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Original Article

Safety of a Traditional Korean Medicine, *Cheonggan* extracts (CGX): A 2-week Single-dose Toxicity Study in SD Rats and Beagle Dogs

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Objectives: To evaluate the acute toxic effects and approximate lethal dose of *Cheonggan* extracts (CGX) in SD rats and beagle dogs.
Methods: Male and female rats were divided into 4 groups (Control, CGX 1250, CGX 2500, CGX 5000) respectively and male and female dogs were divided into two groups respectively (Control, CGX 5000) respectively. A single oral dose of CGX was treated to the rats and dogs. Mortality, signs of gross toxicity, and behavioral changes were observed over 14 days. All animals were observed every hour for 4 hours after administration and once a day thereafter for 14 days. Body weights were determined at 0_{th}, 7_{th}, and 14_{th} days. All surviving animals were sacrificed and necrotized. Major organs were inspected visually for gross findings.
Results: No animals died in any of the groups during the experimental period (2 weeks), rats or dogs. Body weights of rats and dogs during the experiment continuously increased in all groups but there was no significant change. No abnormal clinical signs were observed for 2 weeks after a single administration of CGX in any dose group of CGX, rats or dogs. No abnormal findings in major organs were observed in any group of rats or dogs.
Conclusion: CGX does not have acute toxic effects in rats or dogs. Therefore, an approximate lethal dose is assumed to exceed 5000 mg/kg in both rats and dogs.

Key Words : traditional Korean medicine, herbal medicine, acute toxicity tests

Introduction

Recently, the use of herbal medicinal products has been increasing worldwide. Fogden and Neuberger reported that in 39% and 65% use herbal products to improve their health and diseases in the USA and Germany respectively[1)]. They are generally regarded as safe because of their natural origin and long history of application. However, based on the expanding worldwide usage of these products, the possibility for adverse effects is increasing[2,3)]. Thus, there is strong demand for scientific evidence regarding the safety of herbal products. In fact, many side effects have been reported, mainly associated with contamination by foreign matter, toxic internal ingredients, and drug interactions with Western

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medicines[4,5]. In particular, toxicity must be evaluated in herbal medicines composed of multiple herbs, even when individual herbal components are known to be safe.

CGX is a traditional Korean medicine composed of 13 herbs. It has been prescribed for patients suffering from various chronic liver diseases, such as alcoholic liver disorders, chronic viral hepatitis, and liver cirrhosis[6]. The pharmaceutical effects of CGX have been demonstrated clinically and in various animal models including alcoholic liver injury, fatty liver, CCl4-induced acute injury, and DMN-induced liver fibrosis[7-10]. Because only 10 of the 13 herbs comprising CGX are approved for food supplements by the Korea Food and Drug Administration (KFDA), it cannot be used in dosage form such as tablet, viscous extract or powder without approval by the KFDA. Therefore, oral administration of the medicine are inconvenient. To develop a hepatotherapeutic for widespread use, toxicological information should be provided.

A single-dose toxicity test should be performed prior to a repeated-dose toxicity test. This study investigated acute toxic effects and approximate lethal dose of CGX in SD rats and beagle dogs.

Materials and Methods

1. Preparation of CGX

An initial mixture of dried herbs was made following Table 1. Briefly, a total of 120 kg of the mixture was boiled in 1200 L of distilled water for 4 h at 100℃, then filtered using a 300-mesh filter (50 μm) and then condensed. A 10.71% (w/w) lyophilized CGX aqueous extract was obtained from the dried mixture. The condensed extracts containing 62% of solid were stored under -20℃ until use and administration dosages were prepared on the basis of dry extracts. This manufacturing process for CGX follows the process given in over-the-counter Korean monographs by Samik Pharmacy (Seoul, Korea). Final CGX extract was satisfied with criteria according to the Korean Pharmaceutical Codex including quantity of each herb, contamination including heavy metals, general bacteria, fungi, and specific pathogens, and quantity of ingredients.

2. Fingerprint of CGX

Fingerprint of CGX was made using high-performance liquid chromatography-diode array detector-

Table 1. Herbal Composition of CGX

Herbal name	Scientific name	Voucher specimen number	Ratio
Artemisiae capillaris Herba	*Artemisia capillaris*	AC-2007-01-He	5
Trionycis Carapax	*Trionyx sinensis*	TS-2007-01-Ca	5
Raphani Semen	*Raphanus sativus*	RS-2007-01-Se	5
Atractylodis Rhizoma Alba	*Atractylodes macrocephala*	AM-2007-01-Rh-Al	3
Poria	*Poria cocos*	PC-2007-01-Po	3
Alismatis Rhizoma	*Alisma orientalis*	AO-2007-01-Rh	3
Atractylodis Rhizoma	*Atractylodes chinensis*	AC-2007-02-Rh	3
Salviae Miltiorrhizae Radix	*Salvia miltiorrhiza*	SM-2007-01-Ra	3
Polyporus	*Polyporus umbellatus*	PU-2007-01-Po	2
Ponciri Fructus	*Poncirus trifoliata*	PT-2007-01-Fr	2
Amomi Fructus	*Amomum villosum*	AV-2007-01-Fr	2
Glycyrrhizae Radix	*Glycyrrhiza uralensis*	GU-2007-01-Ra	1
Aucklandiae Radix	*Aucklandia lappa*	AL-2007-01-Ra	1

mass (HPLC-DAD-MS). The main compounds of the five major compositional herbs *Glycyrrhizae Radix, Artemisiae Capillaris Herba, Raphani Semen, Salvia Miltiorrhizae Radix, Atractylodis Rhizoma Alba and Ponciri Fructus* are liquiritin and glycyrrhizin, 6,7-dimethoxycoumarin and scopoletin, naringin, rosmarinic acid, and atractylenolide III and poncirin, respectively. Briefly, after dissolution (20 mg of CGX and 2 mg of 5 herbal extracts in 1 mL of water, 0.01 mg of eight standards in 1 mL of water or 50% methanol) and filtration, these drugs were subjected to HPLC analysis. The HPLC system consisted of a SCL-10A system controller, LC-10AD pump, SPD-10MVP diode array detector and CTO-10AS column temperature controller (Shimadzu, Kyoto, Japan). A Phenomenex Prodigy C18 (2.0 × 150 mm) column was eluted with solvents A (10% acetonitrile in water containing 0.05% formic acid) and B (90% acetonitrile in water) at flow rate of 0.4 mL/min. Solutions 100% A and 0% B changing over 30 min to 25% B, 60 min to 75% B were used (Fig. 1).

3. Animals and management

Six-week-old male and female CrjBgi: CD (SD) rats were obtained from Orient Bio (Gyoenggi-do, Korea). The animal room was maintained at a temperature of 21.8~24.3℃, 49.1~58.2% relative humidity, air ventilation of 10-15 times/h, and ambient light (200-300 lux) controlled to produce a 12-h light/12-h dark cycleed tve rats were housed in separate wire cages (500 × 300 × 200 mm), and identification cards recording the test number and animal number were attached to themedDuring the study, the rats were fed *ad libitum* with Purina ceriified rodent meal sterilized with radiation (2.0 M rad) and UV-sterilized water. The animals were accliioned to laboratory conditions for 1 week before use.

Five-month-old, male and female beagle dogs were obtained from Marshall Beijing (Beijing, China). The animal room was maintained at a temperature of 18.2~20.1℃, 44.1~48.9% relative humidity, air ventilation of 10~15 times/h, and ambient light (200-300 Lux) controlled to produce 12-h light/dark cycle. The dogs were acclimated to laboratory conditions for 3 weeks before use. The dogs were fed with 320 ± 20 g per day of Gold Pet (Purina, Sungnam, Korea) twice a day and filtered water *ad libitum* during the study. The dogs were kept in separate wire cages (900×1200×1000 mm) and identification cards stating test number and animal number were attached to the cage.

This study was performed at the Korea Testing and Research Institute after approval of the Institutional Animal Care and Use Committee according to the "Guidelines for Toxicity Tests of Drugs and Related Materials, Notification No. 2005-60" and "Guidelines for Management for Nonclinical Test, Notification No. 2005-79" prepared by KFDA[11,12].

4. Single–dose toxicity test in rats

Forty male rats and forty female rats were assigned into four groups of 10 individuals each. After 12 h fasting, the rats were given a one-time dose of CGX (1250, 2500, or 5000 mg/kg) or distilled water (control group) orally using an oral Zonde needle. At the start of the experiment, male rats weighed 183.9-209.3 g and female rats weighed 142.6-161.8 g.

5. Single–dose toxicity test in dogs

Four male and four female dogs respectively were divided into 2 groups of 2 animals each. After overnight fasting, CGX (5000 mg/kg) was administered once orally using a stomach tube to the experiment animals while only distilled water was administered to the controls. At the starting time, the dogs weighed 6.996 ~ 7.398 kg (male) and 6.168 ~ 7.116 kg (female).

6. Observation of clinical signs and mortality

Mortality, signs of gross toxicity, and behavioral changes were observed every hour for 4 hours after

administration and once a day after that for 14 days. Body weight was determined at 0th, 7th, and14th days.

7. Necropsy findings

All survival animals were sacrificed and necrotized. Dogs were sacrificed via bleeding under anesthesia of Zoletil (Virbac Lab. Carros, France) and major organs were examined visually.

8. Statistical analyses

Body weight was analyzed for homogeneity of dispersion using Levene's test. One-way analysis of variance (ANOVA) was performed to evaluate the significance of dispersion, and significance was confirmed with Scheffe's multiple comparison test. Dunnett's multiple comparison test was performed for non-homogeneous data. All analyses were perf-ormed using ver. 10.1 of the SPSS program (SPSS Inc., Chicago, IL, USA).

Results

1. Mortality

No animal (dog or rat) died in any of the groups during the experimental period (2 weeks) (Table 2 and 3).

2. Body weight changes

Body weight of rats increased continuously during the experiment in all groups but there was no significant change (Table 4). When compared with controls, there was no significant change in the weight gain of CGX-treated dogs (Table 5).

3. Clinical signs

No abnormal clinical signs were observed for 2 weeks after a single administration in any dose group of rats which were given different doses of CGX (Table 6). In dogs, no abnormal signs were observed in the control or CGX group (5000 mg/kg) (Table 7).

Table 2. Mortality of SD Rats Treated with Single-dose of CGX or DW for 2 Weeks

Group	Dose (mg/kg)	Mortality (dead / total)	
		Male	Female
G1	0	0% (0 / 10)[a]	0% (0 / 10)
G2	1250	0% (0 / 10)	0% (0 / 10)
G3	2500	0% (0 / 10)	0% (0 / 10)
G4	5000	0% (0 / 10)	0% (0 / 10)

Table 3. Mortality of Beagle Dogs Treated with Single-dose of CGX or DW for 2 Weeks.

Group	Dose (mg/kg)	Mortality (dead / total)	
		Male	Female
G1	0	0% (0 / 2)[a]	0% (0 / 2)
G2	5000	0% (0 / 2)	0% (0 / 2)

Table 4. Changes of Mean Body Weights of SD Rats Treated with Single-dose of CGX or DW for 2 Weeks

Group	Dose (mg/kg)	Sex	Number of animals	Day(s) after treatment		
				0	7	14
G1	0	Male	10	196.7±7.2	284.1±13.5	332.4±17.4
		Female	10	149.1±5.1	196.4±10.2	218.8±12.2
G2	1250	Male	10	196.9±5.6	283.2±14.8	333.0±24.1
		Female	10	151.6±4.9	197.3±8.7	221.4±10.4
G3	2500	Male	10	196.0±5.8	286.1±13.9	334.4±19.5
		Female	10	148.1±4.2	194.3±7.5	219.3±10.9
G4	5000	Male	10	196.5±6.3	279.1±18.6	329.6±26.6
		Female	10	149.3±6.8	195.1±9.2	216.7±12.4

Table 5. Changes of Mean Body Weights of Beagle Dogs Treated with Single-dose of CGX or DW for 2 Weeks

Group	Dose (mg/kg)	Sex	Number of animals	Day(s) after treatment		
				0	7	14
G1	0	Male	2	7.188±0.272	7.297±0.341	7.503±0.406
		Female	2	6.642±0.670	6.813±0.604	7.081±0.559
G2	5000	Male	2	7.347±0.072	7.596±0.133	7.751±0.086
		Female	2	6.768±0.223	7.113±0.284	7.300±0.246

Table 6. Clinical Signs of SD Rats Treated with Single-dose of CGX or DW for 2 Weeks

Group	Dose (mg/kg)	Sex	Number of animals	Clinical signs
G1	0	Male	10	No Abnormality Detected
		Female	10	No Abnormality Detected
G2	1250	Male	10	No Abnormality Detected
		Female	10	No Abnormality Detected
G3	2500	Male	10	No Abnormality Detected
		Female	10	No Abnormality Detected
G4	5000	Male	10	No Abnormality Detected
		Female	10	No Abnormality Detected

Table 7. Clinical Signs of Beagle Dogs Treated with Single-dose of CGX or DW for 2 Weeks

Group	Dose (mg/kg)	Sex	Number of animals	Clinical signs
G1	0	Male	2	No Abnormality Detected
		Female	2	No Abnormality Detected
G2	5000	Male	2	No Abnormality Detected
		Female	2	No Abnormality Detected

Table 8. Necropsy Findings of SD Rats Treated with Single-dose of CGX or DW

Findings	G1 0 mg/kg		G2 1250 mg/kg		G3 2500 mg/kg		G4 5000 mg/kg	
	Male	Female	Male	Female	Male	Female	Male	Female
Number examined	10	10	10	10	10	10	10	10
No abnormalities detected	10	10	10	10	10	10	10	10

Table 9. Necropsy Findings of Beagle Dogs Treated with Single-dose of CGX or DW

Findings	G1 0 mg/kg		G4 5000 mg/kg	
	Male	Female	Male	Female
Number examined	2	2	2	2
No abnormalities detected	2	2	2	2

4. Necropsy findings

Major organs were removed and inspected visually. No abnormal findings were observed in any group of rats or dogs (Table 8, 9).

Discussion

Around the world, various medicinal plants and botanical drugs have been widely adopted as primary therapeutics or supplements for treating disease[13-15]. Moreover, many herbal formulae or plants have been

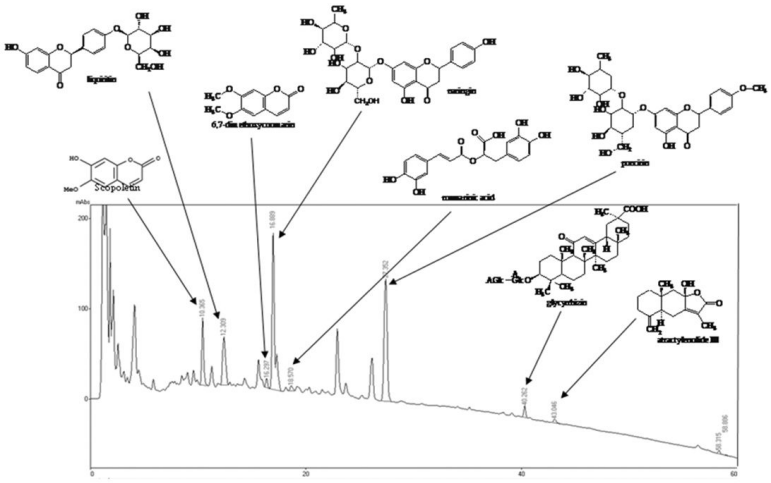

Fig. 1. Fingerprint of CGX and its main components using high-performance liquid chromatography-diode array detector-mass (HPLC-DAD-MS).

After dissolution (20 mg of CGX and 2 mg of 5 herbal extracts in 1 mL of water, 0.01 mg of eight standards in 1 mL of water or 50% methanol) and filtration, these drugs were subjected to HPLC analysis. A Phenomenex Prodigy C18 column was eluted with solvents A (10% acetonitrile in water containing 0.05% formic acid) and B (90% acetonitrile in water) at flow rate of 0.4 mL/min. Solutions 100% A and 0% B changing over 30 min to 25% B, 60 min to 75% B were used.

traditionally prescribed for patients with hepatic disorders[16-18]. Several medicines, including silymarin, glycyrrhizin, *sho-saiko-to, Phyllanthus amarus, Picrorrhiza kurroa*, LIV 52 and so on have been used particularly for liver disease[17,20]. CGX, a traditional Korean medicine, has been used to treat patients with various liver diseases since 2001 at Daejeon Oriental Hospital. Hepatoprotective effects are well demonstrated not only in animal studies but also clinical studies[6-10].

As the use of herbal remedies grows worldwide, standardization and scientific evidence of the safety of such products is demanded[21]. Adverse effects of herbal products such as hepatotoxicity and nephrotoxicity mainly originate from a misidentification of the plant, incorrect preparation, heavy metal contamination, and adulteration[4,22-24]. The standardization of multiple herbal medicines is a very important issue for toxicology. CGX was prepared by a pharmaceutic company according to Good Manufacturing Practices (GMP). All herbs used were in accordance with the Korean Pharmacopoeia. A three-dimensional fingerprint was made using high-performance liquid chromatography-diode array detector-mass (HPLC-DAD-MS) (Fig. 1).

Regulations on herbal products vary country by country, even when these products are consumed as dietary supplements. Three of the 13 herbs used in CGX are not accepted for use as food supplements by the KFDA. Thus, toxicology studies are needed to develop it as general hepatic protective herbal drug.

In this study, we examined the safety of CGX, an herbal-derived hepatotherapeutic composed of 13 herbs, via a 2-week single-dose toxicity study in rats and dogs. For the single-dose toxicity study, we treated the rats and dogs with 50 times the recommended dose of CGX. The highest dose (5000 mg/kg) is the maximum dosage in single dose toxicity test. In general, toxic response is different among test species. Larger species (e.g., dogs or humans) are more sensitive than smaller species (eg, mice or rats) to a short-term toxicity[25]. Lehman reported that a human is 6 times as sensitive as dog and 10 times as sensitive as a rat[26]. However, some organs of some animals show more sensitive-specific toxic effects than humans and sensitivity is different among different species dependent on kind of chemical[27]. Therefore we can provide good information through this study by using two different species.

No abnormal clinical signs such as diarrhea, anorexia, or change in activity were observed and there were no abnormal gross findings. Normal weight gain was shown over the 2 weeks of the experiment in the rats and the dogs. Therefore, an approximate lethal dose is assumed to exceed 5000 mg/kg in both rat and dog. The results indicate that CGX does not have an acute toxic effects in either rats or dogs. However, this result is limited to acute toxic responses. Accordingly, further study is needed to assess the long-term toxicity potential.

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