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Saikosaponin a alleviates depressive-like behavior by regulating the BDNF-TrkB-CREB signaling pathway in a mouse model of chronic unpredictable mild stress

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Highlights

Saikosaponin a alleviated CUMS-induced depressive behaviors in mice.

Saikosaponin a induces the expression of CREB in the hippocampus.

Saikosaponin a may exert antidepressant effects by regulating BDNF-TrkB-CREB signaling pathway.

Abbreviations

SSa, Saikosaponin a; CUMS, Chronic unpredictable mild stress; FST, Forced swimming test; TST, Tail suspension test; SPT, Sucrose preference test; OFT, Open feld test; BDNF, Brain-derived neurotrophic factor; TrkB, Tyrosine kinase B; CREB, cAMP response element binding protein; BW, Body weights; QRT-PCR, Quantitative Real-Time PCR; MAOIs, monoamine oxidase inhibitor; TCA, stricyclic antidepressants; SSRIs, selective 5-HT reuptake inhibitors; AC, adenylate cyclase; ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate.

Abstract

This study aimed to determine the effect of Saikosaponin a (SSa) in a model of depression induced by chronic unpredictable mild stress (CUMS) and to investigate whether SSa plays an antidepressant role by regulating the BDNF-TrkB-CREB signaling pathway. A mouse model of depression was established and treated with different concentrations of SSa (15, 30 and 60 mg/kg). Behavioral tests, including sucrose preference test (SPT), open-field test (OFT), forced swim test (FST) and tail suspension test (TST). mRNA levels of BDNF, TrkB and CREB in the hippocampus of mice were detected by QRT-PCR. Protein levels of BDNF, pTrkB, TrkB, pCREB and CREB were measured by Western blot. Results showed that compared to the model group, the SSa treatment significantly increased the sucrose preference, improved the spontaneous activity in the OFT, and reduced the immobility time in the TST and FST. In addition, SSa effectively inhibited CUMS induced mRNA reductions in BDNF, TrkB and CREB and up-regulated CUMS induced protein expression levels of BDNF, pTrkB, TrkB, pCREB and CREB in the mouse hippocampus. In conclusion, our results indicate that SSa can effectively improve CUMS-induced depressive-like behavior in mice, which may be achieved by activating the BDNF-TrkB-CREB signaling pathway.

Key words: Depression; Saikosaponin a; CUMS; BDNF; TrkB; CREB

1. Introduction

Depression is a common mental disease that affects approximately 16% of the world's population [1]. Patients with depression demonstrate the following symptoms: low spirits, loss of interest, guilt or inferiority, sleep anxiety or loss of appetite, insomnia or drowsiness, fatigue or energy loss, and poor concentration or difficulty in decision making. The World Health Organization (WHO) predicts that depression may become the second leading cause of illness-induced disability by the year 2030 [2]. Because the pathogenesis of depression is still unclear, current treatments are not very effective. Antidepressants treat depression at less than half the rate of its occurrence and have serious side effects, such as fatigue, loss of appetite and sexual dysfunction [3]. Therefore, it is urgent to identify novel effective and safe antidepressants.

In recent years, traditional Chinese medicine has been shown to have advantages of stable effects, fewer side effects, and lasting curative effects to treat depression [4]. Clinical experiments show that Radix Bupleuri has the effect of soothing the liver and relieving depression, Depression treatment with its compound prepared in the clinic was reported in aspects of inhibiting nerve inflammation, regulating monoamine transmitters of the nerves, inhibiting abnormal activity of the HPA axis, and enhancing expression of the BDNF [5]. SSa is a soap-like component extracted from Radix Bupleuri that has various biological activities, such as anti-inflammatory, analgesic, nerve-regulating, anti-fibrotic and anticancer [6]. Chinese Pharmacopoeia shows that the content of SSa in Radix Bupleuri is not less than 0.3%. Data indicate that SSa (50 and 100 mg/kg) improve behavior in perimenopause-like depression induced by CUMS in female Wistar rats, which may be mediated by upregulation of protein expression in the BDNF-TrkB signaling pathway [7]. However, the specific antidepressant mechanism of SSa has not been reported. The BDNF-TrkB-CREB pathway has been proven to be closely related with depression by regulating neurotrophic factors, inflammation, and neuroplasticity, all of which are involved in the occurrence and development of depression. Therefore, the aim of this study was to evaluate the antidepressant effect of SSa in male Kunming mice induced by CUMS and to reveal the possible mechanism of the antidepressant of SSa through a systematic examination of the BDNF-TrkB-CREB pathway to provide favorable evidence for the identification of novel antidepressants.

2. Methods

2.1. Animals and Reagents

Healthy male clean Kunming mice, (18-24 g, 4 weeks of age) were obtained from the Harbin Medical University animal center (SCXK (Hei) 2013-001). Mice were housed 5 per cage with free access to food and water, a 12 h-light/dark cycle at a temperature of 22 ± 1°C and relative humidity of 55±5%. All mice were adapted to their environment for 7 days before the initiation of experimental procedures. Mice were weighed every seven days and were randomly and evenly divided into control and CUMS model groups. All experimental procedures were performed according to the suggestions for the care and use of laboratory animals formulated by the Ministry of Science and Technology of People' Republic of China and were approved by the Animal Ethics Committee at Harbin Medical University, Harbin, China. SSa (Fig. 1) was obtained from Ruifensi Biotechnology Co., LTD (Chengdu, China). Fluoxetine was purchased from Daqing People's Hospital (Daqing, China).

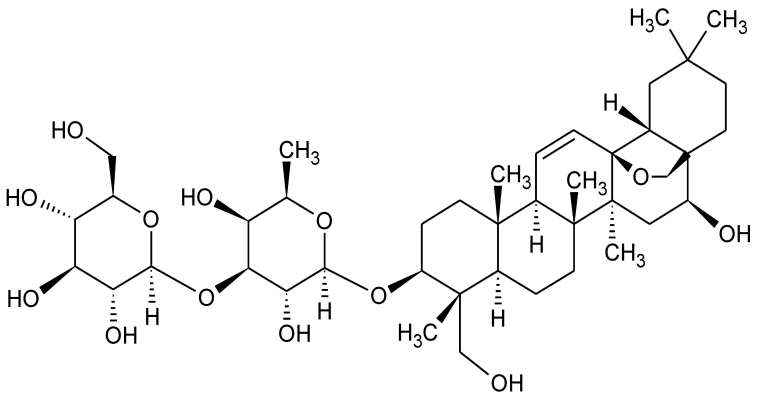

Fig.1. Structural formula of Saikosaponin a

2.2. Experimental design

Kunming mice were randomly divided into six groups (n = 8 per group): Control, Model, CUMS + fluoxetine (3 mg/kg), CUMS + SSa-L (15 mg/kg), CUMS + SSa-M (30 mg/kg) and CUMS + SSa-H (60 mg/kg)（Table 1）. All drugs were dissolved in saline, and mice in control and model groups were treated with saline. After 28 days' exposure to the CUMS procedure, mice were administered drugs intragastrically (0.4 mL/kg) once daily at 9:00 a.m. for a further 28 days. Twenty-four hours after completion of the forced swim test, mice were euthanized by rapid decapitation. Whole brains were rapidly removed and chilled in an ice-cold saline solution. The hippocampus was dissected on a cold plate and immediately frozen in liquid nitrogen. Tissue samples were stored at -80°C until use. The entire experiment lasted 56 days,

and the procedural sequence was as follows: (1) Stress procedure: days 1–56; (2) Drug administration: days 28–56; (3) Body weight test: days 0, 7, 14, 21, 28, 35, 42, 49, 56; (4) Sucrose preference test: days 56; (5) open field test: day 57; (6) Tail suspension test: day 58; (7) Forced swimming test: day 59; (8) Handing of animals: day 60 (Fig. 2).

Table 1. Animal grouping and dosage.

Group	Drug	Dosage	Number
Control	None	None	8
CUMS	NaCl	0.9%	8
CUMS+FLU	FLU	3mg/kg	8
CUMS+SSa-L	SSa-L	15mg/kg	8
CUMS+SSa-M	SSa-M	30mg/kg	8
CUMS+SSa-H	SSa-H	60mg/kg	8

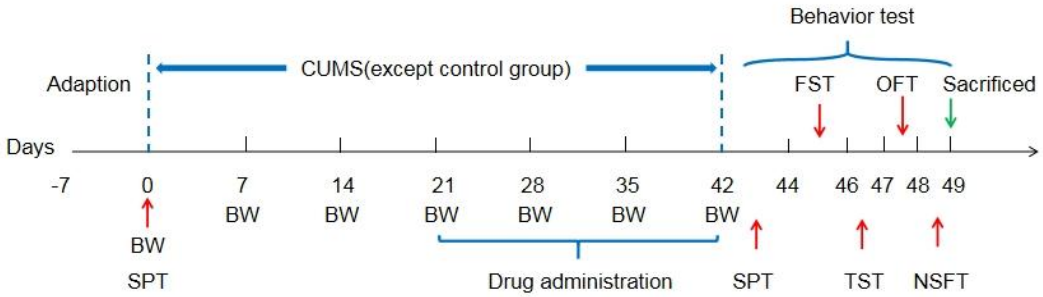

Fig. 2. Schematic representation of experimental procedures for chronic unpredictable mild stress and drug treatment in mice. Mice were exposed to CUMS procedure for 28 days, and drug administration was subsequently performed for an additional 28 days during which CUMS continued.

2.3. Chronic unpredictable mild stress (CUMS)

According to previous methods[8], mice in the model group randomly received 12 types of stimulation (Table 2), including restraint (2h), a cage tilted 45 degrees overnight, cage rotation (1h), overnight isolation overnight, food deprivation, overnight water deprivation, noise pollution (80 dB, 2h), no packing overnight, day and night reversed, stroboscope use overnight, wet bedding overnight and crowding overnight. Two random methods per day

lasted for 56 days, and the same stress pattern was not used for 2 consecutive days. Non-stressed mice were left undisturbed in their cages, except for necessary procedures, such as regular cage cleaning.

Table 2. The chronic unpredictable mild stress (CUMS) stressor procedures.

Stressor	Duration
Restraints	2h
Tilt cage (45°)	Overnight
Cage rotation	1h
Isolation	Overnight
Food deprivation	24h
Water deprivation	24h
Noise pollution(80dB)	2h
Without packing	Overnight
Day and night reversed	12h
Stroboscape	Overnight
Wet bedding	Overnight
Crowding	Overnight

2.4. Behavioral tests

2.4.1. Body weights (BW)

Weighing food intake is an important indicator for measuring animals' depressive state. Depressive mice often lose appetite and reduce their food intake. In this experiment, weights of the mice were measured once a week to observe growth trends.

2.4.2. SPT

The hedonic behavior deficiency, a fundamental symptom of depression [9], can be evaluated by measuring sucrose preference. As such, the SPT was performed at the end of the fourth and eighth week (1h after the last drug treatment) of CUMS exposure to measure success of the model and behavioral changes after administration. Briefly, before the test, mice were adapted to the sucrose solution (1%, w/v). Two bottles of sucrose solution were placed in each cage

for 24h, and then one bottle of sucrose solution was replaced with tap water for 24h. After adaptation, mice were deprived of water and food for 12h. The test was conducted at 8:30 a.m. The mice individually and had free access to two bottles, one containing sucrose solution and the other containing tap water. After 12h, the volumes of the consumed sucrose solution and water were recorded. Sucrose preference (SP) values were calculated by the following formula: SP=sucrose consumption/ (sucrose consumption + water consumption) × 100%.

2.4.3. OFT

Locomotor activity and exploratory behavior of each mouse was assessed using the OFT as illuminated by previous studies [10]. The open-field apparatus consists of a 50 cm × 50 cm × 40 cm square arena with black wall and black base, of which the base is divided into 5 × 5=25 equal squares with legible white lines. Each mouse was gently placed in the center of the open-field floor and then allowed to enjoy independent movement and explore freely for 5 min. Number of crossings, number of rearings and number of groomings were monitored and recorded. After each trial, 75% ethyl alcohol was used to refresh the open-field apparatus to get rid of interfering odor signals.

2.4.4. TST

The TST is another well-known animal behavioral test for assessing antidepressant activity. The total duration of immobility induced by tail suspension was measured according to a described previously method [11]. The length of immobility time reflects the degree of despair in mice. Briefly, after the last open-field test, mice were suspended 50 cm above the floor for 6 min by adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time during the final 4 min of the testing period was recorded. Mice were considered immobile only when they hung passively and completely motionless.

2.4.5. FST

In the forced swim test, an individual mouse was placed softly into a 20 cm diameter glass cylinder filled to 20 cm in depth with 24 ± 1℃ water for 6 min. Mouse immobility and struggling behavior during the last 4 min swim session were recorded and quantified by investigators blinded to group assignment. Struggling was defined as vigorous movements of the forepaws breaking the water, and immobility was defined when mice floated without struggling, making only those movements necessary to keep their head above the water [12]. The glass cylinder was replaced with fresh water after each experiment. The time of immobility reflects the degree of despair in mice.

2.5. Molecular biology tests

2.5.1. Quantitative Real-Time PCR (QRT-PCR)

Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). cDNA was then synthesized from RNA samples using a PrimeScript™ RT reagent Kit with gDNA Eraser (Takara, Harbin, China) [13]. QRT-PCR was performed using SYBR® Premix Ex Taq™ II (Takara, Harbin, China) with a LightCycler 480 apparatus (Roche Molecular Systems, Inc., Pleasanton, CA, USA). GAPDH was used as an internal control. Relative gene expression was calculated by the $2^{-\Delta\Delta Cq}$ method. Primer sequences were as follows:

GAPDH forward, 5'-GGTTGTCTCCTGCGACTTCA-3'

and reverse, 5'-TGGTCCAGGGTTTCTTACTCC-3';

BDNF forward, 5'-TCATACTTCGGTTGCATGAAGG-3'

and reverse, 5'-AGACCTCTCGAACCTGCCC-3';

TrkB forward, 5'-GGCT TGACATCATTGGCTGAC-3';

and reverse, 5'-CATTGGGCCGAACTTTCTGGT-3';

CREB forward, 5'-CCTGTTTGATCGGCAGGAC-3';

and reverse, 5'-CGGGGGACC ATAATGGAGA-3'.

2.5.2. Western blot

The entire hippocampus was homogenized in RIPA lysis buffer containing protease inhibitors and phosphatase inhibitors for protein extraction [14]. Supernatants were collected by centrifugation at 12,000 rpm for 15 min at 4°C, and total protein concentrations were determined by BCA assay (Beyotime, Daqing, China). Protein samples were separated by 10% SDS-PAGE gel electrophoresis and transferred onto NC membranes. After blocking with 5% non-fat dried milk, membranes were incubated with BDNF (Proteintech, 1:500 dilution), TrkB (Proteintech, 1:2000 dilution), pTrkB (Bioss, 1:1000 dilution), CREB (Proteintech, 1:2000 dilution), pCREB (Signalway antibody, 1:500 dilution) and β-actin (CST, 1:5000 dilution) antibodies at 4°C overnight. Subsequently, membranes were rinsed three times with TBS-T followed by incubation with goat anti-rabbit HRP-conjugated IgG (Zhongshan Jinqiao Biotechnology Co., Ltd, dilution at 1:20000) and goat anti-mouse HRP-conjugated IgG (Zhongshan Jinqiao Biotechnology Co., Ltd, dilution at 1:10000) secondary antibodies for 1 h at room temperature. Blots were washed three times with TBS-T and successively visualized

with an enhanced chemiluminescence (ECL) system. Protein bands were quantified using a densitometer (AI600 Imaging System).

2.6. Statistical analysis

All data are expressed as the mean ± S.E.M. (Standard Error of the Mean). One-way ANOVA followed by Dunnett's multiple comparisons test was performed. A value of $P < 0.05$ was considered statistically significant. Figures were created using GraphPad Prism (version 6).

3. Results

3.1. Antidepressant-like effects of SSa in male mice exposed to CUMS

3.1.1. BW

As shown in Fig. 3A, body weight was measured once a week from the beginning of modelling. From 1 to 8 weeks, body weights were significantly decreased in the model group in compared to the control group ($P < 0.001$). Compared with the model group, body weights were significantly increased in SSa ($P < 0.001$) and fluoxetine ($P < 0.001$) at week 8. These results suggest that SSa improves depressive-like symptoms by increasing appetite in mice.

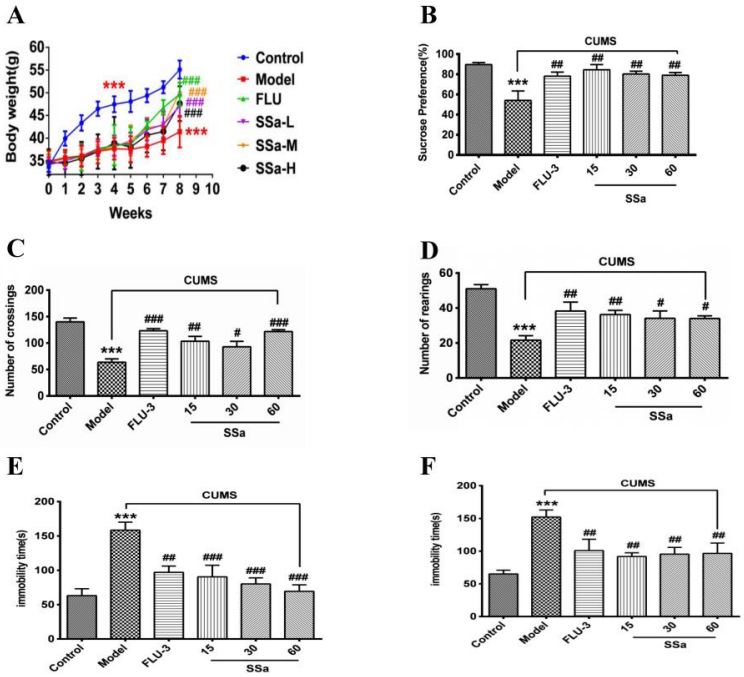

Fig.3. Body weight and behavioural tests. (A) Body weights. (B) Sucrose preference test. (C) Open field test-crossings. (D) Open field test rearings. (E) Tail suspension test. (F) Forced swim test. The results are indicated as the mean ± SD of body weights. The results are indicated as the mean ± S.E.M. of other tests. $^{***}P < 0.001$ vs. Control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. Model group.

3.1.2. SPT

SPT results are shown in Fig. 3B. CUMS mice showed anhedonia behavior with decreased sucrose consumption compared to the control group [F (5, 42) = 5.801, (P < 0.001)]. Four-week treatment with SSa significantly increased sucrose consumption in CUMS mice (SSa 15, 30 and 60 mg/kg, P < 0.01), and this effect was similar to that of fluoxetine (P < 0.01). These results indicate that SSa may play an antidepressant role by improving anhedonia behavior in mice.

3.1.3. OFT

Results of OFT are shown in Fig. 3. Mice exposed to CUMS showed significantly decreased spontaneous locomotor activity in the open field test: crossings [F (5, 36) = 13.12, P < 0.001] and rearings [F (5, 44) = 9.306, P < 0.001] compared to the control group. These results suggest that CUMS effectively leads to decreased movement and exploration in mice. SSa or fluoxetine treatment reversed the decreased number of crossings (SSa 15 mg/kg, P < 0.01, 30 mg/kg, P < 0.05, 60 mg/kg, P < 0.001; fluoxetine 3 mg/kg, P < 0.001; 3C) and rearings (SSa 15 mg/kg, P < 0.01, 30 mg/kg, P < 0.05, 60 mg/kg, P < 0.05; fluoxetine 3 mg/kg, P < 0.01; 3D); however, there was no significant difference in number of groomings (data did not shown). These results reveal the potential antidepressant effects of SSa.

3.1.4. TST and FST

As shown in Fig. 3, we also examined the effects of SSa treatment on TST and FST. CUMS mice displayed desperate behavior as indicated by increased immobility time compared to the control group [F (5, 49) = 10.44, P < 0.001 TST; 3E; F (5, 46) = 7.072, P < 0.001 FST; 3F]. This suggests that CUMS leads to desperate behavior in mice, while SSa or fluoxetine treatment significantly alleviated despair behavior induced by CUMS treat (SSa 15 mg/kg, P < 0.001, 30 mg/kg, P < 0.001, 60 mg/kg, P < 0.001; fluoxetine 3 mg/kg, P < 0.01 TST and SSa 15 mg/kg, P < 0.01, 30 mg/kg, P < 0.01, 60 mg/kg, P < 0.01; fluoxetine 3 mg/kg, P < 0.01 FST). These results suggest that SSa plays an antidepressant role by effectively improving despair behavior in mice.

3.2. The effects of SSa on BDNF-TrkB-CREB in male mice exposed to CUMS

3.2.1. QRT-PCR

The effects of SSa (15, 30 and 60 mg/kg) or fluoxetine (3 mg/kg) on mRNA expression of BDNF, TrkB and CREB are shown in Fig. 4. Results demonstrate that mRNA expression of

BDNF, TrkB and CREB were all decreased compared to the control group [F (5, 12) = 12.81, $P < 0.01$ BDNF; 4A; F (5, 12) = 12.85, $P < 0.01$ TrkB; 4B; F (5, 12) = 24.63, $P < 0.05$ CREB; 4C]. In addition, administration of SSa (SSa 15 mg/kg, $P < 0.001$, 30 mg/kg, $P < 0.001$, 60 mg/kg, $P < 0.001$ BDNF; SSa 15 mg/kg, $P < 0.05$, 30 mg/kg, $P < 0.01$, 60 mg/kg, $P < 0.001$ TrkB; SSa 15 mg/kg, $P < 0.001$, 30 mg/kg, $P < 0.001$, 60 mg/kg, $P < 0.001$ CREB) or fluoxetine at a dose of 3 mg/kg($P < 0.001$ BDNF, $P < 0.001$ TrkB and $P < 0.001$ CREB) had a significantly improved effect. These results show that SSa reverses downregulation of mRNA expression of BDNF, TrkB and CREB induced by CUMS.

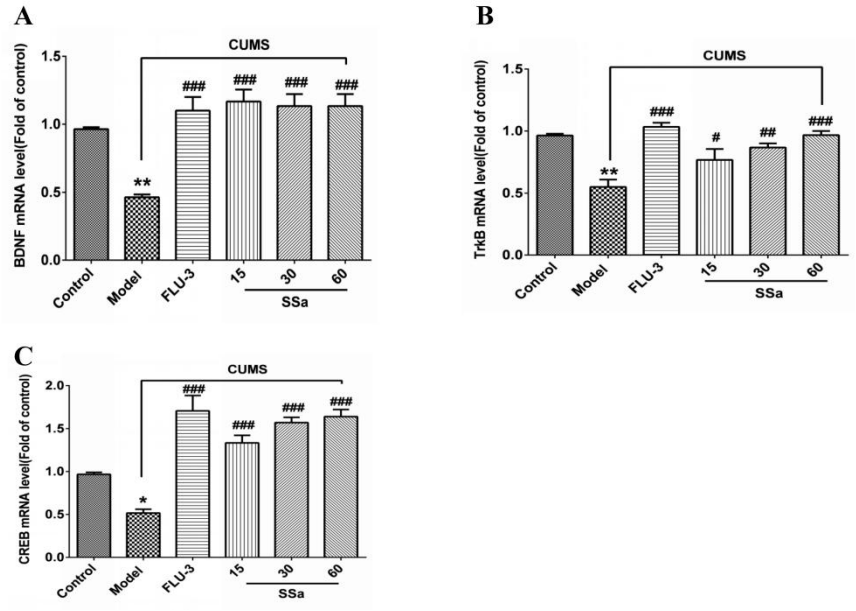

Fig.4. Hippocampal mRNA expression of BDNF, TrkB and CREB. (A) mRNA expression of BDNF. (B) mRNA expression of TrkB. (C) mRNA expression of CREB in hippocampus. The results are indicated as the mean ± S.E.M. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. Control group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. Model group.

3.2.2. Western blot

Fig. 5 shows the effects of SSa administration on BDNF-TrkB-CREB signaling pathways. One-way ANOVA indicated that CUMS significantly reduced BDNF levels [F (5, 12) = 10.46, $P < 0.01$], pTrkB/TrkB ratio [F (5, 12) = 10.13, $P < 0.01$] and pCREB/CREB ratio [F (5, 12) = 12.06, $P < 0.001$] in the hippocampus of CUMS mice compared to controls. Gavage with SSa (SSa 15 mg/kg, $P < 0.01$, 30 mg/kg, $P < 0.01$, 60 mg/kg, $P < 0.001$ BDNF; 5A; SSa 15 mg/kg, $P < 0.05$, 30 mg/kg, $P < 0.01$, 60 mg/kg, $P < 0.001$ pTrkB/TrkB; 5B; SSa 15 mg/kg, $P < 0.01$, 30 mg/kg, $P < 0.001$, 60 mg/kg, $P < 0.001$ pCREB/CREB; 5C) and fluoxetine ($P < 0.01$ BDNF; $P < 0.001$ pTrkB/TrkB; $P < 0.001$ pCREB/CREB) significant improved these effects.

These results suggest that SSa interferes with the downregulation of BDNF, pTrkB/TrkB and pCREB/CREB expression induced by CUMS.

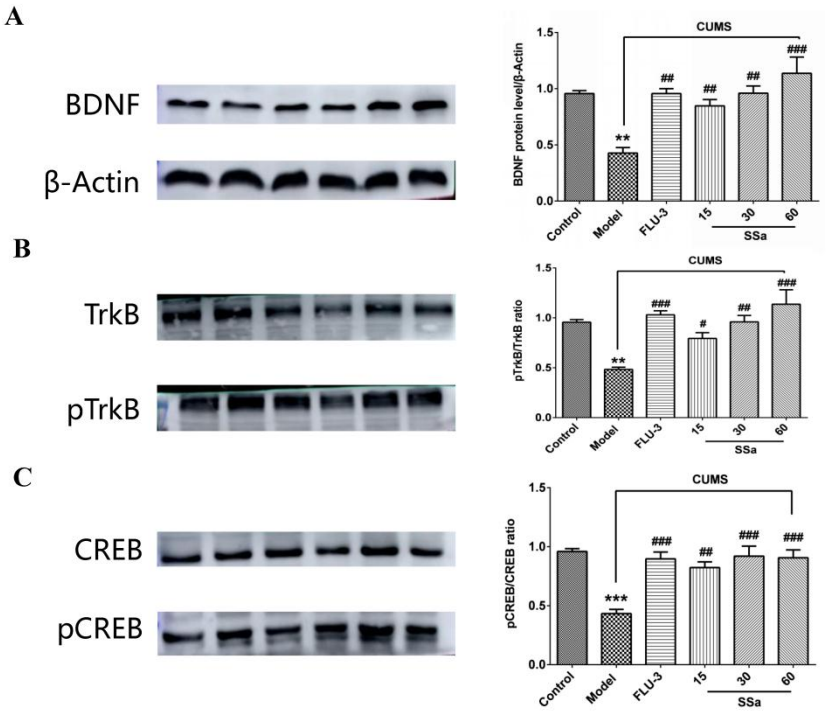

Fig.5. Protein expression of BDNF, p-TrkB/TrkB, p-CREB/CREB in hippocampus. (A) BDNF. (B) p-TrkB/TrkB. (C) p-CREB/CREB. The results are indicated as the mean ± S.E.M. $^{**}P < 0.01$, $^{***}P < 0.001$ vs. Control group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. Model group.

4. Discussion

The present study aimed to examine the depressive-like response following administration of SSa in male Kunming mice subjected to CUMS. The major findings of this study include administration of SSa during CUMS ameliorated depressive-like behaviors, including anhedonia, weight loss, decreased spontaneous locomotor activity and despair conditions, which might be related to upregulation of the BDNF-TrkB-CREB pathway.

Depression is a major clinical problem characterized by low mood. Clinical diagnosis of depression is primarily evaluated using the Hamilton Depression scale, and the severity of depression is judged according to the score of the depression scale. There is a lack of effective molecular diagnostic indicators for depression. It was reported that depression is primarily divided into three stages: the first stage is mild psychotic depression; the second stage of subthreshold depression may be large or small in the middle; and the third stage of severe depression is characterized by clear depression and a lack of pleasure [15]. For these three

phases of depression, clinically, the primary therapeutic measures are drug therapy, psychotherapy and physiotherapy, respectively. Traditional tricyclic antidepressants and newly marketed monoamine neurotransmitter inhibitors are the primary drugs used for depression therapy, psychotherapy is primarily used to guide the emotions of patients. Physiotherapy is primarily electrotherapy, transcranial magnetic stimulation and acupuncture therapy [16]. Extant medicine marketed to treat depression is divided into several subtypes: tricyclic antidepressants (TCAs), monoamine oxidase inhibitors (MAOIs), selective 5-HT reuptake inhibitors (SSRIs) et al. According to clinical data investigation, TCAs exerts significant adverse effects on the cardiovascular system and has limited clinical application. MAOIs have clear toxicity and side effects, including headache, nausea, insomnia and other adverse reactions. Compared to the other two types of drugs, SSRIs are the most widely used clinically, and they selectively inhibit the presynaptic membrane, causing 5-HT content in the synaptic gap to increase. Fluoxetine is a representative SSRI, which has the advantages of small dose, good oral absorption, fewer adverse reactions and good safety [17]. It is the first choice of antidepressant drugs in clinical practice. It has been reported that fluoxetine upregulates BDNF, TrkB and CREB content in depressive model mice and plays an antidepressant role [18]. Therefore, this study selected fluoxetine as a positive control drug to evaluate the antidepressant effects of SSa.

Learning and memory function of mice in depression in depression models is altered, and damage to the limbic system may be the primary cause [19]. The hippocampus is a major part of limbic system. Literature shows hippocampal atrophy and axonal reduction in depressed people and in animal models of depression [20]. Therefore, this study investigated mRNA and related protein expression levels of BDNF-TrkB-CREB in the hippocampus in mice.

The establishment of stable and effective animal model of depression is key to the study of depression. At present, depression models used in pharmacology research are divided into four categories: physical factor induced depression models, drug factor induced depression models, olfactory bulb excision depression models and transgenic animal depression models [21]. Physical factor induced depression models primarily include CUMS and the acquisition helplessness model (LH). Although the LH depression model is similar to clinical depression symptoms in patients, the duration of this model is short, and it cannot be used to screen chronic antidepressant use [22]. Drug-induced depression models are used to evaluate drug interactions by injecting glucocorticoid, reserpine and morphine into animals. The olfactory bulb depression model is similar to humans in physiology and behavior and involves removal

of the olfactory bulb, simulating sensitive symptoms of clinical depression patients. However, it is technically difficult to construct this model, and animal mortality is high. The CUMS model was first proposed by Willner [23], in which animals were placed in unpredictable environments for approximately 3-4 weeks, and mild stimuli were given daily to observe the responses of animals to stimuli. The CUMS model is the most widely used depression model at present, which simulates the primary manifestation of long-term chronic low-intensity stimulation in human daily life and has the following advantages: in accordance with the clinical characteristics of the disease; good repeatability, simple, reliable and maneuverability stable in state, long in duration and convenient for observation[24]. Therefore, this model was used to construct the animal model of depression.

In terms of behavioral testing of experimental animals, we used four classical experiments: SPT, OFT, TST and FST. SPT indirectly reflects the animal's food preference by measuring the rate of consumption of sugar water, which is used to simulate the clinical symptoms of a lack of pleasure in patients with depression; OFT examines the animal's movement and exploration ability by measuring the distance of horizontal movement and the number of uprights and groomings, reflecting the animal's conscious behavior and curiosity about new things[25]. TST and FST examine animal despair by measuring the immobility time in tail suspension and forced swim experiments [26]. Despair is usually consistent with suicidal, desperate behavior in depressed patients [27]. The experimental model creation and drug administration lasted for 56 days. Before modelling, animals had a week to adapt to the environment and were then evaluated by OFT. Mice with similar scores were equally divided into 6 groups: control, model, fluoxetine, and SSa (15, 30 and 60 mg/kg). Results demonstrated that compared to the control group, the 4-week model group had the following significant changes: body weight increased relatively slowly and was lower than the control group, the consumption rate of sugar and water decreased significantly, free movement and exploration decreased significantly and the time of immobility increased markedly, indicating that CUMS produced depression-like behaviors, such as a lack of pleasure, loss of interest, loss of appetite and despair, supporting the success of the model. In the experiments, there were no significant differences in the number of groomings among the three groups, indicating that SSa had no effect on cleaning ability of CUMS-induced depression mice, which was similar to Ke's results [28]. Treatment with SSa showed that compared to the model group, drug administration significantly increased sugar consumption in depressive mice ($P < 0.001$), similar to the effects of fluoxetine and SSa-L (15 mg/kg), which were all significantly

better than the control group ($P < 0.05$). Weight is an important indicator of depression, and depressed patients typically suffer from weight loss, loss of appetite, decreased food intake and other symptoms (Ren et al., 2018). Results showed that compared to the model group, SSa-L (15 mg/kg), SSa-M (30 mg/kg) and SSa-H (60 mg/kg) groups effectively improved weight gain in mice. TST and FST showed that compared to the model group, the three groups given SSa treatment exhibited significantly improved immobility time and improved desperate behavior. Furthermore, the improvement effect of SSa-H was superior to that of fluoxetine. Immobility time of mice approximated levels of the control group. These results suggest that SSa plays an antidepressant role by increasing the weight of depressed mice, increasing sugar consumption rate, improving spontaneous activity and alleviating desperate behavior in depressed mice.

It has been reported that the BDNF-TrkB-CREB signaling pathway is involved in the survival, growth and plasticity of nerve cells in the human brain and is closely related with depression [29]. BDNF is one of the important neurotrophic factors in the central nervous system that plays an important role in the development of the central nervous system and the survival, differentiation, growth and development of neurons. Results have shown that the content of BDNF decreases in both the central and peripheral regions of depression patients, and treatment with antidepressants restores the normal level [30], indicating the pathogenesis of depression is related to BDNF. The synthesis of BDNF occurs from a precursor of proBDNF, which is assembled in the Golgi, stored in vesicles and released in an activity-dependent manner in the presynaptic membrane of axon terminals and further transformation into mature BDNF [31]. Mature BDNF activates high-affinity receptor TrkB to promote receptor TrkB self-phosphorylation (pTrkB) [32]. The BDNF-TrkB signaling pathway is involved in survival, growth, migration, inhibition of nerve excitation, dendritic processes in the central nervous system and physiological processes, such as axon growth and synaptic remodeling [33]. The literature shows that in a model of chronic stress depression, expression of pTrkB/TrkB protein in the brain is downregulated, which induces depression-like behavior [34]. CREB is an important transcription factor induced by adenylate cyclase (AC) that transforms adenosine triphosphate (ATP) into cyclic adenosine monophosphate (cAMP) [35]. In the intracellular pathway, its transcription function is also affected by auto-phosphorylation (pCREB), which can regulate central nervous system functions and participates in the growth, proliferation and differentiation of cells, as well as physiological functions, such as survival and neuroplasticity [36]. Expression CREB and pCREB mRNA and protein was significantly downregulated in a

CUMS-induced depression rat model, and antidepressant treatment reversed this downregulation [37]. In conclusion, the BDNF-TrkB-CREB signaling pathway is involved in the physiological and pathological mechanism of depression through inflammation, apoptosis and neuroplasticity, and it is related to SSa. However, related research on SSa has not yet been reported. Therefore, the aim of this study was to study the mechanism of SSa on BDNF-TrkB-CREB signaling and to evaluate the antidepressant effect of SSa by behavioral and molecular biology assays. The results showed that mRNA expression of BDNF, TrkB and CREB in the model group was downregulated, while expression was significantly upregulated after SSa treatment, whose effect was superior to that of fluoxetine, especially in the SSa-L (15 mg/kg) group ($P < 0.001$). Protein expression of BDNF, pTrkB/TrkB and pCREB/CREB was downregulated in the model group. After SSa treatment, however, protein expression was upregulated to a similar degree as that of fluoxetine. The effect of SSa-H (60 mg/kg) group was better than that of 60 mg/kg group ($P < 0.001$). This indicates that SSA exerts an antidepressant effect on CUMS mice by regulating the BDNF-TrkB-CREB signaling pathway.

5. Conclusions

In conclusion, this study revealed that SSa could improve depressive-like behavior in male Kunming mice induced by CUMS, which induces depression by decreasing the rate of consumption of sugar water, increasing immobility time in tail suspension test and forced swim test, and decreasing of the number of crossings and rearings in the open-field test. SSa may play an antidepressant role through the BDNF-TrkB-CREB signaling pathway by regulating mRNA expression levels of BDNF, TrkB and CREB and protein expression levels of BDNF, pTrkB/TrkB and pCREB/CREB. This experiment laid a theoretical foundation for the development and utilization of Radix Bupleuri, and the mechanism of action of the pathway on depression will be further examined in subsequent studies.

Author contributions

Conceived and designed the experiment: Taiming Wei, Teli Zhang. Performed the experiments: Min Liu, Rong Wang, Biying Shi, Yanan Sun, Qi wang. Analyzed the data: Rong Wang, Chunyue yu, Min Liu, Chun-Ming Zou. Wrote the paper: Min Liu, Rong Wang, Chunyue yu.

Conflicts of Interest

The authors declare that they have no competing interest.

All authors have contributed to and approved the final manuscript.

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