

# Danzhi Xiaoyao Powder Reduced the Scratching Behavior Exaggerated by Chronic Psychological Stress in Mice

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## Research

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# Abstract

**Objective:** To explore the efficacy and possible mechanism of Danzhi Xiaoyao Powder in the treatment of scratching behavior worsened by psychological factors.

**Methods:** Male BALB/c mice were divided into control group (CON group), chronic WAS group (WAS group) and chronic WAS + Danzhi Xiaoyao Powder group (DZX group). All mice excluded control group were exposed to 1 h water avoidance stress (WAS) for ten consecutive days. DZX group were given Danzhi Xiaoyao Powder solution by intragastric administration every day, and the other two groups were given the same amount of saline. Twenty-four hours after the last stress session, the mice were injected into the back of the neck with a condensation product of N-methyl-p-methoxyphenethylamine with formaldehyde (compound 48/80), and their scratching behavior was then observed for 120 min. The scratching number was recorded, the histamine levels in the plasma were examined, and the behavioral changes of depression in mice before and after treatment were observed by open field test and forced swimming test.

**Results:** The scratching number was significantly higher in the WAS group than in the DZX group, which still higher than in the CON group. The peak histamine in the plasma after the compound 48/80 injection were also significantly higher in the WAS group in comparison to the DZX group. In terms of depression behavior, the number of standing in the DZX group was more than that in the WAS group and less than that in the CON group, and the immobility time in the forced swimming test was less than that in the WAS group and more than that in the CON group.

**Conclusion:** Danzhi Xiaoyao Powder can relieve the scratching behavior of mice under the influence of WAS, and its possible mechanism is to control the release of histamine and improve the depressive state of mice.

## 1. Background

In pruritic cutaneous diseases, pruritus sensation is the main symptom which perplex patients profoundly<sup>[1]</sup>. At the same time, patients with pruritus disease have a wide range of adverse psychological states such as anxiety and depression, which can worsen pruritus again and make patients endure great pain both psychologically and physically. A number of independent epidemiological studies at home and abroad have also confirmed the relationship between pruritus and anxiety, depression, mental tension and other adverse psychological state<sup>[2]</sup>. A cross-sectional study in 13 European countries published in 2020 shows that pruritus greatly increases the burden of mental illness on patients with cutaneous diseases<sup>[3]</sup>. Both acute and chronic stress can significantly affect pruritus in healthy people and those diagnosed with pruritic cutaneous and systemic diseases, leading to a vicious circle in which stress exacerbates pruritic and vice versa<sup>[4]</sup>. In terms of treatment, psychotherapy is also of great help in relieving pruritus. After analyzing the existing treatment of pruritus in atopic dermatitis, Pavlis J et al found that patient education, sleep management and stress relief are important means to improve the

curative effect<sup>[5]</sup>. At present, the medical profession of TCM has also noticed the close relationship between psychology and pruritus, and there is a good clinical efficacy in the treatment of skin pruritus according with the liver, and a clinical consensus has been obtained. However, its mechanism is still a blank in the research at home and abroad. From the viewpoint of emotional pathogenesis of traditional Chinese medicine, this study focuses on the worsening effect of psychological factors on pruritus, and chooses Danzhi Xiaoyao Powder, a representative prescription for the treatment of pruritus according with the liver. Using the simple healthy animal model established in the previous study, which can effectively eliminate the interference of non-psychological factors, to explore the efficacy and possible mechanism of the treatment of skin pruritus worsened by psychological factors according with the liver.

## **2. Materials And Methods**

### **2.1 Materials**

#### **2.1.1 Animals**

Male BALB/c mice (8 weeks of age) were individually maintained at a constant temperature (23–25 °C) on a 12-h light/12-h dark cycle with food and water freely available. Experiments were always done in the morning (between 9:00 a.m. and 12:00 a.m.) to minimize variation due to circadian rhythmicity.

#### **2.1.2 Drugs**

The prescription compositions of Danzhi Xiaoyao Powder are mudanpi 3 g, zhizi 3 g, chaihu 6 g, danggui 6 g, fuling 6 g, baizhu 6 g, gancao 3 g. The drugs above were concentrated in water at twice the body weight dose of adult 70 kg to contain the crude drug 0.38 g/ml.

#### **2.1.3 Reagent**

Mouse histamine enzyme-linked Immunoassay Kit, 96T, Shanghai enzyme-linked Kit. compound 48/80 (Sigma, St. Louis, MO).

#### **2.1.4 Instruments**

Standard enzyme labeling instrument (Multiskan MK3, Thermo Scientific). Automatic plate washing machine (DEM- 3, Beijing Top Analytical instrument Co., Ltd). Electric constant temperature incubator (SKP- 02.600, Huangshi Hengfeng Medical equipment Co., Ltd). 8-mm video camera (CCD-TRV86, Sony, Tokyo, Japan).

## **2.2 Methods**

### **2.2.1 Modeling**

The animal model of " the exaggeration of scratching behavior induced by chronic psychological stress" established by the author in 2013 was used<sup>[6]</sup>. Mice were exposed to 1 h water avoidance stress (WAS)

for ten consecutive days. They were placed on a glass platform (diameter, 7 cm) in the middle of a plastic container filled with water to 1 cm below the height of the platform.

## **2.2.2 Grouping and Administration**

The mice were randomly divided into three groups: control group (CON group), chronic WAS group (WAS group), chronic WAS + Danzhi Xiaoyao Powder group (DZX group). The DZX group were exposed to 1 h WAS for ten consecutive days, and given intragastric administration of Danzhi Xiaoyao Powder solution according to 0.04 ml/ g body weight once a day. The WAS group were exposed to 1 h WAS for ten consecutive days, and given intragastric administration of equal volume of normal saline once a day. The CON group were isolated 1 h for ten consecutive days, and given intragastric administration of equal volume of normal saline once a day.

## **2.2.3 Scratching behavior experiment**

Twenty-four hours after the last WAS session, a total of 10 µg of compound 48/80 was dissolved in 50 µl saline and 5 µl solution was injected subcutaneously into the back of the neck. Immediately after the injection, each mouse was put back into an individual cage and the scratching behavior was then recorded for 120 min using an 8-mm video camera under quiet conditions. In general, the mice showed several scratches of the injected site for about 1 s and such scratching behavior was counted as one bout of scratching. Every bout of scratching was counted across each 10 min bin. Any information as to which group was exposed to WAS or treated with drugs was blinded to the observer.

## **2.2.4 Measurement of plasma histamine level**

32 mice were taken from each group to examine time-course changes in plasma histamine level, the mice were sacrificed by a cervical dislocation before, and 15, 30, 60 and 120 min after a subcutaneous injection of compound 48/80. Whole blood was obtained by a cardiac puncture using a heparinized syringe, and then was collected in EDTA-coated sample tubes. The plasma obtained by centrifugation was stored at -80 °C for histamine and corticosterone determination later. The plasma histamine and corticosterone levels were measured using commercially available ELISA kits.

## **2.2.5 Study of depression behavior**

Open field test and forced swimming test were used to detect the degree of depression in mice and analyze the improving effect of Danzhi Xiaoyao Powder on depressive behavior.

Open field test: the mice were put into four buckets of the open field activity test box (the area with the central radius of 7.5 cm in the barrel was divided into the central area), and the antidepressant mode in the open field experiment computer image real-time detection and analysis system was selected. The computer automatically records six indexes, such as the total distance, the exercise time, the average speed, the distance ratio and the time ratio in the central area. At the same time, the number of standing times that the mice had just been placed in the test box in the 5 min was manually recorded (the animals'

forelegs were off the ground at the same time, or both forelegs were placed on the barrel wall as standing once).

Forced swimming test: The animals were placed separately in a cylindrical plastic bucket (water depth 15 cm, diameter 20 cm), and the water temperature was  $(25 \pm 1) ^\circ\text{C}$ . After forced swimming for 6 min, the cumulative immobility time of mice in 4 min was recorded with a stopwatch. (immobility, that is, "the mouse stops struggling or floating, and the limbs move slightly to keep the head on the surface of the water").

## 2.2.6 Statistical analysis

SPSS20.0 was used for statistical analysis, all data are expressed as average  $\pm$  standard deviation ( $\bar{x} \pm s$ ). The analysis of variance was compared between groups, and the statistical differences among groups were determined by single factor analysis of variance. A value of  $P \leq 0.05$  was considered to be significantly different from the corresponding value.

## 3. Result

### 3.1 Scratching behavior experiment

After two-way ANOVA analysis of scratching times among the three groups, it was found that there were statistical differences among the three groups. The scratching times in the DZX group was significantly lower than that in the WAS group, but more than that in the CON group. The single factor analysis of variance of the data in each period showed that there was a significant difference between the DZX group and the WAS group at 0–10 min, 10–20 min, 100–110 min. The results showed that taking Danzhi Xiaoyao Powder significantly reduced the scratching times of WAS model mice, especially in the early stage of observation. As shown in Fig. 1.

### 3.2 Determination of plasma histamine level

There was significant difference in the level of plasma histamine between WAS group and CON group, WAS group and DZX group ( $p < 0.05$ ). The level of plasma histamine in CON group and DZX group was lower than that in WAS group. there was no significant difference between DZX group and CON group. In the analysis of each time point, at 15 min, 30 min and 120 min, the plasma histamine level in the DZX group was significantly lower than that in the WAS group, so Danzhi Xiaoyao Powder could significantly reduce the plasma histamine level in WAS model mice. As shown in Fig. 2.

### 3.3 Depressive behavior test

In the open field experiment, the number of standing in the DZX group was significantly higher than that in the WAS group, and the immobility time was significantly decreased in the forced swimming experiment. The number of standing times in the CON group was the most and the immobility time was the least compared with the other two groups. As shown in Figs. 3 and 4.

## 4. Discussion

An unpleasant experience of severe, long-term, repeated pruritus and the deterioration of local skin lesions bring great psychological pressure to patients, and psychological pressure can also directly worsen pruritus symptoms and local skin lesions; scratching can temporarily relieve the unpleasant experience caused by pruritus and get a sense of euphoria. Over time, scratching is no longer just a way for patients to relieve pruritus, but also a way for patients to alleviate other psychological pressure and get euphoria. Therefore, psychological pressure participates in every link of the vicious circle of pruritus and scratching. Pruritic cutaneous diseases is a psychosomatic disease closely related to psychological stress, which has brought great pain to patients both psychologically and physically for a long time<sup>[7]</sup>. Therefore, starting with regulating emotions and reducing psychological pressure, it will have a good therapeutic effect on pruritus diseases, which can effectively break the vicious circle of psychological pressure of pruritus and scratching<sup>[8-9]</sup>.

Traditional Chinese medicine believes that the liver stores blood, and governs regulating, it likes the smooth movement of qi and hates depression. If being in a bad mood, then the liver qi is stagnated and turns to heat with the passing of time, then the blood will be disturbed. The liver governs the wood, the spleen governs the earth, the liver qi invades spleen, causing spleen deficiency and dampness, and the liver-heat and spleen dampness invades the skin, causing skin pruritus, eczema, dermatitis and other skin diseases<sup>[10]</sup>. It should be treated by soothing the liver, clearing heat and invigorating the spleen to eliminate dampness<sup>[11]</sup>. Danzhi Xiaoyao Powder is published in Xue Xinfu's *Abstract of Internal Medicine*, which is based on the famous prescription "Xiaoyao Powder" plus zhizi and mudanpi, also known as Jiawei Xiaoyao Powder and Bawei Xiaoyao Powder, which has the effect of soothing the liver and relieving depression, invigorating the spleen and Harmonizing Ying-qi, and clearing stagnated heat. It has been followed by doctors of all ages as a classic prescription for the treatment of liver depression and fire syndrome<sup>[12]</sup>.

In this study, healthy mice were selected for modeling, which can effectively eliminate the effect of factors other than psychological pressure on pruritus symptoms. The results showed that after treatment, the number of scratches in the DZX group was significantly less than that in the WAS group, indicating that Danzhi Xiaoyao Powder could effectively improve the skin pruritus under the influence of psychological pressure.

It is well known that histamine is the most common pruritus mediator, which exists in human basophils and mast cells. Endogenous sensitizing substances secreted by keratinocytes, such as cytokines, amines, neuropeptides and nerve growth factors, can stimulate mast cells to release histamine and cause skin pruritus. Mast cells can also stimulate skin C-nerve fiber receptors to sensitize and cause skin pruritus. Histamine binds to specific receptors in the plasma membrane, such as H1R and H4R, and activates TRPV1, to activate downstream target molecules in sensory neurons, including G protein, phospholipase C, phospholipase A2 and 12-lipoxygenase, which leads to pruritus. The results showed that the level of plasma histamine in the DZX group was significantly lower than that in the WAS group, indicating that

Danzhi Xiaoyao Powder could reduce the plasma histamine level in the model mice, suggesting its possible mechanism.

After treatment with Danzhi Xiaoyao Powder, the degree of depression in the model mice was significantly less than that in the WAS group, indicating that Danzhi Xiaoyao Powder could improve the state of depression caused by psychological stress. The author's previous studies have confirmed that changes in mental state can worsen the pruritus symptoms of this model mice, so improving the state of depression is also a possible treatment for pruritus to alleviate the deterioration of psychological stress. Previous studies have shown that the antidepressant mechanism of Danzhi Xiaoyao Powder may be related to the regulation of hypothalamus-pituitary-adrenal (HPA) axis, which can inhibit the abnormal hyperactivity of HPA axis. The authors' previous studies have also confirmed that chronic psychological stress can lead to damage to the HPA axis and delay the peak of corticosterone secretion in plasma. Psychological stress may lead to the change of HPA axis, but the change may be related to the type of stress and other conditions, so the antidepressant effect of Danzhi Xiaoyao Powder and the mechanism of improving pruritus of chronic psychological stress deterioration may be related to the regulation of HPA axis, but the specific mode of action needs to be further explored.

In this study, it was found that there was still a statistical difference in the number of scratches between the DZX group and the CON group, and the scratching times after treatment was still higher than that in the CON group, indicating that Danzhi Xiaoyao Powder only relieved the pruritus caused by the deterioration of chronic psychological pressure, but it still can not be completely cured, which suggests that Danzhi Xiaoyao Powder can not block all the mechanisms of pruritus in the deterioration of chronic psychological pressure. Existing studies have confirmed the existence of a vicious circle of pruritus and psychological stress. Existing studies have shown that the occurrence and deterioration of pruritus are mainly related to nerve conduction pathways and pruritus mediators, and psychogenic pruritus is also one of the causes of pruritus. The authors' previous studies have confirmed that pruritus with worsening psychological stress can be associated with increased mast cells in skin tissue, elevated plasma histamine levels and changes in the HPA axis. In recent years, the research on psychological stress and pruritus has made remarkable progress in the field of neuroscience<sup>[13]</sup>. Su Xinyu of Shanghai Jiaotong University confirmed by photogenetics that two types of neurons in the ventral tegmental area regulate two different components of pruritus, that is,  $\gamma$ -aminobutyric acid neurons mediate pruritus-induced aversion, while dopaminergic neurons mediate pleasure after scratching. It is suggested that the brain science-related mechanism of pruritus is related to psychological stress<sup>[14]</sup>. In addition, a study published by Sanders KM et al in 2018 shows that the vicious circle between pruritus and anxiety exists in healthy people, and the central nervous system plays an important role in the relationship between pruritus and anxiety<sup>[15]</sup>. In this author's further study in 2019, it was found that a subpopulation of amygdala neurons responded to itch stimuli such as histamine<sup>[16]</sup>. Optogenetic activation of histamine-responsive amygdala neurons affected both scratching and anxiety-like behavior<sup>[17]</sup>. Pharmacological and non-pharmacological treatments to reduce anxiety showed good antipruritic effects. In the research of modern traditional Chinese medicine, Danzhi Xiaoyao Powder is effective in the treatment of

neurodermatitis, chronic eczema and senile skin pruritus, so focusing on emotion and treating skin pruritus from the liver is a recognized treatment for skin pruritus<sup>[18]</sup>. This study has confirmed that Danzhi Xiaoyao Powder can relieve skin pruritus caused by chronic psychological stress by controlling histamine release and improving depression. However, the related mechanisms of nerve conduction pathway and non-histamine pruritus medium need to be further studied.

## 5. Conclusion

Danzhi Xiaoyao Powder can relieve the scratching behavior of mice under the influence of WAS, and its possible mechanism is to control the release of histamine and improve the depressive state of mice.

## 6. List Of Abbreviations

Table 1  
List of abbreviations

Full name	Abbreviations
water avoidance stress	WAS
control group	CON group
chronic WAS group	WAS group
chronic WAS + Danzhi Xiaoyao Powder group	DZX group
hypothalamus-pituitary-adrenal axis	HPA axis

## Declarations

### 7.1 Ethics approval and consent to participate

This study is an animal experiment and has been approved by the Ethics Review Committee.

### 7.2 Consent for publication

Not applicable.

### 7.4 Competing interests

No conflict of interest exists in the submission of this manuscript.

### 7.4 Funding

This study has been supported by the university-level project of Beijing University of Chinese Medicine, and the funding comes from Beijing University of Chinese Medicine.

## 7.5 Authors' contributions

Ruoyi Liu analyzed and explained the data on behavior and plasma histamine levels, and was the main contributor to the manuscript. Li Xu participated in the experimental process of modeling and behavioral testing, Yue Wang took samples and determined the level of plasma histamine, and Shujun Zhang participated in modeling and literature retrieval. Peng Zhao directed the design and implementation of this study, directed and revised the writing of the article, and was the correspondent author of this letter.

## 7.6 Acknowledgements

Not applicable.

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## Figures

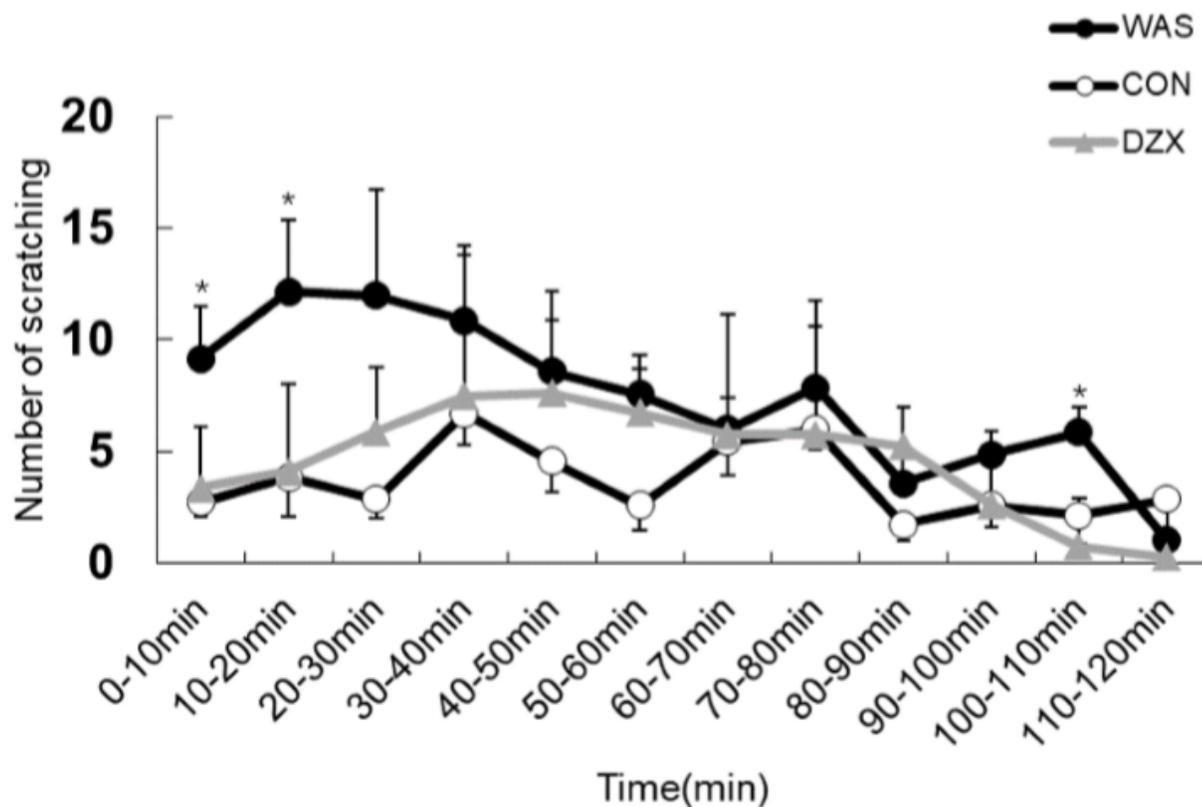


Figure 1

Effect of Danzhi Xiaoyao Powder on scratching behavior induced by compound 48/ 80. Mice exposed to chronic WAS were given an injection of compound 48/80, and their scratching behavior was evaluated. All data are expressed as the average  $\pm$  S.E. \* $P < 0.05$  were considered to be significantly different from the corresponding values between the CON group and DZX group.

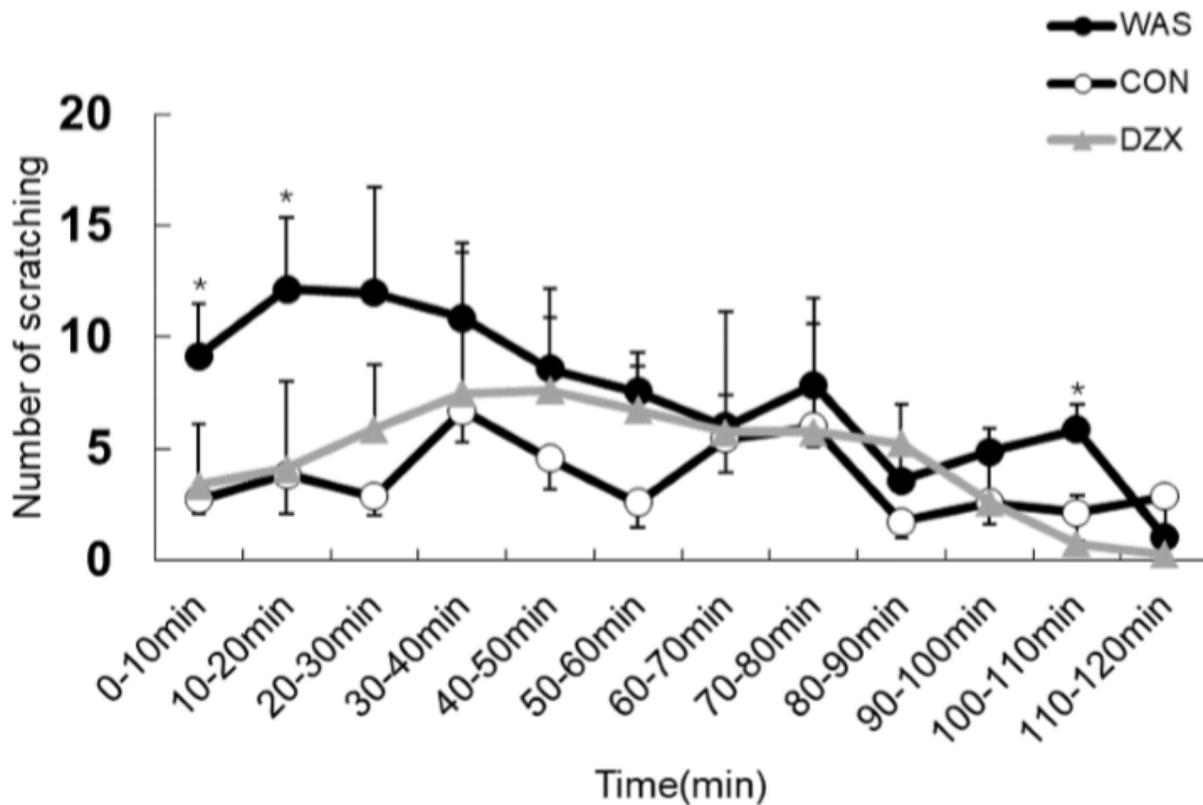
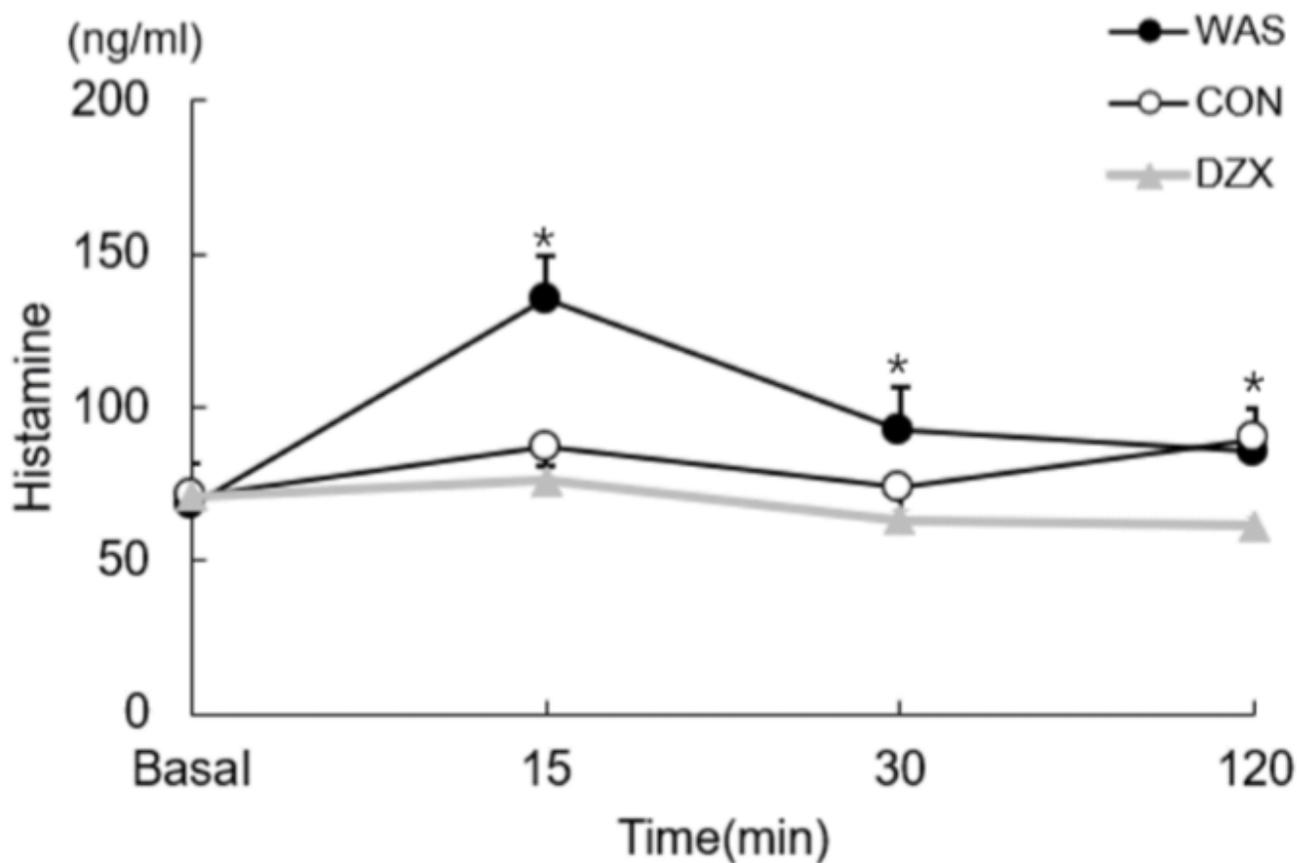


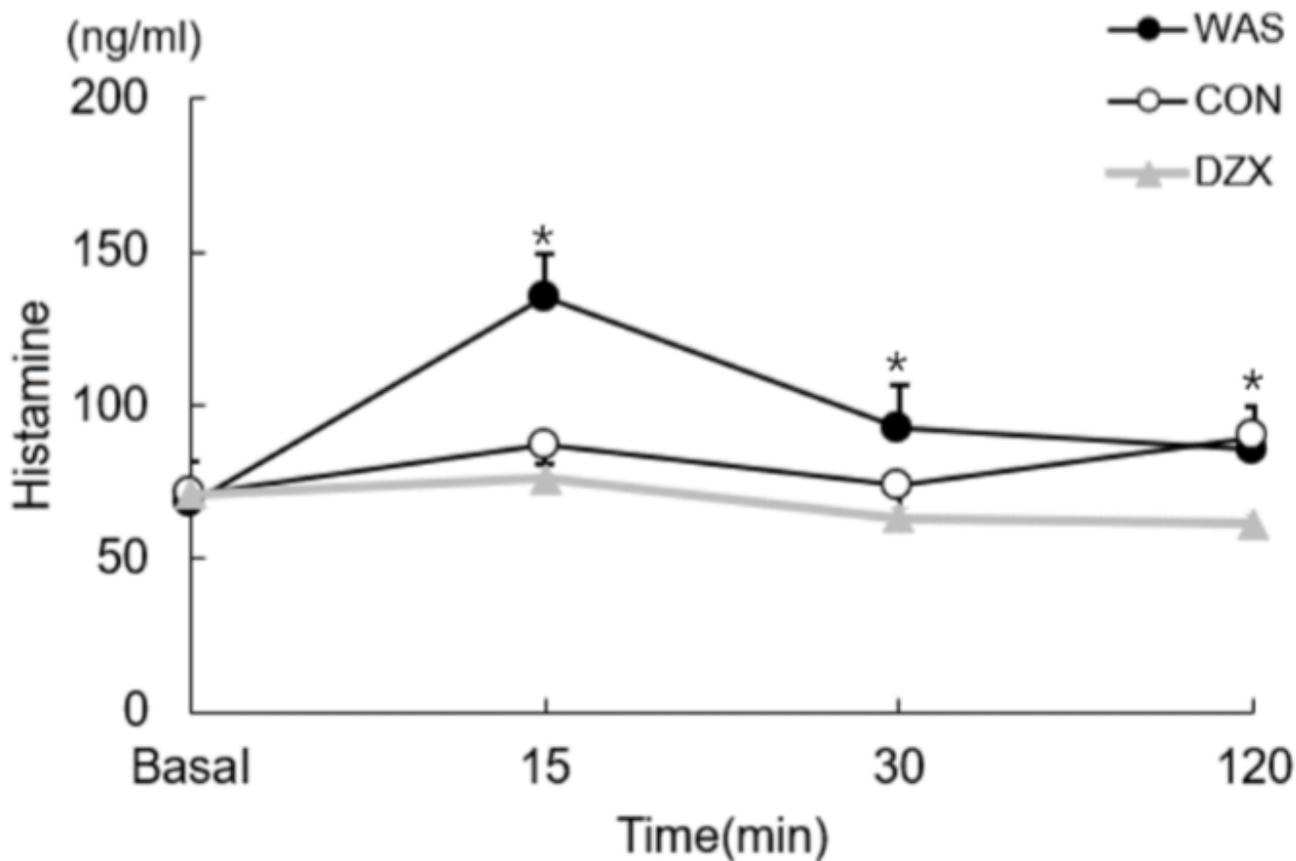
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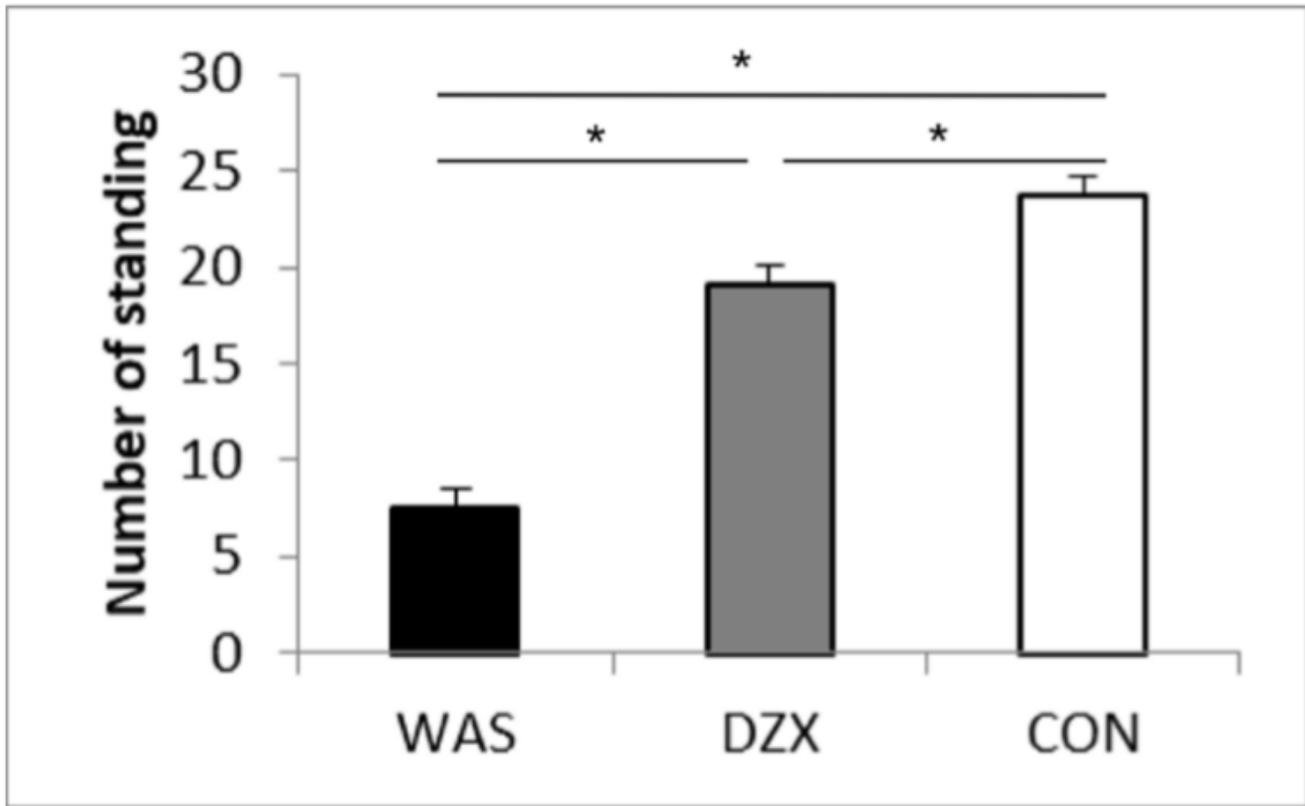
**Figure 2**

Effect of Danzhi Xiaoyao Powder on plasma histamine level. The plasma histamine levels of mice in each group were measured before injection of compound 48pm 80 (basic), 15,30 and 120min after injection of compound 48 / 80, respectively. All data are expressed as average  $\pm$ S.E. (nude 5-10 at each time point). \*  $P < 0.05$  is considered that there is a statistical difference between the traditional Chinese medicine group and the psychological stress group.



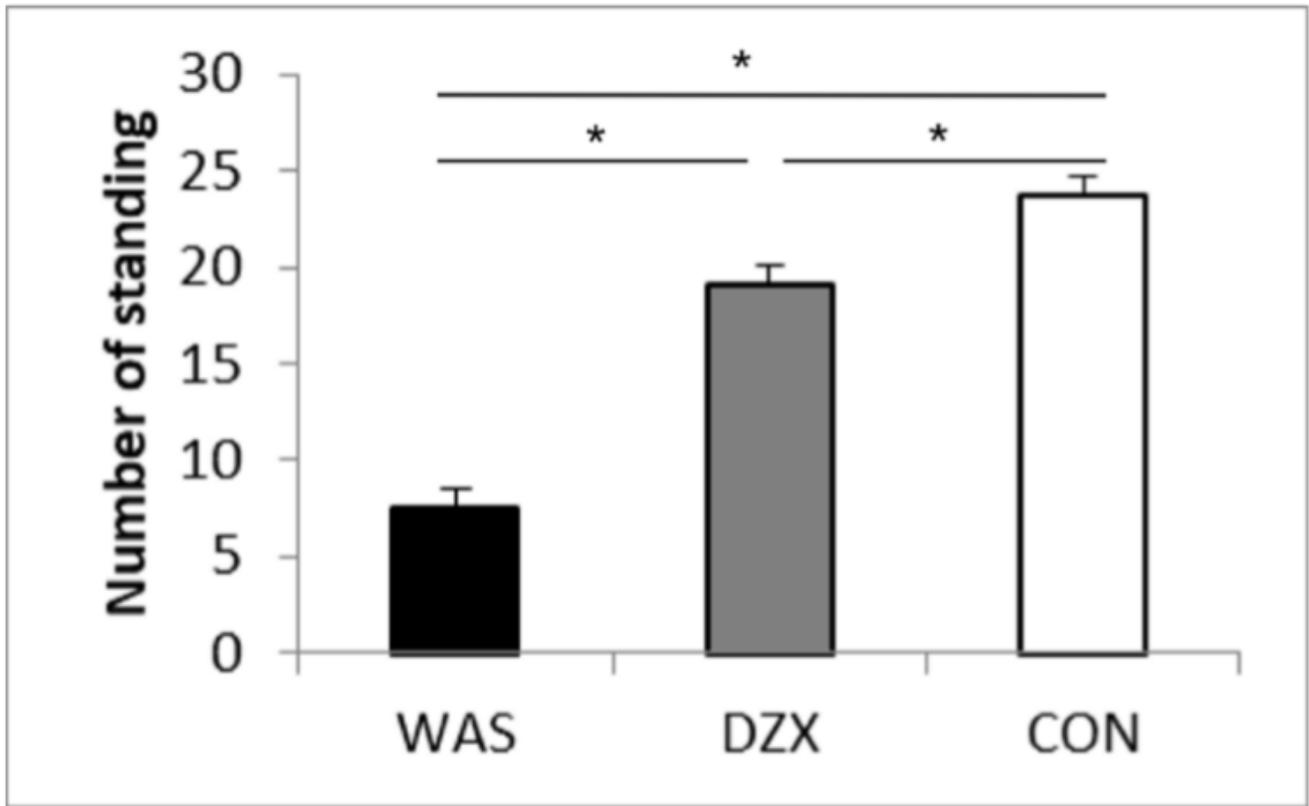
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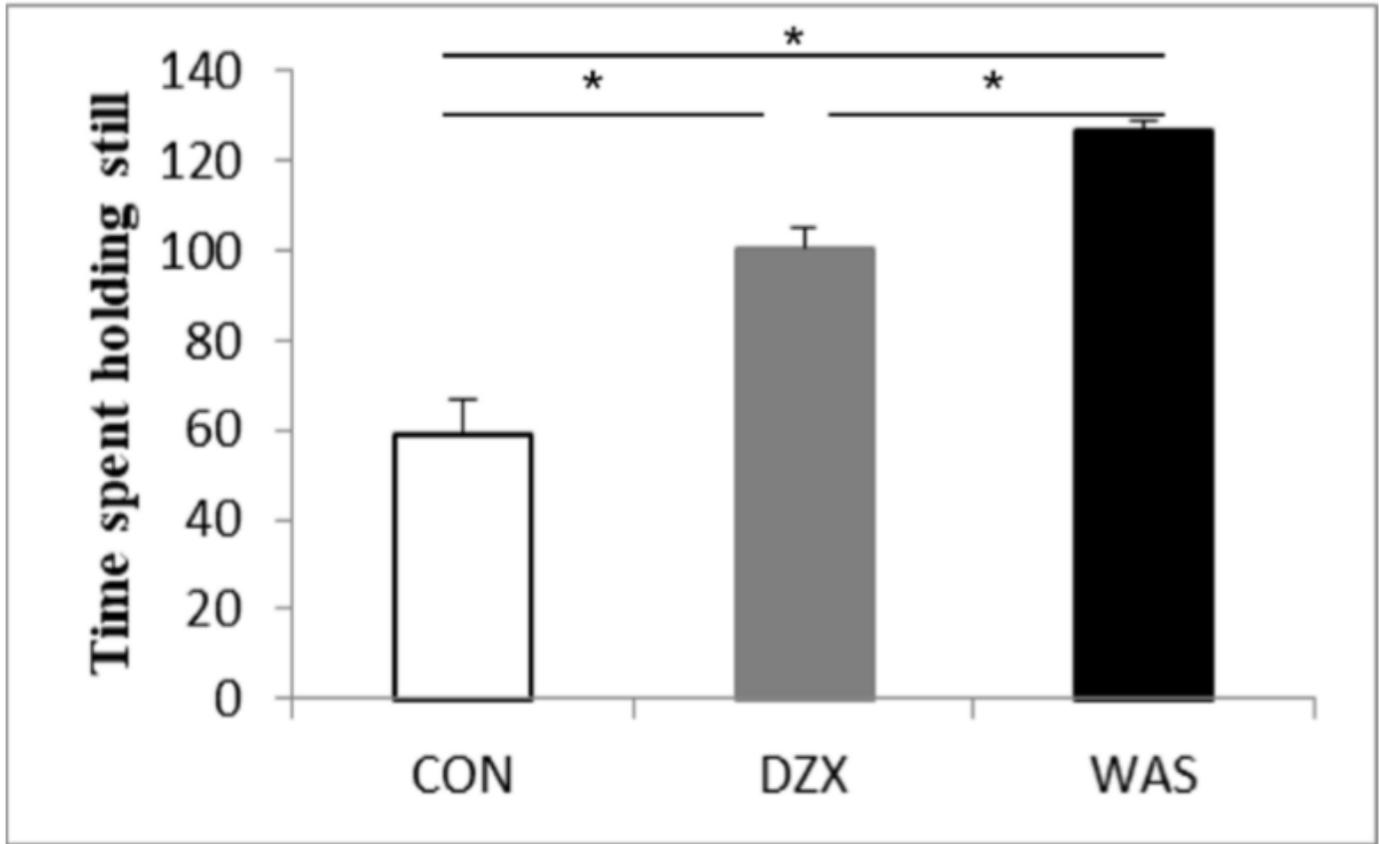
**Figure 3**

Effect of Danzhi Xiaoyao Powder on open field experiment. The standing times of the three groups of mice were counted. All data are expressed as average  $\pm$ S.E. (n = 7 in each group). \* P < 0.05 was considered to be statistically different between the two groups.



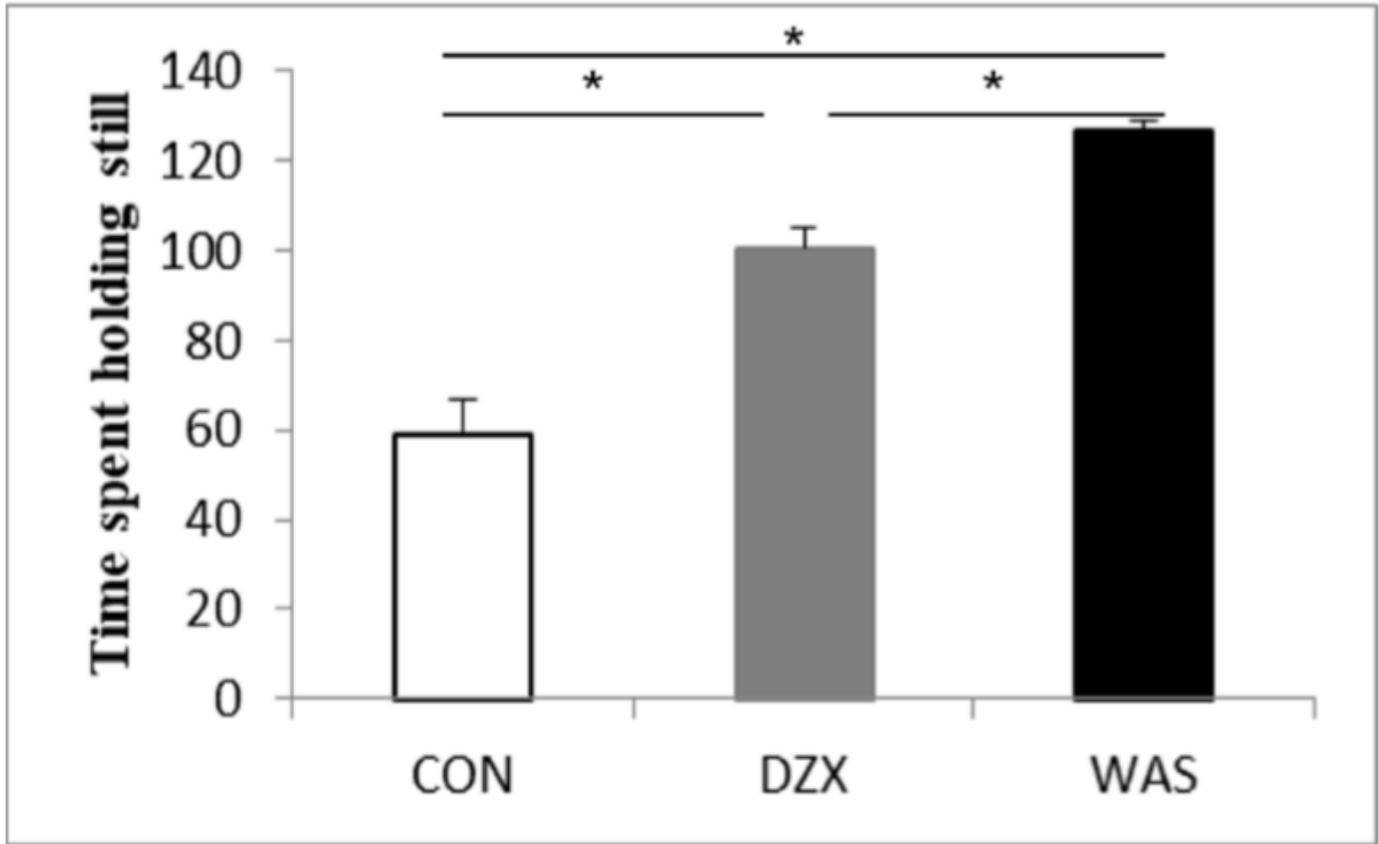
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**Figure 4**

Effect of Danzhi Xiaoyao Powder on forced swimming experiment. The immobility time (minutes) of the three groups of mice was recorded. All data are expressed as average  $\pm$ S.E. ( $n = 7$  in each group). \*  $P < 0.05$  was considered to be statistically different between the two groups.



**Figure 4**

Effect of Danzhi Xiaoyao Powder on forced swimming experiment. The immobility time (minutes) of the three groups of mice was recorded. All data are expressed as average  $\pm$ S.E. ( $n = 7$  in each group). \*  $P < 0.05$  was considered to be statistically different between the two groups.