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# Study on the Regulatory Effect of Acanthopanax senticosus Leaves Extract on Spleen-yang Deficiency and Kidney-yin Deficiency

**Qingping Xia[1], Shuihua Yan[2,*], Chenning He[2], Ge Wu[2], Hui Shen[3]**

[1] *Medical Research Center,Mudanjiang Medical University, Mudanjiang 157011, China*
[2]*College of pharmacy, Mudanjiang Medical University, Mudanjiang 157011, China*
[3]*Institute of medical imaging,, Mudanjiang Medical University, Mudanjiang 157011, China*
[*]*Corresponding author e-mail: 99004790@qq.com*

***ABSTRACT:*** *To investigate the effect of Acanthopanax senticosus extract on Spleen-yang deficiency and Kidney-yin deficiency in mice. Using the quantitative analysis method, it was found in the experiment that Acanthopanax senticosus extract can significantly reduce the β-EP content in the serum, and the reduction range increases and decreases with the increase and decrease of the dose. Acanthopanax senticosus extract can significantly increase the content of cAMP in the serum, and the effect of Acanthopanax senticosus extract on the kidney-yin deficiency group has a significant decrease in ACTH, CORT, CRH content. The results showed that the extract of Acanthopanax senticosus leaves had a regulating effect on spleen-yang deficiency and kidney-yin deficiency in mice.*

***KEYWORDS:*** *Acanthopanax senticosus; Spleen -yang deficiency; Kidney-yin deficiency; β-EP; cAMP; CRH; CORT; ACTH*

## 1. Introduction

With the rapid development of science and technology, people's life rhythm is becoming more and more accelerated. Unhealthy lifestyle is also accompanied, resulting in sub-health status of the body. For sub-health status of people, western medicine biochemical indicators monitoring and imaging examination is difficult to have abnormalities, so it is necessary to diagnose and recuperate by traditional Chinese medicine. Traditional Chinese medicine makes up the blank area of western medicine examination.And it gradually becomes the focus of global medical community. The theory of etiology and pathogenesis of traditional Chinese medicine is an important part of traditional Chinese medicine. Wang bing summarized the

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etiology and pathogenesis of various diseases into four categories when he annotated the yellow emperor's book of internal classics of the yellow emperor, and the four categories were all caused by gas, and the gasification power operated under the operation law of Yin and Yang [1]. The basic theory of traditional Chinese medicine emphasizes the balance between Yin and Yang in human body. However, when Yin and Yang are seriously out of balance, the human body will show serious organic diseases, that is real diseases [2].

In recent years, the extract of acanthopanax leaves has been widely used, such as acanthopanax injection for the treatment of cerebral hemorrhage, coronary heart disease and other diseases, which has a significant effect. A series of symptoms such as skin temperature decreasing, humoral fluid and blood movement slowing down are caused by the weakening of its warm function. In clinical practice, the common syndrome of spleen Yang deficiency presents digestive disorders and other symptoms, while the syndrome of kidney Yin deficiency refers to the syndrome of kidney and other viscera dysfunction, which is caused by deficiency of kidney Yin, loss of nutrition, and ingestion of deficiency heat, resulting in deficiency of essence blood, brain marrow and body fluid, which is one of the basic syndromes of traditional Chinese medicine [3]. For the treatment of leukopenia, agxcanthopanax tablets (or capsules) have been widely used in clinical practice. At present, most of the research and development of acanthopanax leaves are related to the cardiovascular, nervous and immune systems of acanthopanax leaves [4], but there are not many studies on the two-way regulation of spleen Yang deficiency and kidney Yang deficiency in Chinese medicine, so further research is needed.

Acanthopanax senticosus (Rupr. et Maxim.) Harms belongs to the genus Acanthopanax of Araliaceae (Acanthopanax). With the same family as the precious traditional Chinese medicine ginseng, it is also known as "ginseng of Araliaceae", which is often used as root or stem medicine, and is one of the important traditional Chinese medicinal materials [5].

Modern pharmacological studies have confirmed that acanthopanax not only has a protective effect on the body and regulates the immune system, but also can play a role in strengthening the spleen and calming down, anti-tumor, anti-oxidation and anti-aging, hypoglycemic, anti-radiation, anti-fatigue and treatment of neurasthenia. In addition, acanthopanax also has a significant therapeutic effect on the cardiovascular and cerebrovascular system, with small toxic and side effects, which is highly recognized by the global medical community [6-7].Spleen Yang deficiency and kidney Yin deficiency of ICR mice. Through the determination of serum beta-endorphin in beta (EP), adenosine monophosphate (cAMP), adrenocorticotropic hormone (CRH), cortisol (CORT) and adrenocorticotropic hormone (ACTH) content,which explore the acanthopanax leaves extract in mice spleen Yang deficiency and kidney Yin deficiency if there is a regulation,to protect the wild acanthopanax resources and the further research, development and utilization of acanthopanax leaves give full play to its role in promoting.

ISSN 2706-6819 Vol.2, Issue 1: 40-48, DOI: 10.25236/IJFM.2020.020106

## 2·Experimental

### 2.1Experimental materials

#### 2.1.1 Experimental animals

80 healthy ICR mice, aged 4-6 weeks, half male and half female, weighing (20 ± 2) g, were normally raised in SPF animal room of medical research center of Mudanjiang Medical College.

#### 2.1.2 Experimental reagent

*Table 1 Experimental reagents*

| Drug | Batch number | Manufacturer |
|---|---|---|
| Mouse cyclic adenosine monophosphate (cAMP) ELISA Kit instruction | MB18102310 | Lai Er Bio-Tech |
| Mouse beta-endorphin(β-EP)ELISA Kit instruction | MB18102310 | Lai Er Bio-Tech |
| Mouse corticosterone (CORT) ELISA Kit instruction | MB18102310 | Lai Er Bio-Tech |
| Mouse Adrenal cortical hormone (ACTH) ELISA Kit instruction | MB18102310 | Lai Er Bio-Tech |
| Mouse Corticotropin (CRH) ELISA Kit instruction | MB18102310 | Lai Er Bio-Tech |
| Rhubarb slices | 080901 | Sichuan Herbal Pieces Co., Ltd |
| Thyroxine tablet | 20180517 | Shanghai Great Wall Pharmaceutical Co., Ltd |
| Acanthopanax senticosus leaves extract | / | Mudanjiang Medical College |
| Ethyl ether | 20170529 | Tianjin Fuyu Fine Chemical Co., Ltd |

#### 2.1.3Experimental instrument

*Table 2 experimental instruments*

| Instrument | Model | Manufacturer |
|---|---|---|
| Multifunctional marker | M3 | Shanghai Zequan Instrument Equipment Co., Ltd |
| Refrigerated centrifuge | 5804R | Guangzhou Leide Biotechnology Co., Ltd |
| Electrothermostatic water tank | XMTD-4000 | Shanghai billon Instrument Co., Ltd |
| Microoscillator | WZ-2A | Jiangsu Jintan medical instrument factory |
| Electronic balance | FA1004N | Shanghai Precision Scientific Instrument Co., Ltd |
| Refrigerator | BCD-224 | SIEMENS |
| Cryopreservation box | 907 | Thermo Fisher Scientific (China) Co., Ltd |

ISSN 2706-6819 Vol.2, Issue 1: 40-48, DOI: 10.25236/IJFM.2020.020106

### 2.2 experimental methods

#### 2.2.1 animal feeding

The temperature of the feeding environment is about 20℃.And the humidity is about 60%. The animals in each experimental group were fed with nutritious feed for one week before the experimental model is built. The animals can get food and water freely. And the water is changed once a day.

#### 2.2.2 experimental grouping

Spleen Yang deficiency: there were 8 mice in the blank control group, 8 in the model group, and 8 in the Acanthopanax group (high, medium and low concentration), with a total of 40 mice.

Kidney-yin deficiency: there were 8 mice in the blank control group, 8 in the model group and 8 in the Acanthopanax group (high, medium and low concentration), with a total of 40 mice.There were a total of 80 ICR mice.

#### 2.2.3 establish models of spleen Yang deficiency and kidney Yin deficiency

Preparation of spleen Yang deficiency model: add 500ml water to 500ml rhubarb decoction pieces.And then soak them for 30min.After that fry them for 3 times and combine them with filtrate for 3 times. After decompression and concentration to 500ml, that is, the concentration was 1g/ml. The ICR mice were gavaged with 1g/ml rhubarb decoction at a dose of 10ml/kg/d (0.01ml/g/d) every morning (distilled water was given to the normal control group). In the afternoon, mice were gavaged with acanthopanax leaves extract or distilled water of corresponding concentration for 28 consecutive days.

Preparation of kidney Yin deficiency model: thyroxine tablets were prepared with purified water into 0.03g/ml. Mice were given a 0.3g/kg (0.03ml/g/d) dose of thyroxine suspension for modeling every morning (distilled water was given to the normal control group), and the corresponding concentration of acanthopanax leaves extract or distilled water was given to the mice by gavage in the afternoon for 28 consecutive days.The experimental modeling period was 4 weeks.

#### 2.2.4 experimental methods and dose

In the treatment group, spleen Yang deficiency group was treated with rhubarb decoction of 100% rhubarb with 10ml/kg/d (0.01ml/g/d).And the ICR mice were treated with rhubarb decoction in the morning and rhubarb decoction in the afternoon for 28 consecutive days. In the kidney Yin group, mice were given a model of thyroxine suspension with a concentration of 0.3g/kg (0.03ml/g/d) in the morning (distilled water was given to the normal control group), and the volume of gavage was 0.01ml/g. In the afternoon, mice were given corresponding drugs or distilled water for

ISSN 2706-6819 Vol.2, Issue 1: 40-48, DOI: 10.25236/IJFM.2020.020106

28 consecutive days. During administration, the extract of acanthopanax japonicum was administered to the group once a day by regular gavage, and the dose was administered in groups with high, medium and low concentrations of the extract of acanthopanax japonicum japonicum (high concentration was 0.8g/ml, medium concentration was 0.6/ml, and low concentration was 0.4g/ml). In the model group, the volume of distilled water of acanthopanax leaves extract was obtained in the morning after gavage, and in the afternoon after gavage and administration for 28 consecutive days.

#### 2.2.5 determination of substance content in blood of mice

Mice in each group were anesthetized with ether after fasting and dehydration for 12h on the 28th day of administration. Blood was collected from the orbit and placed in a 1.5ml centrifuge tube. Immediately after 0.5h in a 37 ° c incubator, the mice were centrifuged for 10 minutes (3000rpm) to separate the serum from red blood cells. The collected serum was grouped and stored in a -80 degree refrigerator. The serum was taken out at the time of measurement and thawed at room temperature (the serum must be thawed sufficiently evenly). The contents of CORT, cAMP, CRH, endorphin and ACTH in serum were determined according to the instructions of the corresponding ELISA kit.

#### 2.2.6 statistical analysis

SPPS23.0 statistical software was used to analyze and process the data. The data were expressed as mean ± standard deviation.And the statistical method was using one factor analysis of variance. $P<0.05$ was considered statistically significant, and $P<0.01$ was considered extremely significant [8].

### 3.1 Effect of the extract of acanthopanax leaves on Spleen-yang deficiency in mice

#### 3.1.1 Effect of Acanthopanax senticosus leaves extract on serum β - EP in Spleen-yang deficiency mice

The content of β - EP in the study of the effect of Acanthopanax senticosus leaves extract on Spleen-yang deficiency group: The content of β-EP in model group and low dose group was significantly higher than that in blank control group（*P<0.05）。Compared with the model group, the content of β - EP in the middle and high dose groups decreased significantly（ ▲P<0.05）, It can be seen that Acanthopanax senticosus leaves extract can significantly reduce the content of β - EP in serum.。

ISSN 2706-6819 Vol.2, Issue 1: 40-48, DOI: 10.25236/IJFM.2020.020106

*Table 3 Effect of Acanthopanax senticosus leaves extract on β - EP in serum of Spleen-yang deficiency mice（Unit: pg / ml）*

| Group | Number | (x±s) |
|---|---|---|
| Blank control group | 7 | 38.77±8.34 |
| Model group | 7 | 78.05±15.22* |
| Low dose group | 6 | 60.35±5.40* |
| Medium dose group | 6 | 42.03±10.91▲ |
| High dose group | 6 | 41.60±5.39▲ |

Notes：Comparison with blank control group，*P<0.05; Compare with model group，▲P<0.05。

### 3.1.2 Effect of Acanthopanax senticosus leaves extract on cAMP in serum of Spleen-yang deficiency mice

The content of cAMP in the study of the effect of Acanthopanax senticosus leaves extract on Spleen-yang deficiency group: Compared with the control group, the model group and the low dose group were significantly lower（**P<0.01）。The middle dose group and high dose group were significantly higher than the model group（ ▲P<0.05）， It can be seen that Acanthopanax senticosus lea ves extract can significantly increase serum cAMP content

*Table 4 Effect of Acanthopanax senticosus leaves extract on camp in serum of Spleen-yang deficiency mice（Unit：pg/ml）*

| Group | Number | (x±s) |
|---|---|---|
| Blank control group | 7 | 50.48±3.68 |
| Model group | 7 | 35.45±3.98** |
| Low dose group | 6 | 39.63±3.85** |
| Medium dose group | 6 | 51.03±6.34▲ |
| High dose group | 6 | 52.81±4.15▲ |

Notes：Comparison with blank control group，*P<0.01。Compare with model group，▲P<0.05。

### 3.2 Effect of Acanthopanax senticosus leaves extract on Kidney-yin deficiency in mice

### 3.2.1 Effect of Acanthopanax senticosus leaves extract on ACTH in serum of Kidney-yin Deficiency mice

The content of ACTH in the study of the effect of Acanthopanax senticosus leavf extract on Kidney-yin deficiency group: The model group was significantly higher than the blank control group（*P<0.05）， Compared with the model group, the content of ACTH in the middle dose group and high dose group decreased significantly（ ▲P<0.05）， It can be seen that Acanthopanax senticosus leaves extract can significantly reduce the content of ACTH in serum。

ISSN 2706-6819 Vol.2, Issue 1: 40-48, DOI: 10.25236/IJFM.2020.020106

*Table 5 Effect of Acanthopanax senticosus leaves extract on ACTH in serum of kidney-yin deficiency mice（Unit：pg/ml）*

| Group | Number | (x±s) |
|---|---|---|
| Blank control group | 8 | 110.06±6.68 |
| Model group | 6 | 148.83±6.68* |
| Low dose group | 6 | 148.83±12.44 |
| Medium dose group | 7 | 117.41±23.75▲ |
| High dose group | 6 | 113.97±9.85▲ |

Notes：Comparison with blank control group，*P<0.05。Compare with model group，▲P<0.05。

### 3.2.2 Effect of Acanthopanax senticosus leaves extract on serum CORT in Kidney-yin deficiency mice

The content of CORT in the study of the effect of Acanthopanax senticosus leaves extract on Kidney-yin deficiency group: The CORT content of the model group and the low dose group was significantly higher than that of the blank control group（**P<0.01）。Compared with the model group, the content of CORT in the middle dose group and the high dose group decreased significantly（▲P<0.05）, The content of CORT in the middle dose group, the high dose group and the low dose group decreased significantly（##P<0.01）, It can be seen that Acanthopanax senticosus leaves extract can significantly reduce serum CORT content.

*Table 6 Effect of Acanthopanax senticosus leaves extract on CORT in serum of Kidney-yin deficiency mice（Unit：pg/ml）*

| Group | Number | (x±s) |
|---|---|---|
| Blank control group | 8 | 80.31±8.35 |
| Model group | 6 | 114.96±7.87** |
| Low dose group | 6 | 116.24±13.97** |
| Medium dose group | 7 | 80.34±10.29▲## |
| High dose group | 6 | 81.51±11.62▲## |

Notes：Comparison with blank control group，**P<0.01。Compare with model dose group，▲P<0.05。Comparison with dose group，##P<0.01。

### 3.2.3 Effect of Acanthopanax senticosus leaves extract on CRH in serum of Kidney-Yin deficiency mice

The content of CRH in the study of the effect of Acanthopanax senticosus leaves extract on kidney-yin deficiency group: The content of CRH in the model group was significantly higher than that in the blank control group（*P<0.05）。Compared with the model group, the CRH content in the middle dose group and the high dose group decreased significantly（▲P<0.05）, It can be seen that the extract of

ISSN 2706-6819 Vol.2, Issue 1: 40-48, DOI: 10.25236/IJFM.2020.020106

Acanthopanax senticosus leaves can significantly reduce the content of CRH in serum.

*Table 7 Effect of Acanthopanax senticosus leaves extract on CRH in serum of kidney-yin deficiency mice（Unit：pg/ml）*

| Group | Number | (x±s) |
|---|---|---|
| Blank control group | 8 | 55.02±12.97 |
| Model group | 6 | 80.42±12.57* |
| Low dose group | 6 | 80.42±6.37 |
| Medium dose group | 7 | 59.90±15.03▲ |
| High dose group | 6 | 57.62±8.02▲ |

Notes：Comparison with blank control group，*P<0.05.Compare with model group，▲P<0.05。

## 4.Conclusion

Acanthopanax leaves extract produce β-EP to deal with the serious spleen Yang of ICR mice.It can reduce the high expression by regulating effects of cAMP ,adjust its content in mice to normal levels, reduce the high level of CRH, ACTH,CORT in the blood of ICR mice,which content reduces those material levels in the blood of mice to converge with those of normal control mice. It was proved that the extract of acanthopanax can balance Yang of the spleen and Yin of the kidney .

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