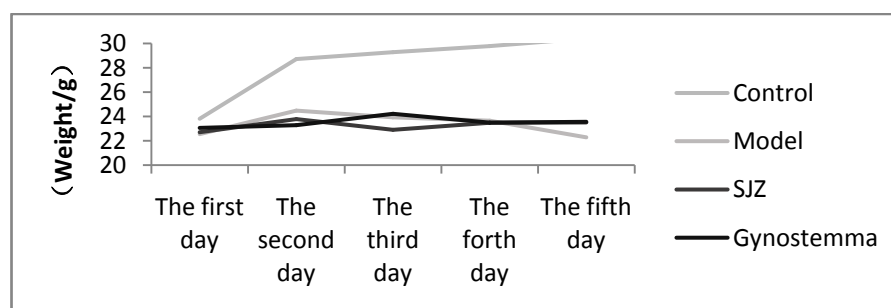
### 3. Results

#### 3.1. Effect of Gynostemma Pentaphyllum on Body Weight of Spleen-Deficiency Mice.

Compared with the normal group, the body weight of model group was significantly decreased. Compared with the model group, the body weight of Gynostemma Pentaphyllum group was significantly increased.

**Table 1.** Effect of Gynostemma Pentaphyllum on Body Weight in Spleen -Deficiency Mice (unit:g)

group	Normal	Model	SJZ	GSM
The first day	23.76 $\pm$ 2.37	24.80 $\pm$ 3.13	22.71 $\pm$ 3.12	25.06 $\pm$ 1.93
The second day	28.70 $\pm$ 3.24	24.48 $\pm$ 4.85	24.66 $\pm$ 2.95	25.28 $\pm$ 3.17
The third day	29.30 $\pm$ 3.44	23.92 $\pm$ 5.04	22.03 $\pm$ 5.09	24.22 $\pm$ 4.24
The forth day	29.77 $\pm$ 3.45	24.65 $\pm$ 5.85	21.48 $\pm$ 6.07	26.55 $\pm$ 4.69
The fifth day	30.43 $\pm$ 4.10	25.23 $\pm$ 7.62	22.50 $\pm$ 5.62	26.16 $\pm$ 5.44



**Figure 1.** Effect of Gynostemma Pentaphyllum on Body Weight in Spleen-deficiency Mice

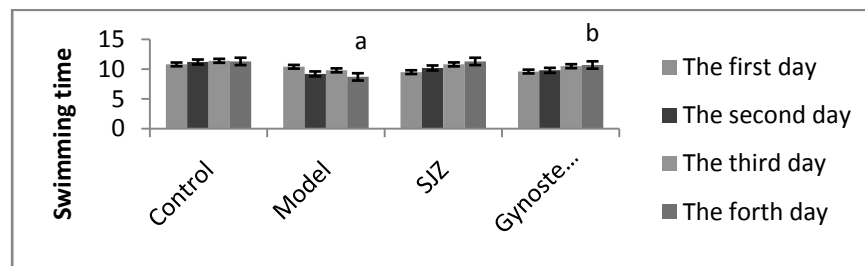
#### 3.2. Effect of Gynostemma Pentaphyllum on Swimming Time of Spleen-Deficiency Mice

Compared with the normal group, the swimming time on the fourth day of the model group was significantly decreased ( $P < 0.05$ ). Compared with the model group, the swimming time of Gynostemma Pentaphyllum group was significantly increased on the forth day ( $p < 0.05$ ).

**Table 2.** Effect of Gynostemma pentaphyllum on Swimming Time of Spleen-Deficiency Mice (unit: min)

Group	The first day	The second day	The third day	The forth day
normal	10.8 $\pm$ 2.75	11.2 $\pm$ 2.96	11.4 $\pm$ 1.79	11.3 $\pm$ 0.63
Model	10.4 $\pm$ 1.23	9.2 $\pm$ 1.14	9.8 $\pm$ 2.84	7.7 $\pm$ 1.28 <sup>a</sup>
SJZ	9.5 $\pm$ 0.86	10.2 $\pm$ 1.37	10.8 $\pm$ 1.53	11.3 $\pm$ 1.54
GSM	9.6 $\pm$ 2.13	9.8 $\pm$ 0.52	10.5 $\pm$ 1.64	10.7 $\pm$ 0.77 <sup>b</sup>

Compared with the normal group, <sup>a</sup> $P < 0.05$ ; compared with the model group, <sup>b</sup> $P < 0.05$ .



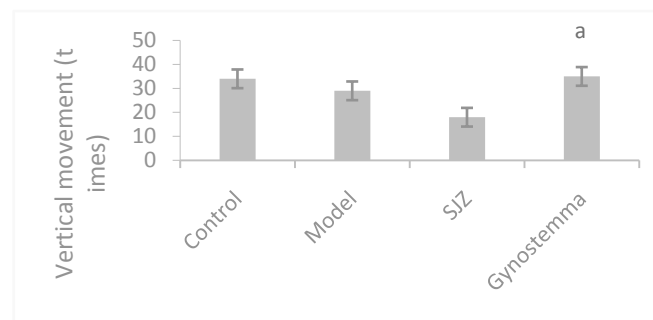
**Figure 2.** Effect of Gynostemma pentaphyllum on Swimming Time of Spleen-Deficiency Mice

### 3.3. Effect of Gynostemma Pentaphyllum on Open Field Test of Spleen-qi Deficiency Mice

Compared with the normal group, the vertical movement time in the model group was decreased. Compared with the model group, the vertical movement time of the mice in the Gynostemma Pentaphyllum group was significantly increased.

**Table 3.** Effect of Gynostemma pentaphyllum on open field of spleen-qi deficiency mice (unit: min)

Group	Vertical movement (times)
Nomal	34±12.05
model	29±12.65
SJZ	18±14.86
GSM	35±14.30 <sup>a</sup>



Compared with the model group, <sup>a</sup>P < 0.05

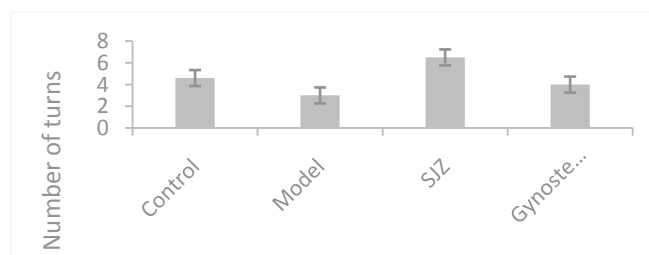
**Figure 3.** Effect of Gynostemma Pentaphyllum on open field test of spleen-qi deficiency mice

### 3.4. Effect of Gynostemma Pentaphyllum on Water Maze Test in Spleen- deficiency Mice

Compared with the normal group, the number of swimming cycles in the mice of model group was decreased. compared with the model group, the number of swimming cycles of Gynostemma Pentaphyllum group was increased.

**Table 4.** Effect of Gynostemma pentaphyllum on water maze test in spleen-deficiency mice (unit: min)

group	Number of turns
normal	4.6±0.57
model	3.0±0.81
SJZ	6.5±0.58
GSM	4.0±0.81



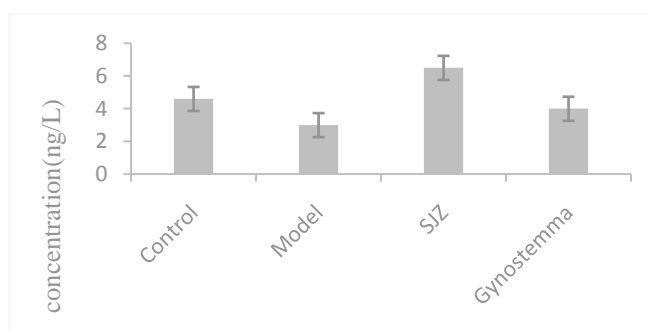
**Figure 4.** Effect of Gynostemma pentaphyllum on water maze test in spleen-deficiency mice

### 3.5. Effect of Gynostemma Pentaphyllum on Immunoglobulin IgG Concentration in Mice with Spleen-deficiency.

Compared with the normal group, the immunoglobulin IgG concentration in the model group was decreased; compared with the model group, the concentration of immunoglobulin IgG in the Gynostemma Pentaphyllum group was increased.

**Table 5.** Effect of Gynostemma Pentaphyllum on Immunoglobulin Concentration in Spleen-Deficiency Mice

group	concentration(ng/L)
normal	1.432±0.083
model	1.428±0.047
SJZ	1.429±0.163
GSM	1.429±0.003



**Figure 5.** Effect of Gynostemma Pentaphyllum on Immunoglobulin Concentration in Spleen-Deficiency Mice

## 4. Discussion

In this experiment, the mice were forced swim, then the mice were given rhubarb decoction at the concentration of 1.56 g/kg by gavage for 5 days to establish the model of spleen-qi deficiency. After the intervention of Gynostemma pentaphyllum, we found that Gynostemma Pentaphyllum could improve the symptoms of spleen qi deficiency, inhibit the weight loss of mice, prolong the swimming time and enhance exercise ability.

Chinese medicine believes that the spleen can elevate essential substances. The spleen function is normal, the five organs are fully supported and cognitive ability will also be normal. If the spleen-qi is weak, the body's cognitive function is reduced.

Open field test results show that Gynostemma Pentaphyllum can improve vertical activity in spleen-qi deficiency mice. Water maze results show that Gynostemma Pentaphyllum can increase the number of swimming cycles in one minute. All these show that Gynostemma Pentaphyllum can improve cognitive function in mice.

Immunoglobulin G is the most important anti-pathogenic microorganism antibody. This experiment shows that Gynostemma Pentaphyllum can increase the immunoglobulin G. This study

shows that *Gynostemma Pentaphyllum* can improve the symptoms of spleen-qi deficiency mice, improve immune function and improve cognitive dysfunction of mice.

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