



Single-Dose Oral Toxicity of Fermented *Scutellariae Radix* Extract in Rats and Dogs

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The aim of this study was to investigate the acute oral toxicity of fermented *Scutellariae Radix* (JKTMHGu-100) in rats and dogs. JKTM-HGu-100 was orally administered at a dose of 2,000 mg/kg in Sprague-Dawley rats. An escalating single-dose oral toxicity test in beagle dogs was performed at doses of 500, 1000, and 2000 mg/kg with 4-day intervals. Clinical signs, changes in body weight, mortality, and necropsy findings were examined for 2 weeks following oral administration. No toxicological changes related to the test substance nor mortality was observed after administration of a single oral dose of JKTM-HGu-100 in rats or dogs. Therefore, the approximate lethal dose (LD) for oral administration of JKTMHGu-100 in rats was considered to be over 2,000 mg/kg, and the maximum tolerance doses (MTDs) in rats and dogs were also estimated to be over 2,000 mg/kg. These results indicate that JKTM-HGu-100 shows no toxicity in rodents or non-rodents at doses of 2,000 mg/kg or less.

Key words: *Scutellariae Radix*, Single oral toxicity, Rat, Dog

INTRODUCTION

As people become more interested in the food safety and well-being in recent years, so the demand for functional food from natural sources is increased (Lee *et al.*, 2003). Therefore, medicinal plants have provided high opportunities for the development of herbal food products, dietary supplements, and functional foods (Chau and Wu, 2006). Toxicological aspect of natural origin-functional foods has been neglected because they have been used for a long time on various purposes. However, medicinal plants have also undesirable side effects as patients on herbal medicine therapy experienced some side effects (Chan and Cheung, 2000).

Scutellaria baicalensis Georgi is a medicinal plant widely distributed throughout Asia. Its dried root, *Scutellariae*

Radix (SR), is recognized as bioactive ingredients to treat various disease conditions accompanying inflammation, fever, allergy, oxidative stress, cardiovascular diseases, respiratory and gastro-intestinal infections (Gong and Sucher, 1999; Kang *et al.*, 2003).

Investigations on the chemical components and pharmacological effects of SR have demonstrated that flavonoids were the main bioactive components. Flavonoids are the most abundant constituents in SR and over 30 of them have been identified and quantified by various analytical techniques (Li *et al.*, 2004). Baicalin, wogonoside, and their aglycones baicalein and wogonin are major effective flavones in dried roots of SR (Li and Sheu, 1995; Choi *et al.*, 2002). Four flavones, baicalin, wogonoside, baicalein and wogonin, possess anticancer, anti-HIV, anti-hepatitis B virus, anti-viral, anti-inflammatory, anti-oxidant, anti-convulsant and anxiolytic effects. Moreover, these compounds are being widely extracted as effective constituents for pharmaceutical, cosmetic and also food industries (Makino *et al.*, 2006; Mustafa and Turner, 2011).

Glucose-conjugated isoflavones are highly polar, water soluble compounds. They are more difficult to be absorbed by the intestinal epithelium than the corresponding aglycones (Miksicek, 1995; Day *et al.*, 1998; Setchell *et al.*, 2002). Bacterial or enzymatic transformation has received

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particular attention because it contains structurally modified components that enhance the herb's beneficial effects (Cheigh and Park, 1994; Blaut and Clavel, 2007; Kim *et al.*, 2008). These modified components (baicalein and wogonin) show better bioactivity and higher bioavailability than their glucuronides (Gao *et al.*, 1999; Lai *et al.*, 2003; Park *et al.*, 2007). However, it is very difficult to obtain baicalein and wogonin directly because of their low contents in *Scutellariae Radix*. Therefore, a better method of obtaining baicalein and wogonin is by enzyme hydrolysis from baicalin and wogonoside, which are present in higher content in aqueous extract of *Scutellariae Radix*.

The purpose of this study was to investigate the acute oral toxicity of fermented *Scutellariae Radix* (JKTM-HGu-100) by *Lactobacillus brevis* in rats and dogs.

MATERIALS AND METHODS

Preparation of standardized *Scutellariae baicalensis* aqueous extract. The radix of *Scutellariae baicalensis* was obtained from a local herbal market in Hwasun, Korea and a voucher specimen (No. 2011-SB-07) was deposited at the herbarium of Jeollanamdo Development Institute for Traditional Korean Medicine. *Scutellariae Radix* (SR, 45 kg) was minced and boiled in a 10-fold volume of twice-filtered water for 4 hr two times. The extractive solution was filtered with 100 mesh sieve and concentrated to 5~10 hPa at 50~60°C. The yield of aqueous extract from SR was 74.8%.

Preparation of JKTM-HGu-100. *Lactobacillus brevis* (KCCM 35464) obtained from the Korean National Genebank

Information Center (Suwon, Korea) was used to transform the flavonoids in aqueous extract of *S. Radix*. *Lactobacillus brevis* were activated by inoculation into de Man-Rogosa-Sharpe (MRS) broth medium (Becton Dickinson Co., NJ, USA) at 37°C for 48 hr. Fermentation using SR extract was prepared according to established procedures (Kim *et al.*, 2010). Fermentation was carried out in a 5 L vertical glass fermenter (Fermentec, Seoul, Korea) with a 3 L working volume of medium (pH 6.0) including 100 g of SR extract at 37°C up to 48 hr. A 5% (v/v, 10⁸ cfu/ml) culture of *L. brevis* grown in MRS broth was used as an inoculum. The cultures were stirred at 20 rpm throughout the fermentation, and stored at -20°C. The frozen samples were freeze-dried for isoflavone analysis.

Profiling the chemical contents of JKTM-HGu-100 by HPLC. Extraction of isoflavones from JKTM-HGu-100 and quantification were performed as reported (Wei *et al.*, 2004). The sample was analyzed using a modified HPLC method (Lai *et al.*, 2001). Sample (10 mg) was dissolved in 1 ml of 70% methanol and filtered through 0.2 µm filter (Simplicity Filtration SystemTM, Millipore, Seoul, Korea) before injection into the high performance liquid chromatography system. HPLC was performed using a Shimadzu LC 20A system (Kyoto, Japan). Separation was achieved on Kinetex C₁₈ reversed phase column (100 mm × 4.6 mm, 2.6 µm, Phenomenex, Aschaffenburg, Germany) with a guard column (4.0 mm × 3.0 mm, 3.0 µm) using a mobile phase composed of A (0.1% formic acid in distilled water) and B (acetonitrile). A gradient elution procedure was used as follows: 0~3 min 20% B, 3~12 min 75% B, 12~15 min

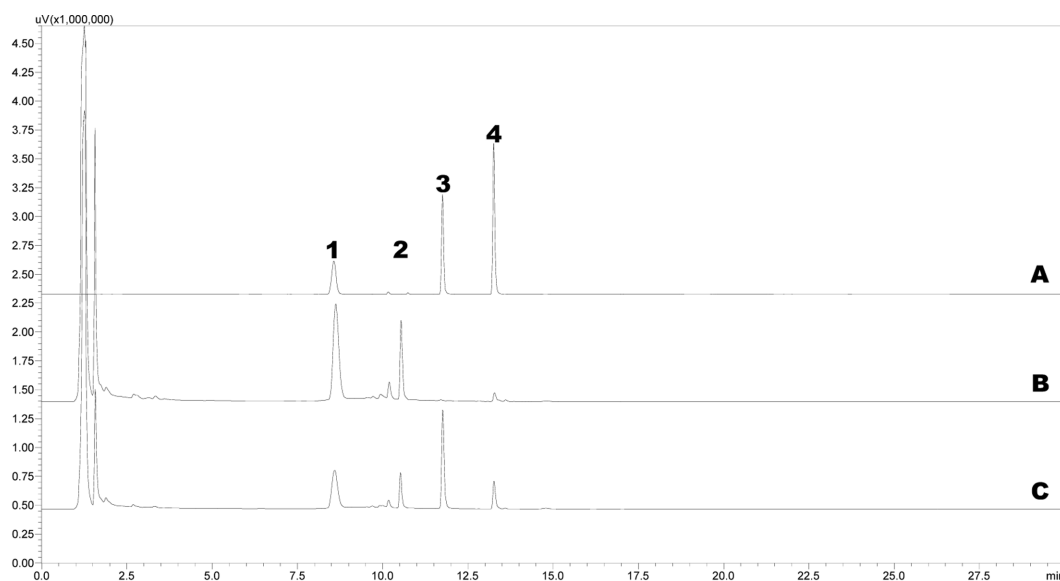


Fig. 1. HPLC Chromatograms of the standard mixture of three major compounds (A), and pre- and post-biotransformation of *Lactobacillus brevis* at 0 hr (B) and 48 hr (C) at 37°C. 1: baicalin (8.607 min), 2: wogonoside (10.530 min), 3: baicalein (11.768 min), 4: wogonin (13.273 min).

75% B, 15~16 min 90% B, 16~21 min 90% B, 21~22 min 20% B and 22~30 min 20% B. The sample injection volume was 10 μ l and the column temperature was maintained at 40°C with a flow rate of 0.8 ml/min and the total time of a single run was 30 min. The detection wavelength was set at 276 nm (Fig. 1). The contents of baicalin, baicalein and wogonin were determined to be 4.42, 6.3% and 1.7% in JKTM-HGu-100, respectively.

Animals. Single oral toxicity study was carried out on 5 weeks old Sprague-Dawley rats of both sexes (male; 139.8~154.1 g, female; 121.3~144.6 g). Rats were obtained from Orient Bio (Sungnam, Korea). Rats were acclimated in the controlled room (temperature, 22.0 \pm 3.0°C; relative humidity, 50.0 \pm 20.0%; air circulating frequency, 10~15 times/hr; artificial light, 300 Lux from 8 am to 8 pm; noise, < 50 db) for 14 days before experimentation. The animals were housed in the polycarbonate cages (27 cm \times 50 cm \times 18 cm) and were supplied with laboratory animal feed pellet (Cargill Agri Purina, Gunsan, Korea) and filtered water *ad libitum*.

Beagle dogs of both sexes aged 5.5 months (male; 4.6~5.4 kg, female; 4.8~5.8 kg) were used for single oral dose-escalating toxicity assessment. Dogs were obtained from Woojung BSC (Suwon, Korea). Dogs were acclimated in the controlled room (temperature, 20.0 \pm 3.0°C; relative humidity, 50.0 \pm 20.0%; air circulating frequency, 10~15 times/hr; artificial light, 300 lux from 8 am to 8 pm; noise, < 50 db) for 14 days before experimentation. The animals were housed in stainless-steel cages (90 cm \times 120 cm \times 100 cm) and were supplied with laboratory animal feed pellet (Cargill Agri Purina, Gunsan, Korea) and filtered water *ad libitum*.

This study was performed according to "Guide for the Care and Use of Laboratory Animals" prepared by the Korea Testing and Research Institute (2010-12-10). Safety evaluation of standardized JKTM-HGu-100 was evaluated by good laboratory practices (GLP) guideline of Korea Food and Drug Administration.

Single dose oral toxicity study in rats. Healthy adult male and female Sprague-Dawley rats (7~8 weeks aged, n = 5 in each group) were assigned to a control group or treatment group. The dosage level was selected as 2,000 mg/kg according to the recommendation by KFDA (2009-116, 2009) and the Organization for Economic Co-operation and Development (OECD) (2001) guidelines. The test chemical was dissolved in water and administered by gavage in a single dose of JKTM-HGu-100 in a volume not to exceed 10 ml/kg body weight at 2,000 mg/kg after overnight fasting (about 12 hr, water was not restricted). The animals in the control group received the vehicle only.

Single oral dose escalating toxicity study in dogs. Healthy adult male and female dogs (n = 2 in each group) were randomly assigned to 2 experimental groups: a treat-

ment group (dose escalation cohorts of 500, 1000 and 2000 mg/kg of JKTM-HGu-100 with 4-day intervals. The dosage level was selected according to the recommendation by the KFDA (2009-116, 2009) guidelines. The test chemical was dissolved in water and administered by gavage in a single dose in a volume not to exceed 5 ml/kg body weight after overnight fasting (about 12 hr, water was not restricted).

Observation of clinical signs. Throughout the single and escalation dose toxicity studies, all animals were observed daily for clinical signs of toxicity, morbidity and mortality. Detailed clinical observations were recorded daily.

Body weight changes. In single oral toxicity study, body weights of each rat were measured at the initiation of treatment and once a week (7th and 14th day) after treatment. In case of single oral dose escalating toxicity studies, body weights of each dog were measured at the initiation of treatment and once a week throughout the treatment and recovery period.

Gross pathological findings. Rats and dogs for the scheduled necropsy were fasted overnight and anesthetized with intramuscular injection of ketamine and tiletamine/zolazepam (5 mg/kg of Ketamin[®], Yuhan Co., Korea; 10 mg/kg of zoletil 50[®], Virbac Lab., Carros, France) on the day of necropsy. Under anesthesia, autopsy was conducted on every animal and all major organs and tissues including heart, lung, liver, stomach, intestine, kidney, adrenal gland, spleen, and ovary or testis were examined for gross lesions. All animals were discarded after macroscopic abnormalities were recorded.

Statistical analyses. Body weight changes were compared using Student *t*-test by SPSS program (version 18.0). Differences at *p* < 0.05 were considered statistically significant.

RESULTS

Single-dose toxicity study in rats. Throughout the experiment, Sprague-Dawley rats appeared healthy, inquisitive and active. No illness or death occurred (Table 1). No observable difference in the animal hair luster was noticed between the groups, and there were no signs of gastrointestinal upsets including diarrhea (Table 1). During the 14 days treatment, no significant difference (*p* > 0.05) in the body weight gains between the experimental and control groups. All tested animals showed the increase in body weight with time (Table 2).

Escalating-dose toxicity study in dogs. Throughout the experiment, beagle dogs appeared healthy, inquisitive

Table 1. Mortality and clinical signs of single oral toxicity study in Sprague-Dawley rats

Sex	Group	No. of animals	Dose (mg/kg B.W.)	Clinical signs	Mortality (dead/total)	Approximate maximum tolerance dose (MTD), (mg/kg)
Male	Vehicle control	5	0	No abnormalities detected	0% (0/5)*	> 2,000
	Treatment	5	2,000	No abnormalities detected	0% (0/5)	
Female	Vehicle control	5	0	No abnormalities detected	0% (0/5)	> 2,000
	Treatment	5	2,000	No abnormalities detected	0% (0/5)	

*No. of dead animals/No. of tested animals.

Table 2. Body weight gains after oral treatment of JKTM-HGu-100
Unit: g

Sex	Group	Intervals		
		Day 0~Day 7	Day 7~Day 14	Day 0~Day 14
Male	Vehicle control	93.6 ± 4.8	75.8 ± 8.7	169.3 ± 11.7
	2,000 mg/kg	95.4 ± 3.4	61.6 ± 3.5	157.1 ± 6.1
Female	Vehicle control	50.8 ± 5.6	28.0 ± 4.6	78.8 ± 10.0
	2,000 mg/kg	42.5 ± 10.3	28.2 ± 6.1	70.7 ± 15.3

and active. No illness or death occurred (Table 3). No observable difference in the animal hair luster was noticed between the groups, and there were no signs of gastrointestinal upsets including diarrhea or vomiting (Table 3). During the 21 days treatment, no statistically significant differences in body weights were noted in males and females treated with the test substance in comparison to controls ($p > 0.05$). All tested animals showed the increase in body weight with time (Fig. 2).

Table 3. Mortality and clinical signs of single oral dose escalating toxicity study in beagle dogs

Sex	Group	No. of animals	Dose-escalating (mg/kg B.W.)	Clinical signs	Mortality (dead/total)	Approximate maximum tolerance dose (MTD), (mg/kg)
Male	Vehicle control	2	0 → 0 → 0	No abnormalities detected	0% (0/2)*	> 2,000
			500	No abnormalities detected	0% (0/2)	
	Treatment	2	1,000	No abnormalities detected	0% (0/2)	
			2,000	No abnormalities detected	0% (0/2)	
Female	Vehicle control	2	0 → 0 → 0	No abnormalities detected	0% (0/2)*	> 2,000
			500	No abnormalities detected	0% (0/2)	
	Treatment	2	1,000	No abnormalities detected	0% (0/2)	
			2,000	No abnormalities detected	0% (0/2)	

*No. of dead animals/No. of tested animals.

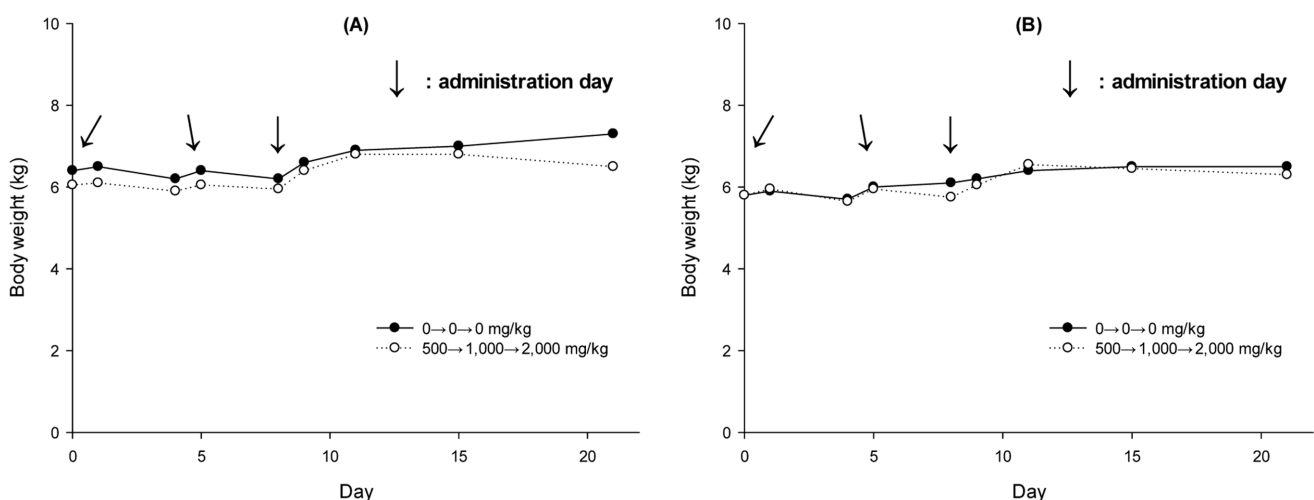
**Fig. 2.** Body weight changes of single oral dose escalating toxicity study in male (A) and female (B) beagle dogs.

Table 4. Gross pathological findings of single oral and dose escalating toxicity study in rats and dogs

Species	Sex	Group	Remarkable organ	Gross finding	Frequency	Type of sacrifice
Rats	Male	Vehicle control	Not remarkable	-	0/5	Schedule (Day 14)
		2,000 mg/kg	Not remarkable	-	0/5	Schedule (Day 14)
	Female	Vehicle control	Not remarkable	-	0/5	Schedule (Day 14)
		2,000 mg/kg	Not remarkable	-	0/5	Schedule (Day 14)
Dogs	Male	Vehicle control	Not remarkable	-	0/1	Schedule (Day 21)
		500 → 1,000 → 2,000	Not remarkable	-	0/2	Schedule (Day 21)
	Female	Vehicle control	Not remarkable	-	0/1	Schedule (Day 21)
		500 → 1,000 → 2,000	Not remarkable	-	0/2	Schedule (Day 21)

-, No abnormalities detected.

Gross pathological findings. At the end of the observation period all animals were sacrificed and autopsied. All major organs including heart, lung, liver, stomach, intestine, kidney, adrenal gland, spleen, and ovary or testis were examined grossly. There were no abnormal lesions in both rats and dogs regardless of the dose of JKTM-HGu-100 (Table 4).

DISCUSSION

Traditional herbal medicines are generally regarded as safe because they have been used for various purposes since a long time ago, but are scientifically not always so. Their efficacy is sometimes suspected on the ground that their traditional remedies contain only very low active principles and rely on just magical-energetic principles (Firenzuoli and Gori, 2007; Jordan *et al.*, 2010). Therefore, consumers still question about their efficacy and safety.

Scutellariae Radix is one of the important components of herbal prescription, such as Hwang Ryun Hag Dok Tang, Hwang Kum Hwang Ryun Tang, Yong Dam Sagan Tang (YDST) and So Siho Tang and is widely used in traditional herbal medicine for the treatment of inflammation, fever, hepatitis, allergic diseases, hypertension and so on (Lu *et al.*, 2007; Wang *et al.*, 2007; Lee and Chang, 2010).

Fermentation is commonly used to break down certain undesirable compounds, induce effective microbial conversion, and improve potential nutraceutical values (Hubert *et al.*, 2008; Oboh *et al.*, 2008). Many studies have reported the pharmacological efficacy of dosage form of *S. Radix* including solvent extract, fermentation and biotransformation of bacteria, but there is no information on its safety, such as single dose toxicity. In the present study, we investigated the acute oral toxicity of fermented *S. Radix* (JKTM-HGu-100) by *Lactobacillus brevis* in rat and dog models. JKTM-HGu-100 was once orally administered at a dose of 2,000 mg/kg in rats and at dose levels of 500, 1,000 and 2,000 mg/kg at interval of 4 days in dogs. Mortality, clinical signs, body weight changes and gross finding were monitored during the study period.

As the results, no mortality was observed in rats and dogs treated with JKTM-HGu-100. During the observation period, no abnormal clinical signs were shown in animals regardless of the dose level used in this study. No significant change on body weight and gains was detected between the experimental (or treatment) and control groups in rats and dogs. The body weight detected in this study was well corresponded to the body weight ranges of the same aged normal rats and dogs (Yu *et al.*, 2005; Kim *et al.*, 2007). Chang and But (1987) reported that oral administration of the aqueous decoction of *S. Radix* alone (10 g/kg body weight) in rabbits caused sedation, but no deaths. And a single oral dose of an aqueous extract (12 or 15 g/kg body weight) in dogs caused no reactions during 48 hr of observation. These results are consistent with our results.

In conclusion, the results obtained in this study suggest that approximate lethal dose (LD) and maximum tolerance dose (MTD) values after treatment with JKTM-HGu-100 in rats and dogs were considered over 2000 mg/kg in both female and male animals. The results on the single-dose oral toxicity of JKTM-HGu-100 indicate that it is not possible to reach oral dose levels related to death or dose levels with any harmful side-effects. Furthermore, the use of fermented herbal medicine can increase the bioactivity and bioavailability of the drug, expecting new herbal prescriptions.

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