

# Effects of Yiqi Fanggan Mixture on Anti-Stress Response and Immunity of Mice

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## Abstract

To study the effect of Yiqi Fanggan Mixture on the anti-stress response and immunity of mice, a Chinese herbal compound (composed of some tonic Chinese medicine, including astragalus flavone, deep-fried atractylodes macrocephala koidz, fortune eupatorium herb, cyrtomium fortunei, white cardamom, windproof, dyers woad root, licorice, jobstears seed) was given to the mice for intragastric administration once a day for 2 weeks. Mice in each group are subjected to normal pressure hypoxia test, swimming test, cold resistance test and high temperature resistance test. Organ index and serum hemolysin content of mice were also monitored. The results indicated that the Yiqi Fanggan Mixture can improve the survival time of mice under the condition of normal pressure hypoxia; prolong the swimming time and high temperature resistance time of mice; reduce the death rate of mice under the low temperature condition; and improve the activity of serum hemolysin and organ index of mice. The results showed that Yiqi Fanggan Mixture could improve the ability of anti-stress response and immune ability in mice.

## Keywords

Yiqi Fanggan Mixture, Stress, Immunity, Mice

## 1. Introduction

Stress response is an adaptive response produced when the body is stimulated by changes of body's internal and external environment. During stress, a series of neuroendocrine reactions will appear, mainly manifested by the excitation of the sympathetic nervous system and the increased secretion of adrenocortical hormones by the hypothalamic-pituitary-adrenaline axis (HPA), which is a protective response. Stress ability is the ability of the body to adapt to various emergency situations. However, just like everything has two sides, persistent and excessively strong stress response will also cause obvious damage to the body [1],

which are mainly manifested in the destruction of the body's defense system, reducing the body's immunity, and ultimately lose resistance against disease, and then lead to disease, especially infectious diseases.

At present, more and more researches on anti-stress ability tend to focus on traditional Chinese medicine. Traditional Chinese medicine and its preparations have been reported to have good anti-stress ability [2] [3] [4] and the effect of improving body immunity. The experiment, with the Chinese herbal compound—Yiqi Fanggan Mixture—consisted of tonic traditional Chinese medicinal such as astragalus flavone, deep-fried atractylodes macrocephala koidz, fortune eupatorium herb, cyrtomium fortunei, white cardamom, windproof, dyers woad root, licorice, jobstears seed, etc., studies the effects of the Yiqi Fanggan Mixture on the anti-stress response and immunity of mice, hoping to provide reference for the development of Chinese medicine immune enhancer under the background of novel coronavirus disease 2019 (COVID-19).

## 2. Materials and Methods

### 2.1. Prescription and Preparation [5]

Preparation: Decoct Prescription: Astragalus flavone 150 g, deep-fried atractylodes macrocephala koidz 100 g, fortune eupatorium herb 100 g, cyrtomium fortune 50 g, white cardamom 100 g, windproof 100 g, dyers woad root 100 g, licorice 50 g, jobstears seed 100 g.

Preparation: Decoct the above 9 herbs with water for three times. After 1.5 h of the first boiling, 1 h of the second boiling and 1 h of the third boiling, combine the decoction, centrifuge, filter, concentrate the filtrate to 850 ml, add 2 g potassium sorbate, mix, fill to 100 ml, and sterilize at 105°C for 30 min.

### 2.2. Animal Grouping and Methods of Administration

Kunming male mice count 350, clean grade, weight ( $20 \pm 2$ ) g, provided by Yangtze University Medical Experimental Animal Center. Of these, 280 mice were first modeled and then divided into four groups at random: the large dose of the Yiqi Fanggan Mixture group (giving Yiqi Fanggan Mixture at the 5.0 ml/100g body weight), and the small dose group of the Yiqi Fanggan Mixture (giving Yiqi Fanggan Mixture at the 2.5 ml/100g body weight equivalent to concentrated crude drug solution), and blank control group (giving the same volume of physiological saline), and the positive control group (Andolin capsules,  $0.64 \text{ g}\cdot\text{kg}^{-1}$ ). The remaining 70 mice were used as sham control group (giving the same volume of physiological saline). Each group is given intragastric administration, 1 time/d for 2 weeks.

The experimental plan has been approved by the Animal Experimental Ethics Committee of Yangtze University.

### 2.3. Test of Anti-Stress Ability of Mice

1) Normal pressure hypoxia test [6]. Taking the five groups of mice, each

group (n = 10) of mice placed in a 250 ml bottle containing 20 g of sodium lime. Seal the cap with petroleum jelly around it. Observe the time when mice died of asphyxiation due to lack of oxygen. The survival time of mice was recorded by the criterion of death based on the cessation of breathing of mice. Experiments were carried on under  $26^{\circ}\text{C} \pm 10^{\circ}\text{C}$ .

2) Cold-resistant test [7]. Taking the five groups of mice, each group has 10 mice. Put the mice in  $-5^{\circ}\text{C} \pm 1^{\circ}\text{C}$  refrigerator. The animals are removed after 1 h, counting the survival amount of mice in each group.

3) High temperature-resistant test [8]. Taking the five groups of mice, each group has 10 mice, and the mice were placed in a constant temperature oven at a temperature of  $45^{\circ}\text{C} \pm 1^{\circ}\text{C}$ . Observe the survival time of the mice.

4) Swimming test [8]. Taking the five groups of mice, each group has 10 mice, attached a weight of 7% by body mass to each mice tail, and then individually place in a constant temperature tank (45 cm × 40 cm × 22 cm), water temperature  $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$ , depth of 20 cm. Observe and record the time of each mouse from swimming to no longer surfacing within 20 seconds of sinking (swimming time).

## 2.4. Immune Testing of Mice

1) Determination of mouse organ index. The mice were sacrificed with cervical dislocation. Weigh the mouse first, then soak the mouse in 75% alcohol for 3 min, move the mouse to the ultra-clean workbench, take out the spleen and thymus, wipe the blood on the organ with filter paper, weigh the wet weight of the organ, and calculate the organ index of the mouse. Mouse organ index = organ weight/body weight (mg/g).

2) Determination of serum hemolytic pigment of mice. Starting from the 6<sup>th</sup> day before the end of administration, the mice were intraperitoneally injected with 0.2 ml of 20% SSRBC every day. After 6 days, the eyeballs were removed for blood collection and placed in an anticoagulant centrifuge tube, centrifugated at 2000 r/min for 10 min at  $4^{\circ}\text{C}$ . The serum was carefully absorbed and diluted 50 times with normal saline for testing. 1 ml of 50 times diluted mouse serum was in turn added to another 10 ml centrifugal tube, rehydrated (guinea pig serum) to 0.5 ml, added 20% SSRBC 0.5 ml, mixed and then placed in  $37^{\circ}\text{C}$  constant temperature water bath for 10 min, and terminated the reaction by ice bath. Blank control tube was filled with physiological saline, and the others were treated in the same way as example tubes. Read the absorbance value at 540 nm wavelength using a photometer.

## 3. Results

### 3.1. Effects of Yiqi Fangan Mixture on Anti-Stress Response of Mice

The effect of Yiqi Fangan Mixture on the anti-stress ability of mice is shown in **Table 1**. As can be seen from **Table 1**, the Yiqi Fangan Mixture can signifi-

cantly prolong the survival time of mice under normal pressure hypoxia, and is related to dose. The Yiqi Fanggan Mixture can improve the survival rate of mice in cold conditions, prolong the survival time of mice in high temperature environment, significantly extend the swimming time of mice, and are all dose-related.

### 3.2. Effects of Yiqi Fanggan Mixture on Immunity in Mice

#### 3.2.1. Effect on Mouse Organ Index

The results of this test are shown in **Table 2**. Compared with the control group, the organ index of the administration group is improved to a certain extent, and the difference between the high-dose group and blank control group is statistically significant blank control group ( $P < 0.05$ ), but the difference between the small dose group and blank control group is not statistically significant ( $P > 0.05$ ).

#### 3.3.2. Effect on Serum Hemolytic Content of Mice

The effect of Yiqi Fanggan Mixture on serum hemolytic pigment content in mice can be seen in **Figure 1**. **Figure 1** shows that compared the high-dose and low-dose groups with blank control group, the content of serum hemolytic hemolyte in mice has a statistical difference ( $P < 0.05$ ), indicating that the Yiqi Fanggan Mixture has a certain effect on improving the activity of serotonin.

## 4. Discussion

Stress is a nonspecific systemic reaction of the body when stimulated by various

**Table 1.** Effects of Yiqi Fanggan Mixture on anti-stress ability of mice.

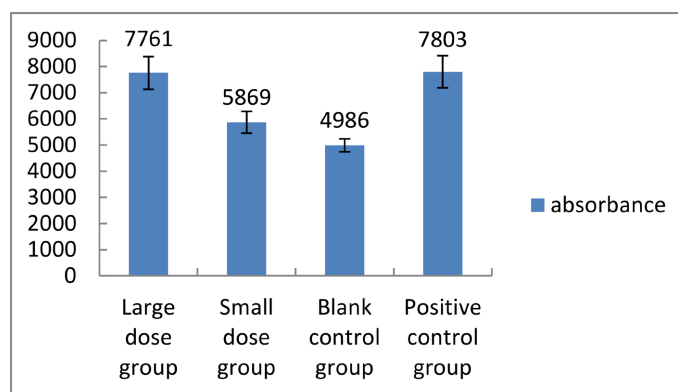
Grouping	Quantity	Survival time under normal pressure hypoxia/min	Number of survivors in the cold	Survival time at high temperatures/min	Swimming time/min
Large dose group	10	34.6 ± 7.5 <sup>Δ</sup>	8 <sup>Δ</sup>	23.6 ± 7.1 <sup>Δ</sup>	29.6 ± 6.5 <sup>Δ</sup>
Small dose group	10	28.4 ± 6.7 <sup>Δ</sup>	7 <sup>Δ</sup>	21.5 ± 5.2 <sup>Δ</sup>	25.3 ± 6.4 <sup>Δ</sup>
Blank control group	10	23.4 ± 7.3	5	17.9 ± 5.4	22.6 ± 6.5
The Positive control group	10	33.7 ± 8.9 <sup>Δ</sup>	8 <sup>Δ</sup>	23.4 ± 7.8 <sup>Δ</sup>	29.3 ± 7.6 <sup>Δ</sup>
Sham control group	10	44.6 ± 11.9*	15*	31.7 ± 9.6*	38.5 ± 11.4*

Note: Compared with blank control group, <sup>Δ</sup> $P < 0.05$ ; Compared with the other groups, \* $P < 0.01$ .

**Table 2.** Effects of Yiqi Fanggan Mixture on immune organ index in mice.

Grouping	Thymus index (mg/g)	Spleen index (mg/g)
Large dose group	2.785 ± 0.735 <sup>Δ</sup>	11.026 ± 4.521 <sup>Δ</sup>
Small dose group	2.521 ± 0.759	10.826 ± 5.264
Blank control group	2.429 ± 0.573	10.563 ± 4.125
Positive control group	2.884 ± 1.065 <sup>Δ</sup>	12.279 ± 6.373 <sup>Δ</sup>

Note: Compared with blank control group, <sup>Δ</sup> $P < 0.05$ .



**Figure 1.** Effect of Yiqi Fanggan Mixture on serum hemolysin content of mice.

internal and external environmental factors, which is closely related to the change of the neuroendocrine system of the body under stimulation, and is mainly manifested in the neuroendocrine reaction of the synonymous-adrenal myelin and hypothalamus-pituitary-adrenal cortex. According to the pathogenesis of traditional Chinese medicine and the theory of pathogenesis, the body's positive gas plays a decisive role in maintaining normal physiological function, anti-disease and exorcism and post-disease self-healing [9]. The pathogenesis of deficiency syndrome is mainly insufficient positive gas, the physiological functions such as viscera and meridian are weakened, and the ability to resist evil gas decreases [10]. Modern pharmacological research also shows that people of deficiency syndrome according to TCM dialect often show the signs of low immune function, while tonic Chinese medicine can mostly enhance the body's non-specific immunity, cellular immunity and body fluid immune function [8], thereby improving disease resistance. This study shows that the Yiqi Fanggan Mixture can significantly prolong the survival time of mice under normal pressure hypoxia and high temperature conditions, prolong the swimming time of mice, reduce the mortality rate of mice at low temperature, and show that the Yiqi Fanggan Mixture can enhance the mice's ability to resist hypoxia, oxygen fatigue and improve mice's ability of anti-stress.

Immunity is the ability of animals to protect themselves against external enemies, and the strength of immunity can measure the body's resistance to pathogenic microorganisms [11]. Serum hemolytic pigment is an important indicator of the immune function of animal bodies, and when hemolytic production content increases, the immunity of mice is also enhanced. The thymus and spleen, as important immune organs, play an important role in the regulation of the body's immune function, and the thymus index and spleen index depend on the effect of traditional Chinese medicine compound on the immune organs. In this experiment, compared with blank control group, there is a statistical difference in the content of serum hemolytic pigment in mice ( $P < 0.05$ ), which shows that the Yiqi Fanggan Mixture has a certain effect on the activity of serosolytic hemolytic pigment. At the same time, the thymus index and spleen index of mice in the treatment group are improved, and the difference between the large dose

group and blank control group is statistically significant ( $P < 0.05$ ), indicating that the Yiqi Fanggan Mixture could effectively improve the immunity of mice.

“The treatment of potential diseases” is an important application of traditional Chinese medicine, “the treatment of potential diseases” emphasizes that treat before the occurrence of diseases, prevention is more important than treatment, in advance to take effective preventive measures can prevent the invasion of diseases and evils, avoid the occurrence and development of diseases. It is an important principle of “the treatment of potential diseases” to support positive gas and remove pathogenic factors. Jade screen dispersion is a commonly used important foundational agent to foster positive gas and drive away evil. There has been studies that has functions such as improving immunity, antiviral effect and so on, and the jade screen dispersion is often used in pneumonia, common cold, viral cold and other respiratory diseases prevention [12] [13]. COVID-19 prevention prescriptions released nationwide show that many prescriptions are based on added or subtracted Yupingfeng Powder. [14] [15]. Yiqi Fanggan Mixture is also on the basis of the classical famous prescription yupinfeng powder and combining with the characteristics of COVID-19 and regional climate, population constitution characteristics of Jiangnan plain to add or subtract. In the prescription, *Astragalus membranaceus* can supply with gas of spleen and lung. Pyobaizhu has the function of replenishing gas to invigorate the spleen, helping astragalus to cultivate soil and produce gold. Fangfeng can dispel evil and relieve the appearance of illness. Perylum and white cardamom play the role of aromatizing dampness and warming up the spleen. Coix seed has the effect of water infiltration and invigorating the spleen. Radix isatidis and cyrtomium fortunei clear away heat and toxic materials. Licorice and all the drugs together play roles in tonifying gas and solid surface, dampness and spleen, heat and detoxification. Most of the medicines in the prescription go through the lung, spleen and stomach, focusing on strengthening the gas of the lung and protecting the spleen and stomach, so as to achieve the purpose of boosting the healthy gas and guarding evil in the outside. At the same time, modern pharmacological research shows that a variety of Chinese medicine in the Yiqi Fanggan Mixture has the effect of enhancing immunity, anti-viral effect, anti-infection effect and so on, such as astragalus flavone polysaccharides, saponins, flavonoids and amino acids in astragalus flavone, which can improve immune function, and have a unique role in anti-viral effect, anti-infection effect and other aspects [16]; Wind protection has anti-microbial, anti-inflammatory and immunomodulatory effects [17]; Volatile oils, endoester Mixtures, polysaccharides, glycosides and other components in white art can achieve anti-inflammatory effects by inhibiting the production of cytokines that mediate inflammatory reactions and regulating immunity [18]. Licorice has many pharmacological activities, such as antibacterial, anti-inflammatory, antiviral, immunomodulation effects, etc. [19]. The various components of radix isatidis have antibacterial, anti-endotoxin, antiviral and immunomodulatory effects, of which the anti-flu virus effect is particularly ob-

vious [20] [21]. Therefore, this prescription has a pharmacological basis in improving positive gas, enhancing immunity and preventing new coronary pneumonia.

### Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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