Effects of Total Saponins from *Panax Notoginseng* Leaves on Brain Monoamine Neurotransmitters and Neurotrophic Factors in a Depressive Rat Model

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Abstract: A chronic unpredictable mild stress (CUMS) rat model of depression was established. The levels of serotonin (5-HT), noradrenaline (NA), and dopamine (DA) as well as the expression of brain-derived neurotrophic factor (BDNF), tropomyosin-related kinase B (TrkB), phosphorylated cAMP response element-binding protein (pCREB), and phosphorylated extracellular signal-regulated kinase 1 and 2 (pERK1/2) were measured using competitive enzyme-linked immunosorbent assay (ELISA) kits and an immunohistochemistry (ICH)-staining method, respectively. The effect of the total saponins from leaves of *Panax notoginseng* on levels of brain monoamine neurotransmitters and neurotrophic factors were evaluated. After the model was established for five weeks, the body weight and the sucrose preference in the animals of the CUMS group were significantly reduced by 17.43% and 39.49%, respectively, compared with the control group. Moreover, CUMS procedure induced a significant decrease in the exploratory locomotor activity, an increased total duration of thigmotaxis, and a significant decrease in the mass percentage of the hippocampus relative to the whole brain. Animals exhibited depression-like symptoms, such as body weight loss, anhedonia, decrease in locomotor activity, and reduction of hippocampal volume, similar to major human depressive symptoms. Compared with the CUMS group, chronic treatment with SLPN could effectively reverse the aforementioned CUMS-induced symptoms. SLPN at a dose of 100 mg/kg significantly increased the NA and DA levels in the brain of CUMS-treated rats by 13.89% and 19.66%, respectively, but had no significant effects on the 5-HT levels as compared with the CUMS group. In addition, SLPN also significantly improved BDNF, TrkB, pCREB, and pERK1/2 expression in the CUMS-treated rats by 16.67%, 47.83%, 20.42%, and 22.84%, respectively. Taken together, these data indicate that SLPN exhibits excellent antidepressant-like properties.

Key words: leaves of *Panax notoginseng*; depression; chronic unpredictable mild stress; monoamine neurotransmitters

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三七叶总皂苷对抑郁大鼠脑内单胺及神经因子的影响

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摘要：建立慢性应激抑郁大鼠模型，采用 ELISA 法测定 5-HT、NA、DA 水平，ICH 法测定 BDNF、TrkB、pCREB、pERK 的表达，评价三七叶总皂苷对脑内单胺及神经因子的影响。建模五周后，与空白组比较，模型组大鼠体重、糖水偏好率分别降低了 17.43%、39.49%，自主活动表现为探索能力降低、趋避能力升高，海马体质量百分比显著减少，呈现出人类抑郁症中体重下降、快感缺失、自主活动下降、海马体萎缩等核心症状。而给药组与模型组比较，上述指标得到有效逆转。与模型组比较，三七叶总皂苷高剂量组（100 mg/kg）可以使脑内 NA、DA 水平分别升高 13.89%及 19.66%，而对 5-HT 水平没影响，并且使脑内 BDNF、TrkB、pCREB、pERK 的表达分别上升 16.67%、47.83%、20.42%、22.84%，表明三七叶总皂苷具有良好的抗抑郁作用。

关键词：三七叶；抑郁；慢性应激；单胺

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Panax notoginseng (Burk.) F.H. Chen (Araliaceae, named as Sanqi or Tianqi in Chinese), a precious medicinal herb in Asia, has a long history of use in Chinese medicine. The total saponins from leaves of P. notoginseng (SLPN) is recorded in the China Pharmacopoeia (edition, 2010) as the main component of the traditional Chinese patent medicine "Qi Ye Shen An tablet," used for the treatment of insomnia, anxiety, and neurasthenia neurosis. Previous studies have demonstrated the antidepressant-like effects of SLPN in several behavioral tests in both mice and rats [1]. SLPN contains a series of bioactive protopanaxadiol-type ginsenosides, having similar aglycones with a dammarane skeleton, such as ginsenosides Rb_1, Rb_2, Rb_3, Rc, Rd, and 20(*S*)-protopanaxadiol (PPD) [2~4]. Ginsenoside Rb_3, one of the major components of SLPN composing over 15% content, possesses excellent antidepressant-like effects [5, 6]. In addition, ginsenosides Rb_1 and PPD have also been reported for their antidepressant effects by other researchers [7, 8]. It seems that protopanaxadiol-type ginsenosides display important role in the antidepressant effects. Although SLPN has been demonstrated to contain several active components, an exact characterization of the pharmacological mechanisms underlying its antidepressant-like effects remains largely to be investigated.

A growing number of studies have demonstrated that ginsenoside extracts or pure ginsenosides have neuroprotective effects with little toxicity. Dang et al. reported that the antidepressant activity of ginseng total saponins (GTS) from *Panax ginseng* C. A. Meyer (Chinese and Korean ginseng) in stress-treated rats might be partially mediated by the enhancement of brain-derived neurotrophic factor (BDNF) expression in the hippocampus [9]. Ginsenoside Rg_1, one of the most abundant and active ingredients of GTS, has already been shown to possess antidepressant activity via activation of the BDNF signaling pathway and up-regulation of hippocampal neurogenesis [10]. Similarly, ginsenoside Rb_3 has exerted antidepressant-like effects by increasing the BDNF expression in the hippocampus [6].

Thus, it was hypothesized that the excellent effects of SLPN on depression-like symptoms were likely to be due to an increase in BDNF expression in the hippocampus. Therefore, to elucidate the detailed molecular mechanisms of SLPN's antidepressant effects, the effects of SLPN on behavioral activities were investigated in the present study by using a chronic unpredictable mild stress (CUMS) rat model of depression. Furthermore, the levels of monoamine neurotransmitters and the hippocampal expression of some important neurotrophic factors, including BDNF, BDNF receptor tropomyosin-related kinase B (TrkB), phosphorylated cAMP response element-binding protein (pCREB), and phosphorylated extracellular signal-regulated kinase 1 and 2 (pERK1/2), were detected.

1 Materials and Methods

1.1 Chemicals and drugs

SLPN was supplied by Guangdong Medi-World Pharmaceutical Co. Ltd. (Foshan, China), meeting the criterion of the Pharmacopoeia of the People's Republic of China, 2010. SLPN contained 78.5% total saponins and 0.34% total flavonoids as determined by ultraviolet spectrophotometry, as well as ginsenosides Rb_3 15.62%, Rc 7.62%, Rb_1 2.98%, Rb_2 3.83%, F1 0.75%, F2 10.52%, notoginsenoside Fa 3.27%, Fc 6.83%, and gypenosides 10.52%, as determined by high performance liquid chromatography (HPLC). However, no alkaloids, polysaccharides, starches, tannins, proteins, amino acids, and peptides were detected [1]. Fluoxetine (FLU, a typical SSRI-type antidepressant) was used as the reference in this study and was purchased from Guangdong South of China Pharmacological Corp. (Guangzhou, China). Anti-BDNF rabbit antibody and anti-TrkB rabbit antibody were purchased from Abcam (Cambridge, UK), and anti-pERK rabbit antibody, and anti-pCREB rabbit antibody were purchased from Cell Signaling Technology (Danvers, MA, USA).

1.2 Animal grouping and drug administration

Male SD rats weighing 180~220 g were purchased from Guangdong Medical Laboratory Animal Center (Foshan, China). The animals were group-housed under standard laboratory conditions (25 ± 2 ℃, 60~70% humidity, 12 h/12 h light/dark cycle, standard food and water available ad libitum). The animals were acclimatized to laboratory conditions for at least 3 days before starting the experiments. Behavioral studies were performed during the light phase of the light/dark cycle.

The procedures were approved by the Animal Welfare Committee of Guangdong Medical Laboratory Animal Center and were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publication no. 85~23, revised 1985).

Prior to the chronic unpredictable mild stress (CUMS) experiments, all the animals were trained to consume 1% (*m/V*) sucrose solution and then subjected to a baseline sucrose preference test. After the baseline test, the animals were divided into five groups based on the baseline sucrose preference score: the control group; the CUMS group; the FLU group (10 mg/kg); the SLPN group (70 mg/kg and 100 mg/kg) (n = 8 for each group). During the CUMS experiment, the healthy control rats were housed together without disturbance in a separate room, except for general handing (e.g., regular cage cleaning and measuring body weight) that matched the CUMS groups, whereas the rats of other groups were housed individually (one cage per rat) and exposed to CUMS. Drugs were administered orally once per day at 8:00~9:00 a.m. The control group and the CUMS group were given 10 mL/kg distilled water daily. All possible efforts were taken to minimize the number of animals used and their suffering at any stage of the experiments.

1.3 CUMS procedure in rat

The CUMS procedure was performed as described by Willner et al. with some modifications [11]. Briefly, the rats were exposed to the following stressors for five consecutive weeks: wet bedding for 24 h (100 mL plain water spilled onto the bedding), behavior restraint in a tube for 2 min (diameter: 8 cm, length: 20 cm), tail nip for 5 min, cage tilting for 24 h (45 °), food deprivation for 24 h, water deprivation for 24 h. Each rat received one of these stressors per day and the same stressor was not applied for 2 consecutive days so that animals could not predict the occurrence of stimulation.

1.4 Sucrose preference test

According to a previous procedure, the sucrose preference test consisted of training and testing trials [12]. Rats were trained to adapt to a 1% sucrose solution (*m/V*): two bottles of sucrose solution were placed in each cage for 24 h, and then one bottle of sucrose and one bottle of tap water were placed for the next 24 h. Before the sucrose preference test, the rats were deprived of water and food for 23 h. Then the rats were housed in individual cages and given free access to two bottles for 1 h, containing 100 mL of sucrose solution and 100 mL of tap water, respectively. The consumption was measured by weighing pre-weighed bottles containing the sucrose solution or tap water at the end of the test. The sucrose preference was calculated according to the following formula: The sucrose preference (%) = sucrose consumption / (sucrose consumption + water consumption). The sucrose preference was monitored to evaluate the CUMS model and the action of drugs in the beginning and end of the CUMS procedure.

1.5 Open field test

Open field test (OFT) was performed according to a previously described method with modifications [13]. Briefly, the animal was individually placed into the corner of the box (45 cm × 45 cm × 35 cm) to adjust to the environment for 1 min followed by recording for 6 min using video camera. Their movement parameters were analyzed by ZH-QPT analytic system, which was purchased from Huaibei Zhenghua bioinstrumentation limited company (Huaibei, China). The apparatus was cleaned with 10 % ethanol and dried during the interval of the test. The total durations of exploration (movement in the central ring) and thigmotaxis (movement in the outer ring, calculated by the time spent in the corners plus that spent along the walls) were regarded as important indexes of the locomotor activity of rats. OFT was conducted 1 h after the last treatment of drugs in a quiet room.

1.6 Enzyme-linked immunosorbent assay analysis

After the last behavioral test, the rats were deeply anaesthetized with pentobarbital sodium and perfused intra-cardially with 0.9% PBS. The whole brains were removed, blotted dry on filter paper and weighed, then total hippocampi were carefully and immediately dissected on glass plates over ice. The levels of the rat brain monoamine neurotransmitters 5-HT, NA, and DA were measured using commercially available, competitive enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions in the Guangdong Medical Laboratory Animal Center (Guangzhou, China). Briefly, hippocampus was homogenized using a Teflon-glass homogenizer. A 30-mL portion of the homogenate was mixed with 100 mL of

normal saline and then centrifuged at 15,000 × g for 15 min at 4 ℃. The supernatant and antigen standard were added to the wells of 96-well plates that were pre-coated with primary antibodies. After adding 50 μL each of a biotin-conjugate reagent and an HRP-conjugated reagent into each well, the plates were incubated at 37 ℃ for 60 min, washed five times with distilled water, and dried. Then, chromogen solution A and chromogen solution B were added to each well and incubated at 37 ℃ for 15 min. Finally, 50 μL of stop solution was added to end the chromogenic reaction and the absorbance of the reaction mixture (final volume of 150 μL) was measured at 450 nm using a microplate reader (Thermo Scientific Multiskan GO, Shanghai, China) within 15 min. All samples were measured in duplicate.

1.7 Immunohistochemistry analysis

The hippocampus was placed into 4% paraformaldehyde and embedded in paraffin. The sections of the hippocampal CA3 area were cut on a rotary microtome using an atlas of rat anatomy [14], and processed for immunohistochemical analyses according to a previously described method [15]. The paraffin-embedded sections were soaked in xylene to remove paraffin and rehydrated through graded alcohol (100%~0%). Then they were rinsed three times for 5 min each with PBS (pH 7.4). After the above pretreatment procedures, all samples were incubated with 3% hydrogen peroxide for 10 min to block the intrinsic peroxidase. The sections were washed three times for 5 min each with PBS (pH 7.4) and then heated in a microwave oven at a low power for 5 min in 0.01 mmol/L citrate buffer (pH 6.0). After rinsing three times with PBS for 5 min, the sections were kept in 5% bovine serum albumin (BSA) for 30 min, followed by incubation with the primary antibodies (BDNF, 1:200, TrkB, 1:100, pCREB, 1:400, pErk1/2, 1:400) overnight at 48 ℃. The sections were washed three times for 5 min with PBS before incubation with biotinylated secondary antibody for 60 min, and then treated with streptavidin-biotin peroxidase for 60 min at 37 ℃. The samples were rinsed three times for another 5 min with PBS and incubated with 3,3′-diaminobenidine (DAB) solution for 10 min. The reaction was stopped by washing with distilled water. PBS was used to replace the primary antibodies in the negative control group. All sections were counterstained with hematoxylin and dehydrated through graded ethanol (0%~100%). They were enveloped with gelatin and observed under bright-field illumination. Images of positive staining in the CA3 region of hippocampus were captured at 400× magnifications by an Olympus BX43 microscope (Olympus, Tokyo, Japan). The expression of BDNF, TrkB, pCREB, and pErk1/2 was measured by detecting the mean optical density (MOD) of positive cells.

2 Results and Discussion

2.1 Effect of SLPN on body weight

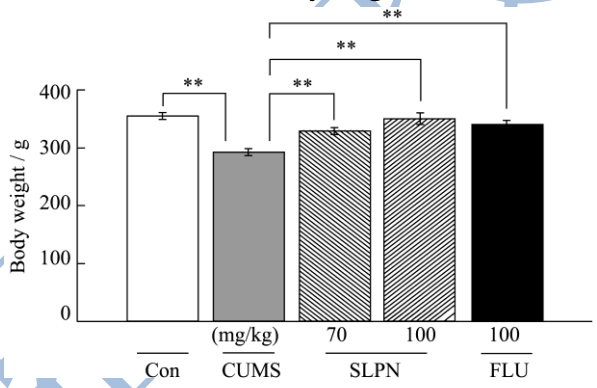

Fig.1 The effects of the total saponins from leaves of *Panax notoginseng* (Burk.) F.H. Chen (SLPN) and fluoxetine (FLU) on the body weight change in rats exposed to the chronic unpredictable mild stress (CUMS) after the 5-week experiment

Note: Con, the control group; * $p < 0.05$, ** $p < 0.01$.

Body weights were recorded weekly during the experiment. Rats in the control group showed a normal increase in the body weight, whereas other groups exposed to CUMS exhibited a relatively small increase. The effects of chronic stress and administration with SLPN and FLU on the body weight after the 5-week experiment are presented in Figure 1. One-way ANOVA indicated statistically significant difference [$F(4, 35) = 12.73$, $p < 0.01$] among the five groups. Compared with the control group, the body weight in the animals of the CUMS group was significantly reduced by 17.43% ($p < 0.01$). This was similar to the loss of appetite experienced by patients with depression. Compared with the CUMS group, the treatment with SLPN (70 mg/kg and 100 mg/kg) produced a significant increase in the body weight by 12.37% and 19.57% respectively (each $p < 0.01$), which was similar to the effect of the positive drug FLU at a dose of 10 mg/kg (15.90%, $p < 0.01$). These

findings suggested that SLPN and FLU could prevent the weight loss caused by CUMS.

2.2 Effect of SLPN on sucrose consumption

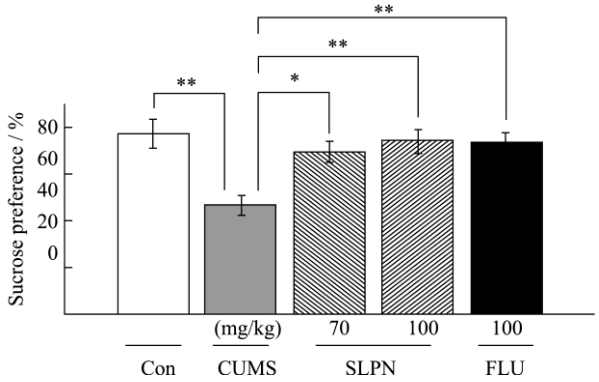

Fig.2 The effects of SLPN and FLU on the sucrose preference in rats exposed to CUMS after the 5-week experiment

Note: Con, the control group; * $p < 0.05$, ** $p < 0.01$.

In the initial baseline test, the sucrose preference showed no significant difference among the groups (data not shown). Effects of SLPN and FLU on the sucrose preference in CUMS-treated rats after the 5-week treatment are shown in Figure 2. One-way ANOVA indicated statistically significant difference [F (4, 35) = 6.18, $p < 0.01$] among the five groups. Compared with the control group, the sucrose preference in the CUMS group was significantly reduced by 39.49% ($p < 0.01$), indicating impairment of hedonic reactivity in model rats. However, compared with the CUMS group, the treatment with SLPN (70 mg/kg and 100 mg/kg) significantly increased the sucrose preference by 48.95% and 57.55%, respectively (each $p < 0.05$), which was similar to the effect of the positive drug FLU at a dose of 10 mg/kg (56.77%, $p < 0.01$). These findings suggested that SLPN and FLU could reverse the anhedonia caused by CUMS.

2.3 Effect of SLPN on locomotor activity

The locomotor activity in the CUMS experiment was observed in the OFT. Effects of SLPN and FLU after the 5-week treatment on the locomotor activity in CUMS-treated rats are shown in Figure 3. One-way ANOVA indicated statistically significant difference in both the total duration of explore [F (4, 35) = 6.31, $p < 0.01$] and the total duration of thigmotaxis [F (4, 35) = 4.21, $p < 0.01$] among the five experimental groups. The post hoc revealed that CUMS led to a significant decrease in the locomotor activity by showing decreased total duration of explore ($p < 0.01$) and increased total duration of thigmotaxis ($p < 0.01$), compared with the control group. By long-term treatment with SLPN (70 mg/kg and 100 mg/kg) or with FLU (10 mg/kg), the above-mentioned behavioral changes in the locomotor activity were significantly reversed, compared with CUMS group (each $p < 0.05$). These findings suggested that SLPN and FLU could improve the decrease in the locomotor activity caused by CUMS.

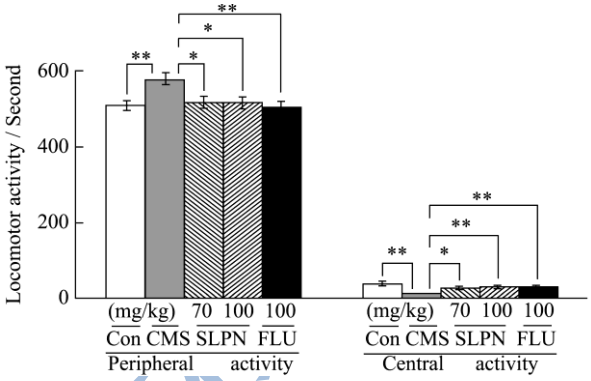

Fig.3 The effects of SLPN and FLU on the locomotor activity in rats exposed to CUMS after the 5-week experiment

Note: Con, the control group; * $p < 0.05$, ** $p < 0.01$.

2.4 Hippocampus weight analysis

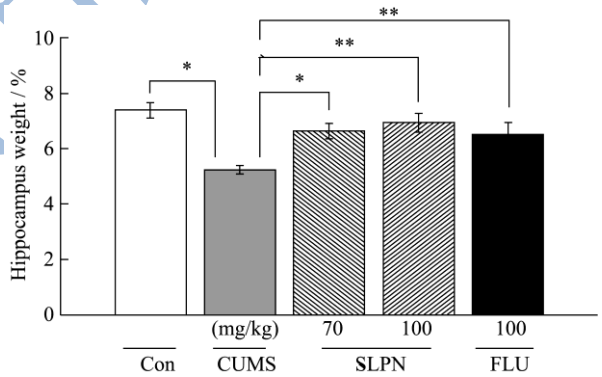

Fig.4 The effects of SLPN and FLU on the hippocampus percentage of rats exposed to CUMS after the 5-week experiment

Note: Con, the control group; * $p < 0.05$, ** $p < 0.01$.

The hippocampus and the remaining cerebrum were rapidly removed and weighed, and the percentage of the hippocampus relative to the whole brain was calculated. Effects of SLPN and FLU after the 5-week treatment on the hippocampus percentage in CUMS-treated rats are shown in Figure 4. One-way ANOVA revealed that there was a statistically significant difference among the experimental groups [F (4, 35) = 5.28, $p < 0.01$]. Compared with the control group, CUMS procedure

induced a significant decrease in the percentage of the hippocampus relative to the whole brain ($p < 0.05$). Compared with CUMS group, SLPN (70 mg/kg and 100 mg/kg) had significantly increased the hippocampus percentage in the CUMS-treated rats (each $p < 0.05$). In addition, FLU at a dose of 10 mg/kg had also markedly improved the hippocampus percentage in the CUMS-treated rats, compared with the CUMS group ($p < 0.01$). These results suggested that the drug-treatment could reverse the hippocampal atrophy induced by CUMS.

2.5 Effects of SLPN on NA, DA, and 5-HT levels

Table 1 The effects of SLPN and FLU on the monoamine neurotransmitters levels in the hippocampi of rats exposed to CUMS

Group	Dose/(mg/kg)	NA/(ng/L)	DA/(ng/L)	5-HT/(ng/mL)
Control	-	40.48±0.88	70.54±2.80	6.59 ±0.12
CUMS	-	38.50±1.11	67.25±1.84	6.21 ±0.12
SLPN	100	43.84±1.45*	80.47±3.82*	6.94±0.25
FLU	10	41.24±1.09	72.74±3.75	7.23±0.25*

Note: Compare with the CUMS group, * $p < 0.05$.

Effects of SLPN and FLU on the NA, DA, and 5-HT levels in the brain of CUMS-treated rats after treatment for 5 consecutive weeks are showed in Table 1. One-way ANOVA revealed that there were statistically significant differences among the experimental groups in the NA, DA, and 5-HT levels [$F(3, 28) = 3.70$, $p < 0.05$, $F(3, 28) = 3.17$, $p < 0.05$, $F(3, 28) = 5.07$, $p < 0.01$, respectively]. Compared with the control group, post hoc test displayed that CUMS procedure exhibited a relatively small tendency to decrease the NA, DA, and 5-HT levels (each $p > 0.05$). Compared with the CUMS group, SLPN at a dose of 100 mg/kg significantly increased the DA and NA levels in the hippocampus of CUMS-treated rats by 19.66% and 13.89% (each $p < 0.05$), respectively, whereas produced no significant effects on the 5-HT levels ($p > 0.05$). Conversely, the positive control drug FLU at a dose of 10 mg/kg remarkably improved the 5-HT levels by 16.34% ($p < 0.05$), compared with the CUMS group, but exhibited no significant effects on the DA and NA levels (each $p > 0.05$).

2.6 Effects of SLPN on BDNF, TrkB, pCREB, and pERK1/2 expression

One-way ANOVA revealed that there was a statistically significant difference in the BDNF expression among the experimental groups [$F(3, 28) = 4.24$, $p < 0.05$]. As shown in Figure 5 and Table 2, CUMS procedure induced a significant decrease in the BDNF expression ($p < 0.05$), compared with the control group. After 5-week treatment, SLPN at a dose of 100 mg/kg as well as FLU at a dose of 10 mg/kg, had significantly improved the BDNF expression in the CUMS-treated rats (each $p < 0.05$) as compared with the CUMS group.

One-way ANOVA showed that there was a statistically significant difference in the TrkB expression among the experimental groups [$F(3, 28) = 9.42$, $p < 0.01$]. As shown in Figure 6 and Table 2, CUMS procedure exhibited a relatively small tendency to decrease the TrkB expression, compared with the control group ($p > 0.05$). After 5-week treatment, ,SLPN at a dose of 100 mg/kg as well as FLU at a dose of 10 mg/kg, had significantly improved the TrkB expression in the CUMS-treated rats (each $p < 0.01$) as compared with the CUMS group.

One-way ANOVA indicated that there was a statistically significant difference in the pCREB expression among the experimental groups [$F(3, 28) = 11.13$, $p < 0.01$]. As shown in Figure 6 and Table 2, CUMS procedure slightly but insignificantly decreased the pCREB expression, compared with the control group ($p > 0.05$). After 5-week treatment, , SLPN at a dose of 100 mg/kg had significantly increased the pCREB expression in the CUMS-treated rats ($p < 0.01$) as compared with the CUMS group. In particular, FLU at a dose of 10 mg/kg had also markedly improved the pCREB expression in CUMS-treated rats compared with both the CUMS group ($p < 0.01$) and the control group ($p < 0.05$).

One-way ANOVA revealed that there was a statistically significant difference in the pERK1/2 expression among the experimental groups [$F(3, 28) = 9.35$, $p < 0.01$]. As seen in Figure 6 and Table 2, CUMS procedure produced no significant effects on the pERK1/2 expression compared with the control group ($p > 0.05$). However, compared with the CUMS group, SLPN at a dose of 100 mg/kg, as well as FLU at a dose of 10 mg/kg, had significantly improved the pERK1/2 expression in the CUMS-treated rats after 5-week

treatment (each $p < 0.01$).

Table 2 The effects of SLPN and FLU on the neurotrophic factor expression in the hippocampi of rats exposed to CUMS

Group	Dose/(mg/kg)	BDNF	TrkB	pCREB	pERK
Control	-	0.250 ±0.008	0.191 ±0.009	0.157 ±0.007	0.177 ±0.008
CUMS	-	0.216 ±0.008$^{\#}$	0.161 ±0.012	0.142 ±0.003	0.162 ±0.002
SLPN	100	0.252 ±0.009*	0.238 ±0.013**	0.171 ±0.005**	0.199 ±0.008**
FLU	10	0.252 ±0.009*	0.239 ±0.016**	0.180 ±0.005**	0.202 ±0.004**
FLU	10	0.252 ±0.009*	0.239 ±0.016**	0.180 ±0.005**	0.202 ±0.004**

Note: The number of rats in each group was 8. Data were expressed as mean ± S.E.M. Differences among groups were assessed using Tukey *post hoc* test. Compared with the CUMS group, * $p < 0.05$, ** $p < 0.01$; compared with the control group, $^{\#}$ $p < 0.05$.

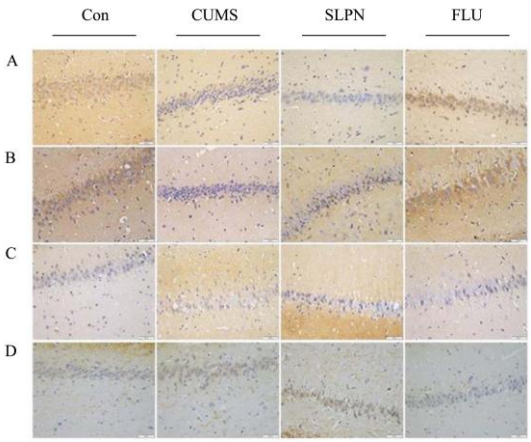

Fig.5 (A-D). The effects of SLPN and FLU on the BDNF (A), TrkB (B), pCREB (C), and pERK1/2 (D) expression in the hippocampus of rats exposed to CUMS after the 5-week experiment

Note: Con, the control group.

2.7 Discussion

The model of depression induced by CUMS is generally thought to be the most valid and the most widely used animal model. Compared with the acute stress-based models, such as the tail suspension test (TST) and the forced swim test (FST), the CUMS model can exclude false positive effects caused by psychostimulant agents [16~18]. Moreover, it is also the suitable animal model for studying the neurobiological basis of depression and the cellular and molecular mechanisms of antidepressant drugs [11, 12]. In the present study, a CUMS-induced rat model with depression-like behavioral changes was successfully established by measuring the change of the sucrose preference, the locomotor activity, the body weight, and the hippocampus percentage. More importantly, the present result showed that the 5-week chronic treatment with SLPN (70 and 100 mg/kg) and FLU (10 mg/kg) could effectively reverse the CUMS-induced aforementioned changes. It could be suggested that SLPN possessed an excellent antidepressant-like effect in the animal model, which is consistent with our previous result [1].

Although multiple mechanisms are responsible for the development of depression, it is well accepted that monoamine neurotransmitters (NA, DA, and 5-HT) and their metabolites in the central nervous system have a close relationship with depression. Most of the currently available antidepressant drugs exert their effects by increasing the content of monoamine neurotransmitters. In particular, it should be noted that a great number of studies have shown that the 5-HT system plays a major role in depression [19]. Previous studies have shown that some ginsenosides, having similar molecular structure to SLPN, exert antidepressant-like effects by acting through monoamines [6, 7, 9], and hence it was speculated that SLPN may possess similar properties. To test this hypothesis, the levels of 5-HT, DA, and NA in the hippocampus of rat exposed to CUMS were measured using ELISA method. Interestingly, the results showed that, unlike the positive control FLU (10 mg/kg), SLPN (100 mg/kg) significantly increased the NA and DA levels in the hippocampus, but it did not affect the 5-HT levels. Previous studies had yielded similar results in different behavioral animal models, including the *L*-*DOPA*-induced running behavior, the Clonidine-induced aggression behavior, and the 5-HTP-induced head-twitch test in mice [1]. Moreover, the data on the monoamine neurotransmitter system are in accordance with other reports showing that ginsenosides can reverse the stress-induced decrease in

the NA and DA levels in the brain [6, 9]. It can be suggested that the antidepressant-like effects of SLPN may primarily involve the function of the noradrenergic and dopaminergic pathways.

However, the monoamine hypothesis cannot completely explain the reduction of hippocampal volume. Many reports have suggested that alterations in hippocampal function and reductions in hippocampal volume may be involved in the etiology of depression [20]. Several factors can contribute to this atrophy, such as decreased neurogenesis, death or atrophy of existing neurons, changes in neuropil, the number of synapses, and synaptic bulk [21]. BDNF, one of the most important neurotrophic factors in mammalian brain, has been implicated in the survival of neurons during hippocampal development, neural regeneration, synaptic transmission, synaptic plasticity, and neurogenesis. Recently, it is well accepted that reduced BDNF expression contributes to depression-like behavior in animal models and may lead to similar effects in humans, and that antidepressant drug treatment increases or reverses this deficit, although there have been some conflicting studies [22]. Therefore, BDNF plays critical roles in depression and antidepressant drug treatment. More evidences have demonstrated that ginsenoside extracts or pure ginsenosides exert strong antidepressant-like effects via up-regulating BDNF expression in the hippocampus [6, 9, 10, 23]. Thus, it was speculated that the recovery of hippocampal atrophy by SLPN involved BDNF. According to the present results, SLPN (100 mg/kg) could significantly recover hippocampal volume and almost normalize the CUMS-induced deficit in the expression of BDNF and its receptor TrkB , an effect that was similar to that of FLU (10 mg/kg), indicating that the neurotrophic mechanism is involved in the antidepressant-like effect of SLPN. CREB, which functions as a transcription factor, is a key mediator of cell survival and cognition function. Notably, the activation of CREB, in turn, leads to increased BDNF gene expression, indicating that the transcription of BDNF is dependent on CREB. Furthermore, complex intracellular signaling pathways are involved in CREB-mediated gene transcription [24]. Among them, the ERK-CREB signal pathway has been thought to be an important intracellular signal mechanism that mediates the antidepressant effect [25]. Especially, ERK1/2 is the most-studied member of the mitogen-activated protein kinase (MAPK) family, regulating a large variety of cellular processes, such as growth, survival, and plasticity of neurons [26]. One of the most important properties of ERK1/2 activation is that it must be phosphorylated to exhibit full enzymatic activity. The activation of the ERK signaling pathways leads to the phosphorylation of the CREB and modulation of their transcriptional activity. Phosphorylation of ERK1/2 and CREB has been studied for their vital role in stress and depression. For example, it has been found that chronic stress reduces pERK1/2 and pCREB expression in rat brain, whereas antidepressant drugs could significantly reverse this decrease [27~29]. In agreement with previous findings, the results demonstrated that chronic stress exposure decreased the pERK1/2 and pCREB expression in the hippocampus of the CUMS-treated rats. It has been reported that the effect of the typical SSRI-type antidepressant drug FLU involves the ERK-CREB signal pathway [30]. Similarly, the data in this study also showed that the chronic treatment with SLPN (100 mg/kg) and FLU (10 mg/kg) could significantly increase the pERK1/2 and pCREB expression in the CUMS-treated rats. Taken together, it is reasonable to speculate that the antidepressant activity of SLPN in the CUMS model appears to be related to the modulation of hippocampal ERK1/2, CREB, and BDNF (TrkB) signaling pathways.

It should be pointed out that ginseng total saponins (GTS) also exerted strong antidepressant-like effects by regulation of neurotrophic factor expression in the hippocampus [9]. Chen et al. further focused on the upstream signaling molecules and transduction pathways of BDNF, and suggested that the effect of GTS on corticosterone-induced depression-like behavior might be mediated partly through interfering with hippocampal GSK-3β-CREB-BDNF signaling pathway and reversing the decrease of some plasticity-related proteins [23]. P. notoginseng has the same genus as P. ginseng, which are popular medicinal herbs in the world. However, the chemical characteristics for GTS and SLPN are obviously different. GTS contains rich protopanaxatriol-type and protopanaxadiol-type saponins, whereas SLPN contains protopanaxadiol-type saponins only [31], which may be responsible for the difference in their mechanisms of action.

3 Conclusion

In conclusion, the present study showed that SLPN exhibited antidepressant-like properties in CUMS model of depression, which might be mediated through up-regulation of the hippocampal noradrenergic and dopaminergic pathways, as well as the ERK1/2, CREB, and BDNF signaling pathway.

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