Preventive Action of Kai Xin San Aqueous Extract on Depressive-Like Symptoms and Cognition Deficit Induced by Chronic Mild Stress

HAIXIA DANG,* LIHUA SUN,* XINMIN LIU,*,[1] BO PENG,* QIONG WANG,* WILLIAM JIA,† YING CHEN,* AIHUA PAN,‡ AND PEIGEN XIAO*

Research Center of Pharmacology and Toxicology, Peking Union Medical College Institute of Medicinal Plant Development, Beijing 100193, China; †Brain Research Center, Faculty of Medicine, University of British Columbia, Vancouver, Canada V6T 2B5; and ‡Sinobioway Group Co. Ltd, Beijing 100085, China

Kai Xin San (KXS), a traditional Chinese herbal medicine, has been used clinically for the treatment of depressive disorders and cognitive impairment for centuries. However, the effects of KXS on cognitive dysfunction induced by depression have not been evaluated scientifically. The present study aimed to explore the antidepressant-like and nootropic effects of an aqueous extract of KXS (at doses of 0.3, 0.9, and 2.7 g/kg/day) using chronic mild stress (CMS) as a model of depression. Depressive symptoms were analyzed using the sucrose-preference and novelty-induced inhibition of feeding tests. Cognitive function was evaluated using a two-way active avoidance task. Serum corticosterone and adrenocorticotropic hormone (ACTH) levels, acetylcholinesterase (AChE) protein expression in the hippocampus, and monoamine neurotransmitter concentrations in the prefrontal cortex and hippocampus were also determined to elucidate the neurochemical mechanisms. Experimental results showed that KXS aqueous extract significantly ameliorated the CMS-induced depressive symptoms, including the reduced preference index and prolonged latency to novelty-suppressed feeding. Simultaneously, KXS significantly reversed the CMS-induced decrease in the numbers of active avoidance and active movement distances and increase in the numbers of the passive avoidance and passive movement distances, thereby by producing nootropic effects in the two-way active avoidance test. KXS also inhibited the increased AChE expression in the hippocampus, up-regulated the decreased monoamine neurotransmitter concentrations of both brain areas and reduced the elevated ACTH concentrations in the serum induced by CMS. Taken together, these results indicate that KXS exerts its antidepressant-like and nootropic effects in the CMS model by modulating the HPA axis, monoamine neurotransmitter and cholinergic systems. Exp Biol Med 234:785–793, 2009

Key words: Kai Xin San; depression; chronic mild stress; cognitive impairment

Introduction

Depressive disorders are among the most prevalent psychiatric disorders, with a lifetime incidence of 15–25% (1). Cognitive dysfunction (including learning and memory, executive function, etc.) is one of the most important features in a depressive patient; it sometimes appears rather earlier and may even precede signs of depressed mood (2–3).

At present, there are three main kinds of classical antidepressants in clinical practice, including tricyclic antidepressants (TCA), selective serotonin reuptake inhibitors (SSRIs), and monoamine oxidase inhibitors (MAOIs). Some of these drugs, however, have undesirable side effects on cognitive function (4–5). The lack of satisfactory treatments for the cognitive deficits accompanying depression presents a constant challenge for psychopharmacology research. Thus search for effective antidepressant agents with the capability of enhancing cognitive function holds the promise of significant clinical advantages.

Kai Xin San (KXS), comprising ginseng (*Panax ginseng* C. A. Meyer), hoelen (*Poria cocos* (Schw.) Wolf), polygala (*Polygala tenaifolia* Willd), and acorus (*Acorus*

This work was financially supported by a grant from the International Technological Collaboration Project (Grant No. 2006DFA21740) and National Support Plan (2006BAI08B05–07).

[1] To whom correspondence should be addressed at Research Center of Pharmacology and Toxicology, No. 151 Malianwa North Road, Haidian District, Beijing 100094, P.R. China. E-mail: liuxinminuae@yahoo.com.cn

Received December 8, 2008.
Accepted March 26, 2009.

DOI: 10.3181/0812-RM-354
1535-3702/09/2347-0785$15.00

Table 1. Experimental Manipulations During the Course of the Present Study

Time (day)	Experimental arrangement
1–3	Animal adaptation
4–10	Baseline sucrose-preference test
11–46	Chronic mild stress, KXS, and fluoxetine treatment for stressed rats; control rats were housed in groups of 5 per cage and kept under normal physical conditions
47–49	Sucrose-preference test, single housing of stressed rats, and fluoxetine and KXS treatment
50–51	Rest, single housing of stressed rat, and KXS and fluoxetine treatment
52–54	NIH test, single housing of stressed rat, and KXS and fluoxetine treatment
55–56	Rest, single housing of stressed rat, and KXS and fluoxetine treatment
57	Shuttle box test, single housing of stressed rat, and KXS and fluoxetine treatment
58	Decapitation

tatarinowii Schott), was originally recorded in 'Bei Ji Qian Jin Yao Fang' in the Tang Dynasty. It was recorded that KXS could cure symptoms including 'desolation, moodiness, forgetfulness etc.,' which are similar to the neuroses such as depression, anxiety, and impairment in learning and memory. Experimental studies demonstrated that KXS exerts antidepressant-like effects in the forced swimming and tail suspension tests (6). In addition, previous studies have demonstrated that KXS ameliorates the learning and memory deficit in amygdala-lesioned mice (7), senescence-accelerated mice (8), mice with learning and memory deficits induced by ethanol and scopolamine (9), as well as mice with thymectomy-induced impairment of learning behaviors (10) in the step-down or Morris water maze test. Furthermore the mechanism of action of KXS that influences learning and memory may be operative via an enhancement of hippocampal long-term potentiation (11–12) and could be exploited for the treatment of cognitive impairment induced by depression.

Chronic mild stress (CMS), an accepted animal model of depression, is a reliable tool for exploring the mechanisms of depression and its co-morbidities because of its combined features of predictive validity, face validity, and construct validity (13). In addition to a wide variety of depressive symptoms parallel to that of human depression, CMS has been shown to impair learning and memory abilities (14). Therefore, in the present study, we used the CMS paradigm to determine if chronic KXS treatment can alleviate CMS-induced depressive symptoms and cognitive impairment. Additionally, the probable mechanisms of the antidepressant-like and nootropic activities of KXS were also explored by analyzing levels of corticosterone and adrenocorticotropic hormone (ACTH) in the serum, monoamine neurotransmitter concentrations in the rat brain, and acetylcholinesterase (AChE) protein expression in the hippocampus, which have been reported to be involved in the pathophysiology of depression and cognitive deficit (2, 15).

Materials and Methods

Drug Preparation. KXS was supplied in the form of a dried powder that had been manufactured as an aqueous extract of a mixture of ginseng, hoelen, polygala, and acorus. These traditional Chinese herbal medicines were purchased from Beijing Tongrentang Drugstore (Beijing, China) and authenticated by Professor Zhao Runhuai (Institute of Medicinal Plant Development, Beijing, China) based on their macroscopic characteristics. The quality of these crude drugs is controlled by the Chinese Pharmacopoeia (2005), and a voucher specimen of each plant (No. 05003, No. 05004, No. 05005, No. 05006, respectively) was prepared and deposited in the herbarium of the Institute of Medicinal Plant Development.

The crude drugs were cut into small pieces and then mixed together at a ratio of 3:3:2:2. Dried samples weighing about 4800 g were boiled in 50 L of distilled water for 2 h. After extraction, the decoction was collected, and the residue was subjected to further extraction in 40 L of boiling water for 2 h. The decoctions collected from the two successive extractions were mixed and passed through filter paper. The filtrate was collected and concentrated in a water bath kept below 60°C under reduced pressure using a rotary evaporator. The percentage (w/w) yield of the water extract of KXS was 14.8%.

Ethical Evaluation. All animal handling procedures were performed in compliance with the 'Principles of Laboratory Animal Care' (NIH publication No. 85–23, revised 1985) and P.R. China legislation for the use and care of laboratory animals.

Experimental Design. After arrival, the animals were habituated to the Specific Pathogen Free animal house for 3 days. Baseline behavioral tests were conducted on the fourth day. All experimental manipulations were performed according to the experimental design presented in Table 1 and described in subsequent sections.

Animal Handling and Grouping. Sprague-Dawley rats of both genders, weighing 190–250 g, were purchased from the Laboratory Animal Center (Beijing, China); 32 male and 32 female rats were used in this experiment. Animals had free access to food and water. They were maintained on a 12-h light/dark cycle (lights on at 08.00) at a temperature of 22–25°C and humidity of 55 ± 10% unless otherwise noted. All animals were initially subjected to the sucrose-preference baseline test. On the basis of the sucrose preference score, the animals were divided into six groups: the CMS group, CMS+Fluoxetine group (10 mg/kg), CMS+KXS group (0.3 g/kg, 0.9 g/kg, 2.7 g/kg), and the control (CON) group ($n = 10 \sim 12$ for each group). Roughly equal numbers of animals of each gender were included in each group. Male and female rats were housed separately. The stressed experimental animals were housed individually

and exposed to CMS. The non-stressed control animals received daily care and were housed in a different room in groups (five rats in one cage).

Drug Administration. The water extract of KXS was dissolved in distilled water, and was administered as 10 ml/kg during both the 5-week stress session and the behavior test periods. The extract was administered by gavage at doses of 0.3, 0.9, and 2.7 g/kg. Simultaneously, the CMS+Fluoxetine group received fluoxetine (10 mg/kg, synthesized by Venturepharm, Beijing, China.). The control group was administered distilled water (10 ml/kg p.o.) daily. The drug or water was given at 10.00.

Stress Procedure. The CMS model was a slight modification of the method described by Willner (13). Ten stressors were used to induce a depressive state, including: food deprivation (23 h); cage tilt (45°, 23 h); continuous overnight illumination; soiled cage (100 ml of water spilled onto the bedding, 23 h); cold water swimming (4°C for 5 min); shaken on a rocking bed (200 Hz for 5 min; HY-4A, manufactured in Shunhua Scientific Instruments Limited, China); exposure to empty water bottles (23 h); 2 h of behavior restraint in a tube (diameter: 8 cm; length: 20 cm); and intermittent illumination (light on and off every 2 h). These stressors lasted for 5 weeks, and each animal received one stress per day. After five weeks, the stressed rats continued to be housed in individual cages to sustain the depressive state. Table 2 describes the detailed stress arrangements.

Sucrose-Preference Test. A sucrose-preference test was employed to define anhedonia (diminished interest or pleasure) operationally (16). After a 3-day adaptation period, two sucrose-preference baseline tests were conducted 4 days apart for all animal groups and the results were averaged. These tests involved 23 h of food and water deprivation, followed by 1% sucrose solution and water for 1 h. Intake was measured by weighing the bottles containing the sucrose solution and water at the end of each test. All tests were carried out in the home cage to minimize extraneous novelty and disturbance. The sucrose-preference test was conducted again after 5 weeks of CMS. The sucrose preference (SP) was calculated as sucrose intake/(sucrose intake + water intake).

Novelty-Induced Hypophagia (NIH) Test. Hyponeophagia refers to the inhibition of feeding produced by exposure to novelty (17). After 48 h of food deprivation, the rats were placed into a new plastic cage. The latency of feeding (time elapsed until the rat started to eat) was recorded manually.

Two-Way Active Avoidance Test. The two-way avoidance test was performed in a 70 cm × 70 cm × 70 cm metallic box, which was divided into two identical two-way shuttle boxes (developed by the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, jointly with Chinese Astronaut Center). Each shuttle box consisted of two identical compartments (30 cm × 35 cm × 70 cm), separated by a black partition with a small opening

Table 2. Procedures for Inducing Chronic Mild Stress in Rats

	Single housing	Dark and light circle	Tilt cage	Cold water swimming	Shaking	Empty water bottles	Behavior restraint	Soiled cage	Continuous lighting	Food deprivation
Monday	9.00→	9.00→								
Tuesday	9.00→		9.00→							
Wednesday	9.00→			10.00→11.00						
Thursday	9.00→				14.00→15.00					
Friday	9.00→					11.00→				
Saturday	9.00→						9.00→11.00			
Sunday	9.00→							14.00→		
Monday	9.00→								9.00→	
Tuesday	9.00→									10.00→
Up to the end of experiment	These stressors lasted for 5 weeks and each animal randomly received one stress per day. After 5 weeks, the stressed rat was housed individually.									

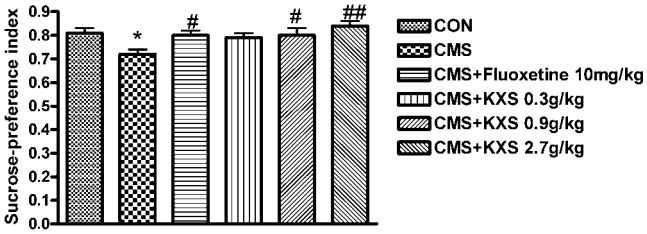

Figure 1. Effects of KXS on the sucrose-preference test in rats. Each column represents the mean ± SEM. * $P < 0.05$ compared with control; # $P < 0.05$, ## $P < 0.01$ compared with CMS.

(10 cm ×10 cm). A camera connected to a computer was fixed onto the center of the ceiling of each shuttle box to monitor the animal's behavior and register on the computer using a frame-grabbing card (DH-CG300). Compartment floors (without any physical separation between them) were independently electrifiable and constructed of stainless steel bars. The conditioned stimulus (CS) light located on both side walls was blue light (10 Lux) of 5 s duration. The unconditioned stimulus (US) consisted of a 0.4 mA electrical foot shock not exceeding 30 s. The avoidance training sessions consisted of 60 trials with 10 s inter-trial. The automated experimental protocol evaluated animal cognitive function: the number of active avoidances (i.e., leaving the compartment after the CS but before the US) and passive escapes (leaving the compartment after the US started), the total distance moved for avoidances and escapes. Prior to the experiments, all the animals received a 5-min adaptation time period.

Serum Corticosterone and ACTH Levels. After performing the shuttle box test, the rats were sacrificed, and blood samples were collected, kept on ice for 1 h, and then centrifuged at 3000 *g* at 4°C for 15 min. The serum samples were stored at –80°C until use. Corticosterone and ACTH levels were measured using commercially available ELISA kits (RapidBio Lab, California, USA). The assay sensitivities of ACTH and corticosterone were 1 pg/ml and 0.7 nmol/L, respectively. The intra- and inter-assay coefficients of variation were about 8%.

Determination of Monoamine Neurotransmitter Levels. The levels of norepinephrine (NE), dopamine (DA), 5-hydroxytrypytamine (5-HT), and their metabolites 3,4-hydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 5-hydroxyindoleacetic acid (5-HIAA) were determined by reverse-phase high-performance liquid chromatography (HPLC) with electrochemical detection. Briefly, a C_{18} reverse-phase column (Waters 150 mm × 4.6 mm, 5 μm), an electrochemical detector (ESA, 5600A, Bedford, MA, USA), and a liquid chromatography work station were employed. Following decapitation, the brains were rapidly removed and dissected on ice; prefrontal cortex and hippocampus were isolated. The tissues were weighed, sonicated for a few seconds in 0.15 M $HClO_4$ (Fisher Scientific, Nepean, ON, Canada) (1 ml/100 mg tissue), and centrifuged. The supernatant was filtered using 0.2 μm and stored at –80°C until HPLC-ED analysis. The mobile phase composed of phosphate buffer (pH of 3.9, 90 mmol/L NaH_2PO_4, 50 mmol/L citric acid, 1.7 mmol/L octanesulfonic acid sodium salt, and 0.05 μmol/L Na_2EDTA (Sigma-Aldrich)); with 0.6 ml/min flow rate. The sample (20 μl) was injected and the oxidation potential was fixed at 450 mv. The peak areas of the external standards were used to quantify the sample peaks.

Immunohistochemistry Analysis for AChE Protein Expression in the Hippocampus. Rats were anesthetized with sodium pentobarbital (100 mg/kg, i.p.) and perfused intracardially with saline, followed by 4% paraformaldehyde in 0.1 M sodium phosphate buffer, pH 7.2. The coronal hippocampus was dissected, fixed with a solution containing 4% paraformaldehyde, embedded in paraffin, and cut into 7-μm thick sections. Following deparaffinization, the sections were rehydrated and blocked with 2% normal goat serum for 2 h. The blocked sections were incubated with rabbit polyclonal anti-AChE antibody (1:200 dilution, Santa Cruz Biotechnology, Inc., USA), and subsequently reacted with the biotinylated secondary antibody. A Histostain-SP kit (Zymed Laboratories, Inc., USA) was used to visualize the immune complexes. Images were captured on a Leica microscope and video camera at ×40 magnification. Three random images from each brain section were captured, and integrated optical density (IOD) within this area was measured. Subsequently, the average of the three measurements was used to represent the immunoreactivity.

Statistical Analyses. SPSS 12.0 was used for all statistical analyses. Values are expressed as the mean ± SEM with analysis of variance (ANOVA). Individual values were compared with Dunnett's post hoc test and *P* value of less than 0.05 was considered to be statistically significant.

Results

Sucrose-Preference Test. The effects of oral administration of KXS and fluoxetine on the sucrose-preference index are presented in Figure 1. The sucrose-preference index at baseline did not differ significantly between the six groups [$F(5,59) = 0.24$, $P > 0.05$]. However, after 5 weeks of stress, the CON group was significantly different from the CMS group, with a higher index of sucrose preference ($P < 0.05$). KXS treatment at 0.9, 2.7 g/kg significantly increased the sucrose-preference index in comparison with the CMS group ($P < 0.05$ vs. the CMS group). Likewise, an increase in the preference index was also noted in the CMS+Fluoxetine group ($P < 0.05$ vs. the CMS group).

Latency of Feeding in Novel Cage. Figure 2 indicated that the latency of feeding was significantly prolonged in the CMS group ($P < 0.001$, vs. control) and 35-day treatment with either fluoxetine or KXS (0.3, 0.9, 2.7 g/kg) it was significantly declined [$F(5,59) = 10.17$, $P < 0.001$ vs. CMS group]. Moreover, between-subjects effects

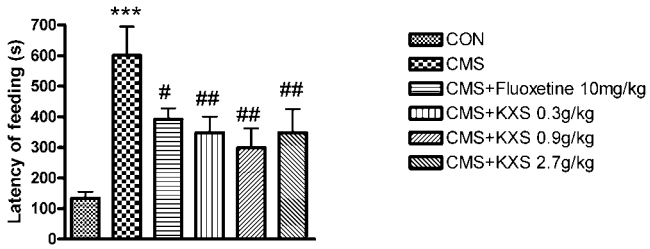

Figure 2. Effects of KXS on latency for feeding in rats. Each column represents the mean ± SEM. *** $P < 0.001$ compared with control; # $P < 0.05$, ## $P < 0.01$ compared with CMS.

showed significant effects of gender [F(1,59) = 29.29, $P < 0.001$] and gender × group [F(5,59) = 0.018, $P < 0.05$] on the latency. The results demonstrate that the male rats displayed a longer latency of feeding than the female rats.

Two-Way Active Avoidance Test. The effects of KXS and fluoxetine on the two-way avoidance performance are shown in Figure 3. After 5 weeks of CMS, the number of passive escapes in the CMS group was significantly increased, with a significant decline in the number of active avoidances, as compared with the CON group ($P < 0.05$) (Fig. 3A). The distance of passive movement of the CMS group was significantly greater than that of the CON animals, but the distance covered during active avoidance was substantially smaller than that in the CON groups (Fig. 3B). The 35-day treatment with the KXS extract (at a dose of 2.7 g/kg) significantly decreased the number of passive escapes [F(5, 59) = 3.13, $P < 0.05$ vs. CMS group] and the passive movement distance [F(5, 59) = 5.86, $P < 0.01$ vs. CMS group], and simultaneously increased the number of active avoidance [F(5, 59) = 3.74, $P < 0.01$] and active movement distances (at doses of 0.9 and 2.7 g/kg) [F(5, 59) = 3.19, $P < 0.05$]. However, 10 mg/kg fluoxetine treatment did not significantly prevent the aforementioned changes induced by CMS. Furthermore, there were no significant between-subject effects of gender, and no interaction effect of gender × group in any of the measures observed in the shuttle box test.

Corticosterone and ACTH Levels. Figure 4 displays the mean serum corticosterone and ACTH levels following 5 weeks of CMS. ANOVA indicated significant treatment effects on the ACTH level [F(5,59) = 10.26, $P < 0.001$]. Further analyses confirmed that exposure to CMS significantly increased the serum ACTH levels relative to the control group ($P < 0.001$). Both fluoxetine and all doses of KXS administration significantly decreased the ACTH level in stressed rats ($P < 0.01$ vs. the CMS group). However, CMS increased corticosterone levels, but there was no significant difference in levels compared with the CON group. Moreover, there was no significant difference between KXS-treated stressed rats and the CON group. A similar phenomenon was also observed in fluoxetine-treated CMS rats.

Between-subject effects showed a significant effect of gender on corticosterone and ACTH levels. There was also an interaction effect of gender × group in ACTH [F(5,59) =

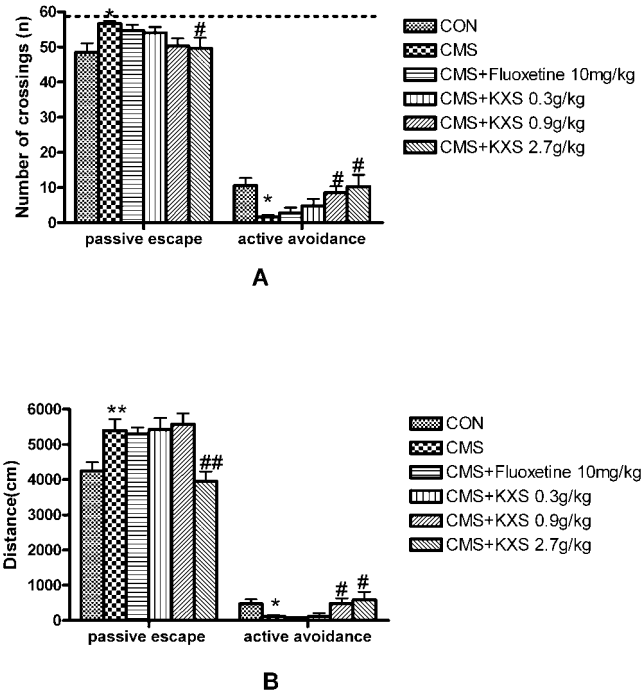

Figure 3. Effects of KXS on the number of passive escapes and active avoidances (A), and on the total distance of movement in passive escapes and active avoidances (B) in the shuttle box test. Each column represents the mean ± SEM. * $P < 0.05$, ** $P < 0.01$ compared with control; # $P < 0.05$, ## $P < 0.01$ compared with CMS.

11.50, $P < 0.001$] and corticosterone levels [F(5,59) = 2.74, $P < 0.05$]. Moreover, the results showed that the male rats displayed higher ACTH concentrations than did female rats.

Monoamine Neurotransmitter Levels. The effects of KXS and fluoxetine treatment on the monoamine neurotransmitter system are summarized in Table 3. In the hippocampus, CMS significantly reduced NE, DA, DOPAC and HVA levels ($P < 0.05$ vs. CON group). Similarly, in the prefrontal cortex, the levels of NE ($P < 0.01$), DA ($P < 0.05$), DOPAC ($P < 0.05$) and HVA ($P < 0.01$) also decreased significantly compared with CON group. However, statistical analysis did not reveal any statistically significant alteration of 5-HT and HIAA in both hippocampus and prefrontal cortex. Fluoxetine treatment was able to reverse the effects of CMS on NE, DA, DOPAC, and HVA levels in both brain regions ($P < 0.01$ vs. CMS group), with the exception of NE in the hippocampus.

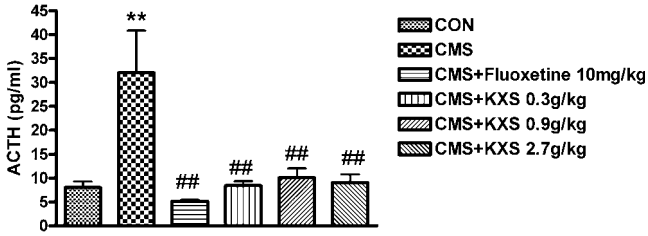

Figure 4. Effects of KXS on ACTH and corticosterone levels. Each column represents the mean ± SEM. * $P < 0.05$, ** $P < 0.01$ compared with control; # $P < 0.05$, ## $P < 0.01$ compared with CMS.

Table 3. The Effects of KXS Administration on Levels of NE, DA, 5-HT, and Their Metabolites (ng/100 mg Brain Tissue) in the Prefrontal Cortex and Hippocampus of Rats[a]

	NE	DA	DOPAC	HVA	5-HT	5-HIAA
Prefrontal cortex						
CON	92.8 ± 6.66	234 ± 32.3	39.6 ± 3.63	20.6 ± 2.75	37.0 ± 9.71	20.1 ± 1.36
CMS	67.8 ± 9.47*	137 ± 22.1*	28.4 ± 3.65*	12.8 ± 1.27*	30.3 ± 3.27	23.0 ± 1.34
CMS + Fluoxetine 10 mg/kg	82.1 ± 5.28	328.5 ± 37.6##	71.0 ± 6.13##	33.4 ± 2.48##	36.3 ± 2.57	36.5 ± 2.20
CMS + KXS 0.3 g/kg	72.1 ± 10.1	309 ± 22.9##	44.1 ± 3.78#	19.4 ± 1.46	40.7 ± 1.78	23.1 ± 1.05
CMS + KXS 0.9 g/kg	91.6 ± 20.6	202 ± 35.7	36.1 ± 3.69	26.8 ± 3.53##	37.0 ± 1.75	21.8 ± 0.66
CMS + KXS 2.7 g/kg	141 ± 12.5##	338 ± 59.8##	56.7 ± 7.28##	26.3 ± 3.83##	42.8 ± 4.11	29.8 ± 1.77
Hippocampus						
CON	120 ± 10.1	9.05 ± 1.23	7.58 ± 0.83	6.64 ± 0.67	20.3 ± 1.25	20.9 ± 1.17
CMS	62.3 ± 9.69**	4.78 ± 1.03*	4.39 ± 0.56*	1.11 ± 1.11**	24.0 ± 1.61	22.0 ± 1.04
CMS + Fluoxetine 10 mg/kg	87.0 ± 5.24	13.8 ± 2.80##	12.5 ± 1.54##	7.68 ± 1.24##	16.8 ± 1.90	25.5 ± 4.30
CMS + KXS 0.3 g/kg	70.7 ± 6.94	6.67 ± 1.20	4.37 ± 0.54	1.20 ± 0.20	25.9 ± 1.75	21.9 ± 1.06
CMS + KXS 0.9 g/kg	90.8 ± 11.6#	14.6 ± 4.91#	5.27 ± 0.69	2.73 ± 0.82	44.2 ± 13.3	25.1 ± 1.22
CMS + KXS 2.7 g/kg	103 ± 9.50##	12.6 ± 2.94#	6.68 ± 0.88	4.49 ± 0.56##	19.5 ± 2.12	18.6 ± 1.14

[a] Values are expressed as means ± SEM.
* $P < 0.05$, ** $P < 0.01$ compared with control.
# $P < 0.05$, ## $P < 0.01$ compared with CMS.

Treatment with KXS at 2.7 g/kg was also effective in reversing the effect of CMS on NE, DA, DOPAC and HVA levels. However, at 0.9 g/kg only able to increase the HVA level of the prefrontal cortex ($P < 0.01$ vs. CMS group), as well as the NE and DA concentrations of the hippocampus ($P < 0.05$ vs. CMS group). Treatment with 0.3 g/kg KXS only showed a significant effect on levels of DA ($P < 0.01$ vs. CMS group) and DOPAC ($P < 0.05$ vs. CMS group) in the prefrontal cortex.

AChE Protein Expression in the Hippocampus. As compared with CON rats (Fig. 5A), CMS caused an increase in AChE protein expression in the hippocampus (Fig. 5B, $P < 0.01$), whereas administration of KXS (0.9, 2.7 g/kg) attenuated the expression of AChE protein (Fig. 5E and 5F, $P < 0.01$) as compared with the CMS model rats. Fluoxetine (10 mg/kg) or KXS (0.3 g/kg) treatment did not significantly prevent the aforementioned changes induced by stress.

Discussion

The present study demonstrated that 35-day treatment with aqueous extract of KXS normalize the depressive-like symptoms produced in a CMS model, including decline in sucrose-preference index and prolonged latency of feeding. These findings further confirm the earlier reports demonstrating that KXS is effective in alleviating depressive symptoms in the forced swimming and tail suspension tests (6). More importantly, our results emphasized that KXS also ameliorates the cognitive deficit induced by CMS.

Attenuation of sucrose preference has been interpreted as anhedonia, a loss of reinforcing capacity, which is a core symptom in the diagnosis of depression (16). Our studies confirmed earlier reports indicating that CMS causes a decrease in sucrose preference (18–19), and this deficit could be effectively reversed not only after chronic fluoxetine treatment (20) but also in KXS-treated rats.

Additionally, the NIH test applied herein, provided an anxiety-related measure that is also sensitive for assessing chronic, but not acute or subchronic, antidepressant treatment (17). It is a conflict-based model which elicits competing motivations: the drive to eat and the fear of venturing into the novel environment. Interestingly, we found that the 35-day CMS procedure caused a significantly prolonged latency for feeding in rats, while administration of the SSRI fluoxetine restored the expected latent feeding pattern. Similar effects on latency to feed were observed with KXS, thereby indicating that KXS also has an anxiolytic-like effect.

In this study, cognitive function was assessed using a two-way avoidance test. It is noteworthy that two-way active avoidance is a complex conditioned reflex task involving different forms of learning, as well as different stages of the acquisition process, which not only represents an operant conditioning paradigm, but also exhibits a form of associative learning (21). In the two-way active avoidance test, the animal learns that a random stimulus (CS) is a reliable predictor for a coming aversive experience (US), and prompts an evasive action in order to avoid by moving to the other side of the shuttle box when the stimuli predict an aversive event. Active avoidance in responses represent learning and memory ability. Here, in accordance with a previous report, the CMS procedure also significantly impairs learning and memory capacity (14). The CMS group exhibited fewer active avoidances and shorter active movement distances than the CON group. However, the aqueous extract of KXS antagonized the CMS-induced cognition impairment in the shuttle box test in a dose-dependent manner, indicating that KXS probably improves the cognitive deficit induced by depression.

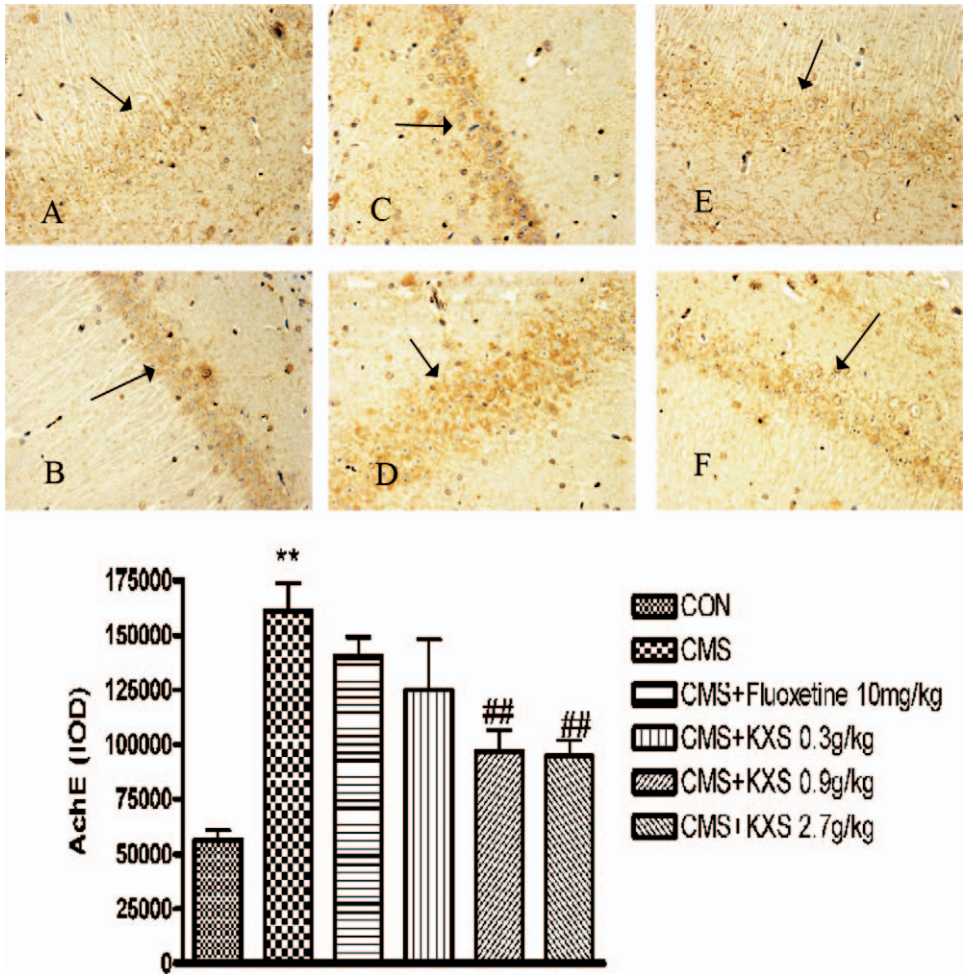

Figure 5. Effects of KXS and fluoxetine on AChE expression in the hippocampus. Histograms showing the mean IOD value of AChE are presented. Immunohistochemical analysis of AChE protein was performed on brain sections of rats in the CON group (A), CMS group (B), CMS+Fluoxetine group (C), CMS+KXS 0.3 g/kg group (D), CMS+KXS 0.9 g/kg group (E), and CMS+KXS 2.7 g/kg group (F). AChE-positive cells are represented as brown spots. Each column represents the mean ± SEM. * $P < 0.05$, ** $P < 0.01$ compared with control; # $P < 0.05$, ## $P < 0.01$ compared with CMS. A color version of this figure is available in the online journal.

The pathophysiology of depression is complex and involves several different biochemical pathways. Since 1965, the monoamine theory of depression advocates that mental depression is due to a deficiency in brain monoaminergic activity, and that antidepressants increase this activity (22). In the present study, in agreement with many reports, CMS induced significant decrease in NE, DA, DOPAC, and HVA concentration in the hippocampus and prefrontal cortex (23–26). Concomitant KXS or fluoxetine treatment restored these changes and showed remarkable dopaminergic activation in both examined brain areas. Moreover, this activation was mainly due to the increase of DA and its metabolite DOPAC and HVA concentration, not the increase of DA turnover ratio. In particular, although a great number of studies showed that serotonergic system plays a major role in the onset and course of depression (27), our results demonstrated there were no significant difference in the levels of 5-HT and 5-HIAA between CMS and CON group. 5-HT is a complex system mediated by 16 types and subtypes of receptors (28). Abnormalities in the 5-HTergic system could occur at one or more of several levels, including the availability of L-tryptophan (TRP), 5-HT synthesis, release, reuptake or metabolism, or at the level of pre- or postsynaptic receptors (29). Thus, it can be presumed that the impairment of CMS on serotonergic system may occur at other sites, but not the concentration of 5-HT.

Hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis is also considered to be a key neurobiological alteration in depression. Elevated serum corticosterone and ACTH levels have been observed in the majority of patients with depression and in the CMS rat model (30). Moreover, the elevation of circulating glucocorticoids exerts an inhibitory influence on learning and memory. Thus, normalization of HPA axis activity is an important factor in alleviating depression. Indeed, in line with previous reports, the stressed rats used also exhibited higher ACTH and corticosterone levels, but the difference in corticosterone levels was not significant relative to CON rats. KXS

administration reduced the CMS-induced increase in serum ACTH and corticosterone levels, returning them to normalization. Thus, the antidepressant-like and nootropic effects of KXS may partly be due to regulation of ACTH and corticosterone levels, thereby normalizing HPA axis hyperactivity.

Notably, KXS treatment appeared to have a beneficial effect on the two-way avoidance performance deficit, whereas fluoxetine failed to exert an effect on this deficit. This is more likely due to additional mechanisms involved in the impairment of two-way avoidance cognitive behavior. It is well established that the central cholinergic system, particularly in the hippocampus, plays a crucial role in the regulation of learning and memory (31), and may also be involved in deficits of executive functions, such as decision making, strategic planning, problem solving, and, in particular, processing speed (32–37). Thus, suggesting that in cognitive impairment induced by depression, the cholinergic system cannot be ignored. In agreement with previous findings (38), a significant rise in AChE protein in the hippocampus (Fig. 5B) of CMS rats was evident, whereas, after KXS treatment a strong anti-AChE activity, caused a concomitant increase in acetylcholine levels in the synaptic cleft consequently increasing the cholinergic activity, leading to enhancement in learning and memory (39). Based on these findings, it is tempting to speculate that the cholinergic system may be able to shed light on the mechanism of action of KXS with regard to its nootropic effect on cognitive deficit in CMS rats.

The antidepressant and nootropic effects of KXS were further extended by neurochemical studies of individual herbs. Smriga reported that hoelen and ginseng are the active components of KXS with regard to the enhancement of Hippocampal LTP (11). Polygala saponin, the main component of polygala, showed antagonistic action on neurotoxicity induced by glutamate (40), anti-stress effect (41) and improving effects on the memory and behavioral disorders (42). Both decoction and volatile oil extract from Acorus exhibited neuroprotective effects by enhancing the GABA level (43).

It is also important to mention that the gender difference in response to 35 days of CMS in male rats was more pronounced and reflected as longer latency for feeding, and higher ACTH and corticosterone levels. This difference might be due to differences in the capacity to respond to stress (44). However, the antidepressant-like and nootropic effects of KXS were equally effective in both in both genders.

Conclusions

The present study therefore demonstrated that the aqueous extract of KXS has two pronounced effects on the chronically induced depression in rats. Firstly, it ameliorates the depressive symptoms and secondly it also improves the cognitive impairment by modulating multiple neural systems such as the HPA axis, and the monoamine neurotransmitter and cholinergic systems. Since KXS is a mixture of compounds, the identification of active compound(s) is warranted and our laboratory is actively involved in it.

1. Sergeyev V, Fetissov S, Mathé AA, Jimenez PA, Bartfai T, Mortas P, Gaudet L, Moreau JL, Hökfelt T. Neuropeptide expression in rats exposed to chronic mild stresses. Psychopharmacology 178:115–124, 2005.
2. Austin MP, Mitchell P, Goodwin GM. Cognitive deficits in depression, possible implications for functional neuropathology. Br J Psychiatry 178:200–206, 2001.
3. Porter RJ, Gallacher P, Thompson JM, Young AH. Neurocognitive impairment in drug-free patients with major depressive disorder. Am J Psychiatry 182:214–220, 2003.
4. Kennedy SH. A review of antidepressant treatments today. Eur Neuropsychopharmacol 16:5619–5624, 2006.
5. Monleón S, Vinader-Caerols C, Arenas MC, Parra A. Antidepressant drugs and memory: insights from animal studies. Eur Neuropsychopharmacol 18:235–248, 2008.
6. Chen YH, Wang DX, Liu P, Wang JL, Dai C, Cheng GF. Antidepression effect of Kai Xin San and research of its compatibility. Chin Med J 118:1214–1216, 2005.
7. Nishiyama N, Zhou Y, Saito H. Beneficial effects of DX-9386, a traditional Chinese prescription, on memory disorder produced by lesioning the amygdala in mice. Biol Pharm Bull 17:1679–1681, 1994a.
8. Nishiyama N, Zhou Y, Saito H. Ameliorative effects of chronic treatment using DX-9386, a traditional Chinese prescription, on learning performance and lipid peroxide content in senescence accelerated mouse. Biol Pharm Bull 17:1481–1484, 1994b.
9. Nishiyama N, Zhou Y, Takashina K, Saito H. Effects of DX-9386, a traditional Chinese preparation, on passive and active avoidance performances in mice. Biol Pharm Bull 17:1472–1476, 1994c.
10. Zhang Y, Saito H, Nishiyama N, Abe K. Effects of DX-9386, a traditional Chinese medicinal prescription, on long-term potentiation in the dentate gyrus in rats. Biol Pharm Bull 17:1337–1340, 1994a.
11. Smriga M, Saito H, Nishiyama N. Hoelen (Poria Cocos Wolf) and ginseng (Panax Ginseng C. A. Meyer), the ingredients of a Chinese prescription DX-9386, individually promote hippocampal long-term potentiation in vivo. Biol Pharm Bull 18:518–522, 1995.
12. Zhang Y, Saito H, Nishiyama N. Improving effects of DX-9386, a traditional Chinese medicinal prescription, on thymectomy-induced impairment of learning behaviors in mice. Biol Pharm Bull 17:1199–2205, 1994b.
13. Willner P, Towell A, Sampson D, Sophokleous S, Muscat R. Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by a tricyclic antidepressant. Psychopharmacology 93:358–364, 1987.
14. Song L, Che W, Min-Wei W, Murakami Y, Matsumoto K. Impairment of the spatial learning and memory induced by learned helplessness and chronic mild stress. Pharmacol Biochem Behav 83:186–193, 2006.
15. Millan MJ. Multi-target strategies for the improved treatment of depressive states: conceptual foundations and neuronal substrates, drug discovery and therapeutic application. Pharmacol Ther 110:135–370, 2006.
16. American Psychiatric Association. DSM IV-Diagnostic and Statistical Manual of Psychiatric Disorders (4th ed.). Washington, DC: American Psychiatric Association, 1994.
17. Dulawa SC, Hen R. Recent advances in animal models of chronic antidepressant effects: the novelty-induced hypophagia test. Neurosci Biobehav Rev 29:771–783, 2005.

18. Katz RJ. Animal model of depression: pharmacological sensitivity of a hedonic deficit. Pharmacol Biochem Behav 16:965–968, 1982.
19. Willner P, Muscat R, Papp M. Chronic mild stress-induced anhedonia: a realistic model of depression. Neurosci Biobehav Rev 16:525–534, 1992.
20. Muscat R, Papp M, Willner P. Reversal of stress-induced anhedonia by the atypical antidepressants, fluoxetine and maprotiline. Psychopharmacology 109:433–438, 1992.
21. Stepanichev MY, Kudryashova IV, Yakovlev AA, Onufriev MV, Khaspekov LG, Lyzhin AA, Lazareva NA, Gulyaeva NV. Central administration of a caspase inhibitor impairs shuttle-box performance in rats. Neuroscience 136:579–591, 2005.
22. Elhwuegi AS. Central monoamine and their role in major depression. Prog Neuropsychopharmacol Biol Psychiatry 28:435–451, 2004.
23. Charney DS. Monoamine dysfunction and the pathophysiology and treatment of depression. J Clin Psychiatry 59:11–14, 1998.
24. Lambert G, Johansson M, Agren H, Friberg P. Reduced brain norepinephrine and dopamine release in treatment-refractory depressive illness. Arch Gen Psychiatry 57:787–793, 2000.
25. Konstandi M, Johnson E, Lang MA, Malamas M, Marselos M. Noradrenaline, dopamine, serotonin: different effects of psychological stress on brain biogenic amines in mice and rats. Pharmacol Res 41: 341–346, 2000.
26. Bekris S, Antonious K, Daskas S, Papadopoulous-Daifoti Z. Behavioural and neurochemical effects induced by chronic mild stress applied to two different rat strains. Behav Brain Res 161:45–59, 2005.
27. Heninger GR, Charney DS, Sternberg DE. Serotonergic function in depression. Prolactin response to intravenous tryptophan in depressed patients and healthy subjects. Arch Gen Psychiatry 41:398–402, 1984.
28. Naughton M, Mulrooney JB, Leonard BE. A review of the role of serotonin receptors in psychiatric disorders. Hum Psychopharmacol 15: 397–415, 2000.
29. Jans L, Riedel WJ, Markus CR, Blokland A. Serotonergic vulnerability and depression: assumptions, experimental evidence and implications. Mol Psychiatry 12:522–543, 2007.
30. Arborelius L, Owens MJ, Plotsky PM, Nemeroff CB. The role of corticotropin-releasing factor in depression and anxiety disorders. J Endocrinol 160:1–12, 1999.
31. Blokland A. Acetylcholine: a neurotransmitter for learning and memory? Brain Res Brain Res Rev 21:285–300, 1995.
32. Christensen H, Griffiths K, Mackinson A, Jacomb P. A quantitative review of cognitive deficits in depression and Alzheimer-type dementia. J Int Neuropsychol Soc 3:631–651, 1997.
33. Drevets WC. Neuroimaging studies of mood disorders. Biol Psychol 48:813–829, 2000.
34. Drevets WC. Neuroimaging and neuropathological studies of depression: implications for the cognitive-emotional features of mood disorders. Curr Opin Neurobiol 11:240–249, 2001.
35. Fossati P, Ergis AM, Allilaire JF. Problem-solving abilities in unipolar depressed patients: comparison of performance on the modified version of the Wisconsin and the California sorting tests. Psychiatry Res 104: 145–156, 2001.
36. Alexopoulos GS, Kiosses DN, Heo M, Murphy CF, Shanmugham B, Gunning-Dixon F. Executive dysfunction and the course of geriatric depression. Biol Psychiatry 58:204–210, 2005.
37. Chen KC, Baxter MG, Rodefer JS. Central blockade of muscarinic cholinergic receptors disrupts affective and attentional setshifting. Eur J Neurosci 20:1081–1088, 2004.
38. Manikandan S, Padma MK, Srikumar R, Jeva. Parthasarathv N, Muthuvel A, Sheela Devi R. Effects of chronic noise stress on spatial memory of rats in relation to neuronal dendritic alteration and free radical-imbalance in hippocampus and medial prefrontal cortex. Neurosci Lett 399:17–22, 2006.
39. Yamaguchi Y, Kawashima S. Effects of amyloid-beta-(25–35) on passive avoidance, radial-arm maze learning and choline acetyltransferase activity in the rat. Eur J Pharmacol 412:265–272, 2001.
40. Li C, Yang J, Yu S, Chen N, Xue W, Hu J, Zhang D. Triterpenoid saponins with neuroprotective effects from the roots of Polygala tenuifolia. Planta Med 72:133–141, 2008.
41. Kawashima K, Miyako D, Ishino Y, Makino T, Saito K, Kano Y. Antistress effects of 3,4,5-trimethoxycinnamic acid, an active constituent of roots of Polygala tenuifolia. Biol Pharm Bull 27:1317–1319, 2004.
42. Chen YL, Hsien CL, Wu PH, Lin JG. Effect of Polygala tenuifolia root on behavioral disorders by lesioning nucleus basalis magnocellularis in rat. J Ethnopharmacol 95:47–55, 2004.
43. Koo BS, Park KS, Ha JH, Park JH, Lim JC, Lee DU. Inhibitory effects of the fragrance inhalation of essential oil from Acorus gramineus on central nervous system. Biol Pharm Bull 26:978–982, 2003.
44. Roca CA, Schmidt PJ, Danaceau MA, Altemus M, Putnam K, Chrousos GP, Nieman LK, Rubinow DR. Sex-related differences in stimulated hypothalamic-pituitary-adrenal axis during induced gonadal suppression. J Clin Endocrinol Metab 90:4224–4231, 2005.