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Decreased absorption of midazolam in the stomach due to low pH induced by co-administration of Banha-sasim-tang

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Objectives Banha-sasim-tang (BST), which consists of seven different herbs, is one of the most popular herbal formulae for treating gastrointestinal disorders in Eastern Asia. The commonly used herbal medicine is often co-administered with other therapeutic drugs, which raises the possibility of herb–drug interactions and may modify the clinical safety profile of therapeutic drugs.

Methods We investigated the potential herb–drug interactions between BST extract and midazolam (MDZ) in mice. The area under the plasma concentration-time curve (AUC) of MDZ and 1′-hydroxymidazolam (1′-OH-MDZ) was evaluated for both oral and intraperitoneal administration of MDZ, following oral administration of BST (0.5 and 1 g/kg).

Results It was found that the AUC of MDZ and 1′-OH-MDZ was lower in case of oral administration of MDZ. Administration of BST extract was not associated with hepatic cytochrome P450 activity. BST extract induced a strong reduction in pH and it has been reported that oral mucosal absorption of MDZ is lower at low pH. The decreased absorption rate of MDZ might be caused by the ingredients of BST and may not be related to other factors such as increased excretion of MDZ by P-glycoprotein.

Conclusions The altered pharmacokinetics of midazolam caused by co-administration with BST *in vivo* could be attributed to a decrease in pH and subsequent reduction of MDZ absorption rate.

Keywords Herb-drug interaction, Pharmacokinetics, Banha-sasim-tang, Midazolam

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Introduction

Herbal medicines are commonly used to treat various diseases worldwide and are often co-administered with therapeutic drugs. However, the co-administration of herbs and drugs raises the potential of herb–drug interactions, which may affect the clinical safety of therapeutic drugs. Herb–drug interactions can be predicted by evaluating pharmacokinetic parameters, including absorption, metabolism, distribution, and excretion of drugs. Several drugs or herbs may affect the activity of drug-metabolizing enzymes, especially cytochrome P450 (CYP), resulting in herb–drug or drug–drug interactions [1]. Among the CYP subfamily, CYP3A is one of the most important drug-metabolizing enzymes in humans. It metabolizes more than 50% of clinically administered drugs [2]. Midazolam (MDZ), a short-acting, water-soluble benzodiazepine, is widely used as a probe substrate for CYP3A in humans and mice [3,4]. MDZ is mainly metabolized to 1′-hydroxymidazolam (1′-OH-MDZ) by the CYP3A subfamily [5]. Therefore, MDZ and 1′-OH-MDZ are typical probes used to measure the activity of CYP3A both *in vivo* and *in vitro* [6,7].

Commonly known as Banha-sasim-tang (BST) in Korea, or Ban-xia-xie-xin tang in China, the herb under study is one of the most popular herbal formulae mentioned in old herbal prescrip-

tion literature [8]. BST consists of seven different herbs, including *Pinellia ternata, Zingiber officinale, Glycyrrhizae uralensis, Coptis japonica, Scutellaria baicalensis, Panax ginseng*, and *Zizyphus jujuba*. In East Asia, BST has been used to treat gastrointestinal disorders [8]. In addition, BST has displayed preventive action against diarrhea induced by irinotecan [9]. BST has been used to treat chronic hypofunction of the gastrointestinal tract (GI) and functional abnormalities of the upper and lower GI system by positively improving GI hormone levels [10]. Recently in China, modified BST was shown to cause symptomatic improvement in patients with functional dyspepsia [11].

To prevent or minimize adverse herb–drug interactions, herbal medicine that interacts with drugs should be investigated in both *in vivo* and *in vitro* systems. Despite extensive clinical research conducted on BST, its potential drug interactions remain to be elucidated [8,12]. BST has been more frequently prescribed in combination therapy with other herbs or drugs than on its own. Moreover, BST can be obtained easily without a prescription in Korea and patients sometimes self-prescribe medicines, which can lead to the co-administration of several different kinds of drugs. Here, a potential herb–drug interaction in BST therapy was investigated by evaluating the pharmacokinetics of model drug MDZ when simultaneously administered with BST on mouse plasma parameters, using an liquid chromatography-tandem mass spectrometry (LC-MS/MS) system.

Materials and Methods

Chemicals and Reagents

BST solid extract was obtained from Hanzung Pharmaceutical (Daejeon, Korea) and prepared in accordance with the Korean Pharmacopoeia (KP), 10th edition. The preparation contained the following ingredients: water extract of *P. ternate* (1.67 g, KP), *Zingiber officinale* (0.83 g, KP), *Glycyrrhizae uralensis* (1.0 g, KP), *Coptis japonica* (0.33 g, KP), *Scutellaria baicalensis* (1.0 g, KP), *Panax ginseng* (1.0 g, KP), and *Zizyphus jujuba* (1.0 g, KP). MDZ and 1′-OH-MDZ were obtained from Bukwang Pharmaceutical (Seoul, Korea) and Cayman Chemical (Ann Arbor, MI, USA), respectively.

Animals

Specific pathogen-free, 5-week-old male ICR mice (24 to 26 g) were obtained from Orient Bio (Seongnam, Korea) and acclimated for at least seven days before the experiment. Upon arrival, the animals were randomly housed in cages, with four or five per cage. The animal quarters were strictly maintained at 23 ± 3°C and 50 ± 10% relative humidity with a 12 hours light/dark cycle (intensity: 150–300 Lux). All animal procedures were performed in accordance with the Society of Toxicology guidelines of 1989. The study was approved by the institutional review board of the Kyungpook National University (2015-0099).

Pharmacokinetics Study

A total of 18 male ICR mice (30 ± 2 g) were randomly divided into a vehicle-treated, and two MDZ-treated groups, which also received BST extract dissolved in saline. In the first group, MDZ (2.0 mg/kg) was orally administered to nine mice after 5 minutes of oral administration of BST extract (0, 0.5 or 1.0 g/kg, n = 3). In the second group, nine mice were intraperitoneally treated with MDZ (2.0 mg/kg) after 5 minutes of oral administration of BST extract (0, 0.5 or 1.0 g/kg, n = 3). After the mice received MDZ, blood samples were collected from the tail vein into heparinized capillary tubes at 0.08-, 0.167-, 0.25-, 0.5-, 1-, and 2 hour time-points. The collected blood was immediately centrifuged at 4000 g for 10 minutes and 10 μL of plasma was obtained for each sample. The samples were stored at −20°C until analysis.

Plasma samples (10 μL) were added to 90 μL of acetonitrile (ACN) with 0.1% formic acid and 5 μM reserpine solution (internal standard [IS], 97.5:2.5, v/v) was added. The solution was mixed and centrifuged at 13000 rpm for 10 minutes at 4°C, and 90 μL of the supernatant was obtained. The supernatants were transferred to autosampler vials, and 5 μL aliquots were injected into the LC system.

Liquid Chromatography-tandem Mass Spectrometry Analysis System

All measurements were performed using an LC-MS/MS system in the selective reaction monitoring (SRM) mode. A Triple Stage Quadrupole Vantage mass spectrometer an HESI-II spray source coupled to an Accela™ LC system (Thermo Fisher Scientific, Waltham, MA, USA) was employed. The vaporizer and capillary temperatures were set to 150°C and 300°C, respectively. Electrospray ionization was performed in the positive mode at a spray voltage of 3500 V. Nitrogen was used as the sheath and auxiliary gas, and was set to 45 and 20 (arbitrary units), respectively. Data were analyzed using the Xcalibur software (Thermo Fisher Scientific). An ACE® 5C18 column (5 μm, 50 mm × 2.1 mm, Advanced Chromatography Technologies, Scotland, UK) and a guard C18 column (2 μm, 2.1 mm ID, Phenomenex, Torrance, CA, USA) were employed for LC separation. The mobile phase consisted of ACN with 0.1% FA (mobile phase A) and water (mobile phase B) at a flow rate of 220 μL/min. The gradient was as follows: 0 minute 5% A, 0.5 minutes 5% A, 1.5 minutes 95% A, 3.0 minutes 95% A, 3.5 minutes 5% A, 5.0 minutes 5% A. Ions monitored in the SRM mode were m/z 326.0 → 291.0 for MDZ,

342.0→203.0 for 1'-OH-MDZ m/z, and 609.4→174.1 for the IS, respectively, at an SRM collision energy of 29 eV for MDZ, 15 eV for 1'-OH-MDZ and 42 eV for IS. Data procurement was controlled using the Xcalibur software (Thermo Fisher Scientific).

Method Validation

The calibration curves of MDZ and 1'-OH-MDZ ranged from 0.05 to 2.0 μg/mL, respectively. Calibration curves were constructed by plotting the peak-area ratios of analyte or IS vs. the concentrations of MDZ and 1'-OH-MDZ in mouse plasma. The equations for the calibration curves of MDZ and 1'-OH-MDZ were $y = 25114\ x - 13.85$ ($r^2 = 0.999$) and $y = 19380x - 9.12$ ($r^2 = 0.998$), respectively. The lower limits of quantification values of MDZ and 1'-OH-MDZ were 50 and 50 ng/mL, respectively. The quality control samples of the methodology were evaluated by analyzing five replicates of mouse plasma spiked with known concentrations of MDZ (0.05, 0.5, 1.0, and 2.0 μg/mL) or 1'-OH-MDZ (0.05, 0.5, 1.0, and 2.0 μg/mL) (Table S1).

PH Determination of the Mixture Containing Banha-sasim-tang Extract

The pH of BST extract was measured using a Metter Toledo S220 SevenCompact™ pH/Ion pH meter (Metter-Toledo International Inc., Columbus, OH, USA). The pH of each concentration of BST extract in the mixture was measured immediately after it dissolved.

Pharmacokinetic Parameters

A non-compartmental model was used to calculate the pharmacokinetic parameters, using WinNonlin version 2.1 (Scientific Consulting Inc., Cary, NC, USA), and includes maximum plasma concentration (C_{max}), time to reach maximum plasma concentration (T_{max}), area under the plasma concentration-time curve (AUC), and half-life.

Statistics

The mean value ± standard error was determined for each treatment group of a given experiment. Dunnett's *t*-test was performed to compare the statistical significance of the data. The *p*-values < 0.05 were considered statistically significant. These are represented by asterisks .

Results

To investigate the potential herb–drug interactions observed during co-administration of BST with MDZ, two different administration methods were evaluated (Figure 1). Firstly, MDZ (2.0 mg/kg) was orally administered after treatment with BST extract (0, 0.5, or 1.0 g/kg) by oral gavage (Figure 1A). By monitoring the plasma concentration of MDZ and 1'-OH-MDZ, first-pass metabolism of BST can be determined. In the other group, MDZ was intraperitoneally administered following oral administration of BST, to focus on the effects of BST in the metabolic system and eliminate the absorption step (Figure 1B).

Mean plasma concentration–time profiles of MDZ and 1'-OH-MDZ were obtained (Figure 2) after simultaneous administration of a single-dose of BST (0, 0.5, or 1.0 g/kg, peroral [PO]) or vehicle control and a single dose of MDZ (2 mg/kg, PO) in mice. The pharmacokinetic parameters of MDZ and

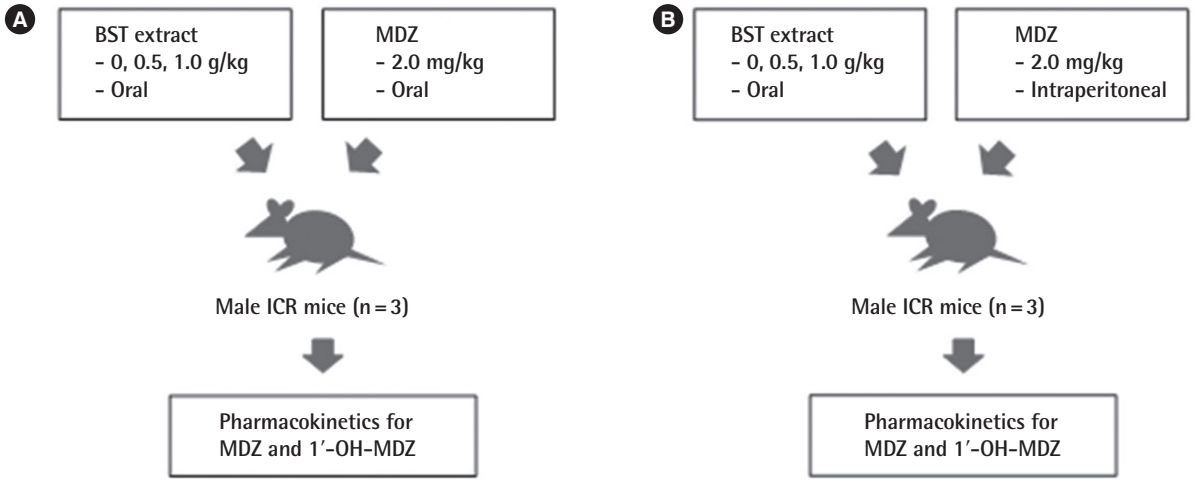

Figure 1. Experimental design. (A) Male ICR mice were orally administered MDZ (2.0 mg/kg) following administration of BST extract (0, 0.5, or 1.0 g/kg) or vehicle (saline) by oral gavage. (B) Male ICR mice were intraperitoneally treated with MDZ (2.0 mg/kg) following administration of BST extract (0, 0.5, or 1.0 g/kg) or vehicle (saline) by oral gavage. The blood samples were obtained and the concentrations of MDZ and 1'-OH-MDZ (metabolite) were determined. MDZ, midazolam; BST, Banha-sasim-tang; 1'-OH-MDZ, 1'-hydroxymidazolam.

1′-OH-MDZ were compared between the vehicle- and BST-treated groups (Table 1). Following oral co-administration of BST and MDZ, the mean C_{max} and AUC values were significantly lower than those observed in the vehicle-treated group. The mean AUC decreased by 76% in the BST co-treated group (1.0 g/kg, PO). This corresponds to the plasma concentration-time profile of 1′-OH-MDZ, in which C_{max} and AUC were significantly decreased by 51% and 53%, respectively in the BST co-treated group (1.0 g/kg, PO). Here, both the AUC and C_{max} of MDZ and its metabolite, 1′-OH-MDZ, decreased in the BST co-administered group, indicating decreased absorption of the parent drug MDZ.

ICR male mice were simultaneously treated with MDZ (2.0 mg/kg, intraperitoneal [IP]) following oral administration of BST (0.5 or 1.0 g/kg), to investigate the inhibitory effect of BST

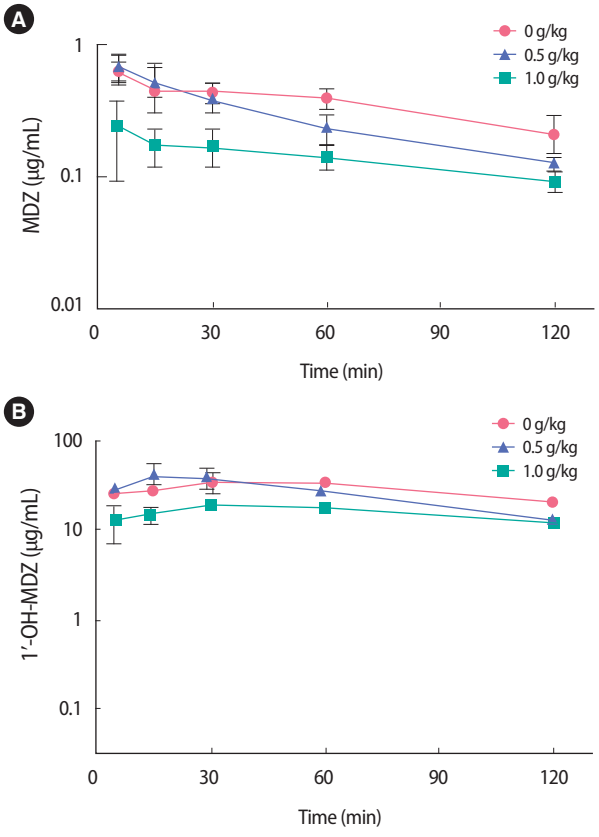

Figure 2. Mean plasma concentration-time profiles of MDZ and 1′-OH-MDZ in mice simultaneously treated with BST (0, 0.5, or 1.0 g/kg, PO) and MDZ (2.0 mg/kg, PO). Data are presented as mean±standard error (n=3). MDZ, midazolam; 1′-OH-MDZ, 1′-hydroxymidazolam; BST, Banha-sasim-tang; PO, peroral.

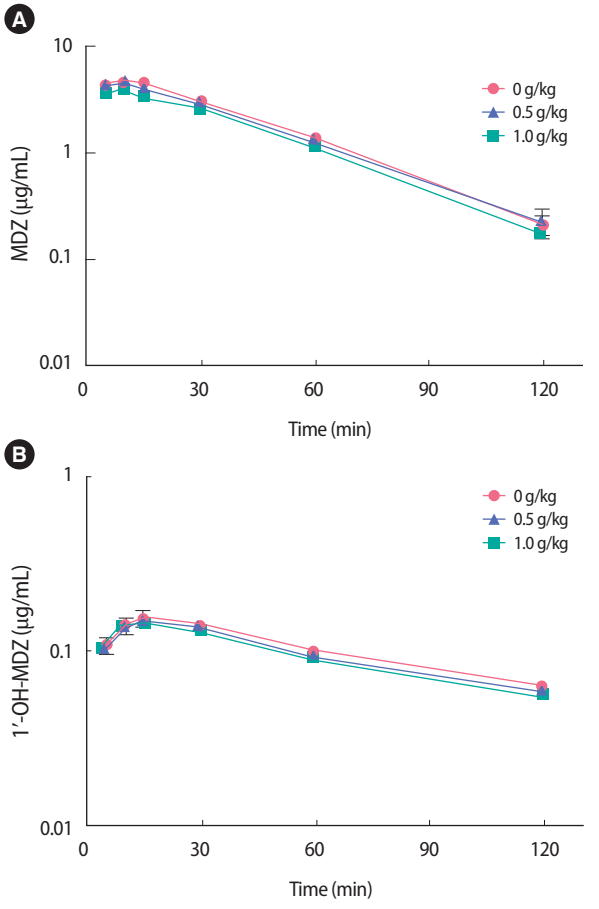

Figure 3. Mean plasma concentration-time profiles of MDZ and 1′-OH-MDZ in mice simultaneously treated with BST (0, 0.5, or 1.0 g/kg, PO) and MDZ (2.0 mg/kg, IP). Data are presented as mean ± standard error (n=3). MDZ, midazolam; 1′-OH-MDZ, 1′-hydroxymidazolam; BST, Banha-sasim-tang; PO, peroral; IP, intraperitoneal.

Table 1. Effects of co-administration of BST extract with MDZ on the pharmacokinetic parameters of MDZ and 1′-OH-MDZ (n=3)

Parameters	BST extract (g/kg)	Oral administration of MDZ				Intraperitoneal administration of MDZ			
		AUC (mg·min/mL)	C_{max} (mg/mL)	Half-life (min)	T_{max} (min)	AUC (mg·min/mL)	C_{max} (mg/mL)	Half-life (min)	T_{max} (min)
MDZ	0.0	45.7±2.9	0.7±0.1	167.3±116.8	5.0±0.0	234.5±9.5	4.5±0.1	23.1±1.5	11.7±1.7
	0.5	33.9±6.5	0.7±0.1	59.3±15.3	8.3±3.3	220.1±26.2	4.5±0.5	25.0±3.1	10.0±0.0
	1.0	16.9±4.1**	0.3±0.1	79.1±1.2	26.7±16.9	197.9±7.8	3.9±0.2	23.1±0.8	9.0±1.3
1′-OH MDZ	0.0	3540.8±312.7	43.1±8.5	55.3±0.0	50.0±10.0	18.9±0.8	0.2±0.01	159.8±11.2	20.0±5.0
	0.5	3189.7±907.0	42.9±12.6	71.8±12.3	16.7±7.3	18.2±0.3	0.1±0.01	166.0±6.1	13.8±1.3
	1.0	1901.0±194.2 *	21.4±2.5	101.1±1.7	31.7±15.9	18.2±0.4	0.1±0.0	169.0±11.3	21.0±3.8

Data are presents as mean±standard errors.
BST, Banha-sasim-tang; MDZ, midazolam; 1′-OH-MDZ, 1′-hydroxymidazolam; AUC, area under the plasma concentration-time curve; C_{max}, maximum plasma concentration; T_{max}, maximum plasma concentration.
*p<0.05, **p<0.01 compared to the vehicle-treated group.

on CYP3A4 activity. The concentration-time profiles of MDZ and 1′-OH-MDZ in plasma are shown in Figure 3. The pharmacokinetics parameters of MDZ and 1′-OH-MDZ in the BST-treated group did not differ significantly from those seen in the vehicle-treated group (Table 1). In addition, BST did not exert an inhibitory effect on CYP1A, 2B, 2C, 2D, or 3A4 at concentrations of 0 to 0.5 g/mL in human liver microsomes, based on the use of a cocktail of probe substrate and LC-MS/MS analysis (data not shown) [7].

Discussion

Our study showed that the pharmacokinetic parameters of MDZ and 1′-OH-MDZ decreased following oral co-administration with BST, but not in case of IP administration. When MDZ was orally administrated, the plasma concentration of MDZ was regulated by two mechanisms: absorption rate and metabolic stability in the liver. The plasma concentration of MDZ was found to be mainly affected by a mechanism involving metabolic conversion of MDZ in the liver. The plasma concentration of MDZ and its metabolites was not changed by BST IP co-administration, which indicates that BST does not have a strong inhibitory action on CYP activities in the liver. Moreover, a decrease in plasma concentration of MDZ and 1′-OH-MDZ is observed following oral co-administration with BST. The findings therefore indicate that BST affects the absorption of MDZ, but not its metabolism.

The reduction in plasma concentration of MDZ and 1′-OH-MDZ in animals co-administered with MDZ and BST (1.0 g/kg, PO) indicates that the absorption of MDZ might be inhibited by BST, and that this inhibition might not be associated with metabolic activity or CYP3A4 enzyme. The reduced absorption of MDZ may be attributed to several phenomena. One is the formation of insoluble particles from the components of BST extract interacting with MDZ at physiological pH conditions (pH 1-2 in the stomach, or pH 5-6 in the intestines). However, the concentrations of MDZ in the supernatants of the BST-MDZ mixture in artificial gastric juice or intestinal juice was not significantly altered (Figure S1). In this way, the ingredients in the extract were found not to form insoluble particles that could reduce the absorption of MDZ in the stomach and intestines.

Another hypothesis is that the reduced pH induced by BST extract in the stomach or intestines affects the oral mucosal absorption of MDZ, since it has been reported that MDZ absorption is strongly inhibited under low pH conditions [13]. Furthermore, changes in local pH affect intestinal absorption of water-soluble, weakly acidic compounds. The bioavailability of MDZ at pH 2.8, 3.2, and 3.9 solutions was 6.2, 18.7, and 22.6%

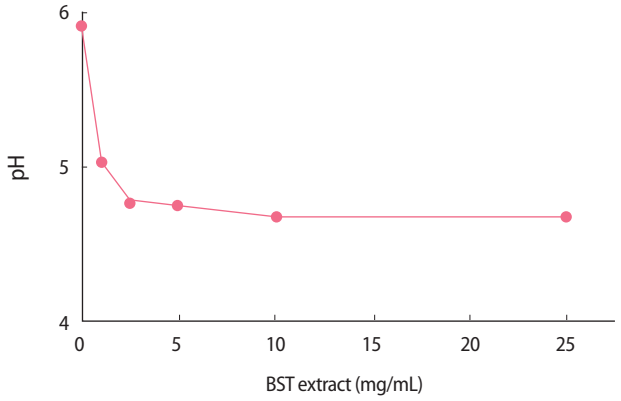

Figure 4. PH values of the Banha-sasim-tang (BST) extract mixtures (0–100 mg/mL).

respectively [14]. As shown in Figure 4, pH significantly reduced with increasing concentrations of BST extract (1-100 mg/mL). The pH value of normal saline was 5.9, whereas that of 1 and 10 mg/mL BST extract was 5.0 and 4.7, respectively. Since BST consists of seven herbs, it exhibits varying concentration ranges for each of its diverse ingredients, and thus might contain acidic compounds that decrease pH.

The other possible mechanism involved in the phenomena under investigation is that the ingredients in BST inhibits the absorption of MDZ in the stomach or intestine. The complex ingredients in BST might compete with the absorption of MDZ. Since MDZ is not a substrate of P-glycoprotein (P-gp), the reduced plasma concentration cannot be related to increased excretion by P-gp [13]. Further, following co-administration with BST (1.0 g/kg, PO), the absorption of caffeine was found to be inhibited, since the bioavailability of caffeine has been shown to be almost 100% *in vivo* [14]. Moreover, we evaluated the change in the pharmacokinetic parameters of caffeine and paraxanthine after BST administration Table S2. The plasma concentration of caffeine and its metabolite, paraxanthine, decreased following the administration of BST (0.5 and 1.0 g/kg) by oral gavage, as was seen in case of MDZ-BST co-administration. In a previous study, Kampo extract used in traditional Japanese medicines, originated from *Glycyrrhiza uralensis*, reduced the C_{max} and AUC of talinolol, a P-gp substrate drug [15]. Decreased intestinal talinolol absorption induced by Kampo extract might be caused by the inhibition of organic anion transporting peptides, and not by inhibition of P-gp. The low plasma concentration of MDZ or caffeine following co-administration with BST indicated that the absorption rate was inhibited by components of the BST extract.

Here, we investigated the herb–drug interactions between BST extract and MDZ *in vivo*, following oral administration of BST extract (1.0 g/kg). BST extract did not exert effects on the

activity of an enzyme crucial to drug interactions, CYP, in the liver of the mouse model studied. Nevertheless, the pharmacokinetic parameters of MDZ were altered when it was orally administered following BST extract treatment (1.0 g/kg), as evidenced by the observed reduction in the total absorption of MDZ and 1′-OH-MDZ. The reduction of pH induced by the administration of BST extract indicates mechanisms behind the decreased rate of mucosal absorption of MDZ. In conclusion, the oral administration of BST extract together with MDZ might cause potential herb–drug interactions *in vivo*.

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Conflict of Interest

The authors have no conflicts of interest associated with material presented in this paper.

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Table S1. The accuracy and RSD of quality control samples of MDZ and 1'-OH MDZ

	Theoretical concentration (μg/mL)	Concentration observed (μg/mL)	Accuracy (%)	RSD (%)
MDZ	0.05	0.06±0.00	114.3	2.0
	0.5	0.49±0.03	97.3	5.1
	1.0	0.96±0.09	96.2	9.1
	2.0	2.02±0.06	101.1	3.2
1'-OH-MDZ	0.5	0.49±0.01	99.0	0.9
	1.0	1.01±0.00	100.8	0.8
	2.0	1.83±0.03	91.6	1.4
	5.0	5.04±0.04	100.8	0.8

RSD, relative standard deviation; MDZ, midazolam; 1'-OH-MDZ, 1'-hydroxymidazolam.

Table S2. Effects of co-administration of BST extract on the pharmacokinetic parameters of caffeine and paraxanthine

Parameters	BST extract (g/kg)	Oral administration of MDZ			
		AUC (μg·min/mL)	C_{max} (μg/mL)	T_{max} (min)	Half-life (min)
Caffeine	0.0	271.4±11.8	3.2±0.2	22.5±7.5	69.1±0.0
	0.5	216.2±18.4	3.1±0.3	30.0±0.0	48.9±6.8
	1.0	169.2±22.7	1.8±0.2	50.0±10.0	88.1±0.0
Paraxanthine	0.0	167.4±2.5	2.0±0.1	90.0±30.0	-
	0.5	174.3±10.7	2.0±0.2	70.0±26.5	-
	1.0	112.1±11.1	1.3±0.1	80.0±20.0	-

Nine male ICR mice were orally administered with caffeine (2.0 mg/kg) after 5 minutes of oral administration of BST extract (0, 0.5 or 1.0 g/kg, n=3); After the mice received caffeine, blood samples were collected from the tail vein into heparinized capillary tubes at 0.08- 0.25- 0.5- 1.0- and 2 hours; The collected bloods were immediately centrifuged at 4000 g for 10 minutes and 10 μL of plasma was obtained for each sample and stored at -20°C until analysis.
BST, Banha-sasim-tang; MDZ, midazolam; AUC, area under the plasma concentration-time curve; C_{max}, maximum plasma concentration; T_{max}, maximum plasma concentration.

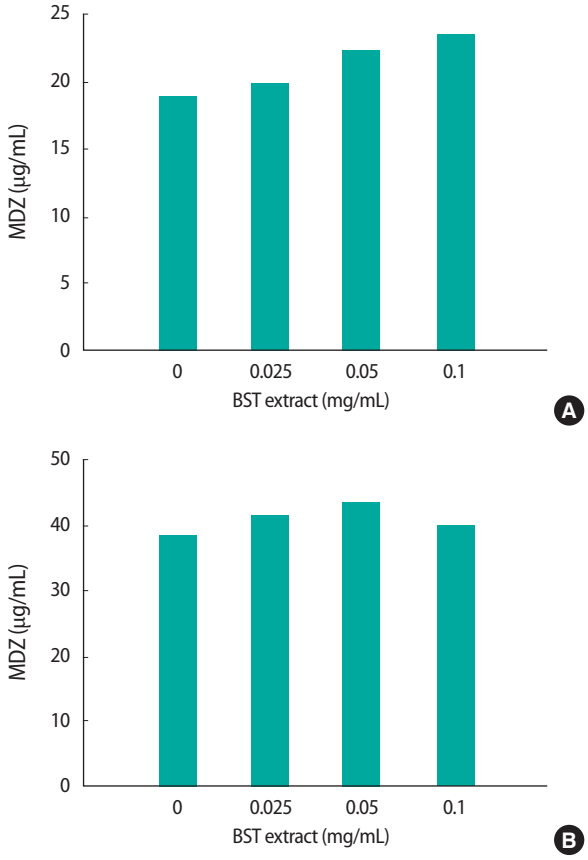

Figure S1. The concentration of MDZ in the supernatant of artificial gastric juice (A) and intestinal juice (B) with MDZ and BST. The data shown are the means of duplicates. MDZ, midazolam; BST, Banha-sasim-tang.