

Full Length Research Paper

Antidepressant-like effects of total saikosaponins of *Bupleurum yinchowense* in mice

Xiuping Sun^{1,2}, Zhe Shi¹, Tengfei Li^{1,3}, Ruile Pan¹, Xinmin Liu^{1*}, Lanlan Bu^{1,4}, Lingti Kong¹ and Qi Chang¹

¹Research Center of Pharmacology and Toxicology, Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100193, P. R. China.

²Department of Pharmacology, Shandong University of Traditional Chinese Medicine, JiNan 250355, P. R. China.

³Guangxi Medical University, NanNing 530021, P. R. China.

⁴Department of Pathology, Hunan University of Chinese Medicine, ChangSha, 410208, P. R. China.

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Bupleurum is known traditionally to effectively treat depressive-like disorders in East Asia. Pharmacological studies have been carried out on several species of *Bupleurum*, such as *Bupleurum chinense* DC. and *Bupleurum falcatum* L. *Bupleurum yinchowense*, the same genus of *Bupleurum* R. H. Shan and Yin Li, is abundantly distributed in the Northwest of China and is widely used in folk medicine, but few reports explore its antidepressant-like activities. In the present study, it was observed that the total saikosaponins isolated from *B. yinchowense* (75 and 150 mg/kg) significantly reduced the immobility time in the forced swim and tail suspension test in mice after 7-day treatment. In the chronic mild stress model, chronic treatment with the total saikosaponins isolated from *B. yinchowense* (50 and 100 mg/kg) increased the sucrose intake in the sucrose preference test and decreased the latency to feed in the novelty-suppressed feeding (NSF) test. In addition, the total saikosaponins isolated from *B. yinchowense* augmented the diminished monoamine neurotransmitter concentrations (5-HT, DA and NE) in the prefrontal cortex induced by chronic mild stress. Taken together, our results suggest that the total saikosaponins isolated from *B. yinchowense* exerts an antidepressant-like action by modulating the 5-HT, DA and NE levels in the prefrontal cortex.

Key words: Chronic mild stress, mice, antidepressant, neurotransmitter, *Bupleurum yinchowense*.

INTRODUCTION

Depression is one of the most prevalent mental illnesses, and is associated with significant morbidity, disability and mortality. About 21% of the world's population is affected by depression (Chopra et al., 2011). The World Health

Organization (WHO) predicts that major depression will be the second leading cause of the global disability burden by 2020 (Murray and Lopez, 1997). However, the current pharmacotherapy treatment of depression is far from satisfactory. Many antidepressants have weaknesses such as low response rate, late onset and undesirable side-effects, including sleep disturbance, sexual dysfunction and cognitive impairment (Hindmarch and Hashimoto, 2010). These unmet medical challenges require more effort to find efficacious and safe antidepressants for the treatment of depression.

Recently, the worldwide use and research on herbal medicine for depression has gained popularity, and significant advances have been made (Sarris, 2007). For example, several medicinal herbs, such as *Hypericum*

*Corresponding author. E-mail: liuxinminuae@yahoo.com.cn or supering_sun@126.com. Tel: +86-13331169087. Fax: +86-10-57833245.

Abbreviations: TBY, Total saikosaponins isolated from *Bupleurum yinchowense*; FST, forced swim test; TST, tail suspension test; CMS, chronic mild stress; SP test, sucrose preference test; OFT, open field test; NSF test, novelty-suppressed feeding test.

perforatum (Linde et al., 2008), *Crocus sativus* (Akhondzadeh et al., 2007) and *Lavandula angustifolia* (Akhondzadeh et al., 2003) have demonstrated antidepressant activities which are supported by clinical evidence. The *Bupleurum* (family Umbelliferae) species is one of the most popular traditional oriental medicine for the treatment of psychosomatic disorders, originally documented in the “Shennong’s Herbal” (Han Dynasty, BC 200) and classified as a high grade herb.

Bupleurum and many traditional prescriptions that include *Bupleurum* as a major ingredient are known to effectively treat depressive-like disorders in clinical practice in East Asia (Jin et al., 2009; Ding, 2005; Han, 2008; Zhang, 2010; Hu and Hong, 2010). Pharmacological studies in depression models have been carried out on several species, such as *Bupleurum chinense* DC. and *Bupleurum falcatum* L. (Dai et al., 2010; Kwon et al., 2010). *Bupleurum yinchowense*, the same genus of *Bupleurum*, is abundantly distributed in the Northwest of China and is widely used in folk medicine. However, so far, no modern scientific evidence is available in the literature concerning its antidepressant pharmacological activities.

Behavioural tests such as the tail suspension and forced swim are widely used for the screening of antidepressant drugs, and they are sensitive to all major classes of antidepressant drugs (Mantovani and Pértille, 2003; Micale et al., 2006; Porsolt et al., 1977; Steru et al., 1985). The chronic unpredictable mild stress (CMS) model, originally developed by Willner et al. (1987), is generally accepted and is the most promising model to study depression in rats and mice because of its combined features of predictive, face and construct validity.

Although, the underlying pathophysiology of depression has not been clearly defined, extensive preclinical and clinical studies have demonstrated the determinant role of the monoamine system (5-hydroxytryptamine (5-HT), dopamine (DA) and nor-epinephrine (NE)) in the pathogenesis and therapy of depression (Nutt, 2008; Bondy, 2002; Bekris et al., 2005; Dang et al., 2009). Several lines of evidence suggest that dysfunction of the prefrontal cortex plays an important role in the pathogenesis of depression (Drevets, 2000; Drevets, et al, 1997; Dolan et al., 1994).

The total saikosaponins from *Bupleurum* are acknowledged to be the principal bioactive components which are known to have antineoplastic (He et al., 2011), antiepileptic (Xie et al., 2016) and anti-inflammatory effects (Liu et al., 2011). In the present study, total saikosaponins were isolated from *B. yinchowense* (TBY). The antidepressant-like effects and mechanisms of TBY are demonstrated using the forced swim test, the tail suspension test, and the chronic mild stress model, in order to explore its potential use as a new antidepressant. The concentrations of three neurotransmitters: 5-HT, NE and dopamine DA, in the mouse prefrontal cortex were simultaneously determined.

MATERIALS AND METHODS

Preparation of TBY and determination of five saikosaponins in *B. yinchowense* by high performance liquid chromatography (HPLC)

Dried roots (500 g) of *B. yinchowense* were milled and extracted with 60% ethanol containing 0.5% ammonia aqua (three volumes, each 1 L) at room temperature for 6 h. The ethanol extracts were combined and dried *in vacuo* (EYELA, OBS-2100, Japan) to yield a dark brown residue (120 g), which was dissolved in H₂O-MeOH (5:95) solution (200 ml) and partitioned with n-hexane (1:1) to afford a n-hexane-soluble fraction. The H₂O-MeOH (5:95) phase was evaporated to remove residual MeOH, and the dried extract was dissolved in distilled water (200 ml). This aqueous solution was loaded on to a column containing D101 macroporous (Cang Bon Adsorber Technology Co., Ltd. China) resin (1 kg) and eluted successively with 2 L of water and ethanol-water (9:1, v/v). The ethanol was evaporated *in vacuo* to yield a pale yellow residue (32 g), which is called TBY.

The contents of the main saikosaponins in TBY were measured using analytical HPLC (Fu et al., 2011) which was performed on waters chromatography including a waters pump control 600E, 2487 ultraviolet (UV) detector and is controlled by Empower software. Five saikosaponins, namely saikosaponins a, c, d, e and f in total saikosaponins of *B. yinchowense*, were determined simultaneously. The separation conditions were the following: the column used was a Lichrocart C₁₈ (dimensions 250 × 4.6 mm; 5 μm pore size) (Merck, Germany); the mobile phase was an acetonitrile (A) step gradient in double distilled water; timetable of elution gradient: 0 min: 5% A, 5 min: 10% A, 15 min: 15% A, 40 min: 33% A, 65 min: 90% A; speed of mobile phase: 1 ml/min; oven temperature 30°C. The wavelength for UV detection was 210 nm. A HPLC fingerprint is obtainable from the authors. The concentrations of saikosaponins a, c, d, e and f in the used extracts were 10.12, 2.84, 14.13, 1.52 and 2.14%, respectively.

Drugs

Amitriptyline, paroxetine and fluoxetine were obtained from the National Institutes for Food and Drug Control (Beijing, China). All drugs were dissolved in distilled water.

Animals

Male pathogen-free C57BL/6J (20 to 22 g) and BALB/c mice (26 to 28 g) were purchased from the Laboratory Animal Institute of the Chinese Academy of Medical Science Center (Beijing, China). The animals were housed in polypropylene cages under standard experimental conditions at 20 to 22°C with 40 to 60% humidity and 12:12 h light/dark cycle. Food and water were available *ad libitum*. Animals acclimatized for one week before the experiments. All experiments were conducted in compliance with the guidelines of the Principles of Laboratory Animal Care (NIH publication No. 80-23, revised 1996) and the legislation of the People’s Republic of China for the use and care of laboratory animals.

Forced swim test (FST), tail suspension test (TST) and open field test (OFT)

Experimental groups and drug treatments for FST and TST

C57BL/6J mice were randomly separated into 4 groups (n = 11 per group): control (distilled water), positive control (10 mg/kg, amitriptyline for FST or paroxetine for TST). The treated groups

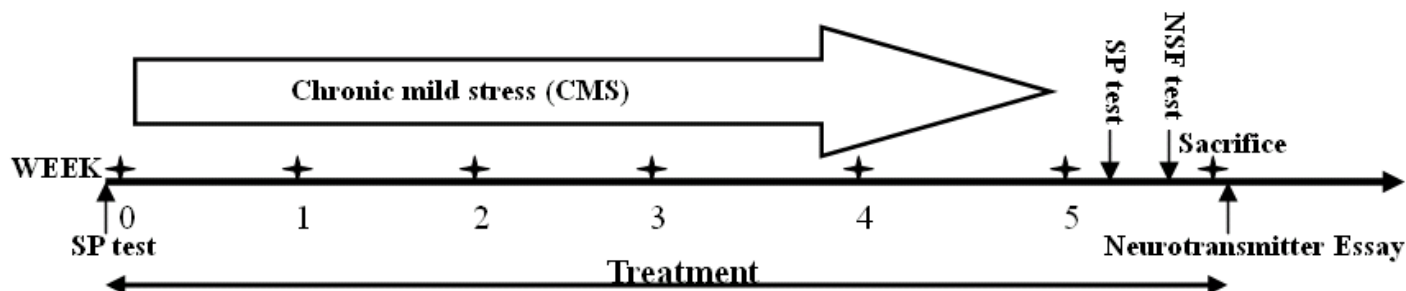


Figure 1. Schematic representation of experimental design. The CMS regimen lasted 5 weeks. TBY and fluoxetine were administered daily by gastric gavages during both the 5-week stress session and the behavior test periods. The sucrose preference baseline test was measured before CMS regimen. After CMS regimen, behavioral tests were carried as follows: sucrose preference test (one day) and novelty-suppressed feeding test (one day). Following the novelty-suppressed feeding test, the mice were sacrificed for neurotransmitter essay.

were dosed 75 and 150 mg/kg of TBY, based on our preliminary screening result. All drugs and water were administered by gastric gavages (20 ml/kg) once daily for 1 week between 9:00 a.m. to 11:00 a.m. The behavioural tests in mice were conducted on the 7th day after the last administration.

FST procedure

The FST was carried out on mice according to the method of Porsolt (1977). Briefly, mice were individually placed into a glass cylinder (20 cm in height, 14 cm in diameter) filled with 12 cm high water ($25 \pm 1^\circ\text{C}$). The total duration of immobility (seconds) was measured during the last 4 min of a single 6 min test session. Mice were considered immobile when they made no attempts to escape except for the movements necessary to keep their heads above the water.

TST procedure

The TST was carried out according to the method of Steru et al. (1985), using a computerized device (developed by the Institute of Medicinal Plant Development, the Chinese Academy of Medical Sciences, jointly with Chinese Astronaut Center, China). The apparatus consisted of eight chambers ($18 \times 18 \times 16$ cm) and enabled eight mice to be tested simultaneously. Each mouse was suspended by the tail using adhesive tape to a hook connected to a strain gauge.

The strain gauge picked up all movements of the mouse and transmitted these to a central unit which calculated the total duration of immobility for the 6 min test. Animals that climbed their tails during testing were excluded from the analysis.

OFT procedure

Prior to the tail suspension test, the effect of TBY on locomotor activities was evaluated in the open-field paradigm using a computerized video-tracking system, which was the same as described earlier (Wang et al., 2010). It consisted of four circular black metal containers enclosed in a metallic box with a light source of 120 lx. After 1 h of TBY administration, mice were placed individually in one of the four metal containers and locomotor activity was measured for 10 min. The total distance traveled by mice was recorded to evaluate the locomotor activity.

Chronic mild stress experiment

Experimental groups and drug treatments

Sixty male BALB/c mice were used in the CMS experiment. Prior to the other experiments, all the animals were subjected to the sucrose preference baseline test and were divided into five matched groups (n=12 per group): the control group, the CMS group, the CMS + 10 mg/kg fluoxetine group, the CMS + 50 mg/kg TBY group and the CMS + 100 mg/kg TBY group. During the CMS experiment, the mice of the control group were left undisturbed in the cages in a separate room with the exception of general handling (e.g. regular cage cleaning and measurements of body weight), whereas the mice from the other four groups were housed in individual cages (one cage per mouse) and exposed to CMS. TBY or fluoxetine was administered daily by gastric gavages during both the 5-week stress session and the behaviour test periods. The control group and the CMS group of mice were given distilled water daily (Figure 1).

CMS procedure

The CMS regimen used in this study was based on the procedure originally designed by Willner (1997) and adapted to mice (Ducottet et al., 2003). Mice were submitted daily to 2 to 3 different stressors for 5 weeks in a chronic and unpredictable way. Stressors included: without sawdust, damp sawdust, soiled cage (aversive odour due to old rat sawdust), social stress (switching the cages), predator sounds for 30 min (cat and dog), inversion of light/dark cycle, exposure to a rat for 30 min and food or water deprivation for 12 h (Table 1). The animals were subjected to these stressors randomly at any time of night or day. To make the stress procedure unpredictable, the stressor sequence was changed every week.

Sucrose preference test (SP test)

The sucrose preference test was carried out at the beginning and the end of the 5-week period of CMS exposure. The test was performed as described previously, with minor modifications (Strekalova and Steinbusch, 2010). The procedure composed of training and testing courses. In the training course, mice were trained to experience and drink a sucrose solution (1%) for 24 h, by presenting them simultaneously with two identical bottles: one containing a sucrose solution (1%) and the other containing tap water. In the testing course, mice were given, for 15 h, a free choice

Table 1. Schedule of CMS procedure.

Day	Forced swimming	Sawdust free	Food deprivation	Exposure to a rat	Damp sawdust	Inversion light/dark cycle	Water deprivation	Paired housing	Old rat sawdust	Switching the cage	Stroboscopic illumination	Sounds of predators
Monday	9:00 ↓ 9:20		20:00 ↓ 8:00					17 : 00 ↓ 8 : 00				11:30 ↓ 12:00
Tuesday		9:00 ↓ 12:00				17 : 00 ↓ 17:00			12:00 ↓ 15 : 00			
Wednesday				8:00 ↓ 8:30			20 : 00 ↓ 8 : 00			14 : 00 ↓ 17 : 00		
Thursday	8:00 ↓ 8:30										9:00 ↓ 17:00	
Friday				16:00 ↓ 16:30	20 : 00 ↓ 6 : 00			17:00 ↓ 8:00				8:00 ↓ 9:00
Saturday		14:00 ↓ 17:00	20:00 ↓ 8:00				20:00 ↓ 8:00			9:00 ↓ 12:00		
Sunday						17:00 ↓ 17:00						14:00 ↓ 15:00

between two bottles, with sucrose solution (1%) or tap water. The test began at the start of the dark phase in the mouse home cage. To prevent possible effects of side preference in drinking behavior, the position of the bottles in the cage was switched in the mid-point of testing. No previous food or water deprivation was applied before the test. The sucrose preference was calculated according to

the following formula:

$$\text{Sucrose preference (SP)} = \frac{\text{sucrose intake (g)}}{\text{sucrose intake (g)} + \text{water intake (g)}} \times 100\%.$$

Following the baseline test, animals were divided into five groups, each consisting of 12 mice.

Novelty-suppressed feeding (NSF) test

The NSF test was similar to the version used by Vollenweider et al. (2011). After 24 h food deprivation period, a single pellet of food (regular chow) was placed in the center of the box (42 × 31 × 20 cm). A mouse was placed in a corner of the test box and the latency to bite

was recorded. Mice were removed from the apparatus after they began eating, or a maximum latency of 6 min, and returned to their home cages.

Determination of neurotransmitter levels

The concentrations of three neurotransmitters, 5-HT, NE and DA, in the mouse prefrontal cortex were simultaneously determined by liquid chromatography mass spectrometry/mass spectrometry (LC-MS/MS). Twenty-four hours after the NSF test, the mice were sacrificed by decapitation, the brains were rapidly removed on ice and dissected for prefrontal cortex collection. The tissues were weighed and homogenized in ice-cold 0.2% aqueous formic acid, then mixed with 0.2% formic acid in acetonitrile for protein precipitation. After centrifugation at 13,800 g for 10 min at 4°C, an aliquot (200 µl) of the supernatant was collected and mixed with 20 µl of internal standard solution (300 µg/ml 3,4-Dihydroxybenzylamine (DHBA) hydrobromide). A mixture of 50 µl was injected into the LC-MS/MS system for assay. The LC-MS/MS instrument comprised an Agilent 1200 HPLC system (Palo Alto, CA, USA) and an Applied Biosystem 3200 Q-Trap mass spectrometer (Foster City, CA, USA) with an electrospray ionization source. The source temperature was 450°C and the needle voltage was set at 4.5 kV; the nebulizer gas (GS1), auxiliary nitrogen gas (GS2) and curtain gas (CUR) were set at 50, 50 and 10 psi, respectively. The mobile phases were 6 mM ammonium formate in acetonitrile-water (67.5:32.5, pH 5.50) with a flow rate of 200 µl/min. The neurotransmitters and internal standard were detected by multiple reaction monitoring mode, at m/z 177.2→160.0 (5-HT), 170.3→152.2 (NE), 154.2→137.1 (DA), and 140.1→123.1 (DHBA, IS), respectively. The peak area ratios of the analyte versus internal standard were used to quantify the neurotransmitter concentrations.

Statistical analyses

All tests were analyzed using the Statistical Package for Social Sciences (SPSS) statistic 16.0 (SPSS Inc., Illinois, Chicago, USA). Data were expressed as mean ± standard error of mean (SEM) and analyzed by one-way analysis of variance (ANOVA) followed by least significant difference (LSD) post hoc tests for inter-group comparisons. A value of $P < 0.05$ was considered statistically significant.

RESULTS

Effects of TBY on immobility time in the mouse FST, TST and OFT

As shown in Figure 2a (FST), the immobility time in control animals (143 min) was significantly reduced with similar magnitude (29%, $P < 0.01$; 27%, $P < 0.01$; 27%, $P < 0.01$, respectively) after treatment with TBY (75 and 150 mg/kg) and amitriptyline (10 mg/kg). As shown in Figure 2b (TST), the oral administration of TBY (75 and 150 mg/kg) significantly reduced the immobility time (35%, $P < 0.05$ and 14%, $P < 0.05$), as compared to the control group (132 min). The positive control, paroxetine (10 mg/kg), more significantly reduced the immobility time by 75%.

To verify that the changes in immobility time in the TST were not attributable to non-specific side-effects, the

open field locomotion test was performed. The locomotor activities of the animals were observed 60 min after oral administration of TBY for 7 days (75 and 150 mg/kg). Compared with the control animal (1318 cm), there was no significant alteration in the total distance in the presence of TBY ($P > 0.05$) (Figure 2c).

Effect of TBY on the percentage of sucrose consumption in CMS mice

Before CMS treatment was given, there was no significant difference in the percentage of sucrose consumption among all mice (data not shown). After 5 weeks of exposure to stress, as shown in Figure 3, the percentage of sucrose consumption was significantly reduced in CMS mice (30%, $P < 0.01$), as compared to the control mice. TBY (50 and 100 mg/kg) and fluoxetine (10 mg/kg) showed a significant improvement in the percentage of sucrose consumption when compared with that of the CMS group administered with vehicle (19%, $P < 0.05$; 21%, $P < 0.01$; 20%, $P < 0.05$, respectively).

Effect of TBY on latency to feed in CMS mice

As shown in Figure 4, a post hoc comparison revealed that the CMS group mice exhibited a longer latency to feed than the control group (235%, $P < 0.01$). Chronic treatment with TBY (50 and 100 mg/kg) and fluoxetine (10 mg/kg), produced a significant recovery of the latency to feed, as compared to the CMS group (55%, $P < 0.05$; 67%, $P < 0.01$; 27%, $P < 0.05$, respectively).

Effect of TBY treatment on the 5-HT, DA and NE concentrations in the prefrontal cortex

As shown in Figure 5, the levels of 5-HT, DA and NE were significantly reduced in the CMS model group when compared with the control group (67%, $P < 0.05$; 29%, $P < 0.05$; 39%, $P < 0.05$, respectively). Fluoxetine produced a notable increase of the 5-HT level (191%, $P < 0.05$ versus CMS), but not of DA and NE. Treatment with 50 mg/kg TBY produced a significant increase of 5-HT (207%, $P < 0.01$) level as compared to CMS group. Treatment with 100 mg/kg TBY produced a significant increase of 5-HT (151%, $P < 0.05$) and DA (36%, $P < 0.05$). The mice also tended to have higher NE levels after chronic TBY (50 and 100 mg/kg) and fluoxetine (10 mg/kg) treatment.

DISCUSSION

FST and TST are based on the fact that animals subjected to short-term, inescapable stress will develop an immobile posture. This immobility, referred to as

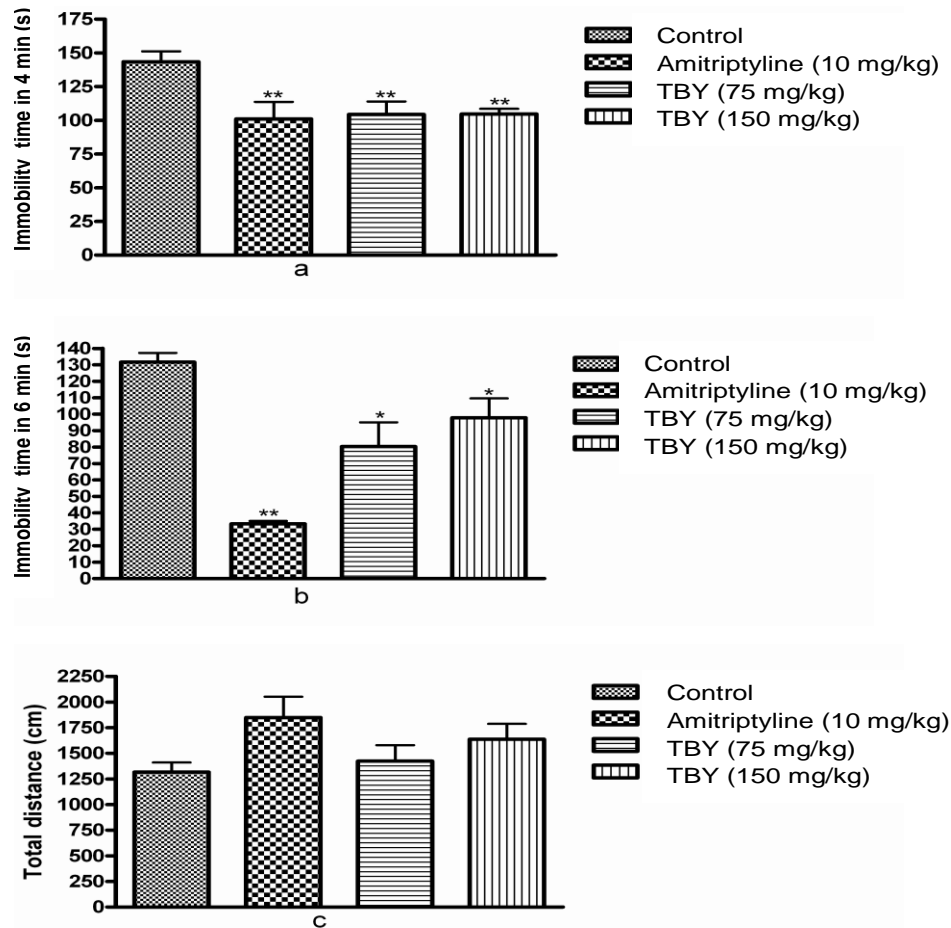


Figure 2. Effect of TBY on the immobility time in the forced swim test (a), tail suspension test (b) and the total distance in the open field test (c). Distilled water, TBY (75 and 150 mg/kg) and amitriptyline or paroxetine (10 mg/kg) were orally administered for 7 days, respectively. Values given are the mean \pm SEM (n=11). For statistical significance, *P < 0.05, **P < 0.01, as compared with the control group.

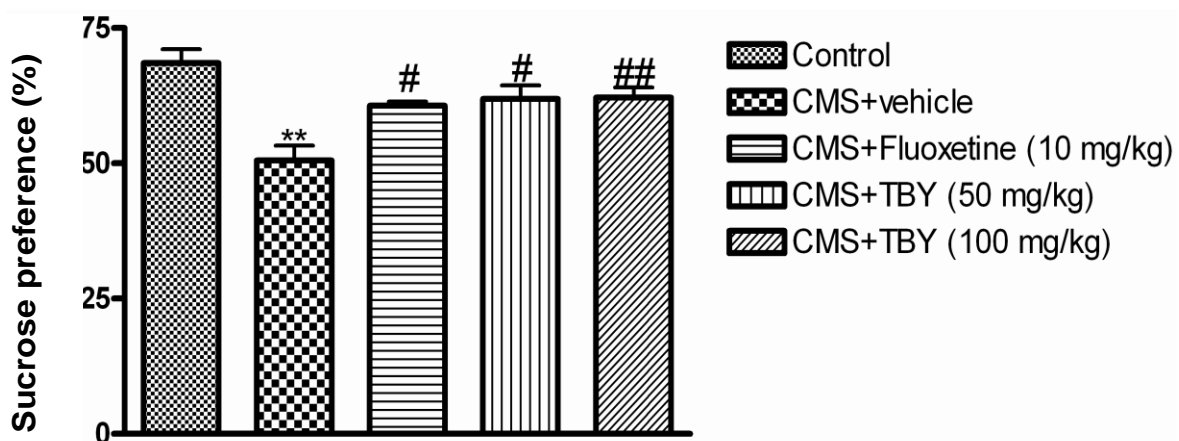


Figure 3. Effect of TBY treatment on the percentage of sucrose consumption in CMS mice. Percentage of sucrose preference was measured after five weeks of CMS. Distilled water (control and CMS group), fluoxetine (10 mg/kg) or TBY (50 and 100 mg/kg) were administered daily by gastric gavage during both the 5-week stress session and behaviour tests periods, respectively. Values given are the mean \pm SEM (n=12). For statistical significance, *P < 0.05, **P < 0.01, as compared with the control group and #P < 0.05, ##P < 0.01, as compared with the CMS group.

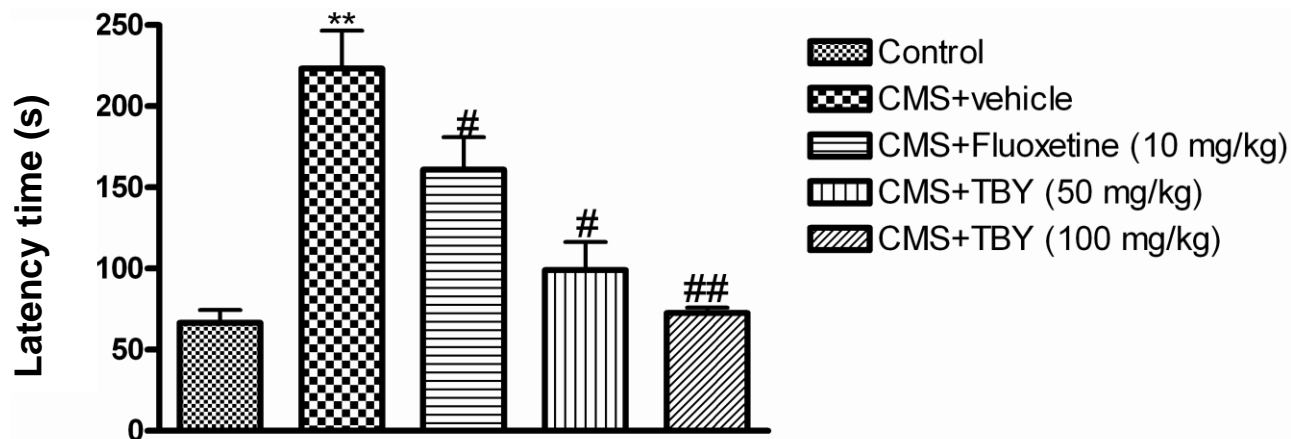


Figure 4. Effect of TBY on the latency time in the NSF test in CMS mice. Distilled water (control and CMS group), fluoxetine (10 mg/kg) or TBY (50 and 100 mg/kg) were administered daily by gastric gavage during both the 5-week stress session and the behavior test periods, respectively. Values given are the mean \pm SEM (n=12). For statistical significance, **P < 0.01, as compared with the control group; #P < 0.05, ##P < 0.01, as compared with the CMS group.

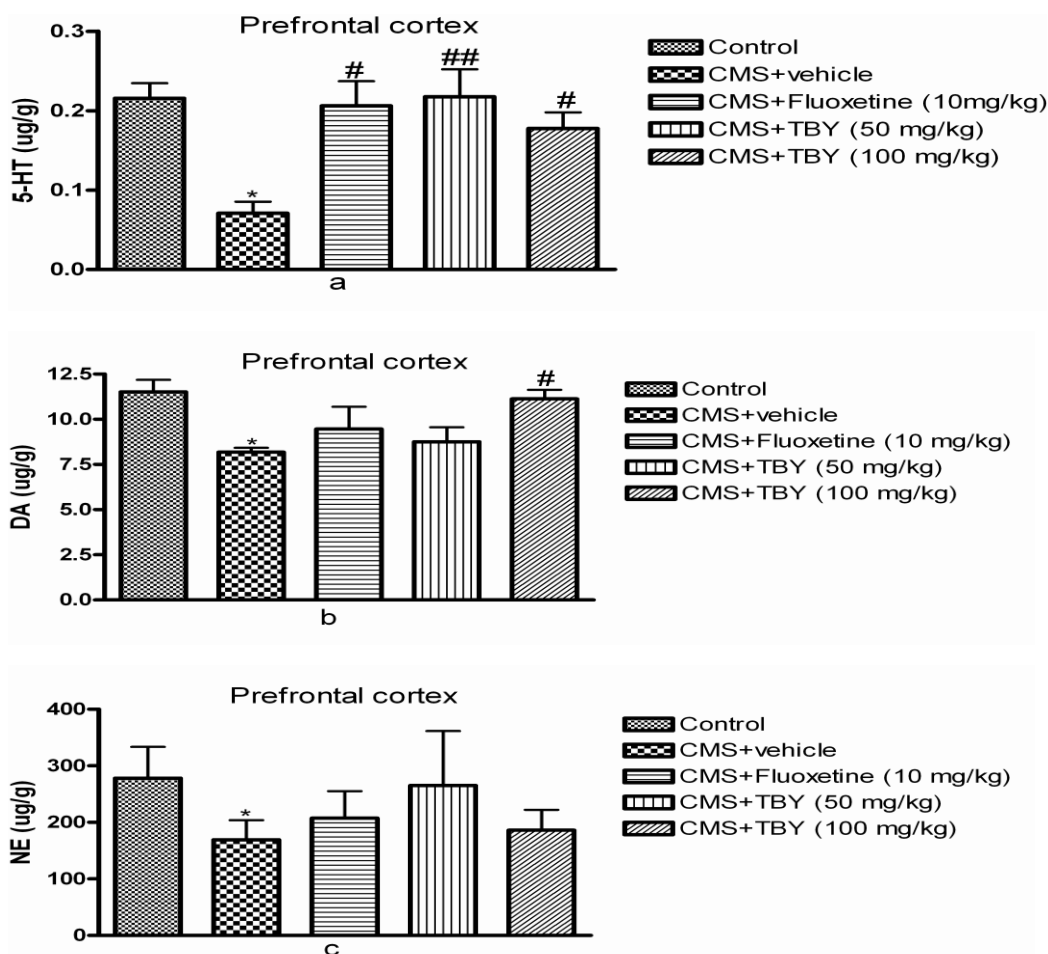


Figure 5. Effect of TBY on the 5-HT, DA and NE levels in the prefrontal cortex in CMS mice. Distilled water (control and CMS group), fluoxetine (10 mg/kg) or TBY (50 and 100 mg/kg) were administered daily by gastric gavage during both the 5-week stress session and the behavior test periods, respectively. Values given are the mean \pm SEM (n=12). For statistical significance, *P < 0.05, **P < 0.01, as compared with the control group; #P < 0.05, ##P < 0.01, as compared with the CMS group.

behavioral despair in animals, is claimed to reproduce a condition similar to human depression (Cryan and Holmes, 2005). Our present study indicates that TBY (75 and 150 mg/kg) during 7 days, significantly reduces the duration of immobility in both tests after being administered orally for 7 days. There was no change in the locomotor activity of animals treated with TBY, implying that TBY could have the antidepressant-like effect without affecting locomotor activity.

CMS model induces various long-term behavioural alterations resembling depressed patients, and can be used for evaluating the efficacy of antidepressants through behavioral tests, such as the SP test and the NSF test. The SP test is usually employed to define anhedonia (a loss of responsiveness to pleasant events), which is a core symptom in the diagnosis of depression and could be modeled by inducing a reduction of sucrose consumption in CMS (Matthews et al., 1995). The NSF test is often used as a measure of depression-like behaviors (Stedenfeld et al., 2011). This test elicits competing motivations: the drive to eat and the fear of novel open spaces. In the CMS paradigm, the latency can be elevated by stress, while chronic treatment with antidepressants can reverse this effect (Barguen-Vargas et al., 2008). Our results showed that CMS induced significant reduction of sucrose intake in the sucrose preference test and increase of latency to feed as compared to the control mice, which were all reversed by fluoxetine treatment. Chronic treatment with TBY (50 and 100 mg/kg) was found to prevent changes induced by CMS. The results from our study demonstrate the antidepressant-like effect of TBY in the CMS model.

Decrement of monoamine neurotransmitters in the prefrontal cortex was thought to contribute to some depressive symptoms (Fibiger, 1995; Cummings, 1992; Owens and Nemeroff, 1994). Our results showed that the levels of 5-HT, DA and NE in the prefrontal cortex, diminished significantly in the CMS model group when compared with the control group, which was in line with previous studies in CMS (Kwon et al., 2010; Mizoguchi et al., 2003). Chronic treatment with TBY (50 and 100 mg/kg) restored these changes in the prefrontal cortex. In addition, mice also showed a tendency for increased NE level in the prefrontal cortex after chronic TBY treatment. Our results suggest that the beneficial action of TBY on the CMS-induced depressive state is based on the prevention of dopaminergic and serotonergic dysfunction in the prefrontal cortex. Further studies should be performed to address the detailed mechanism of TBY action on the monoamine neurotransmitter level.

Conclusion

Conclusively, TBY induced an antidepressant-like action in the depression models (FST, TST and CMS). The antidepressant action of TBY may be mediated by modulating the monoamine neurotransmitter level in the

prefrontal cortex.

To the best of our knowledge, this is the first investigation showing that TBY exerts an antidepressant-like action in experimental mice. TBY could be used as a potential effective antidepressant candidate drug, after further research has been done.

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