Available online at www.sciencedirect.com
ScienceDirect
Pharmacology, Biochemistry and Behavior 89 (2008) 572–580
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Role for serotonin in the antidepressant-like effect of a flavonoid extract of Xiaobuxin-Tang

Lei An [a,b], You-Zhi Zhang [b,*], Neng-Jiang Yu [b], Xin-Min Liu [a], Nan Zhao [b], Li Yuan [b], Yun-Feng Li [b]

[a] *Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100094, China*
[b] *Beijing Institute of Pharmacology and Toxicology, Beijing 100850, China*

Received 4 November 2007; received in revised form 7 February 2008; accepted 7 February 2008
Available online 25 March 2008

Abstract

Xiaobuxin-Tang (XBXT), a traditional Chinese herbal decoction, has been used for the treatment of depressive disorders for centuries in China. Herein, we explored the antidepressant-like effect and its monoaminergic mechanism of the total flavonoids (XBXT-2) isolated from the extract of XBXT. In present study, single XBXT-2 (25, 50, 100 mg/kg, p.o.) administration significantly potentiated the mouse head-twitch response induced by 5-hydroxytryptophan (5-HTP, a metabolic precursor to serotonin), and also, decreased the immobility time in mouse tail suspension test, which was completely prevented by p-chlorophenylalanine (PCPA, an inhibitor of serotonin synthesis) pretreatment. However, single treatment with XBXT-2 had no effect on yohimbine toxicity and high dose of apomorphine-induced hypothermia in mice. These results indicated that acute treatment with XBXT-2 produced serotonergic, but not noradrenergic activation. In addition, chronic XBXT-2 (25, 50 mg/kg, p.o., 28 days) treatments significantly reversed the depressive-like behaviors in chronically mildly stressed (CMS) rats, including the reduced sucrose preference, deficient locomotor activity and prolonged latency to novelty-suppressed feeding. Furthermore, XBXT-2 normalized the neurotransmitter changes, including the decreased serotonin (5-HT) and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) levels in hippocampus and prefrontal cortex in CMS rats. These findings confirm the antidepressant-like effect of XBXT-2 in CMS model of rats, which may be primarily based on its serotonergic activation.

Keywords: Xiaobuxin-Tang; Flavonoids; Antidepressant; Monoamine; Serotonin

1. Introduction

Depression is a common, debilitating, life-threatening mental disorder with high morbidity and mortality. Up to now, the current antidepressants in use, including tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs), monoamine oxidase inhibitors (MAOIs), all exert their antidepressant effect by increasing the levels of monoamines 5-HT and/or noradrenaline (NE). However, they largely have weaknesses such as slow onset, severe side effects (Gumnick and Nemeroff, 2000; Rosen and Marin, 2003). Therefore, it is urgent to explore more promising antidepressant (Adell et al., 2005). Recently, the usage of traditional herbs has provided us a prospective alternative in the treatment of depression because of its better compliance and lower side effects (Mqller, 2003; Xu et al., 2004; Xia et al., 2007). Among them, Chinese traditional herbal prescriptions, which represent the core of Traditional Chinese Medicine (TCM) theory, are becoming more and more attractive, but they are still lack of wide scientific research (Luo et al., 2000; Li et al., 2003; Kim et al., 2005).

Xiaobuxin-Tang (XBXT), comprising Haematitum, Flos Inulae, Folium Phyllostachydis Henonis and Semen Sojae Preparatum, four Chinese medicines, was originally recorded in the silk scroll manuscript of "Fuxinjue Zangfu Yongyao Fayao", written one thousand years ago and discovered in Mogao Caves of Dunhuang. It was recorded that XBXT could cure "sadness and weeping" which was called "Yu-syndrome" in TCM and had been widely used to alleviate depressive illness from ancient clinic.

* Corresponding author. Tel.: +86 10 66874606; fax: +86 10 68211656.
E-mail address: zhyouzhi@yahoo.com.cn (Y.-Z. Zhang).

0091-3057/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2008.02.014

In previous studies, we found that the ethanolic extract of XBXT significantly decreased the immobility time in tail suspension test (TST) and forced swimming test (FST) in mice and rats by single administration (Zhang et al., 2007). Furthermore, we found that the total flavonoids (XBXT-2) isolated from XBXT exerted antidepressant-like effects in the behavioral despair models, indicating that XBXT-2 may be the most active components in the antidepressant-like effects of XBXT. We also demonstrated that subchronic XBXT-2 administration reversed the behavioral alterations and elevated serum corticosterone level in learned helplessness rats (Zhang et al., 2008). However, until now, its action mechanism is still not clear.

As monoaminergic system is one of the most important targets in the pathophysiology and therapy of depression (Elhwuegi, 2004; Millan, 2004), in this study, we used four behavioral and pharmacological models to investigate the possible monoaminergic participations in the antidepressant-like effect of single XBXT-2 administration; then we applied chronic mild stress (CMS) model of rats, a well validated animal model of depression (Willner, 1997), to assess the behavioral effects, as well as the brain monoaminergic neurotransmitter changes after chronic XBXT-2 administration.

2. Materials and methods

2.1. Preparation of the total flavonoids from Xiaobuxin-Tang extract

2.1.1. Materials

Traditional Chinese medicines Haematitum, Flos Inulae, Folium Phyllostachydis Henonis and Semen Sojae Preparatum were purchased from Beijing Tongrentang Drugstore (Beijing, China) and were identified by Prof. Lian-Sheng Shen (School of Chinese medicine, Beijing University of Chinese Medicine) as calcined product of ochery hematite, flowers of *Inula japonica* Thunb., leafs of *Phyllostachys nigra* (Lodd.) Munro var. *henonis* (Mitf.) Stapf ex Rendle, fermented product of *Glycine max* (L.) Merr., respectively. The voucher specimens (No. 02002, No. 02003, No. 02004, No. 02005, respectively) are deposited in the Laboratory of Phytochemistry, Beijing Institute of Pharmacology and Toxicology, China.

2.1.2. Extraction

The medicines Haematitum, Flos Inulae, Folium Phyllostachydis Henonis and Semen Sojae Preparatum (2:2:1:1, w/w) (6 kg) were mixed and extracted three times with 70% alcohol at 100 °C. The combined extracts were filtered and evaporated by a rotary evaporator under reduced pressure to obtain a viscous alcoholic extract (784 g), which was dispersed in 1 L water and partitioned with petroleum ether. Subsequently, the water fraction was diluted with 4 L water and the water solution was centrifuged (4000 rpm, 30 min). Then, the solution was passed by column chromatography on AB-8 macroporous resin (7 L). After eluting with 14 L water, the column was eluted with 21 L 70% alcohol and the 70% alcohol elution was evaporated and dried *in vacuo* to obtain the total flavonoids (320 g). Using lutin as standard substance, the apparent flavone content of the extract was determined as 76.03% by colorimetric method (Xu et al., 2000).

2.2. Animals

Male ICR mice weighing 18–22 g and male Sprague Dawley rats weighing 180–220 g (Beijing Vital Laboratory Animal Technology Company, Beijing) were used for the experimental procedures. The animals were group housed in polypropylene cages under standard experimental conditions: room temperature 21±2 °C, humidity 40–60%, 12 h:12 h-light/dark cycle (lights on at 8:00 am). Food and water were available ad libitum. Animals were allowed to have a period of acclimation before any experimentation. In behavioral tests, to minimize circadian changes and subjective influence, animals in each group were intermixed during the observation (8:00–12:00 a.m.) and the observers were unaware of the treatment conditions. All animal experiments were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH publication No. 86-23, revised 1996).

2.3. Drugs and reagents

Imipramine hydrochloride (IMI, a tricyclic antidepressant), fluoxetine hydrochloride (FLU, a serotonin reuptake inhibitor), 5-hydroxy-L-tryptophan (5-HTP, a metabolic precursor to 5-HT), DL-p-chlorophenylalanine (PCPA, an inhibitor of serotonin synthesis), yohimbine hydrochloride (α_2-adrenoceptor antagonist) and apomorphine hydrochloride were obtained from Sigma (St. Louis, MO, USA). Drugs administered orally were dissolved in distilled water, others for injections were dissolved in 0.9% saline, while PCPA was first dissolved in small volume of 0.1 N NaOH and then neutralized to pH 7 with the same volume of 0.1 N HCl. The chemical standards used in HPLC-ECD, including norepinephrine (NE), serotonin (5-HT) and its metabolite 5-hydroxyindoleacetic acid (5-HIAA), dopamine (DA) and its metabolites homovanillic acid (HVA), 3,4-dihydroxyphenylacetic acid (DOPAC), were purchased from Sigma (St. Louis, MO, USA) and were dissolved in the mobile phase.

2.4. Four pharmacological models

2.4.1. 5-HTP-induced mouse head-twitch test

To investigate the possible serotonergic mechanism in the antidepressant-like effect of XBXT-2, we performed 5-HTP-induced head-twitch test (Corne, 1963). Mice were administered with XBXT-2 (25, 50 and 100 mg/kg), FLU (30 mg/kg) or distilled water orally 1 h before 5-HTP (120 mg/kg, i.p.). Immediately after the injection, mice were placed into plastic cages and the cumulative number of head twitches (rapid movements of the head with little or no involvement of the trunk) was recorded in 20 min.

2.4.2. The depletion of serotonin in mouse tail suspension test

Mice were pretreated with PCPA (300 mg/kg, i.p.) or saline once a day for 3 consecutive days (Redrobe et al., 2005). Then, on the fourth day, mice received XBXT-2 (50, 100 mg/kg), FLU

(30 mg/kg) or distilled water orally 1 h prior to testing. The TST procedure was performed essentially as described by Steru et al. (1985). Mice were considered immobile only when they hung passively and completely motionless. Immobility duration was recorded for the last 4 min of the total 6-min observation period.

2.4.3. Yohimbine toxicity potentiation test

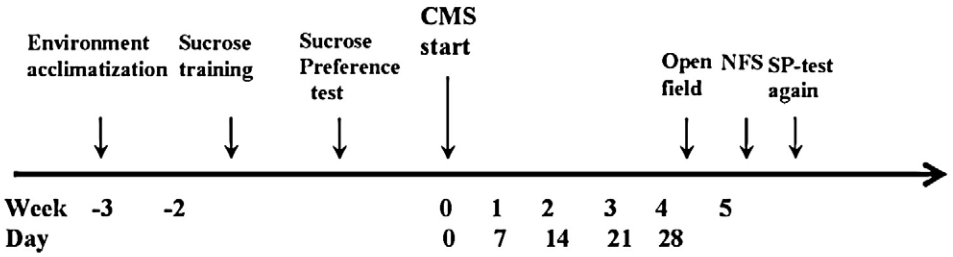
To reveal whether noradrenergic system is involved in the antidepressant-like effect of XBXT-2, yohimbine toxicity potentiation test was performed (Lapin, 1980). Mice were treated with XBXT-2 (25, 50 and 100 mg/kg), IMI (30 mg/kg) or distilled water orally 1 h prior to yohimbine (25 mg/kg, s.c.). The number of dead mouse was calculated during a 20-hour period after the injection of yohimbine.

2.4.4. Antagonism of apomorphine-induced hypothermia test

To further evaluate the possible effect of XBXT-2 on noradrenergic system, high dose of apomorphine-induced hypothermia test was adopted (Puech et al., 1981). Mice were administered with XBXT-2 (25, 50 and 100 mg/kg), IMI (30 mg/kg) or distilled water orally 30 min before apomorphine (16 mg/kg, s.c.). Rectal temperature was measured for three times: firstly, before administration of any drugs, as initial body temperature; Secondly, 30 min after the administration of drugs and just before the injection of apomorphine, in order to evaluate the effect of the drugs on basal body temperature; thirdly, 30 min after apomorphine injection, to evaluate the effect of the drugs on apomorphine-induced hypothermia. During the experiment, the ambient temperature was strictly kept at 20±1 °C.

2.5. Chronic mild stress (CMS) procedure

The CMS procedure was performed mainly according to Papp et al. (1996) and Grønli et al. (2005) with some modifications. The CMS groups were exposed to the following stressors for four consecutive weeks: (1) food or water deprivation; (2) restricted access to food; (3) exposure to empty water bottles following a period of water deprivation; (4) paired housing; (5) cage tilt (45°); (6) overnight illumination; (7) soiled cage (200 ml water spilled onto 100 g sawdust bedding); (8) stroboscopic lighting (100 flashes/min); (9) white noise (approx. 110 dB); (10) forced swimming (5 min, water temperature 15 °C); (11) Restraint. The CMS procedure of the first week was presented in Fig. 1 and repeated with unpredictable sequence during the following 3 weeks. Rats in control group were left undisturbed in the home cages in a separate room.



Day	Food deprivation	Water deprivation	Restricted food	Empty bottles	Paired housing	Cage tilt	Overnight illumination	Soiled cage	Stroboscope	White noise	Forced swimming	Restraint
Monday								17:00 ↓ 8:00	9:00 ↓ 10:00	9:00 ↓ 10:00		
Tuesday												9:00 ↓ 10:00
Wednesday							20:00 ↓ 8:00				9:00 ↓ 10:00	
Thursday	15:00 ↓ 8:00	15:00 ↓ 8:00							9:00 ↓ 10:00			
Friday			10:00 ↓ 8:00	9:00 ↓ 10:00	10:00 ↓ 8:00							
Saturday						9:00 ↓ 8:00						
Sunday												

Fig. 1. An outline of the design for the experiment (top: with time course for CMS and post-CMS tests; bottom: CMS protocol).

2.5.1. Sucrose preference test

Sucrose preference test (Willner et al., 1987; Bekris et al., 2005) was employed herein to determine anhedonia, one of the core symptoms of major depression in human. The procedure was composed of training and testing courses. After 1 week of acclimatization, rats were trained to consume 1% (w/v) sucrose solution before the start of the CMS protocol. In training course, rats were deprived of food and water for 48 h and only exposed to 1% (w/v) sucrose solution. Three days later, after 23 h food and water deprivation, 1 h baseline test was performed, in which rats could select between two pre-weighted bottles, one with 1% (w/v) sucrose solution and the other with tap water. Then the sucrose preference (SP) was calculated according to the following formula:

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

Then rats were divided into five parallel groups based on their sucrose preference result: Control (no stressor); Stress-Vehicle; Stress-IMI (10 mg/kg, p.o.); Stress-XBXT-2 (25 or 50 mg/kg, p.o.). Each group included 10 rats. Drug treatments performed orally once a day between 8:00–9:00 a.m. from the beginning of stress regime. Finally, at the end of the experiment, the sucrose preference test was performed again to evaluate the CMS model and drugs action.

2.5.2. Open field test

Locomotor activity was assessed by open field test (Kulkarni and Dandiya, 1973) after 4 weeks of stress procedure. The open field apparatus was an arena 80 cm in diameter with a white, opaque wall 30 cm high, which was divided into 18 approximately equal sectors. Rats were individually placed in the center of the arena 24 h after the last drug treatments and locomotor activity including the number of crossings and rearings were scored within 5 min.

2.5.3. Novelty-suppressed feeding (NSF) test

The NSF test was performed according to Bodnoff et al. (1988) with minor modification. Rats were placed into the testing apparatus, a lit plastic box (76×76×46 cm), from the corner after 48 h food deprivation. Thirty food pellets with equal size were placed in the center of the box. The first latency to eat the food in a 5-min period was recorded (defined as the rat biting the pellet, not only smelling or toying with it). Immediately after this test, the animal was transferred to its home cage, and food consumption in each rat was measured within 5 min.

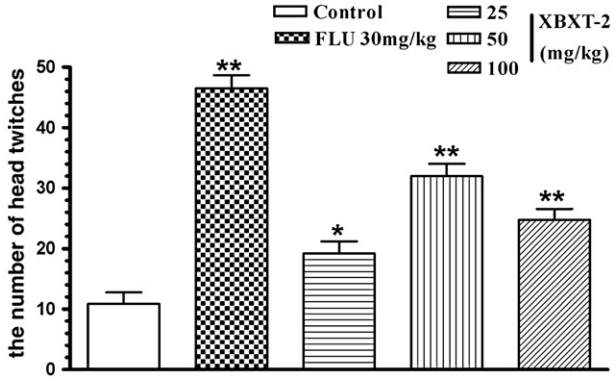


Fig. 2. Effect of XBXT-2 (25, 50 and 100 mg/kg, p.o.) or FLU (30 mg/kg, p.o.) on number of 5-HTP-induced head twitches in mice. Drugs or distilled water were administered 1 h prior to injection of 5-HTP (120 mg/kg, i.p.). Each column represents as the mean±S.E.M., $n=10$. *$P<0.05$, **$P<0.01$ compared with control (ANOVA followed by Dunnett's t-test).

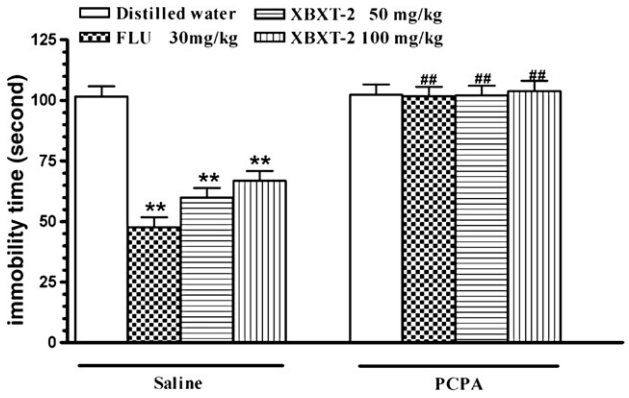


Fig. 3. Effect of pretreatment with PCPA on anti-immobility induced by XBXT-2 (50,100 mg/kg, p.o.) or FUL (30 mg/kg, p.o.) in mouse TST. Mice were pretreated with PCPA (300 mg/kg, i.p.) or saline for 3 consecutive days. On the fourth day, drugs or distilled water were administered 1 h before the test. Each column represents as the mean±S.E.M., $n=10$. **$P<0.01$ compared with control (ANOVA followed by Dunnett's t-test); $^{\#\#}P<0.01$ compared with the same group pretreated with saline (Student's t-test).

2.5.4. Measurement of monoamines by high-performance liquid chromatography with electrochemical detection (HPLC-ECD)

Following decapitation, the brains were rapidly removed, dissected on an ice-chilled glass plate and subsequently prefrontal cortex, striatum, hypothalamus and hippocampus were isolated. The tissues were weighed, sonicated in 0.4 M $HClO_4$ containing 0.5 mM Na_2-EDTA and 0.01% L-cys and centrifuged (12,000 rpm, 30 min). Then, NE, 5-HT, 5-HIAA, DA, HVA and DOPAC were assayed by HPLC-ECD. The HPLC system consisted of a microbore reverse-phase column (particle size 5 μm, 150×4.6 mm; Model C-18, DIKMA Technologies Ltd., Beijing, China), an Agilent 1100 pump (flow rate 1.0 ml/min; Agilent Technologies, Palo Alto, CA, USA) and a Hewlett-Packard HP 1049A glassy carbon amperometric detector (Agilent Technologies, Palo Alto, CA, USA). The mobile phase consisted of 85 mM citrate, 100 Mm sodium acetate, 0.9 mM octyl-sodium sulfate, 0.2 mM EDTA, and 15% methanol, pH 3.7. External standard curves were used to quantify the amounts of NE, 5-HT, 5-HIAA, DA, DOPAC and HVA in each sample calculated by area under curve (AUC). The volume of injection was 50 μl. The detection limit of the assay was 20 pg/sample.

2.6. Statistical analysis

All data were expressed as means±S.E.M. Fisher's exact test and repeated measures ANOVA were used to analyze the data in the yohimbine toxicity potentiation test and the body weight evaluation of CMS procedure, respectively. In the other tests, Student's t-test was used to compare the differences between

Table 1
Effect of XBXT-2 (25, 50 and 100 mg/kg, p.o.) or IMI (30 mg/kg, p.o.) on yohimbine-induced lethality in mice

Groups	Doses (mg/kg)	Yohimbine (mg/kg)	Lethality	
			Total	Died
Control		25	12	4
IMI	30	25	12	10*
XBXT-2	25	25	12	4
	50	25	12	3
	100	25	12	5

Drugs or distilled water were administered 1 h prior to yohimbine (25 mg/kg, s.c.). Data are represents in the blank, n=12. *$P<0.01$ compared with control (Fisher's exact test).

two groups, while ANOVA followed by Dunnett's *t*-test was used to compare the differences among three or more groups. A value of $P<0.05$ was considered significant.

3. Results

3.1. Effect of single XBXT-2 treatment on four behavioral tests

3.1.1. Effect of XBXT-2 on 5-HTP-induced head-twitch response

The results (Fig. 2) showed that both XBXT-2 (25, 50 and 100 mg/kg, p.o.) and FLU (30 mg/kg, p.o.) significantly increased the cumulative number of head twitches in mice (F (4,45)=45.94, $P<0.001$, vs control). The data also showed us a U-shaped dose–effect curve that coincided with the antidepressant-like effects of XBXT-2 in other animal models (Zhang et al., 2008).

3.1.2. Effect of pretreatment with PCPA on the behavioral effect of XBXT-2 in TST

XBXT-2 (50,100 mg/kg, p.o.) or FLU (30 mg/kg, p.o.) treatment induced a significant reduction (F (3,36)=32.69, $P<0.001$, vs control) in the immobility time in mouse TST (Fig. 3). However, pretreatment with PCPA abolished the anti-immobility effect of both XBXT-2 and FLU (F (3,36)=0.04978, $P>0.9$, vs control). PCPA alone did not affect the immobility time.

Results from these two tests indicated that there was an indispensable involvement of the serotonergic system in the antidepressant-like effects of XBXT-2.

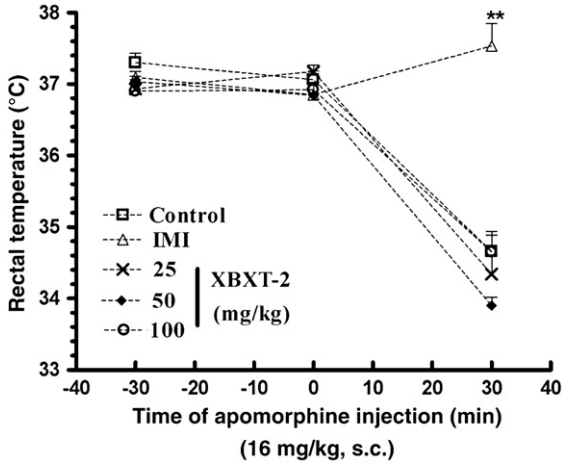


Fig. 4. Effect of XBXT-2 (50, 100 mg/kg, p.o.) or IMI (30 mg/kg, p.o.) on hypothermia induced by high dose of apomorphine in mice. Drugs or distilled water were administered 30 min before the injection of apomorphine (16 mg/kg, s.c.). Rectal temperature of mice was measured at −30, 0, 30 min after apomorphine injection. Each plot represents as the mean±S.E.M., $n=10$. **$P<0.01$ compared with control (ANOVA followed by Dunnett's *t*-test).

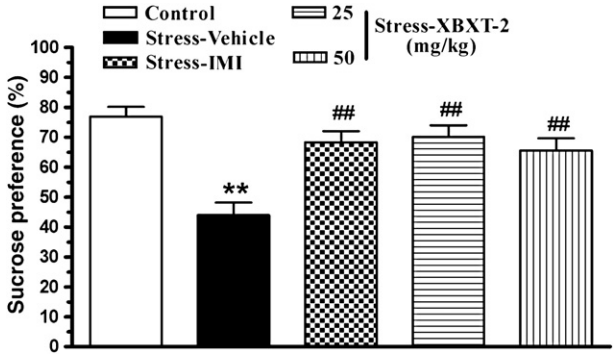


Fig. 5. Sucrose preference of rats after four weeks of CMS. Percentage of sucrose preference was measured for a period of 1 h after 23 h food and water deprivation. Each column represents as the mean±S.E.M., $n=10$. **$P<0.01$ compared with control; ##$P<0.01$ compared with stress-vehicle (ANOVA followed by Dunnett's *t*-test).

3.1.3. Effect of XBXT-2 on the yohimbine toxicity potentiation test

Pretreatment with IMI (30 mg/kg) significantly enhanced the mouse lethality ($P<0.05$, vs control) induced by yohimbine, whereas pretreatment with XBXT-2(25, 50 and 100 mg/kg) had no such effect (Table 1).

3.1.4. Effect of XBXT-2 on the hypothermia induced by apomorphine

The time–temperature curve of each drug-treated group was plotted in (Fig. 4). Temperature variation among groups was not observed at −30 or 0 point. Hypothermia developed as a result of the injection with apomorphine, and the maximum response occurred at 30 min point. Pretreatment with IMI significantly attenuated the hypothermia response in mice ($P<0.01$, vs control), while there was no such effect with XBXT-2 pretreatment.

Results from the above two tests indicated that there was no noradrenergic action with single XBXT-2 administration.

Table 2
Locomotor activity of rats after 4 weeks of CMS

Groups	Drug dose (mg/kg/d)	Crossings	Rearings
Control	-	44.2±5.0	20.7±3.1
Stress-Vehicle	-	11.3±2.3**	12.7±2.5
Stress-IMI	10	33.0±4.9##	18.4±2.7
Stress-XBXT-2	25	28.2±4.4#	17.2±2.7
	50	27.0±3.2#	16.1±2.7

Crossings (the number of sector borders rats crossed with at least three paws) and rearings (the number of rats stood on its hind legs) were calculated within 5 min. Data are represented as the means±S.E.M., n=10. **$P<0.01$ compared with control; # $P<0.05$, ## $P<0.01$ compared with stress-vehicle (ANOVA followed by Dunnett's t-test).

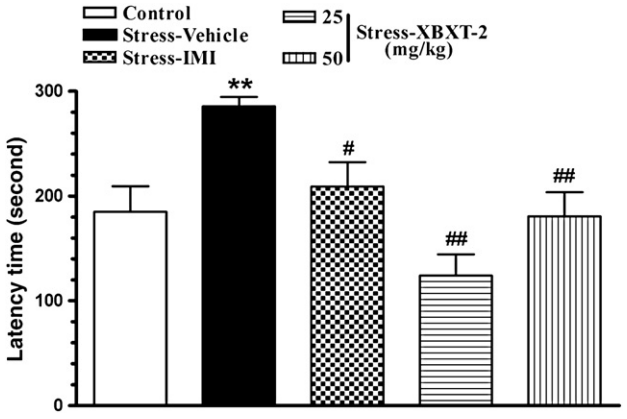


Fig. 6. The feeding latency of rats after 4 weeks of CMS. Latency to feed in 5 min was recorded after 48 h food deprivation. Each column represents as the mean±S.E.M., $n=10$. $^{**}P<0.01$ compared with control; $^{\#}P<0.05$, $^{\#\#}P<0.01$ compared with stress-vehicle (ANOVA followed by Dunnett's *t*-test).

3.2. Results in CMS model of rats

3.2.1. Effect of XBXT-2 on sucrose preference

In the initial baseline test, the mean basal intakes of sucrose solution and tap water were 9.33±0.36 g and 2.14±0.14 g, respectively, and the sucrose preference was 80.02±1.46% (not shown). After 4 weeks of exposure to stress, sucrose intake (4.64±0.71 g) and sucrose preference (43.90±4.25%) were significantly decreased in stress-vehicle group ($P<0.01$, vs control) (Fig. 5), while total water consumption did not change obviously. Sucrose preference was significantly restored by the administration of IMI (10 mg/kg) to 68.26±11.77% and XBXT-2 (25, 50 mg/kg) to 70.08±12.03% and 65.55±12.93%, respectively (F (3,36)=9.407, $P<0.001$, stress-drugs vs stress-vehicle).

3.2.2. Effect of XBXT-2 on locomotor activity

The number of crossings and rearings were observed in open field test (Table 2). The crossing number in stress-vehicle group were significantly reduced ($P<0.01$, vs control) after 4 weeks of CMS. Rats treated with IMI or XBXT-2 crossed more sectors (F (3,36)=6.052, $P<0.002$, stress-drugs vs stress-vehicle). Additionally, the number of rearings showed decreased tendency after CMS and increased tendency with drug treatments without statistical significance.

3.2.3. Effect of XBXT-2 on latency to feed

In NSF paradigm (Fig. 6), the latency to feed was significantly prolonged in stress-vehicle rats ($P<0.01$, vs control). 28-day treatment of either IMI or XBXT-2 significantly decreased the latency time (F (3,36)=11.65, $P<0.001$, stress-drugs vs stress-vehicle). However, there was no difference in home cage food consumption among groups (not shown).

Results from these behavioral tests demonstrated that XBXT-2 exerted significant antidepressant-like effects in CMS model and the effects were comparable to those of IMI.

Table 3
The levels of monoamine and its metabolite (ng/g tissue) in striatum, hypothalamus, hippocampus, prefrontal cortex of rats after 4 weeks of CMS

Groups	Control	Stress-Vehicle	Stress-IMI	Stress-XBXT (mg/kg)	
				25	50
Striatum					
NE	184.8±23.2	182.4±21.0	182.4±30.9	188.2±36.7	186.8±31.3
DA	14984.0±1152	14198.0±1745	14249.0±1911	14284.0±684.3	14301.0±1112
DOPAC	3952.0±566.6	3673.0±548.9	3953.0±490.7	3705.0±630.2	3939.0±602.6
HVA	1037.0±60.9	922.5±120.4	841.6±99.8	834.1±75.9	874.4±161.7
5-HT	294.6±35.3	339.2±54.9	288.8±55.7	287.6±36.0	266.0±52.5
5-HIAA	201.3±24.0	276.1±32.4	190.9±22.3	207.9±34.0	198.4±15.7
Hypothalamus					
NE	2453.0±69.9	2602.0±132.1	2359.0±181.7	2344.0±177.0	2470.0±93.7
DA	608.9±70.0	658.2±104.1	666.7±80.0	614.2±15.1	623.0±44.7
DOPAC	367.1±38.1	350.4±66.2	384.5±60.0	375.0±49.1	368.8±73.4
5-HT	343.2±42.5	286.9±105.3	307.1±19.6	300.9±26.4	301.9±58.5
5-HIAA	193.6±23.3	196.2±72.2	223.0±30.6	219.0±42.5	216.5±29.8
Hippocampus					
NE	447.5±33.4	364.6±30.2	490.3±40.0$^{\#}$	427.1±39.9	442.3±40.9
5-HT	278.4±23.0	121.8±24.1*	275.8±46.8$^{\#}$	322.0±63.6$^{\#\#}$	314.5±35.6$^{\#\#}$
5-HIAA	300.4±38.1	146.0±24.6*	278.0±49.4$^{\#}$	298.7±39.5$^{\#}$	291.6±29.3$^{\#}$
Prefrontal cortex					
NE	254.7±18.5	206.8±10.3	315.7±38.2$^{\#}$	282.2±40.6	260.5±36.1
DA	100.8±17.5	78.3±16.1	87.7±13.1	91.6±17.6	90.4±14.6
DOPAC	62.8±11.9	60.0±10.8	50.2±7.3	66.1±8.3	56.7±9.4
5-HT	170.4±10.4	21.4±9.5**	127.7±31.5$^{\#}$	171.1±26.9$^{\#\#}$	157.8±29.2$^{\#\#}$
5-HIAA	137.8±10.8	87.8±15.8	151.7±14.1$^{\#}$	127.4±18.2	137.0±23.6

Data represents as the mean±S.E.M., n=10. *$P<0.05$, **$P<0.01$ compared with control; $^{\#}P<0.05$, $^{\#\#}P<0.01$ compared with stress-vehicle (ANOVA followed by Dunnett's t-test).

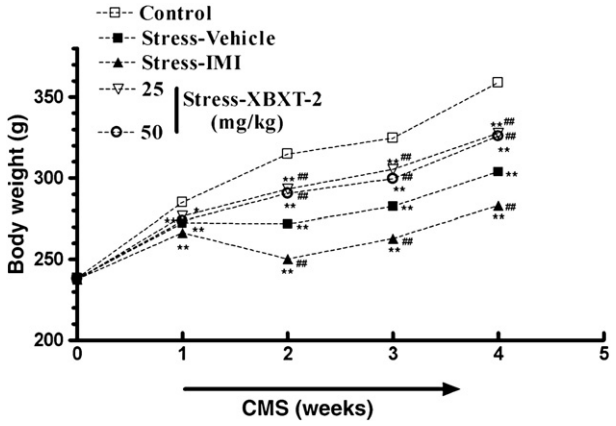


Fig. 7. Body weight of rats during the 4 weeks of CMS. Each plot represents as the mean±S.E.M., $n=10$. *$P<0.05$, **$P<0.01$ compared with control at each time point; $^{\#\#}P<0.01$ compared with stress-vehicle at each time point (repeated measures ANOVA followed by Dunnett's t-test).

3.2.4. Effect of XBXT-2 on monoamine neurotransmitter

The levels of NE, 5-HT, 5-HIAA, DA, HVA and DOPAC in different brain sections of rats were measured in the end of the CMS experiment, and data were shown in (Table 3). Statistical analysis did not reveal any significant alteration of monoamine neurotransmitter and their metabolites in striatum and hypothalamus. However, in hippocampus, 28-day CMS induced significant reduction of 5-HT and 5-HIAA levels ($P<0.05$, vs control) and a decreased trend of NE level. Similarly, in prefrontal cortex, the level of 5-HT decreased significantly ($P<0.01$, vs control), while the reduction of 5-HIAA or NE level didn't satisfy statistical significance. Chronic administration of IMI or XBXT-2 reversed these reductions. XBXT-2 produced notable increase of 5-HT level in both hippocampus and prefrontal cortex ($P<0.01$, vs stress-vehicle), and also, elevated its metabolite 5-HIAA level in hippocampus ($P<0.05$, vs stress-vehicle). In addition, there also showed increased tendency of NE level in these two brain sections with chronic XBXT-2 treatment. These results confirmed and extended the serotonergic activation of XBXT-2, and also indicated noradrenergic regulation in chronic XBXT-2 treatment. The positive control IMI produced significant increase of NE, 5-HT and 5-HIAA levels in hippocampus and prefrontal cortex ($P<0.05$, vs stress-vehicle). Some monoamine or its metabolite level in these brain sections such as the concentration of HVA in hippocampus, which was too low to do proper comparison, was not listed.

3.2.5. Body weight

The body weight of rats was also observed weekly during the experiment. CMS induced a significantly decrease in body weight from the first week. The administration of XBXT-2 attenuated the decrease, while IMI induced even more weight loss during its administration (Fig. 7).

4. Discussion

Serotonergic neurotransmission in the central nervous system is believed to be involved in the pathogenesis and therapy of depression. Decrease in brain concentrations of 5-HT and 5-HIAA (the major metabolite of 5-HT) were commonly observed in animals and in patients experiencing stress and depression, suggesting a dysfunction of serotonergic system (Risch and Nemeroff, 1992; Spreux-Varoquaux et al., 2001; Southwick et al., 2005; Mitani et al., 2006). Clinical findings showed that most therapeutic agents in use induced increased availability of serotonin, which was in line with their antidepressant response (Willner, 1985; Bourin et al., 2002; Blier and Ward, 2003; Trivedi et al., 2004; Daszuta et al., 2005).

In present study, the immediate serotonergic activation of XBXT-2 was demonstrated in 5-HTP-induced head-twitch test, which coincides with the antidepressant-like effect of XBXT-2 in mouse and rat FST (Zhang et al., 2007). Meanwhile, it was also suggested the integrity of 5-HT neural system might be requisite in the antidepressant-like effect of XBXT-2, because the anti-immobility effect of XBXT-2 in mouse TST was completely prevented by pretreatment of PCPA (an irreversible inhibitor of the enzyme tryptophan hydroxylase). It was reported that PCPA treatment at dose of 300 mg/kg for 3 consecutive days, which is the same used in the present study, produced partial but highly significant reductions (over 60%) in brain 5-HT level, while noradrenaline and dopamine levels were not affected (Redrobe et al., 1998).

Yohimbine can produce excessive noradrenaline releasing by its antagonistic action on presynaptic α_2-adrenoceptor, and high dose of apomorphine can induce hypothermia by its interaction with noradrenergic system (Lapin, 1980; Puech et al., 1981). These two models are usually used for the evaluation of noradrenergic effect in antidepressants (Luo, 2005). In present study, single XBXT-2 administration had no effect on the yohimbine or apomorphine induced the noradrenergic action, indicating that noradrenergic system might not be involved in the antidepressant-like effect of single XBXT-2 administration.

Chronic mild stress model is one of the best validated animal models of depression in pre-clinical antidepressant evaluation, for its good etiological validity, face validity and predictive validity (Willner, 1997; Vollmayr and Henn, 2003). In CMS experiment, HPLC-ECD depicted us an elaborate monoaminergic profile with chronic XBXT-2 treatment. 28-day CMS induced significant decrease of 5-HT and its metabolite 5-HIAA concentrations in hippocampus and prefrontal cortex. Concomitant XBXT-2 administration restored these changes and showed remarkable serotonergic activation in both prefrontal cortex and hippocampus which was consistent with its acute action. In particular, this activation was mainly due to the increase of 5-HT concentration, not the increase of 5-HT turnover ratio. On the other hand, there also showed increased tendency of NE level in both hippocampus and prefrontal cortex after chronic XBXT-2 treatment, which was not shown in the acute pharmacological models.

The modern monoamine theory has suggested that the acute increase in the levels of the monoamines at the synapse may be only an early step in a potentially complex cascade of events that ultimately results in antidepressant activity. The adaptive changes such as their receptors density and functions in certain brain regions were responsible for the therapeutic effects which depend on the availability of the specific monoamine at the synapse

(Elhwuegi, 2004). According to this theory, we could speculate that the behavioral effects of XBXT-2 in CMS model (we will talk below) might result from long-term adaptive changes in the monoamine system, which may primarily rely on its serotonergic activation in hippocampus and prefrontal cortex, and that the noradrenergic participation, which may be related to long-term adaptive changes, might not be mainly involved or required in these effects. Therefore, from these findings in HPLC-ECD and the data obtained in acute pharmacological models, it could be suggested that the monoaminergic action of XBXT-2 might be more similar to that of SSRIs than TCAs. In addition, further investigations on the presumptive adaptive changes such as serotonin receptors in hippocampus and prefrontal cortex, may deserve further research.

After 28-day CMS procedure, rats in stress-vehicle group showed a wide variety of symptoms seen in depressive disorder such as anhedonia, motility deficits and anxiety-related behaviors. Chronic administration of XBXT-2 or IMI reversed the aforementioned changes. These findings extended our previous results, which have demonstrated notable antidepressant-like effects of XBXT-2 in acute and subchronic animal models of depression (Zhang et al., 2007). Additionally, the NSF test applied herein, which was previously used for assessing anxiolytic-like behaviors, is also validated to assess chronic (not acute) antidepressant-like efficacy (Bodnoff et al., 1988; Santarelli et al., 2003; Dulawa and Hen, 2005). It is a conflict-based model that elicits competing motivations: the drive to eat and the fear of venturing into the novel environment. Interestingly, we found that the 28-day CMS procedure caused a significantly prolonged feeding latency in rats, while co-administration of tricyclic antidepressant IMI restored it. Meanwhile, similar effect of XBXT-2 on latency to feed was also observed, which may further indicate its antidepressant-like efficacy, or even a potential anxiolytic-like effect. Moreover, chronic treatment of IMI aggravated weight loss in CMS rats, due to its severe side effects, whereas XBXT-2 alleviated the weight loss.

As a traditional Chinese herbal decoction comprising multiple ingredients, XBXT-2 has also been studied focusing on its chemical components. By now, 22 compounds have been isolated from XBXT-2 including 21 flavone compounds: japonicins A and B, onpordin, 3′-O-methylorobol, glycitein, nepetin, patuletin, genistein, luteolin, daidzein, quercetin, apigenin, isoquercitrin, genistin, nepitrin, quercimeritrin, daidzin, patulitrin, quercetagitrin, 3-glucosyl isorhamnetin, isoorientin and an organic acid protocatechuic acid. Additionally, the rough HPLC fingerprint of the extract demonstrated that the major constituents of XBXT-2 were flavones, flavonols, isoflavones and their glycosides. HPLC analysis also revealed that the flavones and flavonols were primary derived from Flos Inulae, as well as the isoflavones were derived from Semen Sojae Preparatum, and the rest of flavones came from Folium Phyllostachydis Henonis. However, as the components are very complicated, this work is still carrying on and deserves further research.

Traditional Chinese Medicine is becoming increasingly popular in pharmacotherapy of depression (Zhang, 2004). Investigations in our study indicate that XBXT-2 possesses excellent antidepressant efficacy in CMS model of rats, which is primarily related to its serotonergic activation. However, further research is needed to elucidate the exact mechanism of XBXT-2.

Acknowledgement

This work was supported by the National Natural Science Foundation of China (No. 30400600, 30472018), the National Basic Research Program of China (No. 2007CB512307) and the National High Technology Research and Development Program of China (No. 2007AA02Z400).

References

Adell A, Castro E, Celada P, Bortolozzi A, Pazos A, Artigas F. Strategies for producing faster acting antidepressants. Drug Discov Today 2005;10: 578–85.
Blier P, Ward NM. Is there a role for 5-HT1A agonists in the treatment of depression? Biol Psychiatry 2003;53:193–203.
Bodnoff SR, Suranyi-Cadotte B, Aitken DH, Quirion R, Meaney MJ. The effects of chronic antidepressant treatment in an animal model of anxiety. Psychopharmacology (Berl) 1988;95:298–302.
Bourin M, David DJ, Jolliet P, Gardier A. Mechanism of action of antidepressants and therapeutic perspectives. Therapie 2002;57:385–96.
Bekris S, Antoniou K, Daskas S, Papadopoulou-Daifoti Z. Behavioural and neurochemical effects induced by chronic mild stress applied to two different rat strains. Behav Brain Res 2005;161:45–59.
Corne SJ, Pickering RW, Warner BT. A method for assessing the effects of drugs on the central actions of 5-hydroxytryptamine. British Journal of Pharmacology 1963;20:106–20.
Daszuta A, Ban Sr M, Soumier A, Hery M, Mocaer E. Depression and neuroplasticity: implication of serotoninergic systems. Therapie 2005;60:461–8.
Dulawa SC, Hen R. Recent advances in animal models of chronic antidepressant effects: the novelty-induced hypophagia test. Neurosci Biobehav Rev 2005;29:771–83.
Elhwuegi AS. Central monoamines and their role in major depression. Prog Neuropsychopharmacol Biol Psychiatry 2004;28:435–51.
Grønli J, Murison R, Fiske E, Bjorvatn B, Sørensen E, Portas CM, et al. Effects of chronic mild stress on sexual behavior, locomotor activity and consumption of sucrose and saccharine solutions. Physiol Behav 2005;84:571–7.
Gumnick JF, Nemeroff CB. Problems with currently available antidepressants. J Clin Psychiatry 2000;61:5–15.
Kim SH, Han J, Seog DH, Chung JY, Kim N, Hong Park Y, Lee SK. Antidepressant effect of Chaihu-Shugan-San extract and its constituents in rat models of depression. Life Sci 2005;6:1297–306.
Kulkarni SK, Dandiya PC. Effects of antidepressant agents on open field behaviour in rats. Psychopharmacologia 1973;33:333–8.
Lapin IP. Adrenergic nonspecific potentiation of yohimbine toxicity in mice by antidepressants and related drugs and antiyohimbine action of antiadrenergic and serotonergic drugs. Psychopharmacology 1980;70:179–85.
Li JM, Kong LD, Wang YM, Cheng CH, Zhang WY, Tan WZ. Behavioral and biochemical studies on chronic mild stress models in rats treated with a Chinese traditional prescription Banxia-houpu decoction. Life Sci 2003;74:55–73.
Luo L, Nong Wang J, Kong LD, Jiang QG, Tan RX. Antidepressant effects of Banxia Houpu decoction, a traditional Chinese medicinal empirical formula. J Ethnopharmacol 2000;73:277–81.
Luo ZP. Anti-depressive pharmacology. In: Zhang JT, Zhang QZ, editors. Technology and method on neuropharmacological research. Beijing: People's Medical Publishing House; 2005. p. 368.
Millan MJ. The role of monoamines in the actions of established and "novel" antidepressant agents: a critical review. Eur J Pharmacol 2004;500:371–84.
Mitani H, Shirayama Y, Yamada T, Kawahara R. Plasma levels of homovanillic acid, 5-hydroxyindoleacetic acid and cortisol, and serotonin turnover in depressed patients. Prog Neuropsychopharmacol Biol Psychiatry 2006;30: 531–4.

Mqller WE. Current St. John's wort research from mode of action to clinical efficacy. Pharmacol Res 2003;47:101–9.

Papp M, Moryl E, Willner P. Pharmacological validation of the chronic mild stress model of depression. Eur J Pharmacol 1996;296:129–36.

Puech AJ, Chermat R, Poncelet M, Doaré L, Simon P. Antagonism of hypothermia and behavioral response to apomorphine: a simple, rapid and discriminating test for screening antidepressants and neuroleptics. Psychopharmacology (Berl) 1981;75:84–91.

Redrobe JP, Bourin M, Colombel MC, Baker GB. Psychopharmacological profile of the selective serotonin reuptake inhibitor, paroxetine: implication of noradrenergic and serotonergic mechanisms. J Psychopharmacol 1998;12:348–55.

Redrobe JP, Dumont Y, Fournier A, Baker GB, Quirion R. Role of serotonin (5-HT) in the antidepressant-like properties of neuropeptide Y (NPY) in the mouse forced swim test. Peptides 2005;26:1394–400.

Risch SC, Nemeroff CB. Neurochemical alterations of serotonergic neuronal systems in depression. J Clin Psychiatry 1992;53(Suppl):3–7.

Rosen RC, Marin H. Prevalence of antidepressant-associated erectile dysfunction. J Clin Psychiatry 2003;64:5–10.

Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, et al. Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. Science 2003;301:805–9.

Southwick SM, Vythilingam M, Charney DS. The psychobiology of depression and resilience to stress: implications for prevention and treatment. Annu Rev Clin Psychol 2005;1:255–91.

Spreux-Varoquaux O, Alvarez JC, Berlin I, Batista G, Despierre PG, Gilton A, et al. Differential abnormalities in plasma 5-HIAA and platelet serotonin concentrations in violent suicide attempters: relationships with impulsivity and depression. Life Sci 2001;69:647–57.

Steru L, Chermat R, Thierry B, Simon P. The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacology(Berl) 1985;85:367–70.

Trivedi MH, Pigotti TA, Perera P, Dillingham KE, Carfagno ML, Pitts CD. Effectiveness of low doses of paroxetine controlled release in the treatment of major depressive disorder. J Clin Psychiatry 2004;65:1356–64.

Vollmayr B, Henn FA. Stress models of depression. Clin Neurosci Res. 2003;3:245–51.

Willner P. Antidepressants and serotonergic neurotransmission: an integrative review. Psychopharmacology (Berl) 1985;85:387–404.

Willner P. Validity, reliability and utility of the chronic mild stress model of depression: a 10-year review and evaluation. Psychopharmacology(Berl) 1997;134:319–29.

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 (Berl) 1987;93:358–64.

Xu G, Zhang H, Hu J. Leaching method of flavone from Bamboo leaves. Chinese J Anal Chem 2000;28:857–9.

Xu C, Luo L, Tan RX. Antidepressant effect of three traditional Chinese medicines in the learned helplessness model. J Ethnopharmacol 2004;91:345–9.

Xia X, Cheng G, Pan Y, Xia ZH, Kong LD. Behavioral, neurochemical and neuroendocrine effects of the ethanolic extract from *Curcuma longa* L. in the mouse forced swimming test. J Ethnopharmacol 2007;110:356–63.

Zhang ZJ. Therapeutic effects of herbal extracts and constituents in animal models of psychiatric disorders. Life Sci 2004;75:1659–99.

Zhang YZ, Li YF, Yu NJ, Yuan L, Zhao YM, Xiao WB, et al. Antidepressant-like effect of the ethanolic extract of Xiaobuxin-Tang, a traditional Chinese herbal prescription in animal models of depression. Chin Med J (Engl) 2007;120:1792–6.

Zhang YZ, Yu NJ, Yuan L, An L, Zhao YM, Xiao WB, et al. Antidepressant-like effect of the total flavonoids extracted from Xiaobuxin-Tang, a traditional Chinese herbal decoction, in forced swimming tests and learned helplessness in rats and mice. Chin J Pharmacol Toxicol 2008;22:1–8.