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PHARMACOLOGICAL EXPERIMENTAL STUDY OF THE ANTI-DEPRESSANT EFFECT OF TOTAL SAIKOSAPONINS.

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Abstract

Background: Chai Hu has the hepato-protective, choleretic, anti-tussive, analgesic, anti-inflammatory, anti-viral, hypotensive, hypolipidemic, and anti-tumor pharmacological effects. In this study, the objective of this paper was to study the anti-depressant effect of total saikosaponins.

Materials and Methods: Total saikosaponins were extracted by reflux method, and were identified by thin layer chromatography (TLC). The anti-depressant effect of total saikosaponins was investigated *in vitro* by tail suspension test, forced swimming test, and reserpine antagonism test in mice.

Results: Two times of reflux extraction, temperature of 70℃, and extraction time of 4hrs, for each extraction could improve the yield of saikosaponins. Each treatment group (100, 200, and 300 mg/kg), could significantly shorten the immobility time of mice in the tail suspension test in a somewhat dose-dependent manner. The total saikosaponins antagonized the reserpine-induced akinesia, and ptosis in mice.

Conclusion: Total saikosaponins have an anti-depressant effect.

Key words*:* total saikosaponins; tail suspension test; forced swimming test

Introduction

Chai Hu, which was formerly known as Zi Hu, was originally recorded in the "Shen Nong's Herbal Classic" as a medium-grade drug. It was renamed in the Song Dynasty to Chai Hu in the "Maps of Materia Medica". Chai Hu comes from Bupleurum chinensis DC. or Bupleurum scorzonerifolium, of family Umbelliferae; the former is named "Bei Chai Hu", and the latter named "Nan Chai Hu" according to the medication habits. Chai Hu is bitter in taste, slightly cold, enters the liver and gallbladder meridians. It has the effects of releasing exterior and curing fever, soothing liver and relieving liver depression, and ascending yang qi; and used in the treatment of alternating episodes of chills and fever, chest fullness, rib pain, bitter taste in the mouth, deafness, headache, dizziness and other symptoms (Liu et al., 2006). Scholars at home and abroad have conducted in-depth researches on the chemical constituents of Chai Hu, and have isolated saponins, flavonoids, lignans, coumarins, phenylpropanols, polyacetylenes, volatile oils, polysaccharides, polyols, fatty acids, sterols and amino acids from different parts of Chai Hu. Modern pharmacological studies have shown that Chai Hu has the hepatoprotective, choleretic, antitussive, analgesic, anti-inflammatory, anti-viral, hypotensive, hypolipidemic and anti-tumor pharmacological effects (Zhang., 2000; Liu et al., 2002; Shao.,2002; Wang et al., 2002; Zhu et al., 2005) . In this study, total saikosaponins were extracted and separated from Chai Hu and identified, and the anti-depressant effect and mechanism of saikosaponins investigated.

Materials and Methods

Reagents and Instruments

Prozac capsules (Eli Lilly Suzhou Pharmaceutical Co., Ltd.), reserpine (Guangdong Bangmin Pharmaceutical Co., Ltd.]; TC-15 thermostat (Wuxi Shengze Physical and Chemical Instruments Co., Ltd.); circulating water multipurpose vacuum pump (Tianjin Zhaoyuan Vacuum Equipment Manufacturing Co., Ltd.); far-infrared drying oven (Shanghai Pudong Rongfeng Scientific Instrument Co., Ltd.); tail suspension device, mouse rectal temperature probe.

Animals

Kunming mice, male, 18-22g, were purchased from the China Medical University, and adaptively fed for four days. All experimental procedures were approved by the Animal Research Ethics Committee.

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Preparation and identification of total saikosaponins

Preparation of total saikosaponins

500g of Bei Chai Hu was weighed out, ground into coarse powder, added with methanol containing 5% pyridine, and soaked overnight, then extracted twice under reflux, reflux temperature was 70℃, and reflux time was 4hrs, for each extraction, followed by filtration. The solvent was then recovered to obtain a concentrated solution. The concentrated solution was diluted, and extracted by addition of saturated n-butanol for a total of three times, the extracts were combined, recovered, and the resulting residue was the total extract containing saikosaponins and sapogenins. The total extract was then added with adequate amount of ether and extracted under reflux for 30min., the ether insoluble matter was collected, and dissolved by adding appropriate amount of hot ethyl acetate, then ethyl acetate was evaporated, and the residue was dried, and crushed to obtain total saikosaponins.

TLC identification of saikosaponins

Appropriate amount of the above total saikosaponins was weighed out, and dissolved in methanol as the test solution. TLC test was performed, developing conditions were: ethyl acetate-ethanol-water (8:2:1), the plates were developed, taken out, air dried, sprayed with 2% solution of p-dimethylaminobenzaldehyde in 40% sulfuric acid, and heated at 60℃ till well-defined pink spots were produced, which indicated the presence of total saikosaponins.

Tail suspension test in mice

The mice were randomly divided into five different groups; namely the blank control group, fluoxetine group (7 mg/kg), and saikosaponin groups (100, 200, and 300 mg/kg). The mice were intra-gastrically administered for seven consecutive days, 1hr, after the last administration, the tail of mice 2cm from the tip was fixed on a horizontal surface, so that the mice were hung upside down, and the immobility time of mice within 6min., was recorded.

Forced swimming test in mice

After the pre-test, the mice were placed in a 15cm deep bucket, the observation began after the mice had swum for 2min., and the immobility time of mice within 4min., was accumulated, that is, the duration of time in which mice stops struggling, with only small movements to remain not submerged under water, mice with the immobility time exceeding 120sec., or less than 60sec., were removed from the test. The qualified mice were randomized, and intra-gastrically administered for seven consecutive days, 1hr, after the last administration, forced swimming test was performed according to the pre-test methods, and the immobility time of mice within 4min., was accumulated.

Reserpine antagonism test (Zheng et al., 2005)

1hr, after the last administration, 4mg/kg reserpine was injected intra-peritoneally to the mice in each experimental group, 4hr, later, electronic thermometer probe was inserted approximately 1cm, into the rectum of mice to measure rectal temperature for 10sec., and differences in the change of rectal temperature of mice in each group before and after 4hrs, were recorded. 1hr, after the intra-peritoneal injection of reserpine, the mice were placed in a circle 8cm, in diameter and observed for 20sec., the out-of-circle rate of mice in each group was calculated, in order to observe the akinetic state of mice in each group. 1hr, after the intra-peritoneal injection of reserpine, the number of mice with ptosis was counted, and the percentage was calculated.

Statistical analysis

Experimental data were processed using SPSS 11.5, statistical software, and were expressed as $\bar{x}\pm s$; comparison among groups was performed by the t-test.

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Results

Effect of saikosaponins on tail suspension test in mice

The experimental results showed that each experimental group could significantly shorten the immobility time of mice in the tail suspension test (P<0.01 or P<0.05), in a somewhat dose-dependent manner, as shown in Figure 1.

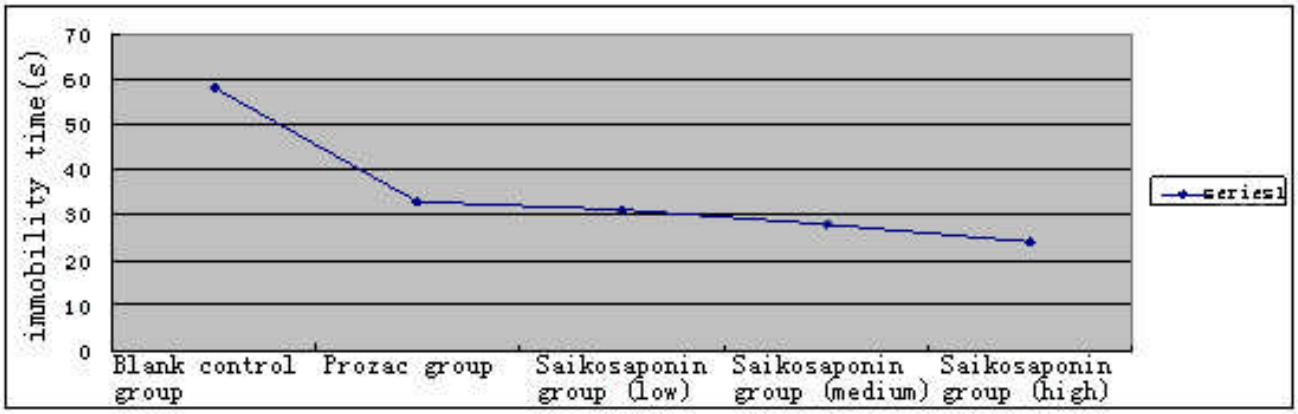

Figure 1: Effect of saikosaponins on tail suspension test in mice

Effect of saikosaponins on forced swimming test in mice

As can be seen from the data, compared with the blank control group, the Prozac group could significantly shorten the immobility time of mice, the saikosaponin medium- and high-dose groups, had similar effects as well, but the saikosaponin low-dose group had no statistically significant effect (see Table 1).

Table 1: Effect of saikosaponins on immobility time of mice in the forced swimming test ($\bar{x}$±s, n=10)

Group	Dose (mg/kg)	Immobility time (s)
Blank control group		95.2±35.6
Prozac group	7	53.4±23.8*
Saikosaponin group (low)	100	86.4±30.9
Saikosaponin group (medium)	200	56.8±35.5*
Saikosaponin group (high)	300	46.3±29.1**

Note: Comparison with the blank control group, * P<0.05, ** P<0.01.

Reserpine antagonism test

In the reserpine-induced akinesia, and ptosis antagonism test in mice, the saikosaponin medium- and high-dose groups antagonized the reserpine-induced akinesia and ptosis in mice; the antagonistic effect was not obvious in the low-dose group. The three dose groups could all antagonize the reserpine-induced hypothermia in mice (Tables 2, 3 and Figure 2)

Table 2: Antagonistic effect of saikosaponins on reserpine-induced ptosis in mice ($\bar{x}$±s, n=12)

Group	Dose (mg/kg)	Number of mice with ptosis	Ptosis percentage (%)
Blank control group		0	0
Reserpine group	4	11	91.67
Saikosaponin group (low)	100	8	66.67
Saikosaponin group (medium)	200	5	41.67*
Saikosaponin group (high)	300	4	33.33*

Note: Comparison with the reserpine group, * P<0.05

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Table 3: Antagonistic effect of saikosaponins on reserpine-induced akinesia in mice ($\bar{x}\pm s$, n=12)

Group	Dose (mg/kg)	Number of mice moving out of the circle	Out-of-circle rate (%)
Blank control group		0	100.00
Reserpine group	4	1	8.33
Saikosaponin group (low)	100	8	66.67
Saikosaponin group (medium)	200	6	50.00*
Saikosaponin group (high)	300	4	33.33*

Note: Comparison with the reserpine group, * P<0.05

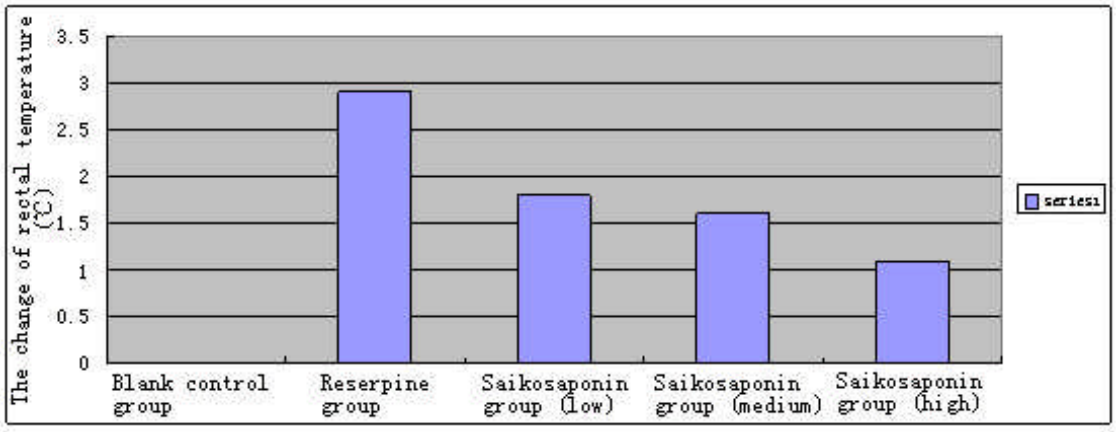

Figure 2: Effect of saikosaponins on reserpine-induced hypothermia in mice

Discussion

Chai Hu has a complex chemical composition, its main constituents are saponins and volatile oils, and it also contains flavonoids, fatty acids, polyols, sterols, lignans, coumarins, polysaccharides, trace elements and other types of constituents. Scholars at home and abroad have done extensive researches on saikosaponins, and have isolated more than 100, kinds of saponin compounds from it; of which the contents of saikosaponin d, a, c were relatively high (Luo et al., 1996; Ebata et al., 1990; Liang et al., 1998; Jin et al., 1996; Ebata et al., 1996). Saikosaponins d, a, c, are all thermally unstable substances, changes occur easily during their extraction and separation process, so the extraction time and temperature should be strictly limited. It can be seen through the analysis of preliminary experimental results that the extraction conditions of two times of reflux extraction, temperature of 70℃, and extraction time of 4hrs, for each extraction can effectively reduce the degradation of saikosaponins, and improve its yield.

Depression is a mood disorder caused by one or multiple reasons which is mainly characterized by low mood, emotional lowness is its core symptom, and its main clinical manifestations are despondency, reduced verbal and physical actions, and retardation of thinking. Depression can be classified into primary depression and secondary depression, and can generally be divided into exogenous depression, endogenous depression and bipolar disorder depression (Thase et al., 2009; Filakovic etal., 2009).

The mouse models of forced swimming and tail suspension used in this study are sensitive to most anti-depressant drugs, and are widely used in the preliminary screening of these drugs.

Our experimental results showed that each treatment group (100, 200, and 300 mg/kg), could significantly shorten the immobility time of mice in the tail suspension test in a somewhat dose-dependent manner. In the reserpine-induced akinesia and ptosis antagonism test in mice, the saikosaponin medium- and high-dose groups could antagonize the reserpine-induced akinesia and ptosis in mice; the antagonistic effect was not obvious in the low-dose group.

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