Antidepressant-like effects and mechanism of action of SYG in depression model in rats

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Abstract

OBJECTIVES: The present study aimed to evaluate whether SYG, a Chinese herbal formula, could produce antidepressant-like effects in learned helplessness (LH) model and chronic mild stress (CMS) model in rats. The mechanism underlying the antidepressant-like action was investigated by exploring BDNF signaling way in the hippocampus.

MATERIAL AND METHODS: SYG was administrated for 5 consecutive days (100 and 200 mg/kg/day, intragastrically) in the learned helplessness model; SYG was administered daily by gastric gavages during both the 5-week stress session and behavior tests periods in the chronic mild stress model (100 and 200 mg/kg). The serum corticosterone level was measured in the learned helplessness model. Levels of BDNF and Tyrosine-related kinase B (TrkB), were evaluated in the hippocampus of chronic mild stress model.

RESULTS: A deficit in avoidance learning and higher corticosterone level were observed in learned helplessness rats. SYG significantly reduced this deficit and reversed the corticosterone alteration. CMS induced significant reduction of sucrose intake in the sucrose preference test, an increased latency to feed in the novelty-suppressed feeding test and an increased immobility time in the forced swim test as compared to the control. It was also found that BDNF and TrkB levels were decreased in CMS model. Chronic treatment of SYG significantly suppressed the behavioral changes and up-regulated the BDNF signal pathway in the hippocampus.

CONCLUSION: Our results suggest that SYG alleviates depression induced by LH and CMS model. The antidepressant-like activity of SYG is likely mediated by activation the BDNF signal pathway in the hippocampus.

ORIGINAL ARTICLE

Abbreviations:

BDNF	- Brain-derived neurotrophic factor
TrkB	- Tyrosine-related kinase B
LH	- Learned helplessness
CMS	- Chronic mild stress
NOT test	- Novel object test
NSF test	- Novelty-suppressed feeding test;
FST	- Forced swim test
SYG	- the mixture of total saponins of *Panax ginseng* and total oligosaccharide esters of *Polygala tenuifolia* (in a ratio of 2:1)

INTRODUCTION

Depression is one of the most prevalent types of mental illness. It currently affects about 10–15% of the population worldwide, and is considered one of the ten leading causes of morbidity and mortality (Lepine & Briley 2011). It is predicted that major depression will be the second leading contributor to the global disability burden by 2020 (Murray & Lopez 1997).

Chronic exposure to stress has long been believed to play an important role in the onset and relapse of depression. Therefore, stress paradigms in laboratory animals are commonly used for studying the pathophysiology and treatment of depression. Learned helplessness (LH) is a widely accepted animal model in which animals are exposed to an uncontrollable and unpredictable stressor such as inescapable shock in order to induce an escape deficit state (Seligman 1972; Maier 1984). The chronic unpredictable mild stress (CMS) model, originally developed by Willner (Willner *et al.* 1987), is a validated and widely used model of depressive disorders (Papp 2001; Bielajew *et al.* 2002). In this model, animals are subjected to a period of mild and unpredictable stressors (e.g., food or water deprivation, tilted cage, paired housing, stroboscopic illumination, damp sawdust, etc.). A few weeks of exposure to CMS produce a variety of behavioural and neurochemical alterations resembling some changes observed in human depression.

The pathobiology of depression is multifactorial (Chopra *et al.* 2011). Recent evidence has supported the notion that brain-derived neurotrophic factor (BDNF) is involved in the pathogenesis of depression (Yu & Chen 2011). Preclinical studies have indicated that stress decreases BDNF level in the hippocampus and chronic antidepressant administration reverses the decreased level of BNDF induced by animal depression model (Hashimoto 2010). Clinical studies have shown the similar alteration in serum BDNF activity occurs in depressive patients and increases of BDNF levels after antidepressant treatment (Brunoni *et al.* 2008). As these, the stimulation of the BDNF signaling pathway could provide new target for the development of antidepressants.

Recent evidence has showed that herb preparations have many benefits in the treatment of depression such as improved tolerability and fewer side-effects (An *et al.* 2009; Sarris 2007). SYG prescription is composed of total saponins of ginseng (GTS) and total oligosaccharide esters of polygala tenuifolia (PTG) (in a ratio of 2:1). Our previous study demonstrated that the antidepressant effect of SYG prescription was better than that of GTS or PTG in the tail suspension and forced swim test (sun *et al.* 2012). In the present study, the antidepressant-like effect of SYG treatment (100 and 200 mg/kg) was investigated in a learned helplessness model and a chronic mild stress (CMS) model in rats. BDNF signaling pathway was explored in the hippocampus of CMS in rats.

MATERIALS AND METHODS

Preparation of SYG and drugs

SYG is the mixture of total saponins of *Panax ginseng* and total oligosaccharide esters of *Polygala tenuifolia* according to the ratio of 2:1. The preparation of total saponins of Panax ginseng and total oligosaccharide esters of Polygala tenuifolia was the same as described earlier (Sun *et al.* 2012). Imipramine and citalopram were purchased from the National Institutes for Food and Drug Control (Beijing, China). All drugs were dissolved in distilled water.

Animals

The experiments were carried out on male Wistar rats (180–200 g). The animals were housed under standard experimental conditions (20–22 °C and 12 h light / 12 h dark cycle). Animals had free access to water and food *ad libitum* and were acclimatized for one week before the experiments. The experiments were approved by the ethical committee for the use of experimental animals of the Institute of Medicinal Plant Development (China).

Learned helplessness (LH) paradigm

Experimental groups and drug treatments

Forty male Wistar rats were used in the LH experiment. All the animals were divided into five matched groups (n=8 per group): the control; the LH model; the LH + 20 mg/kg imipramine; the LH + 100 mg/kg SYG; the LH + 200 mg/kg SYG. On day 1, drugs were administered after the end of inescapable electric foot-shocks. On days 2–5, drugs were administered in two half-doses, one in the morning (1 h before the training) and one in the afternoon.

LH procedure

The procedure was conducted in accordance with the method of Bertaina-Anglade (Bertaina-Anglade *et al.* 2006). It consists of two phases: escape deficit induction and active avoidance performance.

Escape deficit induction: On days 1, rats were subjected to 60 inescapable electric foot-shocks [0.8 mA, 15 s duration, at random intervals (averaging 45–75 s)]. Control animals were exposed to the same apparatus

but did not receive foot-shocks. After escape deficit induction, rats were returned to their home cages.

Active avoidance performance: On days 3–5 (48 h following the escape deficit induction), active avoidance sessions were performed using a computerized video-tracking system, which was the same as described earlier (Dang *et al.* 2009). The apparatus consisted of two identical two-way shuttle boxes. Each shuttle box comprised two identical compartments. Animals were allowed 5 min to explore the entire apparatus. Next, animals were exposed 15 min to 30 stimulus-shock trials. Each trial (30 s) included: Conditioned stimulus period (3s, in the presence of a blue light signal), conditioned and unconditioned stimulus period (3s, presence of a blue light signal and foot shocks), rest period (24s, without any stimulus). The numbers of escape failures and error area time in each of 30 trials were recorded by the system.

Serum corticosterone level measurement: The rats were sacrificed after the learned helplessness test. Blood samples were collected and centrifuged at 3000 g for 15 min at 4 °C. Corticosterone level was measured using commercially available ELISA kits (Enzo, Catalog No. ADI-900-097). All assays were performed in duplicate. The intra- and interassay coefficients of variation were less than 10% for all comparisons.

Chronic mild stress (CMS) paradigm

Experimental groups and drug treatments

Sucrose preference baseline test was performed before the onset of CMS. Following the baseline test, the animals were assigned into five matched groups (n=9 per group): the control; the CMS model; the CMS + 10 mg/kg citalopram; the CMS + 100 mg/kg SYG; the CMS + 200 mg/kg SYG. SYG or citalopram was administered by gastric gavages once a day. Control and CMS animals were given distilled water daily (Figure 1).

CMS procedure

The procedure of CMS was performed as described by Willner (Willner 1997) and Bekris (Bekris *et al.* 2005) with some modifications. Rats were submitted to the following kinds of stressors (2–3 stressors a day): forced swimming, damp sawdust, food or water deprivation, social stress (switching the cages), white noise, inversion of light/dark cycle, cage tilting, paired housing, stroboscopic illumination (details in Table 1). To prevent habituation and make the stress procedure unpredictable, the timing of stressors and stressor sequences was changed every week.

Sucrose preference test

The sucrose preference test was performed as described previously with minor modifications (Tacchi *et al.* 2008). Rats were first trained to drink 1% sucrose solution. Three days later, rats received sucrose preference test, preceded by 23 h food and water deprivation. Each rat was presented simultaneously with both sucrose (1%) and water. Sucrose intake was measured by weighing the bottle at 60 min. The test began at the start of the dark phase (17.00–18.00 h) in the rat home cage. Finally, at the end of CMS, the sucrose preference test was conducted again.

Novel object test (NOT)

Novel object test was performed from previously described studies (Bienkowski *et al.* 2001). On the first day, the rats were habituated individually to the open field (diameter: 80 cm; height: 50 cm) for 30 min. On the second day, a cylinder (diameter: 4 cm; height: 4 cm) was placed in the center of the open field. The cylinder could not be moved by the rat. A rat was considered to be investigating the object if its snout was in contact with the object (the animal's nose within 2.5 cm of the cylinder). Latency to begin investigating the object was recorded for 5 min.

Novelty-suppressed feeding test (NSF test)

The methods for novelty-suppressed feeding test were modified from previous descriptions (Stedenfeld *et al.* 2011). Rats were food-deprived 48 h prior to the test. A food pellet was placed in the center of the open field apparatus (52×42×30 cm) and rats were placed in one corner. Latency to begin eating was recorded with a

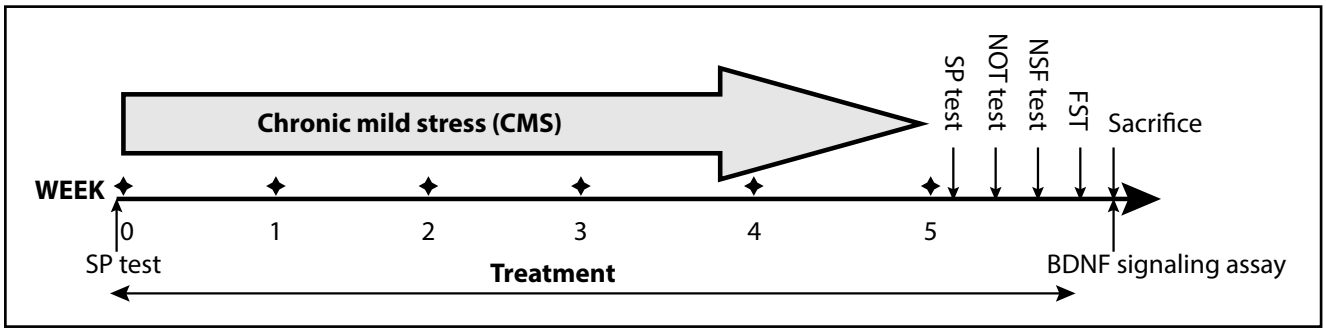

Fig. 1. the schematic representation of experimental design. SP test: sucrose preference test; NOT test: novel object test; NSF test: novelty-suppressed feeding test; FST test: forced swim test. The CMS regimen lasted 5 weeks. SYG or citalopram was administered daily by gastric gavages during both the 5-week stress session and behavior tests periods. The sucrose preference baseline test was measured before CMS regimen. After CMS regimen, behavioral tests were carried as follows: sucrose preference test, novel object test, novelty-suppressed feeding test, and forced swim test. Following the forced swim test, the rats were sacrificed for BDNF signaling essay.

Tab. 1. Schedule of CMS procedure.

Day	Forced swimming	Cage tilting	Food deprivation	Restraint stress	Damp sawdust	Inversion light/dark cycle	Water deprivation	Paired housing	White noise	Switching the cage	Stroboscopic illumination
Monday	9:00 ↓ 9:20		20:00					17: 00			
Tuesday		9:00 ↓ 12:00	↓ 8:00			17: 00		↓ 8:00	12:00 ↓ 15: 00		
Wednesday				8:00 ↓ 12:00		↓ 17:00	18: 00			14: 00 ↓ 17: 00	
Thursday	8:00 ↓ 8:30				20:00		↓ 8: 00	17:00			9:00 ↓ 17:00
Friday				16:00 ↓ 17:00	↓ 8: 00		18:00	↓ 8:00	10:00 ↓ 12:00		
Saturday		14:00 ↓ 17:00	20:00			17:00	↓ 8:00			9:00 ↓ 12:00	
Sunday			↓ 8:10			↓ 17:00					9:00 ↓ 17:00

limit of 10 min. After the rat commenced eating, the rat was returned to its home cage and the amount of food consumed during the subsequent 5 min was quantified to evaluate home food consumption.

Forced swim test (FST)

The FST was carried out on rats according to the method of Kompagne and Porsolt (Kompagne *et al.* 2008; Porsolt *et al.* 1977). The cylinders (18 cm in diameter, 46 cm in height) contained water (25 °C) to a depth of 30 cm. The rats were placed into the water for a 15-min forced swim session. 24 h later, the rats were put in the cylinders again. Immobility was measured during a 5 min test (the animal is judged to be immobile when it stays still or make only movements necessary not to submerge).

Measurements of BDNF protein levels in the hippocampus

Immediately following behavioural test, animals were decapitated and the hippocampus was dissected, weighted and frozen in liquid nitrogen. Protein levels of BDNF were detected by a two-site ELISA kit (Rapidbio, USA). Samples (or standard) were added to each well and incubated for 90 min at 37 °C. After several times washing, anti-rat BDNF bitotin was added to each well and the plate was incubated for 60 min at 37 °C. After the incubation of HRP-conjugate for 30 min at 37 °C, the substrate TMB one solution was added. The reaction was stopped and absorbance recorded at 450 nm in ELISA plate reader. The sensitivity of the assay for BDNF was 40 pg/ml.

Western Blotting

Following decapitation, rat hippocampus was isolated and stored at –80 °C for western blotting detection. The protein concentration was determined by BCA assay. The proteins were transferred onto a PVDF membrane which were incubated overnight at 4 °C with primary antibodies to Tyrosine-related kinase B (Trk B, 1:1 000, Santa Cruz). HRP-conjugated goat anti-rabbit IgG (1:5 000) was used as secondary antibody. The immunoreactive bands were detected by a chemiluminescent reaction (ECL kit, Santa).

Statistical analyses

All data were analyzed using the SPSS statistic 16.0. All values were expressed as mean ± SEM. A one-way analysis of variance (ANOVA) was performed, followed by least significant difference (LSD) post hoc tests for inter-group comparisons. A value of $p<0.05$ was considered statistically.

RESULTS

Effect of SYG on the escape failure in learned helplessness rats

Figures 2 and 3 illustrated that LH model group exhibited significantly more escape failures and error area time than Control group over the 3 days of testing ($p<0.01$, compared with control group). Oral administration of SYG at 100 and 200 mg/kg/day reversed the escape deficit and error area time observed in LH model group on all 3 days ($p<0.01$, compared with the

LH model group). Imipramine at 20 mg/kg decreased the escape failures and error area time significantly only on day 2 and 3 of the avoidance task ($p<0.01$, compared with the LH model group). A slight but not significant decrease in escape failure and error area time was detected in imipramine group compared with SYG group on day 2 and 3 of testing.

Effect of SYG on the serum corticosterone level in learned helplessness rats

As shown in Figure 4, ELISA analysis demonstrated that LH model group significantly increased the serum corticosterone concentration ($p<0.01$, compared with control group). Chronic SYG (100, 200 mg/kg) or imipramine (20 mg/kg) treatment, attenuated this alteration and decreased serum corticosterone concentration ($p<0.01$, compared with the LH model group).

Effect of SYG on the sucrose preference index in CMS rats

Figure 5 showed the effect of SYG on the sucrose comsumption in CMS rats. Before CMS, there was no remarkable difference in the sucrose intake among all rats (data not shown). Sucrose consumption decreased significantly after 5 week CMS exposure ($p<0.05$, vs the controls). SYG treatment (100 or 200 mg/kg) increased sucrose intake in the CMS rats ($F(4,36)=6.645$, $p<0.05$, $p<0.05$, vs CMS model group). Citalopram (10 mg/kg) also significantly reversed the reduction of sucrose consumption in CMS rats ($p<0.05$).

Effect of SYG on the latency of investigating the object in novel object test of CMS rats

Figure 6 indicated that prolonged latency of investigating the object was induced by the CMS treatment ($p<0.01$ vs. control) and long-term treatment with either citalopram or SYG (100 or 200 mg/kg) significantly decreased the latency time ($F(4,36)=12.356$, $p<0.01$, $p<0.01$, $p<0.01$ vs. CMS group).

Effect of SYG on the latency of feeding in novelty-suppressed feeding test of CMS rats

Figure 7 indicated that elevated latency of feeding was induced by the CMS treatment ($p<0.01$ vs. control) and long-term treatment with either citalopram or SYG (100 or 200 mg/kg) significantly decreased the latency time ($F(4,36)=6.443$, $p<0.01$, $p<0.05$, $p<0.01$ vs. CMS group).

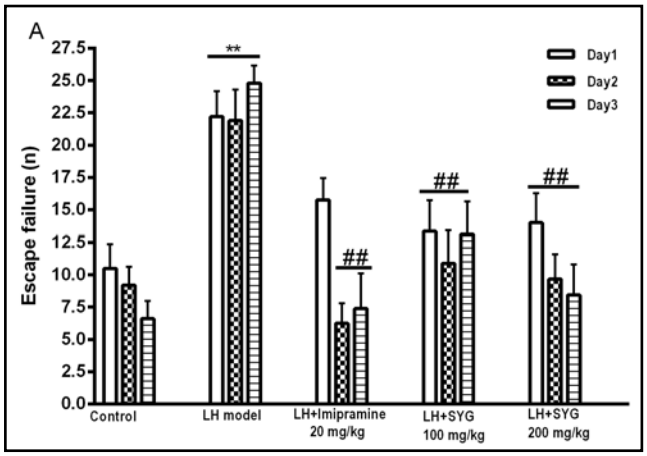

Fig. 2. The numbers of escape failure in control group, LH group, imipramine group and SYG group were expressed as mean ± SEM (n=8). **$p<0.01$, as compared with the control group and ##$p<0.01$, as compared with the LH group.

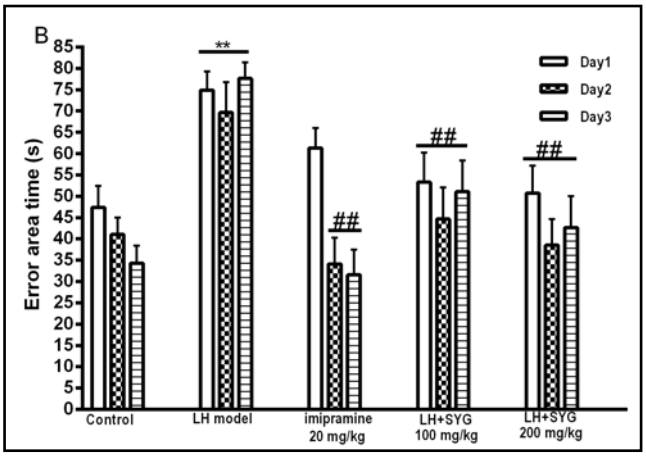

Fig. 3. The error area time in control group, LH group, imipramine group and SYG group was expressed as mean ± SEM (n=8). **$p<0.01$, as compared with the control group and ##$p<0.01$, as compared with the LH group.

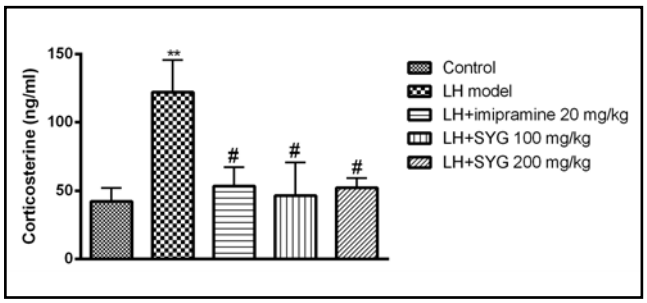

Fig. 4. The serum corticosterone level in control group, LH group, imipramine group and SYG group was expressed as mean ± SEM (n=8). **$p<0.01$, as compared with the control group and #$p<0.05$, as compared with the LH group.

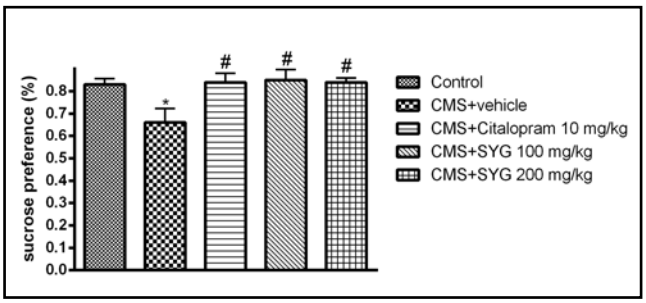

Fig. 5. The percentage of sucrose consumption in control group, CMS group, imipramine group and SYG group was expressed as mean ± SEM (n=9). *$p<0.05$, as compared with the control group and #$P<0.05$, as compared with the CMS group.

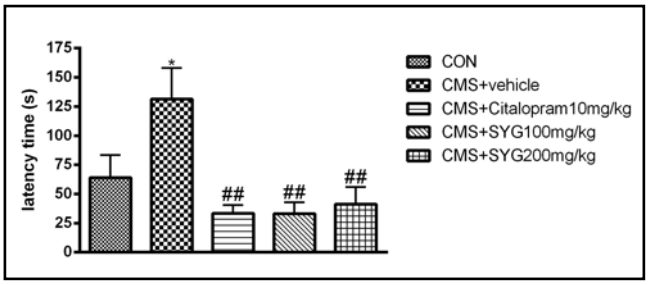

Fig. 6. The latency time in control group, CMS group, imipramine group and SYG group was expressed as mean ± SEM (n=9). $^{**}p<0.01$, as compared with the control group and $^{\#}p<0.05$, $^{\#\#}p<0.01$, as compared with the CMS group.

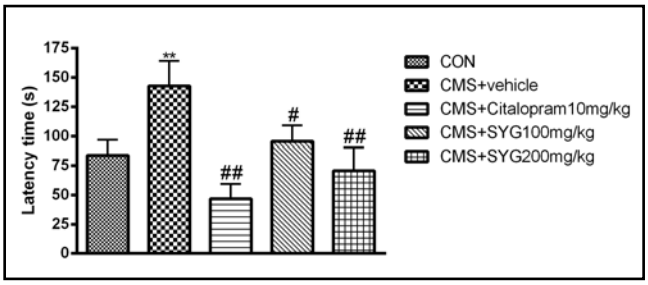

Fig. 7. The latency time in control group, CMS group, imipramine group and SYG group was expressed as mean ± SEM (n=9). $^{**}p<0.01$, as compared with the control group and $^{\#}p<0.05$, $^{\#\#}p<0.01$, as compared with the CMS group.

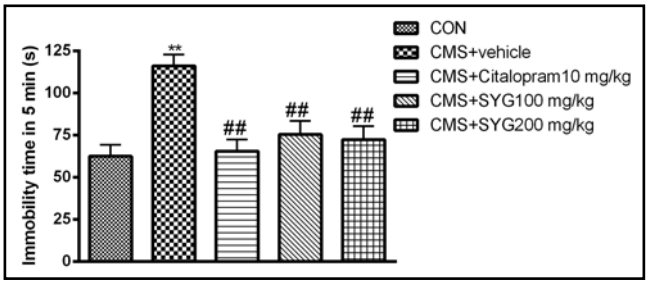

Fig. 8. The immobility time in control group, CMS group, imipramine group and SYG group was expressed as mean ± SEM (n=9). $^{**}p<0.01$, as compared with the control group and, $^{\#\#}p<0.01$, as compared with the CMS group.

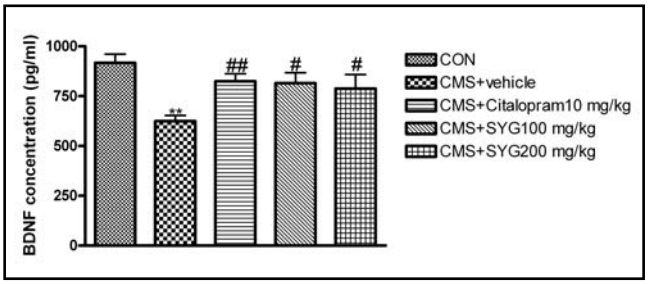

Fig. 9. The level of hippocampus BDNF in control group, CMS group, imipramine group and SYG group was expressed as mean ± SEM (n=9). $^{**}p<0.01$, as compared with the control group and $^{\#}p<0.05$, $^{\#\#}p<0.01$, as compared with the CMS group.

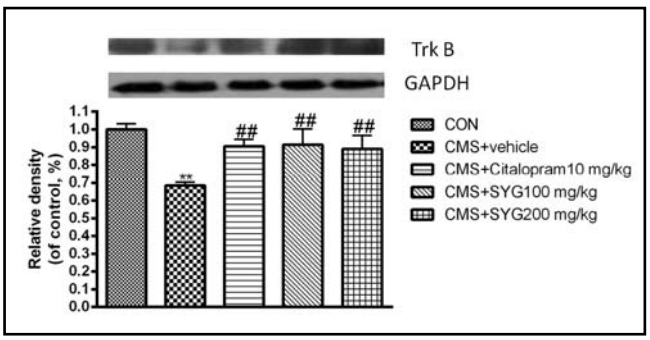

Fig. 10. The level of hippocampal TrkB in control group, CMS group, imipramine group and SYG group was expressed as mean ± SEM (n=9). $^{**}p<0.01$, as compared with the control group and $^{\#\#}p<0.01$, as compared with the CMS group.

Effect of SYG treatment on the immobility time in the FST test in CMS rats

Figure 8 indicated that the immobility time during the forced swim stress was significantly prolonged in the CMS group ($p<0.01$ vs. control) and long-term treatment with either citalopram or SYG (100 or 200 mg/kg) significantly decreased the immobility time ($F(4,36)=8.841$, $p<0.01$, $p<0.01$, $p<0.01$ vs. CMS group).

Effect of SYG treatment on the BDNF expression in the hippocampus of CMS rats

Figure 9 showed BDNF protein level was decreased significantly in the hippocampus by CMS paradigm ($p<0.01$, vs. the control). Citalopram or SYG (100, 200 mg/kg) treatment significantly increased BDNF protein levels in the hippocampus ($p<0.01$, $p<0.05$, $p<0.05$ vs. CMS group).

Effect of SYG treatment on the TrkB expression in the hippocampus of CMS rats

As shown in Figure 10, western blot analysis demonstrated that the TrkB expression in the hippocampus was decreased by 5-week stress regime ($p<0.01$ vs. control). Chronic SYG (100, 200 mg/kg) or citalopram (10 mg/kg) treatment, reversed these alterations and significantly increased hippocampal TrkB expression ($p<0.01$, $p<0.01$, $p<0.01$ vs. CMS group).

DISCUSSION

In the learned helplessness model, increases in escape failures and error area time are suggested to correspondent to depressive symptoms in human (Maier 1984). Chronic treatment with SYG decreased the number of escape failures and error area time. HPA axis Hyperactivity involving elevated levels of circulating glucocorticoids is related to the pathophysiology of depression (Naert *et al.* 2011). Our study indicated that the basal corticosterone level of learned helplessness rats was higher than that of non-learned helplessness rats. SYG and imipramine decreased the corticosterone level, which was in line with the previous study (Nankai *et al.* 1995).

CMS induces some behavioural changes that parallel depression-like symptoms in human, and can be used for evaluation of antidepressant drugs through behavioral tests, such as sucrose preference test, novel object test, novelty-suppressed feeding test and forced swim test (Yan *et al.* 2010). The sucrose preference test is usually employed to measure anhedonia state (a loss of responsiveness to pleasant events) in the CMS model (Matthews *et al.* 1995). Stress-induced anhedonia may be related to deficits in exploration and novel object test is commonly used to evaluate the exploratory behavior of animals when exposed to an unknown object (Labrie *et al.* 2009). Novelty-suppressed feeding test (NSF) is often used as another measure of depression-like behavior and chronic administration of antidepressants decreases the latency to consume food (Stedenfeld *et al.* 2011). The forced swim test has been extensively used for screening antidepressants (Bourin *et al.* 2005). Chronic stress has been shown to increase the immobility time of animals in forced swim test. Consistently, in the present study, CMS induced significant reduction in sucrose consumption and increase in duration of latency to investigate the novel object. CMS also significantly increased latency to feed in the novelty-suppressed feeding test and immobility time in the forced swim test as compared to the control, which were all reversed by citalopram treatment. Chronic treatment with SYG (100 and 200 mg/kg) was also found to prevent changes induced by CMS. Taken together, results obtained from behavioral studies demonstrated that SYG treatment produced an antidepressant-like action in CMS model in rats.

Tropomyosin-related kinase B (TrkB) is the primary receptor for BDNF (Lee & Kim 2010). BDNF-trkB signaling pathway down-regulation may be involved in the pathogenesis of major depression (Wu *et al.* 2012). Activation of BDNF-trkB signaling may alleviate depressive symptoms (Castren & Rantamaki 2010). Consistent with the BDNF/TrkB hypothesis, the present study showed that the levels of BDNF and TrkB in the hippocampus were decreased in CMS model rats, and SYG chronic treatment (100 mg/kg, 200 mg/kg) reversed these CMS-induced changes. Our findings suggest that the activation of BDNF/TrkB signaling may be responsible for the antidepressant-like effects of SYG. Further study should be done to investigate the effect of SYG on signaling transduction pathways that BDNF-TrkB activation can regulate, including the phospholipase C γ (PLC γ), the phosphatidylinositol 3-kinase (PI3K) and the extracellular signal related kinase (ERK, or mitogen-activated protein kinase, (MAPK)) pathways.

In conclusion, our results provide the first evidence that SYG treatment (100 or 200 mg/kg) not only had an antidepressant-like effect in learned helplessness and CMS model in rats but also modulated the corticosterone level of LH model and the BDNF signaling protein levels in the hippocampus of CMS model in rats. Together with previous results, our findings suggest that SYG had a potent antidepressant-like effect in tail suspension test, forced swim test, learned helplessness and CMS model. SYG may be a promising effective drug for depression treatment, after further research has been done.

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