Effect of tribulus terrestris saponins on behavior and neuroendocrine in chronic mild stress depression rats

Zhe Wang, Dongdong Zhang, Shan Hui, Yingjin Zhang, Suiyu Hu

Abstract

OBJECTIVE: To observe the effect of tribulus terrestris saponins (TTS) on behavior and neuroendocrine of chronic mild stress (CMS) depression rats.

METHODS: Thirty male Sprague-Dawley rats were randomly allocated to six groups: vehicle group, CMS group, CMS + fluoxetine group and CMS + TTS of low-dosage (0.375 g/kg), medium-dosage (0.75 g/kg) and high-dosage (2.25 g/kg) groups. All rats except the vehicle group singly housed and exposed an unpredicted sequence of mild stressors. The behavior of rats was detected by open-field test (OFT) and sucrose preference test (SPT). The concentration of corticotropin-releasing factor (CRF) and adrenocorticotropic hormone (ACTH) in serum of the rats were detected by radioimmunoassay. The concentration of cortisol (CORT) in serum was detected by enzyme immunoassay.

RESULTS: CMS procedure not only significantly decreased the scores of crossing, rears and grooming in OFT and the sucrose preference in SPT (all \(P < 0.01\)), but also markedly increased serum CRH and CORT levels (both \(P < 0.05\)). Treatment with TTS (0.75 and 2.25 g/kg) could significantly prevent all of these abnormalities induced by CMS (\(P < 0.05, P < 0.01\)).

CONCLUSION: CMS can affect rat behavior and neuroendocrine and cause depression. TTS has the antagonism on CMS and produce antidepressive effects.

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Key words: Tribulus; Saponins; Stress; Neurobehavioral manifestations; Neuroendocrinology

INTRODUCTION

Depression is a disorder that has high incidence in the world population with impact in life quality of patients. The treatment of depression with conventional antidepressants provides a complete remission just for 50% of the individuals, and produces side effects that may reduce adhesion of patients to the treatment. Thus, traditional drug (including natural medicine, Chinese herbal medicine) or additional treatments with low side effects and costs are of particular interest. Lots of evidence has suggested that an association between depressive disorders and neuroendocrine alterations, hyperactivity of the hypothalamic-pituitary-adrenal (HPA) is thought to be involved in the pathogenesis of depression. It has also been demonstrated that antidepressant treatment could normalize the HPA axis hyperactivity in depressed patients.
leaving factor (CRF), adrenocorticotropic hormone (ACTH) and cortisol (CORT) are key mediators of the mammalian response to stress stimuli, involving in the HPA axis dysfunction. Our previous studies indicated that traditional Chinese herbal decoction which mainly contain tribulus terrestris had an antidepressant-like activity in the depression animals and patients. The present study observe the effect of tribulus terrestris saponins (TTS) which is main effective components from tribulus terrestris on behavior and neuroendocrine of chronic mild stress (CMS) depression rats.

 MATERIALS AND METHODS

Drugs and instruments
TTS was purchased from Hunan Sanwei Medical Technology Co. Ltd. (Hunan, China). The purity of Saponins was 80%. Fluoxetine hydrochloride was from Changzhou Siyao Pharmaceuticals Co., Ltd. (Changzhou, China, lot No. 211021). CRF and ACTH radioimmunoassay kits were purchased from Beijing North Institute of Biological Technology (Beijing, China). CORT enzyme immunoassay (magnetic solid phase) kits were purchased from Beijing Bio-Ekon Biotechnology Co. Ltd. (Beijing, China). The procedure was performed as described by the kit's manufacturer, respectively. Other reagents were analytical grades made in China.

Experimental animals
Sprague Dawley (SD) male rats, six month old, weighing (200±20) g, were provided by Sino-British Sippr/bk Lab. Animal Co. Ltd., with certificate number: SCXK (Shanghai) 2008-0003. Animals were allowed to adapt one week before the experiment started. All rats were individually housed (cage size: 30 cm × 20 cm × 16 cm), with food and water freely available, and maintained on a 12 h dark-light cycle (with the lights on at 07:00 h locate time) under regulated temperature conditions (22°C ± 2°C), except as described below. The study was approved by the institutional Animal Care Committee at the Central South University.

CMS
The CMS procedure was slightly modified from that previously described. Briefly, the weekly stress regime consisted of food and water deprivation, strobescopic illumination (150 flashes/min), white noise, light/dark succession every 2 h, overnight illumination, 45° cage tilt, soiled cage and pair-housing. All stressors were applied individually and continuously. The vehicle rats were housed in a separate room and had no contact with stressed animals. These rats were deprived of food and water for 18 h preceding each sucrose preference test (SPT), but otherwise food and water were freely available in the home cage. The CMS procedure was last for 4 weeks.

The animals were randomly divided into 6 groups (5 rats per group): vehicle group, CMS group, CMS-fluoxetine group (1.8 mg/kg, corresponding to an adult clinical dose), and CMS-TTS at dose of low-dosage (0.375 g/kg), medium-dosage (0.75 g/kg, corresponding to an adult clinical dose) and high-dosage group (2.25 g/kg). Five groups except vehicle were exposed to the CMS procedure for 4 weeks. All drugs were suspended in fresh water, and administered by gavage once daily at 11:00 am for 4 weeks.

SPT
Special experimental procedures were conducted in accordance with the previous literature. SPT as employed previously was used to operationally define anhedonia. Specifically, anhedonia was defined as a reduction in sucrose intake and sucrose preference. Rats were first trained to consume 1% (v/v) sucrose solution for 48 h without food and water supply. Three days later, the sucrose baseline test was performed following 18 h of food and water deprivation. Rats were allowed to choose from two bottles, one with 1% sucrose solution and the other with water, for 1 h. Sucrose and water intake was recorded, and sucrose preference (SP) was calculated as SP (%)=sucrose intake (mL)/[sucrose intake (mL)+water intake (mL)]×100%. Following exposure to 4 weeks of stress, SPT was repeated using the same procedure.

Open-field test (OFT)
The ambulatory behavior was assessed in an OFT as previously described. Open field arena used was a wooden box (77 cm × 77 cm × 40 cm) with the floor divided into 49 equal squares. At the start of each trial, a mouse was placed in the central square of the field and was allowed to freely explore the arena. The scores of crossing, scores of rears, time of staying in the central square and time of grooming were counted in a 6 min session. The arena floor was cleaned between the trials with a 10% ethanol solution and the test was carried out in a temperature, noise and light controlled room. OFT as employed previously was used to define locomotor activity.

Blood sample collection
After the CMS period and post-CMS sucrose intake test, rats were left without any treatment until the following morning. To avoid fluctuations on hormone concentrations due to circadian rhythms, animals were sacrificed via decapitation between the hours of 09:00 and 10:00 on two consecutive days. Blood was collected in pre-iced tubes with protease inhibitor aprotinin and centrifuged at 3000 rpm at 4°C for 20 min. The separated serum samples were stored at −80°C until the assay of CRF, ACTH, and CORT.

Statistical analyses
SPSS 18.0 software (SPSS Inc, Chicago, IL, USA) was used to analyze the data. All data are expressed as
RESULTS

Effect of TTS on body quality and OFT
As shown in Table 1, before CMS (day 0), there was no difference between vehicle group and drug (TTS or fluoxetine)-treated groups in the body quality and each index scale measurement in OFT (all P > 0.05). After exposed to chronic mild stressors (day 29), compared with vehicle group, the CMS group rats decreased body weight significantly (P < 0.01), markedly reduced scores of crossing, rears and time of grooming, increased time of staying in central square in OFT (all P < 0.01), which suggested that chronic stress could affect body weight and behavior of rats. After 4 weeks treatment, the body weight and all records in the OFT of the CMS-treated rats in TTS at 0.75 and 2.25 g/kg groups have significantly improved (P < 0.01). Moreover, this parameter was also significantly increased by the chronic administration of fluoxetine. TTS at 0.375 g/kg could slightly but not remarkably improve body weight and the records of OFT in the CMS-treated rats (all P > 0.05). It is suggested that TTS at 0.75 and 2.25 g/kg could improve body weight and behavior of depressive rats.

Effect of TTS on SPT
As shown in Table 1, there was no difference between vehicle group and drug (TTS or fluoxetine)-treated groups before CMS (P > 0.05). After exposed to CMS for 4 weeks (day 29), compared with vehicle group, the CMS group rats decreased sucrose preference significantly (P < 0.01). In the CMS-TTS treated groups (0.75 and 2.25 g/kg), it significantly elevated the sucrose preference of stressed rats after 4-week treatment (P < 0.01). Fluoxetine remarkably improved CMS-induced sucrose preference (P < 0.01), but TTS at 0.375 g/kg failed to significantly alter this (P > 0.05).

Effect of TTS on serum CRF levels
As shown in Table 2, Compared with vehicle group, the CMS procedure induced serum CRF levels of model rats a significantly higher (P < 0.05). After 4-week treatment, TTS at 0.75 and 2.25 g/kg significantly reduced serum CRF levels in the stressed animals (P < 0.05). Fluoxetine also produced a significant reduce in serum CRF levels (P < 0.05). However, TTS at 0.375 g/kg was failed to alter serum CRF levels in the stressed animals.

Effect of TTS on Serum ACTH Levels
As shown in Table 2, CMS produced no change in serum ACTH concentrations between the stressed animals and corresponding vehicle groups (P > 0.1). There was no significant interaction between the TTS treatment and CMS groups (P > 0.1), and same as fluoxetine treatment.

Effect of TTS on serum CORT levels
As shown in Table 2, Compared with vehicle group, TTS at 0.75 and 2.25 g/kg significantly reduced serum CORT levels of stressed animals (P < 0.05). Fluoxetine also produced a significant reduce in serum CORT levels (P < 0.01). However, TTS at 0.375 g/kg was failed to alter serum CORT levels in the stressed animals.
stress.

Depression are similar to those observed in response to Chronic stress is an important factor in depression, necessary to further explore TTS antidepressant effect. 

nents isolated from tribulus terrestris. Thus, it is neces-

As a result, these symptoms induced by CMS procedure, animals are exposed to different kinds of mild events and results in anhedonia and locomotor activity decreasing, the core symptoms of human depression. 

DISCUSSION

Depression is a common, debilitating, life-threatening illness with an increasing morbidity and mortality. World Health Organization revealed that depression is the fourth leading cause of disability worldwide. Depression belongs to the category of Yu-disease in Traditional Chinese Medicine (TCM). Analysis of TCM different syndromes composition ratio in depression patients find that the most common TCM syndromes of depression is called stagnation of liver-Qi. Sentiment abnormality and stagnation of liver-Qi are thought to be involved in pathogenesis of depression in TCM, and the basic principles of treatment is dispersing stagnated liver Qi for relieving Qi stagnation. Tribulus terrestris is a flowering plant in the family zygophyllaceae, and its dry fruit is widely used in traditional Chinese formulations for dispelling wind-evil and melancholy. Traditional Chinese herbal decoction which mainly contains tribulus terrestris has been previously confirmed to contribute to several depressive symptoms, and augmented activity of this axis is considered a key neurobiological alteration in major depression. The hypothalamic-pituitary-adrenal (HPA) axis is one of the important neuroendocrine axes, and enhanced activity of this axis is considered a key neurobiological alteration in major depression. In the present study, the CMS procedure resulted in increases of serum CRF and CORT in rats, indicating that CMS might cause HPA axis hyperactivity. Thus normalization of HPA axis activity is a cure key and an indication of depression relief. In the present study, the CMS procedure resulted in increases of serum CRF and CORT in rats, indicating that CMS might cause HPA axis hyperactivity. CRF is the most potent ACTH secretagogue, but in the present study, the CMS procedure rendered no relative differences on serum ACTH concentrations in rats. This dissociation between CRF and ACTH was found in other similar studies. These may be due to the origin of serum CRF was a confounding factor in these determinations, hence peripheral concentrations of CRF were difficult to interpret. In this study, we found TTS at 0.75 and 2.25 g/kg but not at 0.375 g/kg significantly attenuated the CMS-induced serum CRF and CORT levels. It supported the hypothesis that TTS could exert its antidepressant effect by normalizing HPA axis hyperactivity through CRF and
CORT levels regulation.
In conclusion, the present study demonstrated that TTS had potentially antidepressant-like activity in CMS rats. The mechanism involved can be mediated by HPA axis. TTS may be a favorable alternative to currently available antidepressant drugs. Further preclinical and clinical experiments are needed to clarify the role of TTS in contributing to the treatment of depressive disorders.

REFERENCES