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Investigation of the synergistic effects of haloperidol combined with *Calculus Bovis Sativus* in treating MK-801-induced schizophrenia in rats

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Abstract: Clinical studies that focused on treating schizophrenia showed that *Calculus Bovis Sativus* (CBS), a substitute of *Calculus Bovis*, when used in combination with haloperidol could significantly lower the dosage of haloperidol compared with treatment with haloperidol alone, whereas efficacy was maintained. The aim of this study was to investigate the synergetic anti-schizophrenia effects in rats using CBS in combination with haloperidol. An open field test was conducted to verify the pharmacodynamic effects of a combination treatment of CBS and haloperidol on MK-801-induced schizophrenic rats. Rat plasma concentrations of intragastric haloperidol and intravenous haloperidol were determined after oral administration of a single dose or 1-week of pretreatment with CBS (50 mg/kg). The pharmacodynamic data showed a significant decrease in locomotor activity and an increase in the percentage of the central distance when haloperidol was concomitantly administered with CBS compared with haloperidol administration alone. The $AUC_{0\text{-}\infty}$ and C_{max} of haloperidol in the orally coadministered groups were significantly higher compared with the oral treatment with haloperidol alone. In conclusion, oral coadministration of CBS with haloperidol resulted in a synergistic effect in rats. The enhanced oral bioavailability of haloperidol when combined with CBS might be attributed to the interaction between them.

Key words: *Calculus Bovis Sativus*, haloperidol, pharmacodynamics, pharmacokinetics, synergism

Introduction

Schizophrenia is a debilitating disorder that afflicts roughly 1% of the world population. Haloperidol is a well-known dopamine antagonist and a typical antipsychotic drug, which is widely used to treat schizophrenia and manic states [18]. However, with regard to the clinical outcome of haloperidol treatment, extensive interindividual variabilities have been observed, and clinical application of haloperidol is restricted by extrapyramidal side effects [17, 34, 35]. Numerous studies have shown improvement of clinical outcome and reduction of the adverse drug reactions of haloperidol treatment [11, 35, 36]; however, the results are limited.

Calculus Bovis (CB, known as *Niuhuang* in China) is a traditional Chinese medicine that has long been used

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for alleviating symptoms of neurological diseases [4]. *Calculus Bovis Sativus* (CBS) is a substitute of CB with a definite chemical profile [4, 41], which has already been included in the Pharmacopoeia of the People's Republic of China [6]. Bile acids, bilirubin, and taurine are considered the main effective components in CBS [38, 41]. In our previous work, we simultaneously quantified taurine and 12 bile acids using high-performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS) to control the quality of CBS [10].

Hitherto, traditional medicine (TM) has played an important role in the treatment of neurological diseases. Moreover, a combination of TM and western medicine can greatly improve clinical efficacy and is accepted by various countries, such as China, Japan, India, and South Korea [5, 27]. Previous pharmacological studies have shown that CBS was beneficial in treating neurological diseases, including hyperlipemia vascular dementia [43]. Recently, a clinical study demonstrated that a combination of CBS and haloperidol significantly reduced the dosage of haloperidol in treating schizophrenia compared with haloperidol treatment alone. In addition, the anti-schizophrenic efficacy was maintained, and side effects, such as extrapyramidal reactions and tachycardia, were reduced [39]. The mechanism underlying the synergy between haloperidol and CBS is unclear and needs to be further investigated. Therefore, in this study, we investigated the synergic effects of haloperidol and CBS in MK-801-induced schizophrenia in rats. Our findings suggested that CBS enhanced the anti-schizophrenic efficacy of haloperidol by increasing its bioavailability.

Materials and Methods

Chemicals and reagents

Haloperidol was obtained from Hunan Dongting pharmaceutical Co., Ltd., (Changde, China), midazolam (internal standard, IS) was obtained from Sigma-Aldrich (St. Louis, MO, purity≥98%), and dizocilpine maleate (MK-801) was obtained from Cayman Chemical Co., Ltd., (Ann Arbor, USA). MK-801 was dissolved in sterile normal saline prior to use. HPLC-grade acetonitrile was purchased from Thermo Fisher Scientific India Pvt. Ltd., (Mumbai, India). Analytical-grade solvents, chemicals, and reagents were used and were purchased locally.

Calculus Bovis Sativus (Lot: 2016–05-16) was provided by Wuhan Jianmin Dapeng Pharmaceuticals Co., Ltd., (Wuhan, China), and dissolved in normal sterile saline. The effective components (taurine and bile acids) in CBS were quantified by a HPLC-MS/MS approach described in our previous study [10].

Animals

Male Sprague Dawley rats (6–8 weeks old, weighing 180–200 g) were obtained from the Experiment Animal Center of Tongji Medical College, Huazhong University of Science and Technology (SCXK2015-0018, Hubei, China). The animals were housed under temperature- (22 ± 2°C), humidity- (50 ± 10%), and light-controlled conditions (inverted 12-h light/dark cycle), and food and water were available and given ad libitum. All studies were approved by the Animal Ethics Committee of Tongji Medical College, Huazhong University of Science and Technology. All procedures and experiments conducted were consistent with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Pharmacodynamic study

Drugs and dosage: Based on previous studies [1, 28], a single administration of 0.1 mg/kg MK-801 was used to generate a schizophrenia-like animal model. The dosage schedules of haloperidol and CBS were converted by means of body surface area to conform to the human clinical schedule as described in the commercial product data sheet [29, 30, 39]. Animals were randomly assigned to nine experimental groups (n=10 per group). Treatment groups were designated as the control group, model group, haloperidol (1.4 mg/kg) group, haloperidol combined CBS groups (50, 100, 150 mg/kg), and CBS groups (50, 100, 150 mg/kg). In the haloperidol combined CBS groups and the CBS groups, rats were administered one daily dose of CBS intragastrically for 7 consecutive days. On the 7th day, haloperidol was administered by oral gavage 30 min after sterile normal saline or CBS administration, in both the haloperidol group and haloperidol combined CBS groups. The control and model groups were treated with a similar volume of normal saline (1 ml/100 g of weight). Except for those in the control group, all rats were injected intraperitoneally with MK-801 on the 7th day after the haloperidol and CBS administration.

Open field test: Open fields (100 cm × 100 cm × 40 cm) equipped with a video-based EthoVision system (Noldus Information Technology, Wageningen, Nether-

lands) were placed in a light- and sound-attenuated room provided with indirect and homogenous illumination. A video camera (Apple Inc., Cupertino, CA, USA) that was hung connected one meter above the arena floor was used to monitor the animals' activities. After the drug treatment, the rats were transferred immediately to a holding cage and transferred to the testing room. After 10 min of acclimation, they were placed in a central start position in the open arena and allowed to explore for 5 min. After each session, in which the rats were tested once, the arena was cleaned with 70% ethanol. The changes in distance of locomotor activity and percentage of the distance travelled in the center zone were measured as the difference between groups [37].

Evaluation of malonaldehyde, nitric oxide, and superoxide dismutase levels in cerebral tissue: After the open field test, the animals were deeply anesthetized using isoflurane anesthesia delivered in 1.5% oxygen and decapitated with a guillotine. Brains were removed and placed on a 4°C ice plate and dissected for the following purposes: Brains were prepared as 10% homogenates using precooled normal saline (which is 9 times as heavy as the brain) and centrifuged at 3,500 rpm for 10 min at 4°C. The supernatant was collected to determine the activity of the superoxide dismutase (Superoxide Dismutase Detection Kit, Nanjing Jiancheng Bioengineering institute, Nanjing, China) and malonaldehyde (Malonaldehyde Detection Kit, Nanjing Jiancheng Bioengineering institute, Nanjing, China) and nitric oxide (Nitric Oxide Detection kit, Nanjing Jiancheng Bioengineering institute, Nanjing, China) contents according to the manufacturers' instructions.

Pharmacokinetic study

Drug administration and blood sampling: Rats were randomly divided into six groups (n=7 per group). Three groups were orally given haloperidol (1.4 mg/kg), whereas the other three groups were intravenously administered haloperidol (0.7 mg/kg). CBS (50 mg/kg) was coadministered orally with haloperidol in either a single dose or multiple doses (7 days, once a day). Animals were anesthetized by very brief exposure (1–2 min) to isoflurane anesthesia delivered in 1.5% oxygen. Blood samples were collected immediately following evaluation of reflexes and physiological parameters. This procedure was used at all individual time points. Blood samples (approximately 0.3 ml each) were collected via retro-orbital puncture at 0, 0.13, 0.25, 0.50, 0.75, 1.0, 1.5, 2.0, 4.0, 6.0, 10, and 24 h in plastic tubes containing 200 U/ml heparin after intragastric (i.g.) or intravenous (i.v.) administration of haloperidol. The supernatants were obtained after centrifugation at 4,000 rpm for 10 min at 4°C and stored at −20°C until further use.

In vitro study: Isolated everted gut sac experiments were performed to evaluate the effects of CBS treatment on the absorption of haloperidol. Animals were exsanguinated by femoral artery blood sampling after being anesthetized with isoflurane, and the entire ileum of each rat was flushed with 50 ml of ice-cold saline. Subsequently, the ileum was isolated and divided into two segments of equal length. Each segment was everted, and a 10-cm-long everted sac was prepared. The sac was immersed in Krebs-Ringer buffer (artificial intestinal juice) in which CBS (0.2, 0.4, or 0.6 mg/ml) and haloperidol (0.005 mg/ml) were dissolved, and the solution was bubbled with a mixture of 95% O_2 and 5% CO_2 at 37°C [15]. The artificial intestinal juice was collected at 20, 40, 60, 80, 100, and 120 min from the serosal side of the ileum and analyzed by HPLC-MS/MS.

Sample preparation: Aliquots of plasma or artificial intestinal juice (100 μl) were added to 10 μl of IS (500 ng/ml) solution and 1.1 ml of ethyl acetate. The solution was mixed and vortexed for 2 min. After centrifugation at 12,000 rpm for 10 min at 4°C, the supernatant was transferred to a clean microtube, and the solvent was evaporated to dryness under a gentle stream of nitrogen gas at 35°C. The residue was reconstituted with 200 μl of methanol, and a 10 μl aliquot was injected into the HPLC-MS/MS system for analysis. Quality control (QC) samples were prepared in bulk at concentrations of 2, 20 and 180 ng/ml (a single batch at each concentration) in the same manner based on independent weighing of standard drugs.

HPLC-MS/MS analysis: A rapid, sensitive, and selective HPLC-MS/MS approach was established and validated for the analysis of haloperidol in rat plasma. Briefly, after being extracted and redissolved, haloperidol and IS were subjected to HPLC-MS/MS analysis using positive electrospray ionization under multiple reaction monitoring mode. The mass spectrometer was set to monitor the transitions of the precursors to the product ions as follows: *m/z* 376.2 to 165.1 for haloperidol and *m/z* 326.1 to 291.2 for midazolam. The chromatographic separation was achieved using a Symmetry® C_{18} (2.1 mm×50 mm, 3.5 μm) column with 10 mmol/l ammonium acetate (pH 7.4 adjusted with formic

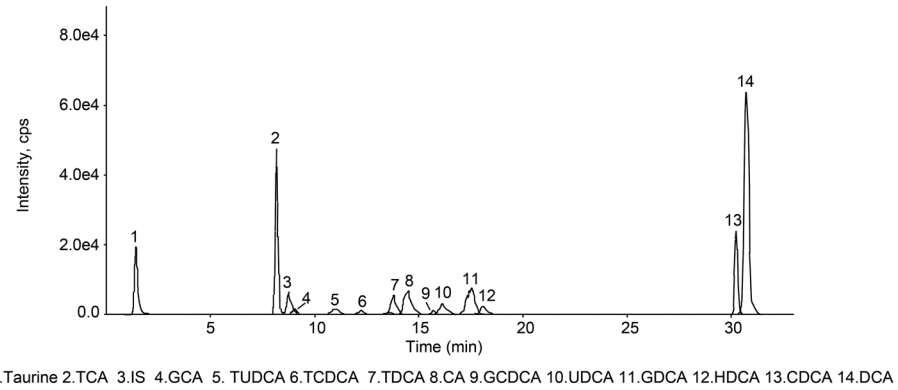

Fig. 1. Multiple reactions monitoring chromatogram of a representative sample of *Calculus Bovis Sativus*.

acid)-acetonitrile as a mobile phase and a gradient elution. The flow rate was kept at 0.3 ml/min. A 10 μl aliquot of each sample was loaded onto the column, separated, and eluted using the following gradient (mins,% mobile phase B): 0 min, 5%; 1 min, 5%; 2 min, 95%; 4 min, 95%; 4.01 min, 5%; and 6 min, 5%. The column temperature was maintained at 40°C. The lower limit of quantification (LLOQ) for haloperidol was 1 ng/ml.

Statistical analysis

Data are presented as the mean ± standard deviation (SD). Pharmacodynamic parameters were calculated by Microsoft Excel 2013 (Microsoft Inc., Redmond, WA, USA) and a video-based EthoVision system. Pharmacokinetic parameters were calculated using a non-compartment model, and non-compartmental analysis was performed using the proprietary DAS (Drug and Statistics) computer software package (version 3.2.8, Chinese Pharmacology Society, Beijing, China). The pharmacokinetic parameters included area under the blood concentration-time curve ($AUC_{0-\infty}$), half-life ($t_{1/2}$), apparent plasma clearance (CL/F), maximum blood drug concentration (C_{max}), and time to C_{max} (t_{max}), and bioavailability (F). The rate of drug transport in the rat's isolated everted gut sacs was expressed as the apparent permeability coefficient (P_{app}). Pharmacokinetic parameters between different groups were subjected to one-way analysis of variance (ANOVA) using the IBM SPSS 19.0 software (IBM Corp, Armonk, NY, USA) followed by *post hoc* comparison tests. Rat plasma concentration time profiles and haloperidol absorption through the ileum were analyzed by using repeated measures ANOVA. A P value<0.05 was considered statistically significant.

Results

Effective components of the CBS

The effective components in CBS in this study were quantified by an HPLC-MS/MS approach described in our previous study [10]. The CBS used in this study contained 61.13 mg/g taurine, 69.04 mg/g cholic acid (CA), 25.52 mg/g deoxycholic acid (DCA), 0.30 mg/g ursodeoxycholic acid (UDCA), 6.88 mg/g chenodeoxycholic acid (CDCA), 0.2 mg/g hyodeoxycholic acid (HDCA), 14.36 mg/g taurocholic acid (TCA), 3.58 mg/g tauroursodeoxycholic acid (TDCA), 0.3 mg/g tauroursodeoxycholic acid (TUDCA), 0.46 mg/g taurochenodeoxycholic acid (TCDCA), 10.23 mg/g glycocholic acid (GCA), 3.11 mg/g glycodeoxycholic acid (GDCA), and 2.89 mg/g glycochenodeoxycholic acid (GCDCA) (Fig. 1).

Pharmacodynamics study

Synergistic effects of haloperidol combined with CBS decrease the locomotor activity of MK-801-treated rats: Initially, pharmacodynamic studies in rats were conducted to investigate the synergistic effect of oral coadministration of haloperidol and CBS. In the model group, a significant increase in locomotor activity was observed compared with the control group (Fig. 2A). Administration of CBS (50, 100, 150 mg/kg) did not induce significant changes compared with the model group (Fig. 2A). Furthermore, the increase in horizontal locomotion was significantly blocked by the combined use of haloperidol (1.4 mg/kg, i.g.) and CBS (50, 100, 150 mg/kg) (P<0.01) (Fig. 2A). Combined treatment of haloperidol and CBS reduced MK-801-induced locomotion by ap-

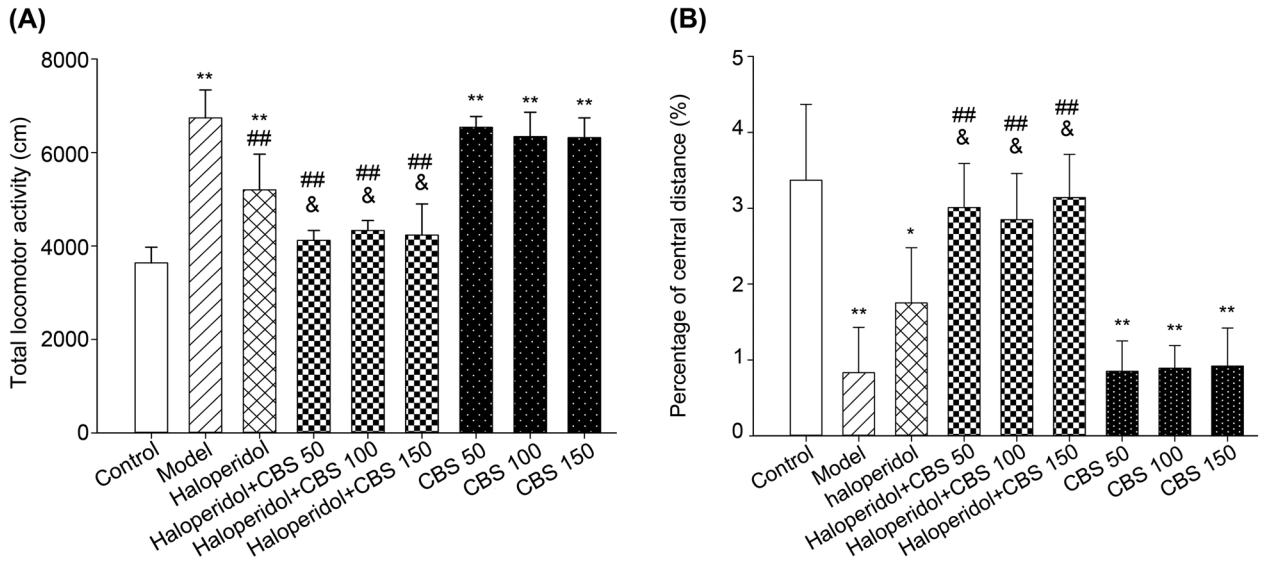

Fig. 2. Effect of CBS and haloperidol on MK-801-induced behavior in the open field test in rats. Total locomotor activity (A) and the percentage of the central distance (B) are shown. All rats were evaluated for 5 min after a 10 min period of acclimation. $^{**}P<0.01$ vs. control group; $^{\#\#}P<0.01$ vs. model group; $^{\&}P<0.05$ vs. haloperidol group. Data are presented as the mean ± SD (n=10).

Table 1. Effects on superoxide dismutase, malonaldehyde, and nitric oxide in cerebral tissue of rats with MK-801-induced schizophrenia in rats

Groups	Dose (mg/kg)	Superoxide dismutase (U/mg)	Malonaldehyde (nmol/mg)	Nitric oxide (μmol/g)
Control	–	54.63 ± 5.36	1.80 ± 0.21	2.51 ± 0.34
Model	–	41.21 ± 4.35**	5.61 ± 0.35**	1.24 ± 0.28**
Haloperidol	1.4	48.21 ± 6.32##	2.51 ± 0.51##	2.05 ± 0.55##
Haloperidol + CBS	1.4 and 50	53.21 ± 5.32&	2.10 ± 0.21&	2.34 ± 0.51&&
	1.4 and 100	53.61 ± 7.61&	2.13 ± 0.24	2.46 ± 0.21&&
	1.4 and 150	55.62 ± 9.21&&	2.11 ± 0.35&	2.34 ± 0.24&&
CBS	50	43.21 ± 6.22	5.42 ± 0.54	1.25 ± 0.23#
	100	48.21 ± 4.61#	5.14 ± 0.61#	1.42 ± 0.68#
	150	49.51 ± 6.51#	4.55 ± 0.61##	1.51 ± 0.56#

$^{**}P<0.01$ vs. control group. $^{\#}P<0.05$ or $^{\#\#}P<0.01$ vs. model group. $^{\&}P<0.05$ or $^{\&\&}P<0.01$ vs. haloperidol group. Data are presented as the mean ± SD (n=10 per group).

proximately 25%. Groups that showed significant changes in locomotor activity were compared, and the results are shown in Fig. 2A.

A significant decrease in the percentage of the central distance was observed in the model group (Fig. 2B). Administration of CBS (50, 100, 150 mg/kg) did not induce significant changes compared with the model group (Fig. 2B). In addition, the increase in anxiety level was significantly reduced by combined treatment of haloperidol (1.4 mg/kg, i.g.) and CBS (50, 100, 150 mg/kg) ($P<0.01$) (Fig. 2B). Moreover, the combination of haloperidol and CBS increased the percentage of the central distance by 2.5-fold. Groups that showed a significant change in the percentage of the central distance were compared, and the results are shown in Fig. 2B.

Combined use of CBS and haloperidol can improve oxidative stress in brain tissue: We also tested the effects of haloperidol treatment alone and the combination of haloperidol and CBS on changes in biochemical markers in cerebral tissue. Compared with the model group, malonaldehyde levels were significantly decreased, and the activity of superoxide dismutase and nitric oxide contents were significantly increased in the cerebral tissue of rats treated with in haloperidol or CBS (Table 1). Moreover, the antioxidant effect in the cerebrum was better after coadministration of haloperidol with CBS compared with haloperidol treatment alone (Table 1).

Pharmacokinetic study

HPLC-MS/MS spectrum of haloperidol: A pharmaco-

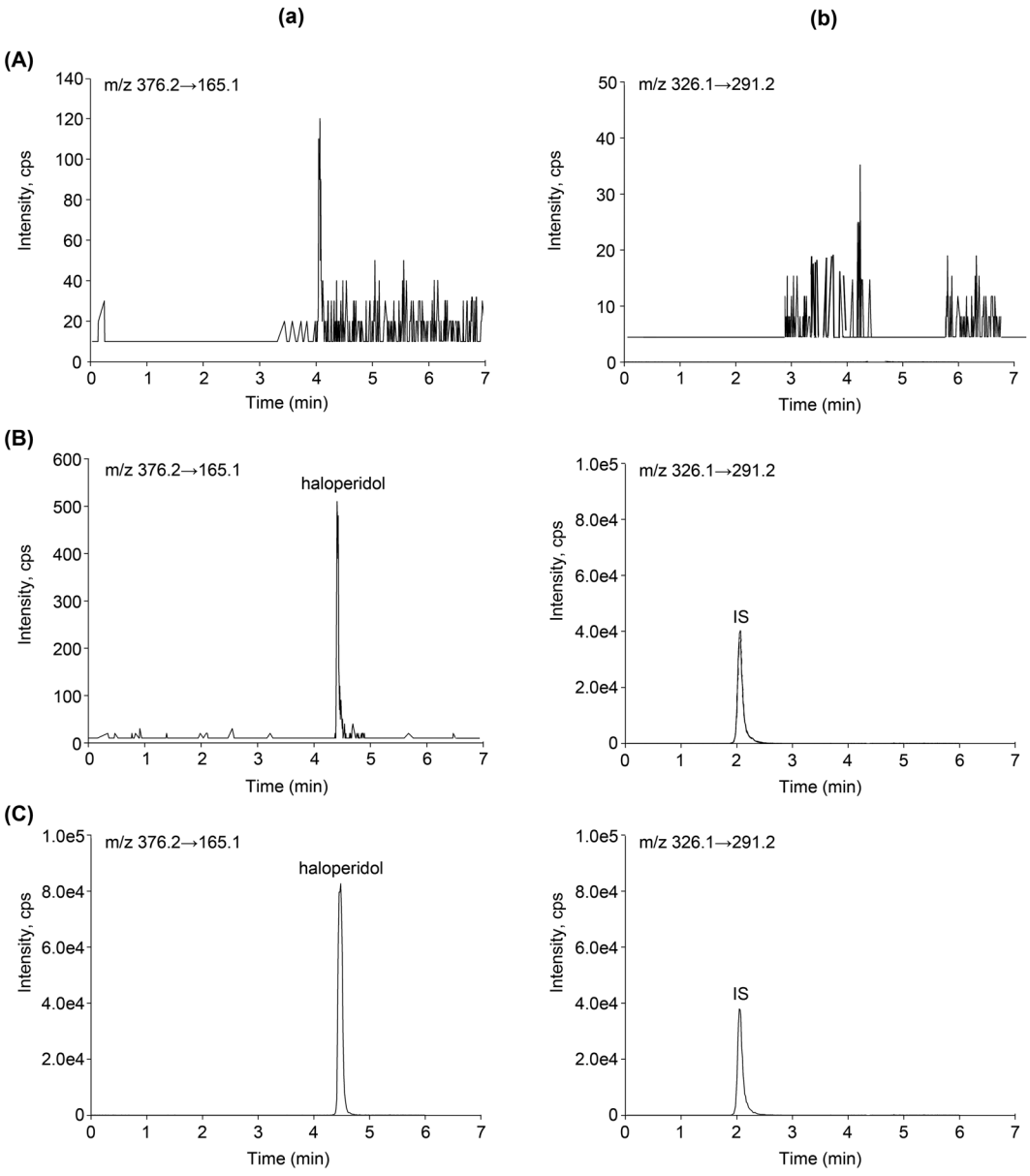

Fig. 3. Representative multiple reactions monitoring chromatograms for haloperidol (a) and IS (b) in rat plasma samples. (A) Blank plasma sample, (B) plasma spiked with haloperidol at the LLOQ level and the IS, and (C) rat plasma sample from a rat 15 min after intravenous administration of haloperidol.

kinetic study was employed to investigate the intrinsic pharmacokinetic interaction between haloperidol and CBS. The HPLC-MS/MS approach as described above was selective and specific. Representative chromatograms obtained from blank plasma, plasma spiked with the LLOQ standard (1 ng/ml), and plasma harvested from a rat 15 min after i.v. administration of haloperidol are shown in Fig. 3. In blank rat plasma, no interfering peaks from endogenous compounds were observed at the retention times of haloperidol or the IS obtained from six different lots. The retention times of haloperidol and the IS were 4.48 and 2.04 min, respectively. As shown in Fig. 3, the proposed HPLC-MS/MS approach clearly determined haloperidol in rat plasma. Therefore, this approach was chosen for investigating the pharmacokinetic interaction in rats after i.g. and i.v. administration of haloperidol.

Effect of CBS on the pharmacokinetics of haloperidol: The mean plasma concentration-time profiles of haloperidol following i.g. (1.4 mg/kg) or i.v. (0.7 mg/kg)

Table 2. Pharmacokinetic parameters of haloperidol after a single dose or a 1-week pretreatment of CBS in rats

Parameter	Oral administration			Intravenous administration		
	Haloperidol	Haloperidol + CBS-single	Haloperidol + CBS-multiple	Haloperidol	Haloperidol + CBS-single	Haloperidol + CBS-multiple
$AUC_{0-\infty}$(ng.h/ml)	43.3 ± 10.8	75.6 ± 12.6**	79.2 ± 12.4**	188.4 ± 20.5	168.5 ± 35.8	174.0 ± 32.6
$t_{1/2}$ (h)	5.9 ± 1.7	6.8 ± 1.8	6.6 ± 1.7	2.9 ± 0.7	2.6 ± 0.7	2.5 ± 0.7
Tmax (h)	0.9 ± 0.2	0.9 ± 0.2	0.7 ± 0.3	–	–	–
C_{max} (ng/ml)	6.8 ± 2.2	11.1 ± 2.5**	12.3 ± 3.0**	–	–	–
CL/F (l/h/kg)	17.1 ± 4.3	10.2 ± 12.4**	12.9 ± 2.7**	3.8 ± 0.4	4.6 ± 1.1	4.1 ± 0.8
F (%)	23.0 ± 5.7	40.1 ± 7.9*	42 ± 7.1*	–	–	–

*P<0.05 or **P<0.01 compared with haloperidol group. Data are presented as the mean ± SD (n=7 per group).

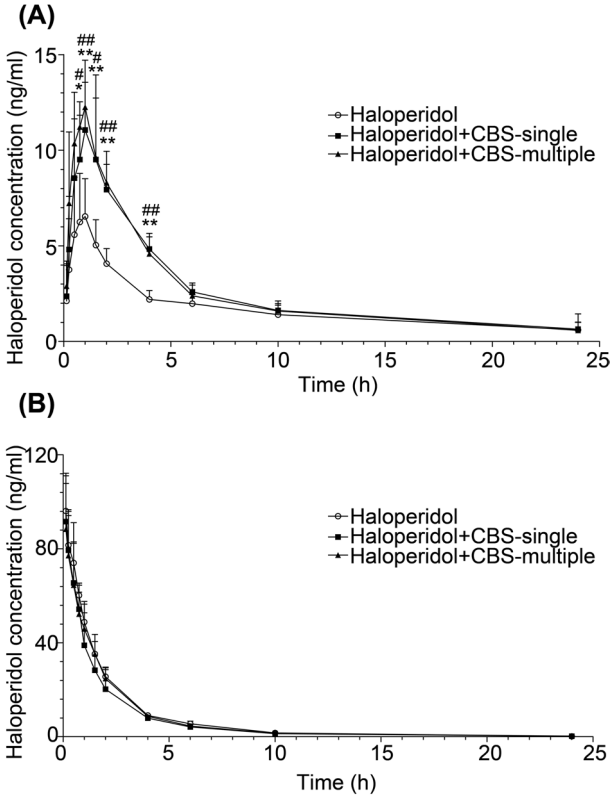

Fig. 4. Rat plasma concentration-time profiles of i.g. haloperidol (A) and i.v. haloperidol (B) after administration of a single dose or a 1-week pretreatment of CBS (50 mg/kg). Data are presented as the mean ± SD (n=7 per group). *P<0.05 or **P<0.01 for haloperidol + CBS-single group vs. haloperidol group. $^{\#}P$<0.05 or $^{\#\#}P$<0.01 for haloperidol + CBS-multiple group vs. haloperidol group. Data are presented as the mean ± SD (n=10).

administration to rats in the presence or absence of CBS (50 mg/kg) are shown in Fig. 4. The corresponding pharmacokinetic parameters are shown in Table 2. Compared with the haloperidol group, in which rats were orally given haloperidol alone, short-term CBS treatment significantly (P<0.01) increased the $AUC_{0-\infty}$ of haloperidol by 74.6% and C_{max} of haloperidol by 63.2%. Similarly, long-term CBS treatment significantly (P<0.01) increased the $AUC_{0-\infty}$ of haloperidol by 82.9% and C_{max} of haloperidol by 80.9% (Table 2). In addition, CBS significantly increased the absolute bioavailability (F) of haloperidol when compared with the haloperidol group (P<0.05) (Table 2). Moreover, CBS treatment slightly prolonged the $t1/2$ of haloperidol, and the *CL/F* of haloperidol was decreased (P<0.05) (Table 2).

When haloperidol was administered i.v., $AUC_{0-\infty}$, C_{max}, and $t_{1/2}$ were not statistically different between groups (Table 2). In addition, the pharmacokinetics of i.v. administration of haloperidol were not affected by CBS treatment. These results suggested that when haloperidol and CBS were administered simultaneously, a possible pharmacokinetic interaction may have occurred during the process of intestinal absorption; however, this does not affect the phase of metabolism or renal elimination.

In vitro everted gut sac experiment: The rat ileum was employed to investigate whether the absorption of haloperidol was affected by CBS treatment in the intestine. We determined the transport of haloperidol from mucosal to serosal surfaces across the everted rat ileum in the absence or presence of CBS. In the presence of CBS, the absorption of haloperidol through the everted rat ileum was significantly altered compared with the control (Fig. 5). Table 3 shows the apparent permeability coefficient (P_{app}) of haloperidol in the ileum. Thus, our results indicated that CBS treatment significantly increased the P_{app} of haloperidol in the ileum (P<0.05).

Discussion

Presently, combination therapies of TM and western

Table 3. Apparent permeability coefficient of haloperidol in everted rat ileum

Groups	Haloperidol	Haloperidol + CBS-low	Haloperidol + CBS-medium	Haloperidol + CBS-high
P_{app} (10^{-4} cm/s)	13.35 ± 1.54	17.83 ± 3.17*	19.99 ± 1.93**	21.86 ± 2.13**

The concentrations of CBS were low, medium, and high (0.2, 0.4, 0.6 mg/ml). *P<0.05 or **P<0.01 compared with haloperidol group. Data are presented as the mean ± SD (n=4 per group).

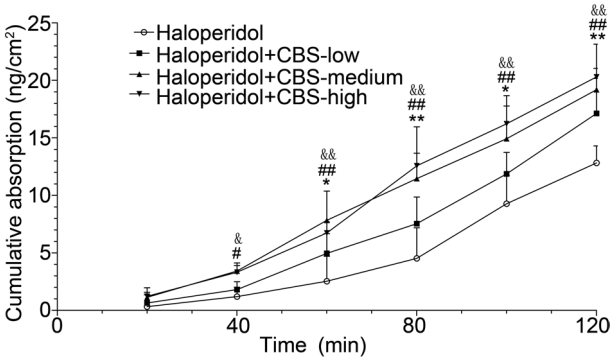

Fig. 5. Haloperidol absorption through the ileum. The concentrations of CBS used were low, medium, and high (0.2, 0.4, 0.6 mg/ml). *P<0.05 or **P<0.01 for haloperidol + CBS-low group vs. haloperidol group. $^{\#}P$<0.05 or $^{\#\#}P$<0.01 for haloperidol + CBS-medium group vs. haloperidol group. $^{\&}P$<0.05 or $^{\&\&}P$<0.01 for haloperidol + CBS-high group vs. haloperidol group. Data are presented as the mean ± SD (n=4 per group).

medicine are increasingly applied in clinical practice. Over the past decade, a number of significant TM-drug interactions have been reported [12, 13, 23]. Therefore, numerous studies have focused on understanding underlying mechanisms of action involved. In general, two types of TM-drug interactions exist: pharmacodynamic and pharmacokinetic interactions. Pharmacodynamic interactions arise as the pharmacological effects of TM synergize or antagonize the pharmacological actions of a prescribed drug. For example, naringenin did not affect the pharmacokinetics of pioglitazone; however, it attenuated the hypoglycemic actions of pioglitazone via pharmacodynamic interactions that affect adipokine expression and/or PPARγ ligand-binding activity [20]. Most importantly, various TM-drug interactions are of pharmacokinetic origin [3] and arise from the ability of TM to regulate the activity of metabolic enzymes or transporters or influence the gastrointestinal environment.

Compound preparations that contain CBS have been extensively used as a single therapy or combined with antipsychotics in treating schizophrenia [19, 32, 42]. Recently, Li *et al.* [19] demonstrated that efficacy against human schizophrenia was enhanced through the combined use of a Niuhuang Ningong tablet (which primarily contains CB) and risperidone. Moreover, when used as a combination of CBS and haloperidol, the dosage of haloperidol used could be significantly lowered, while maintaining efficacy and reducing the incidence of side effects in the clinic [39].

To evaluate the possible pharmacodynamic interactions of haloperidol and CBS, an anti-schizophrenia study was performed in rats, followed by evaluation of biochemical indicators in cerebral tissue. The open field test showed that the extent of locomotor activity was higher in the rat model group compared with the control and treatment groups (Fig. 2). These results were in agreement with previous studies that described specific behavioral characteristics of rats, such as high levels of exploration in a novel environment [1, 7, 9, 16]. Data analysis showed that haloperidol alone or the combined use of haloperidol and CBS attenuated psychosis-like behaviors such as hyper-locomotor activity and anxiety (evaluated by the percentage of the central distance) in MK-801-induced rats (Fig. 2). Moreover, the combined use of haloperidol and CBS showed better inhibition of the locomotor activity and anxiety compared with treatment with haloperidol alone. These results implied that for schizophrenia resistance, CBS played a synergic role with haloperidol.

Oxidative stress is one of the key players in nerve impairments induced by schizophrenia. Studies have shown that current treatments that impact oxidative pathways may reverse pro-oxidative states in schizophrenia [21, 31]. Our results showed that in the model group, the levels of malonaldehyde increased, whereas the activity of superoxide dismutase and nitric oxide content decreased. After single or combination treatment of haloperidol (1.4 mg/kg, i.g.) and CBS, the above indexes significantly improved (Table 2), which indicated that both haloperidol and CBS have antioxidant activity. CBS had an antioxidant effect, which was consistent with our previous report indicating that CBS alleviated oxidative damage in rats with cholestasis [40].

To investigate possible pharmacokinetic interaction, an HPLC-MS/MS approach was applied to determine plasma concentrations of haloperidol in rats with or without oral administration of a single dose or a 1-week pretreatment of CBS (50 mg/kg). Our results indicated that CBS treatment changed the pharmacokinetic characteristics of haloperidol when haloperidol was taken orally; however, it did not affect metabolism and clearance of haloperidol after i.v. administration. Compared with the oral control group that was given haloperidol alone, the $AUC_{0\text{-}\infty}$ and C_{max} of haloperidol were significantly increased when the rats were pretreated with CBS (Fig. 4A; $P<0.05$). This indicated that CBS was effective in promoting the absorption of haloperidol. In addition, our data showed that CBS had little effect on pharmacokinetic characteristics of haloperidol when given i.v. (Fig. 4B). This may be explained by the fact that the i.v. route bypasses the oral absorption phase, and therefore the elimination phase cannot be affected by CBS. Therefore, we conclude that after oral administration of haloperidol, CBS treatment affects absorption rather than distribution, metabolism, and excretion.

Haloperidol is mainly absorbed and passively transported in the ileum segment of the small intestine of rats [22, 26]. Therefore, we used an isolated approach by vascular and luminal perfusion of the ileum of rats to assess the intestinal absorption of haloperidol. Our data from analysis of P_{app} indicated that the three concentrations of CBS used significantly increased the uptake of haloperidol in the ileum (Fig. 5) [2, 14, 33]. Combined with the pharmacokinetic interactions, these results indicated that CBS may increase the absorption of haloperidol, thereby increasing its bioavailability. Our findings suggested that CBS enhanced the anti-schizophrenia efficacy of haloperidol by increasing its bioavailability.

The oral absorption of haloperidol not only depends on the characteristics of the compound but also depends on the gastrointestinal environment. The promotion of the absorption of haloperidol in the gut caused by CBS treatment could be attributed to bile acids and salts. Bile acids and salts present in CBS have been demonstrated to be endogenous surfactants, which have been widely used as absorption enhancers to increase drug transport across various biological barriers, such as the blood-brain barrier, skin, mucosa, nasal, cornea, pulmonary, and intestinal membranes [8, 24, 25]. Therefore, bile acids may contribute to increased absorption of haloperidol into the bloodstream.

Conclusions

To our knowledge, our study is the first to show that coadministration of haloperidol and CBS changes pharmacodynamics and pharmacokinetics of haloperidol in rats. The data indicated that combined use of haloperidol and CBS may provide synergistic effects on the pharmacodynamics of haloperidol due to alterations in the intestinal absorption of haloperidol. This phenomenon may increase the body's exposure to haloperidol, and therefore, the dose of haloperidol can be reduced when used in combination with CBS. The combination of haloperidol and CBS may provide a therapeutic benefit in that CBS enhances bioavailability and lowers the dose of administration of haloperidol. In conclusion, the knowledge gained in this study regarding possible interactions of haloperidol may be helpful for physicians as well as patients who receive CBS treatment. Therefore, human studies should be conducted that assess the clinical relevance of the synergistic effects between haloperidol and CBS.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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References

1. Andiné, P., Widermark, N., Axelsson, R., Nyberg, G., Olofsson, U., Mårtensson, E., and Sandberg, M. 1999. Characterization of MK-801-induced behavior as a putative rat model of psychosis. *J. Pharmacol. Exp. Ther.* 290: 1393–1408. [Medline]
2. Arellano, C., Philibert, C., Vachoux, C., Woodley, J., and Houin, G. 2007. The metabolism of midazolam and comparison with other CYP enzyme substrates during intestinal absorption: in vitro studies with rat everted gut sacs. *J. Pharm. Pharm. Sci.* 10: 26–36. [Medline]
3. Butterweck, V. and Derendorf, H. 2008. Potential of pharmacokinetic profiling for detecting herbal interactions with drugs. *Clin. Pharmacokinet.* 47: 383–397. [Medline] [CrossRef]
4. Cai, H., Guang, Y., and Liu, L. 2007. The protective effects of in vitro cultivated calculus bovis on the cerebral and myo-

cardial cells in hypoxic mice. *J. Huazhong Univ. Sci. Technolog. Med. Sci.* 27: 635–638. [Medline] [CrossRef]
5. Batista, C., Jesus, N.R., Silva, C.M., Silva, T.P., and Campos, M.G. 2017. Herb-drug Interactions: An insight into cardiovascular diseases based on case reports. *Cardiovasc. Hematol. Agents Med. Chem.* 14: 142–149. [Medline] [CrossRef]
6. Chinese Pharmacopoeia Commission 2015. Chinese Pharmacopoeia of 2015 Edition, vol. 1 pp. 173–175. The Medicine Science and Technology Press of China, Beijing (in Chinese).
7. Dubiela, F.P., Messias, M.F., Moreira, K.D., Zanlorenci, L.H., Grassl, C., Filho, R.F., Nobrega, J.N., Tufik, S., and Hipólide, D.C. 2011. Reciprocal interactions between MK-801, sleep deprivation and recovery in modulating rat behaviour. *Behav. Brain Res.* 216: 180–185. [Medline] [CrossRef]
8. Enright, E.F., Joyce, S.A., Gahan, C.G., and Griffin, B.T. 2017. Impact of Gut Microbiota-Mediated Bile Acid Metabolism on the Solubilization Capacity of Bile Salt Micelles and Drug Solubility. *Mol. Pharm.* 14: 1251–1263. [Medline] [CrossRef]
9. Feinstein, I. and Kritzer, M.F. 2013. Acute N-methyl-D-aspartate receptor hypofunction induced by MK801 evokes sex-specific changes in behaviors observed in open-field testing in adult male and proestrus female rats. *Neuroscience* 228: 200–214. [Medline] [CrossRef]
10. Feng, C.Y., Li, X.P., Zhang, C.L., He, G.Z., Xu, Y.J., Li, W., Yu, Z.Q., Cai, H.J., and Liu, D. 2015. Development of a rapid and simple LC-MS/MS method for identification and quality control of natural Calculus bovis and Calculus bovis sativus. *Anal. Methods-UK.* 7: 7606–7617.
11. Ferraz Gonçalves, J.A., Almeida, A., Costa, I., Silva, P., and Carneiro, R. 2016. Comparison of Haloperidol Alone and in Combination with Midazolam for the Treatment of Acute Agitation in an Inpatient Palliative Care Service. *J. Pain Palliat. Care Pharmacother.* 30: 284–288. [Medline] [CrossRef]
12. Fong, S.Y., Wong, Y.C., Xie, C., and Zuo, Z. 2015. Herb-drug interactions between Scutellariae Radix and mefenamic acid: Simultaneous investigation of pharmacokinetics, anti-inflammatory effect and gastric damage in rats. *J. Ethnopharmacol.* 170: 106–116. [Medline] [CrossRef]
13. Gödtel-Armbrust, U., Metzger, A., Kroll, U., Kelber, O., and Wojnowski, L. 2007. Variability in PXR-mediated induction of CYP3A4 by commercial preparations and dry extracts of St. John's wort. *Naunyn Schmiedebergs Arch. Pharmacol.* 375: 377–382. [Medline] [CrossRef]
14. Gurunath, S., Nanjwade, B.K., and Patila, P.A. 2014. Enhanced solubility and intestinal absorption of candesartan cilexetil solid dispersions using everted rat intestinal sacs. *Saudi Pharm. J.* 22: 246–257. [Medline] [CrossRef]
15. Gustafsson, A.B., Gottlieb, R.A., and Granville, D.J. 2005. TAT-mediated protein transduction: delivering biologically active proteins to the heart. *Methods Mol. Med.* 112: 81–90. [Medline]
16. Jacobs, P.S., Taylor, B.M., and Bardgett, M.E. 2000. Maturation of locomotor and Fos responses to the NMDA antagonists, PCP and MK-801. *Brain Res. Dev. Brain Res.* 122: 91–95. [Medline] [CrossRef]
17. Kamble, P., Chen, H., Sherer, J.T., and Aparasu, R.R. 2009. Use of antipsychotics among elderly nursing home residents with dementia in the US: an analysis of National Survey Data. *Drugs Aging* 26: 483–492. [Medline] [CrossRef]
18. Kühl, J.O.G., Laursen, T.M., Thorup, A., and Nordentoft, M. 2016. The incidence of schizophrenia and schizophrenia spectrum disorders in Denmark in the period 2000-2012. A register-based study. *Schizophr. Res.* 176: 533–539. [Medline] [CrossRef]
19. Li, G.W. 2016. Treatment of schizophrenia with risperidone combined with niuhuang ninggong tablet is feasible. *J Med Theor. &. Prac.* 29: 886–888 (in Chinese).
20. Lu, X., Yang, X., Li, X., Lu, Y., Ren, Z., Zhao, L., Hu, X., Jiang, J., and You, X. 2013. In vitro activity of sodium new houttuyfonate alone and in combination with oxacillin or netilmicin against methicillin-resistant Staphylococcus aureus. *PLoS One* 8: e68053. [Medline] [CrossRef]
21. Magalhães, P.V., Dean, O., Andreazza, A.C., Berk, M., and Kapczinski, F. 2016. Antioxidant treatments for schizophrenia. *Cochrane Database Syst. Rev.* 2: CD008919. [Medline]
22. Milanés, M.V., Vargas, M.L., and Puig, M.M. 1984. Haloperidol treatment increases the biosynthesis and release of endorphins in guinea-pig ileum. *J. Pharm. Pharmacol.* 36: 446–449. [Medline] [CrossRef]
23. Minocha, M., Mandava, N.K., Kwatra, D., Pal, D., Folk, W.R., Earla, R., and Mitra, A.K. 2011. Effect of short term and chronic administration of Sutherlandia frutescens on pharmacokinetics of nevirapine in rats. *Int. J. Pharm.* 413: 44–50. [Medline] [CrossRef]
24. Miyake, M., Minami, T., Hirota, M., Toguchi, H., Odomi, M., Ogawara, K., Higaki, K., and Kimura, T. 2006. Novel oral formulation safely improving intestinal absorption of poorly absorbable drugs: utilization of polyamines and bile acids. *J. Control. Release* 111: 27–34. [Medline] [CrossRef]
25. Moghimipour, E., Ameri, A., and Handali, S. 2015. Absorption-Enhancing Effects of Bile Salts. *Molecules* 20: 14451–14473. [Medline] [CrossRef]
26. Murray-Brown, F. and Dorman, S. 2015. Haloperidol for the treatment of nausea and vomiting in palliative care patients. *Cochrane Database Syst. Rev.* 11: CD006271. [Medline]
27. Ogbogu, U. and Necyk, C. 2016. Community Pharmacists' Views and Practices Regarding Natural Health Products Sold in Community Pharmacies. *PLoS One* 11: e0163450. [Medline] [CrossRef]
28. Pesić, V., Popić, J., Milanović, D., Loncarević-Vasiljković, N., Rakić, L., Kanazir, S., and Ruzdijić, S. 2010. The effect of MK-801 on motor activity and c-Fos protein expression in the brain of adolescent Wistar rats. *Brain Res.* 1321: 96–104. [Medline] [CrossRef]
29. Pilla Reddy, V., Kozielska, M., Johnson, M., Mafirakureva, N., Vermeulen, A., Liu, J., de Greef, R., Rujescu, D., Groothuis, G.M., Danhof, M., and Proost, J.H. 2013. Population pharmacokinetic-pharmacodynamic modeling of haloperidol in patients with schizophrenia using positive and negative syndrome rating scale. *J. Clin. Psychopharmacol.* 33: 731–739. [Medline] [CrossRef]
30. Reagan-Shaw, S., Nihal, M., and Ahmad, N. 2008. Dose translation from animal to human studies revisited. *FASEB*

J. 22: 659–661. [Medline] [CrossRef]

31. Sadowska-Bartosz, I., Galiniak, S., Bartosz, G., Zuberek, M., Grzelak, A., and Dietrich-Muszalska, A. 2016. Antioxidant properties of atypical antipsychotic drugs used in the treatment of schizophrenia. *Schizophr. Res.* 176: 245–251. [Medline] [CrossRef]
32. Song, H., Ma, J.D., Chen, Y.X., Huang, S.P., and Zhang, R.L. 2013. Study on efficacy of olanzapine combined niuhuang ninggong tablet in treatment-resistant schizophrenia. *Chin. J. Exp. Trad. Med. Form* 19: 306–310 (in Chinese).
33. Sridhar, V., Surya Sandeep, M., Ravindra Babu, P., and Naveen Babu, K. 2014. Evaluation of first-pass cytochrome P4503A (CYP3A) and P-glycoprotein activities using felodipine and hesperetin in combination in Wistar rats and everted rat gut sacs in vitro. *Phytother. Res.* 28: 699–705. [Medline] [CrossRef]
34. Testad, I., Auer, S., Mittelman, M., Ballard, C., Fossey, J., Donabauer, Y., and Aarsland, D. 2010. Nursing home structure and association with agitation and use of psychotropic drugs in nursing home residents in three countries: Norway, Austria and England. *Int. J. Geriatr. Psychiatry* 25: 725–731. [Medline] [CrossRef]
35. Ulrich, S., Wurthmann, C., Brosz, M., and Meyer, F.P. 1998. The relationship between serum concentration and therapeutic effect of haloperidol in patients with acute schizophrenia. *Clin. Pharmacokinet.* 34: 227–263. [Medline] [CrossRef]
36. van Strien, A.M., Vermeulen Windsant-van den Tweel, A., Leliveld-van den Heuvel, M., di Biase, M., van den Brule, A.J., and van Marum, R.J. 2014. Correlation of haloperidol concentration in blood and cerebrospinal fluid: a pharmacokinetic study. *J. Clin. Psychopharmacol.* 34: 516–517. [Medline] [CrossRef]
37. Violle, N., Rozan, P., Demais, H., Nyvall Collen, P., and Bisson, J.F. 2017. Evaluation of the antidepressant- and anxiolytic-like effects of a hydrophilic extract from the green seaweed Ulva sp. in rats. *Nutr. Neurosci.* 19: 1–9. [Medline] [CrossRef]
38. Wan, T.C., Cheng, F.Y., Liu, Y.T., Lin, L.C., and Sakata, R. 2009. Study on bioactive compounds of in vitro cultured Calculus Suis and natural Calculus Bovis. *Anim. Sci. J.* 80: 697–704. [Medline] [CrossRef]
39. Weng, S.H., Wang, G.H., Wang, X.P., Xiao, W.D., and Cai, H.J. 2010. Calculus bovis sativus combined with haloperidol in the treatment of schizophrenia: A Randomized double-blind controlled study. *Chin. J. Integr. Trad. W. Med.* 30: 1213–1215 (in Chinese).
40. Wu, T., Chang, M.J., Xu, Y.J., Li, X.P., Du, G., and Liu, D. 2013. Protective effect of Calculus Bovis Sativus on intrahepatic cholestasis in rats induced by α-naphthylisothiocyanate. *Am. J. Chin. Med.* 41: 1393–1405. [Medline] [CrossRef]
41. Yan, S.K., Wu, Y.W., Liu, R.H., and Zhang, W.D. 2007. Comparative study on major bioactive components in natural, artificial and in-vitro cultured Calculus Bovis. *Chem. Pharm. Bull. (Tokyo)* 55: 128–132. [Medline] [CrossRef]
42. Zhang, Y.L., Wang, Y.F., Du, J., Su, J.M., and Ding, Y. 2006. Meiluo niu huagn ning gong tablet combined with risperidone in the treatment of schizophrenia. *Shanghai Jingshen Yixue* 18: 33–35 (in Chinese).
43. Zhong, X.M., Ren, X.C., Lou, Y.L., Chen, M.J., Li, G.Z., Gong, X.Y., and Huang, Z. 2016. Effects of in-vitro cultured calculus bovis on learning and memory impairments of hyperlipemia vascular dementia rats. *J. Ethnopharmacol.* 192: 390–397. [Medline] [CrossRef]