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Original Article

Single Oral Dose Toxicity Study of Cheongmokpye in Mice

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ABSTRACT

Background: Acute oral safety studies of herbal medicines are needed not only to identify dose ranges but also to reveal any possible side effects. Although Cheongmokpye has shown greater therapeutic effects than the individual herbs, the toxicological profile of Cheongmokpye is not available. **Objectives:** To assess the acute single oral dose toxicity of Cheongmokpye, a poly herbal complex, in female and male ICR mice, which represents a crucial step in the development of health-related functional food ingredients. **Materials and Methods:** The experiment evaluated the toxicity of Cheongmokpye orally administered to mice at doses of 5,000, 2,500, 1,250 and 0 (vehicle control) mg/kg in a 10 mL/kg volume of distilled water. The maximum dosage was set to 5,000 mg/kg based on preliminary tests and substance solubility. The study aimed to identify the potential toxicity and target organs susceptible to toxicity under the maximum dose after a single oral administration of Cheongmokpye. **Results:** The result of this study showed no treatment-related changes in mortality, clinical parameters, body weight, organ weight, hematological parameters, or gross necropsy findings. These results demonstrated the non-toxic nature of Cheongmokpye in rodents and indicated that it is safe even at the highest tested dose. **Conclusion:** The findings of this study showed that Cheongmokpye is non-toxic for rodents at doses up to 5,000 mg/kg. This implies its potential safety for use as a functional health food ingredient. These findings may be significant in the development of health-related functional foods and can provide valuable insights for research and related industries.

Keywords: Cheongmokpye, *Codonopsis lanceolata*, Functional food, *Platycodon grandifloras*, Single oral dose toxicity.

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INTRODUCTION

Asthma is an immune response disorder that affects approximately 10% of the total population in developed countries,[1] and it is characterized by airway hyper-responsiveness, chronic airway and lung tissue inflammation, coughing and breathlessness.[2] In multiple recent studies, Th2 cells, which produce cytokines IL-4, IL-5 and IL-13, were shown to contribute significantly to the development[3] and maintenance of chronic asthma.[4] Steroid medications represent a long-term therapy for asthma; however, when used at high doses for prolonged periods, they cause serious systemic side effects, such as hyperglycemia, dyslipidemia and cardiovascular disease.[5] In contrast, anti-inflammatory herbal medicines may offer benefits comparable to those of steroid medications without serious side effects.

Traditional and herbal medicines are used for the prevention, diagnosis and treatment of diseases and the maintenance of health. Numerous studies have reported the benefits of traditional herbal medicines.[6-8] The polyherbal complex Cheongmokpye contains twenty-three kinds of herbal extracts and its main components are *Platycodon grandifloras, Codonopsis lanceolata* and *Panax ginseng,* which have demonstrated capacities for immune enhancement and respiratory protection.[9-11] Its additional components *Morus alba* L., *Perilla frutescens* (L.), *Britt. Var. acute* (Thunb.), *Kudo* and *Lonicera japonica Thunberg* have been reported to enhance respiratory immunity and suppress cough.[12-14] Recent research revealed that treatments with Cheongmokpye extract regulated pro-inflammatory cytokines (TNF-α, IL-1β and IL-6) and phagocytosis levels in RAW 264.7 cells, a murine macrophage-derived cell line.[15]

Despite the widespread use of traditional medicines, there are concerns about the lack of safety information for such

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treatments. Therefore, toxins that may cause adverse effects through interactions between plant chemicals and cells must be identified.[16] Hence, effective safety studies are necessary to determine whether a new herbal medicine should be adopted for clinical use. Therefore, acute oral safety studies of herbal medicines are needed not only to identify dose ranges but also to reveal any possible side effects.

The favorable effects of the polyherbal complex Cheongmokpye are greater than those of the individual herbs. Nevertheless, the toxicological profiles of Cheongmokpye have not been reported. Therefore, we examined the acute oral toxicity of Cheongmokpye at limited dosages in female and male ICR mice. To clarify the safe threshold intake level of Cheongmokpye, the rodents were monitored during the post administration observation period for any signs of toxicity, such as death, skin or fur changes, respiratory system issues and diarrhea, based on the guidelines of the Organization of Economic Cooperation and Development (OECD)[17] and the Ministry of Food and Drug Safety of the Republic of Korea.[18]

MATERIALS AND METHODS

Animals

Pathogen-free six-week-old ICR female and male mice (18-20 g) were purchased from a commercial animal breeder (KOATECH, Gyeonggi-do, Korea). A total of 40 mice were separated randomly into five mice per polycarbonate cage and acclimatized in a room at 20-24°C and 45-55% humidity for 1 week. The mice were exposed to a 12 hr light/dark cycle and provided free access to standard rodent chow and water. All experimental procedures were approved by the Institutional Animal Care and Use Committee of the Scripps Korea Antibody Institute (Chuncheon, Korea) (Approval No. SIACUC-21-2-3-1).

Cheongmokpye

We obtained 23 different herbs after confirming their morphology under a microscope. *Cheongmokpye* was prepared by Koreabestone Co., Ltd., (Chuncheon, Korea) (Table 1). Briefly, the herbs were boiled in 4 L of distilled water for 24 hr at 95°C twice and subsequently filtered. The resulting filtrate was decompressed using a rotary vacuum evaporator (Rotavapor R-144; Buchi, Flawil, Switzerland) and lyophilized in a programmable freeze dryer (FreeZone 1 Liter Benchtop; Labconco Corporation, Kansas City, MO, USA). Eventually, the volume of Cheongmokpye reached 173.24 g and it was in the form of a light-brown powder (12.2% yield). The main medicinal components of Cheongmokpye were 2.25 mg of paeoniflorin, 0.63 mg of lancemaside A, 0.94 mg of glycyrrhizin and 0.05 mg of 6-gingerol per gram by HPLC-PDA analysis. The powdered Cheongmokpye was stored at 4°C in the dark until needed.

Groups and treatments

All animals were fasted overnight (approximately 18 hr) prior to treatment and terminal necropsy. After the 7-day acclimatization period, the animals were distributed into eight groups based on body weight, with five mice per group (males: 30.14±1.49 g, range 32.43-26.85 g; females: 23.18±1.13 g, range 25.16-21.07 g). The highest dose used in the present study was 5,000 mg/kg body weight (bw) in a volume of 10 mL (Group 1), which is the limited dosage for rodents and the recommended oral dose volume in mice and distilled water was used as the vehicle (Group 0). For the middle- and lower-dose groups, 2,500 (Group 2) and 1,250 mg/kg (Group 3) bw were selected, respectively, as recommended by the OECD and MFDS Guidelines.[17,18] Female and male vehicle controls were also included in the study. The test article was orally administered once using a sonde attached to a 1-mL syringe. In the vehicle control mice, 10 mL/kg bw of distilled water was administered once by gastric gavage instead of the test solution.

Clinical signs and body weight

Clinical signs were recorded using the Functional Observational Battery at least twice daily before and after dosing.[8] Body weights were measured prior to treatment on the day of vehicle and drug administration (Day 0), Days 1, 2, 3, 4, 5, 6, 7, 10 and 14 after administration and the day of euthanasia (Day 15).

Necropsy

All animals that died spontaneously were observed immediately after discovery and those that survived dosing were subjected to terminal necropsy. Mice were euthanized with carbon dioxide at a rate of 30-70% of the chamber capacity per minute and gross necropsies were performed on day 15 after overnight fasting (approximately 18 hr; water was not restricted).

Plasma sampling

Blood (more than 500 µL) was drawn from the inferior vena cava of the mice under anesthesia with 2-3% isoflurane in a mixture of 70% N_2O and 28.5% O_2. A portion of the samples was collected in 1.5 mL micro-centrifuge tubes containing EDTA-2K at 1.8 mg/mL for hematology and other samples were allowed to clot for 15 min at room temperature and immediately centrifuged at 11,400×g for 10 min at 4°C. The supernatants were then stored as 200 µL aliquots at -70°C until serum biochemistry analysis.

Hematological analyses

Thirteen hematological abnormalities were examined: number of total leukocytes and differential numbers {Neutrophils (Neu), Eosinophils (Eos), Basophils (Bas), Lymphocytes (Lym) and Monocytes (Mon)}, erythrocytes, Platelets (PLT), Hemoglobin (HGB), Hematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC). Hematological

examinations were performed using an auto hematology analyzer (BC-5000 Vet, Mindray Co., Ltd., China).

Blood biochemistry

Serum levels of Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) and Alkaline Phosphatase (ALP) (for liver injury) and Blood Urea Nitrogen (BUN) and Creatinine (CREA) (for kidney injury) were measured using an automated chemistry analyzer (AU480, Beckman Coulter, Inc., USA).

Histological analyses

The lungs**,** hearts, thymuses, kidneys, adrenal glands (Adrenal Gs), spleens, livers, pancreases, brains, lymph nodes, urinary bladders, testes, epididymis, ovaries and uteruses were fixed in 10% neutral-buffered formalin, dehydrated, embedded in paraffin and cut into sections of 4 µm. Sections were stained with hematoxylin and eosin. A blinded histopathological examination was performed.

Statistical analyses

All data are represented as the means±Standard Deviation (SD). The variance in homogeneity was examined using Levene's test. If Levene's test indicated no significance, then the data were analyzed using an independent *t*-test. If Levene's test indicated significance, then the data were analyzed using the Mann-Whitney U test. Statistical significance was set at $p<0.05$.

RESULTS

Mortality and body weight

No mortality occurred in the Cheongmokpye treatment and vehicle control groups after a single oral administration in female and male mice. No significant changes in body weight were detected in any of the Cheongmokpye-treated female or male mice compared to the vehicle control group. All mice were fasted overnight on day 0 and euthanized on day 15 (Figure 1 and Table 2).

Necropsy findings

No Cheongmokpye treatment-related gross abnormalities were observed in the present study. We sporadically detected slight [1+] to moderate [2+] lung congestion, splenic atrophy or hypertrophy, edematous uterine changes and hypertrophy of submandibular lymph nodes in all experimental groups after oral administration, including the sex-matched vehicle controls (Table 2).

Organ weight changes related to toxicity

No meaningful changes to the absolute and relative weights of the 13 principal organs were observed in any of the Cheongmokpye treatment groups compared with the vehicle group (Tables 3-6). Although the males of some groups (G1 and G3) showed significant weight changes in the pancreas compared to those in G0, significant weight change were not observed in G2, concentration-dependent changes were not observed and across-sex uniformity was not observed (Tables 3-6).

Histopathological changes of thirteen principal organs

We sporadically detected slight [1+] to moderate [2+] lung congestion spots (thickening of the alveolar septa with inflammatory cell infiltration and focal hemorrhages), cyst formation in the kidney, decreased splenic white pulp in lymphoid cells, hyperplasia of splenic red pulp in lymphoid cells, uterine mucosa desquamation and diffuse hyperplasia of lymphoid cells in the submandibular lymph nodes in all experimental groups, including sex-matched vehicle controls. We also observed focal inflammatory cell infiltration in the liver parenchyma with or without focal hepatocyte necrosis in two

Table 1: Composition of herbs in the aqueous extracts of Cheongmokpye.

Herbs	Scientific Names	Ratio (%)
Wildflower honey	-	27.0
Pear	*Pyri pyrifoliae* Fructus	21.0
Balloon flower	*Platycodonis* Radix	15.0
Lance asiabell	*Codonopsis lanceolata* Radix	10.0
Ginseng	*Ginseng* Radix Rubra	1.8
Milk vetch	*Astragali* Radix	1.4
Dong quai	*Angelicae gigantis* Radix	1.4
Morus bark	*Mori radicis* Cortex	1.4
Mulberries	*Ophiopogonis* Radix	1.4
Root of an arrowroot	*Puerariae* Radix	1.4
Root of an aster	*Asteris* Radix	1.4
Dried orange peel	*Citri Unshius Pericarpium*	1.4
Fruit of *Schisandra chinensis*	*Schisandrae Fructus*	1.4
Mulberry leaf	*Mori folium*	1.4
Ovate-leaf atractylodes	*Atractylodes japonica*	1.4
Poncirus	*Ponciri fructus* Immaturus	1.4
Ginger	*Zingiberis rhizoma* Recens	1.4
Cassia bark	*Cinnamomi cortex*	1.4
Schizonepeta	*Schizonepetae spica*	1.4
Scutellaria	*Scutellariae* Radix	1.4
Lilly	*Lilii bulbus*	1.4
Liquorice	*Glycyrrhizae* Radix	1.4
Peppermint	*Menthae herba*	1.4

Twenty-three herbs were used to prepare the aqueous extracts of Cheongmokpye in the indicated amounts.

Table 2: Mortality, clinical signs and gross and histological findings of animals exposed to single-dose Cheongmokpye.

Groups	Male vehicle control	CMP treated male mice (mg/kg) 5,000	2,500	1,250	Female vehicle control	CMP treated female mice (mg/kg) 5,000	2,500	1,250
Mortality	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Clinical signs								
Abnormal signs	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Gross findings								
Lung focal congestion 1+	2/5	1/5	1/5	1/5	1/5	0/5	1/5	1/5
Spleen atrophy 1+	1/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5
Spleen hypertrophy 1+	0/5	0/5	0/5	0/5	1/5	1/5	0/5	0/5
LN hypertrophy [a)] 1+	1/5	1/5	0/5	1/5	1/5	1/5	2/5	0/5
Uterus edema 1+	-	-	-	-	2/5	2/5	1/5	0/5
Uterus edema 2+	-	-	-	-	0/5	0/5	0/5	1/5
Histopathological findings								
Lung focal congestion 1+	2/5	1/5	1/5	1/5	1/5	0/5	1/5	1/5
Kidney cyst 1+	1/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5
Spleen wDE [b)] 1+	1/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5
Spleen rHP [c)] 1+	0/5	0/5	0/5	0/5	1/5	1/5	0/5	0/5
Liver focal inflammation 1+	0/5	0/5	0/5	0/5	0/5	0/5	1/5	1/5
Lymph node HP [a, d)] 1+	1/5	1/5	0/5	1/5	0/5	0/5	1/5	1/5
Lymph node HP [a, d)] 2+	0/5	0/5	0/5	0/5	1/5	1/5	1/5	0/5
Uterus DM [e)] 1+	-	-	-	-	2/5	1/5	1/5	1/5

The mice received a single administration of Cheongmokpye or distilled water. Mortality and clinical signs were measured daily and values were recorded as animals/total observed animals.Degree: 1+, slight; 2+, moderate.[a)] Submandibular Lymph Nodes [b)] White pulp lymphoid cell count decreased. [c)] Red pulp lymphoid cell hyperplasia. [d)] Diffuse hyperplasia of the lymphoid cells. [e)] desquamation.

Table 3: Absolute organ weights of male animals exposed to Cheongmokpye in the single-dose toxicity study.

Group	Absolute organ weights (male)						
	Lung (mg)	Heart (mg)	Thymus (mg)	Kidney (mg)	Adrenal G (mg)	Spleen (mg)	Liver (g)
G0M	191.2±15.8	141.4±16.6	49.1±6.1	220.6±23.3	2.42±0.70	90.7±7.3	1.39±0.16
G1M	196.0±11.9	141.6±14.2	49.3±6.2	225.2±21.2	2.18±0.27	93.5±14.3	1.47±0.09
G2M	180.4±16.8	137.7±11.6	50.2±13.0	216.3±18.6	1.80±0.41	84.8±17.1	1.33±0.20
G3M	185.6±15.5	139.7±10.9	40.7±7.3	209.2±12.7	1.86±0.59	96.4±18.2	1.30±0.11
	Pancreas (mg)	**Brain (mg)**	**LN [a)] (mg)**	**Urinary B (mg)**	**Testis (mg)**	**Epididymis (mg)**	
G0M	159.2±16.1	432.3±20.1	4.48±0.31	28.4±2.1	108.7±5.7	40.3±4.3	
G1M	139.8±12.7 [a)]	457.9±23.0	5.32±1.65	30.7±4.5	111.6±10.2	38.4±6.1	
G2M	135.3±22.8	427.1±10.7	4.58±1.20	28.7±5.8	115.2±4.7	37.2±0.8	
G3M	131.2±17.5 [a)]	444.4±16.6	4.22±0.90	27.3±4.4	124.1±17.9	39.8±4.6	

The mice received a single administration of Cheongmokpye or distilled water. The samples were measured for absolute organ weights (13 organs in both genders) and values are expressed as means±SD in 5 mice. [a)], $p<0.05$; [b)], $p<0.01$; [c)], $p<0.005$.

female Cheongmokpye-treated mice: one (1/5; 20%) treated with 2,500 mg/kg and one (1/5; 20%) treated with 1,250 mg/kg Cheongmokpye (Table 2).

Changes in hematological parameters

No meaningful changes in hematological parameters were observed among all Cheongmokpye-treated male mice relative to the equivalent vehicle control mice, except for a significant increase in neutrophil number and eosinophil number in male 1,250 mg/kg-treated mice; a significant increase in White Blood Cell (WBC) count, lymphocyte number, neutrophil number, eosinophil number, Red Blood Cell (RBC) count, HCT and HGB in male 2,500 mg/kg-treated mice; and a significant increase in neutrophil number, RBC, HCT and HGB in male 5,000 mg/kg-treated mice (Tables 7 and 8). Furthermore, no significant changes were observed in any of the Cheongmokpye-treated female mice compared to the equivalent vehicle control, except for a significant decrease in the number of monocytes. in female mice treated with 2,500 mg/kg, whereas there was no decrease in monocytes in female 5,000 mg/kg-treated mice (Tables 7 and 8).

Enzymes relevant to tissue injury

There were no significant differences in the levels of AST, ALT, ALP indicating liver injury between the control mice and those treated with Cheongmokpye (Figure 2) and there were no significant differences between the control mice and those treated

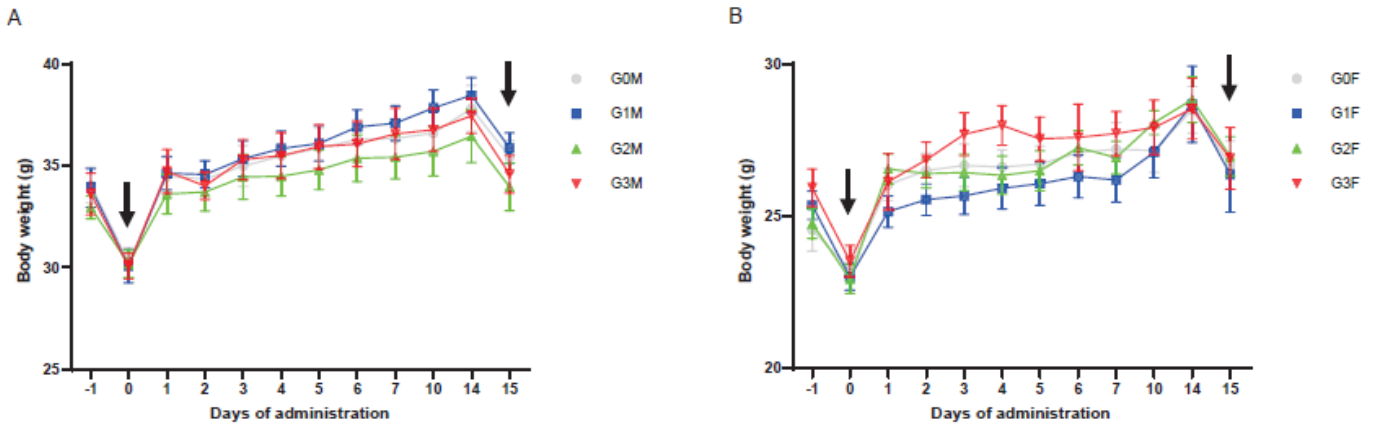

Figure 1: Changes in body weight for 14 days (approximately 2 weeks) in male (A) and female (B) mice after a single oral dose of Cheongmokpye. No significant changes in body weight were detected in any of the Cheongmokpye-treated mice compared with vehicle control mice of equal sex. Values are expressed as mean±SD of five mice. Cheongmokpye, test material; arrow, all mice fasted overnight; before indicates 1 d before administration. Zero indicates the day of administration.

Table 4: Absolute organ weights of female animals exposed to Cheongmokpye in the single-dose toxicity study.

Group	Absolute organ weights (female)						
	Lung (mg)	Heart (mg)	Thymus (mg)	Kidney (mg)	Adrenal G (mg)	Spleen (mg)	Liver (g)
G0F	165.8±15.5	112.2±10.0	67.6±13.9	159.0±32.6	3.14±0.87	90.7±7.3	1.00±0.07
G1F	159.9±7.3	108.9±9.3	61.0±13.5	143.4±12.4	3.68±0.79	93.5±14.3	0.99±0.12
G2F	162.4±12.2	112.7±10.6	54.0±9.7	142.9±15.0	3.56±0.57	84.8±17.1	1.08±0.10
G3F	152.7±17.7	110.2±7.1	67.7±8.5	131.5±6.5	3.44±0.39	96.4±18.2	0.99±0.08
	Pancreas (mg)	Brain (mg)	LN (mg)	Urinary B (mg)	Ovary (mg)	Uterus (mg)	
G0F	122.7±17.5	453.0±22.7	3.02±1.43	15.8±4.0	9.4±2.8	88.1±13.3	
G1F	129.4±23.8	439.5±18.1	2.12±1.13	14.5±3.5	11.7±3.4	126.7±68.3	
G2F	128.6±9.8	443.8±20.4	4.12±1.74	17.1±3.4	17.9±10.0	97.7±22.6	
G3F	123.2±21.8	437.9±17.0	4.40±0.91	13.8±2.9	9.4±4.0	73.1±14.2	

The mice received a single administration of Cheongmokpye or distilled water. The samples were measured for absolute organ weights (13 organs in both genders) and values are expressed as means±SD in 5 mice. [a], $p<0.05$; [b], $p<0.01$; [c], $p<0.005$.

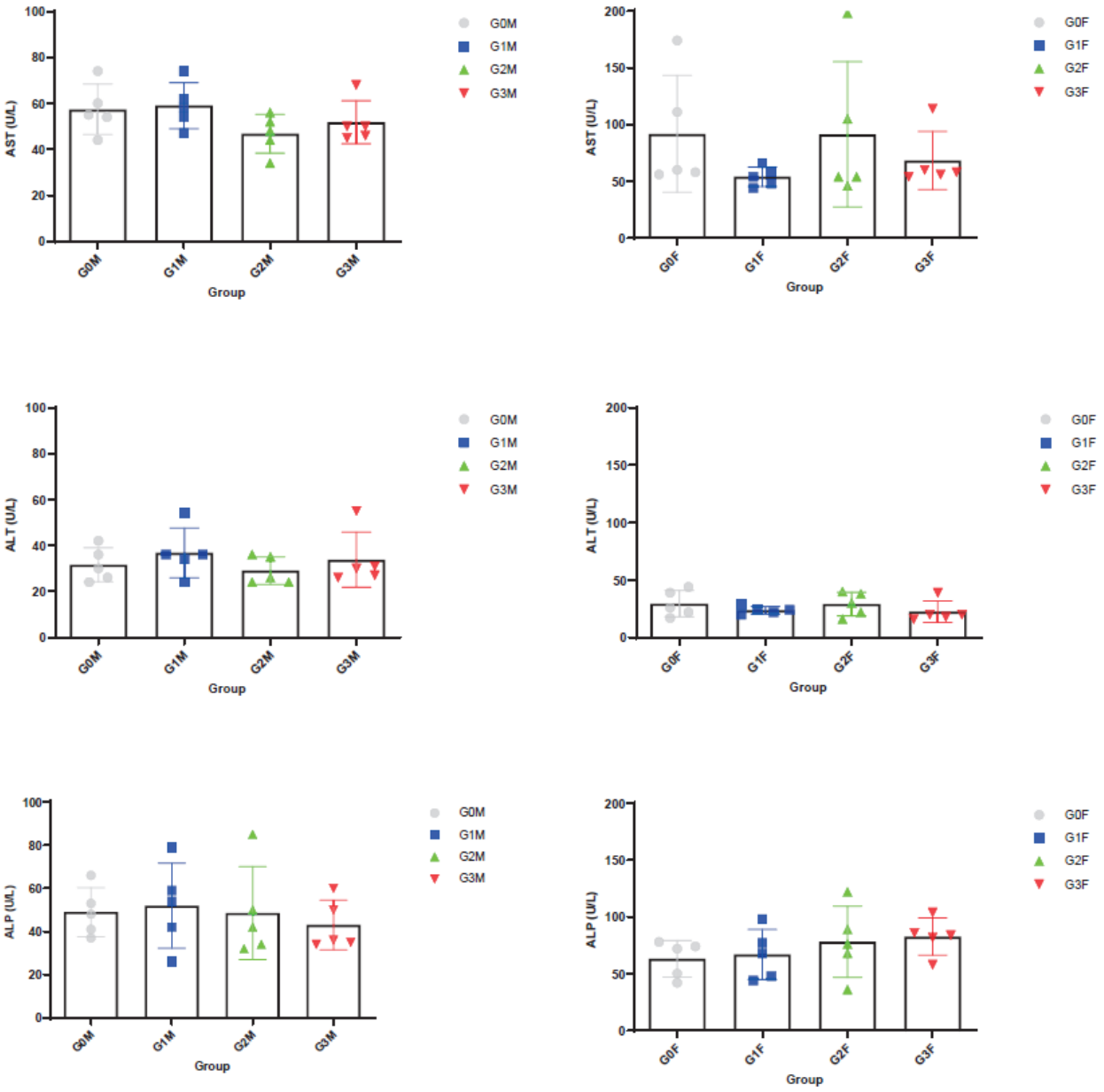

Figure 2: Serum concentrations of AST, ALT and ALP after administration of Cheongmokpye and sacrifice to evaluate liver injury. After a single oral treatment for a 14-day observation period, serum levels of AST, ALT and ALP were measured. Values are expressed as mean±S.D. of five mice. AST, aspartate transferase; ALT, alanine transaminase; ALP, alkaline phosphatase.

with Cheongmokpye in the levels of BUN and CREA indicating kidney injury (Figure 3).

No Observed Adverse Effect Level (NOAEL)

The lethal dose (LD_{50}) of Cheongmokpye was more than 5,000 mg/kg based on a single dose toxicity study. NOAEL was more than 5,000 mg/kg in male and female mice (Table 9).

DISCUSSION

To aid the development of natural medicinal ingredients and/or functional foods based on Cheongmokpye, the present study investigated the toxicity of a single oral dose of Cheongmokpye in female and male ICR mice. To investigate the toxicity and identify the target organs, Cheongmokpye was orally administered to female and male ICR mice at doses of 5,000, 2,500, 1,250 and 0 mg/kg bw (10 mL/kg body weight, dissolved in distilled water). We then measured treatment-induced mortality and body weight changes. Furthermore, clinical signs were monitored for 14 days after treatment via gross observation and organ weight changes and principal organ histopathological changes were determined compared with sex-matched vehicle control mice based on the recommendations set forth by the OECD Guidelines.[17] Although the males in some groups (G1 and G3) showed significant weight changes in the pancreas compared to the G0, significant weight

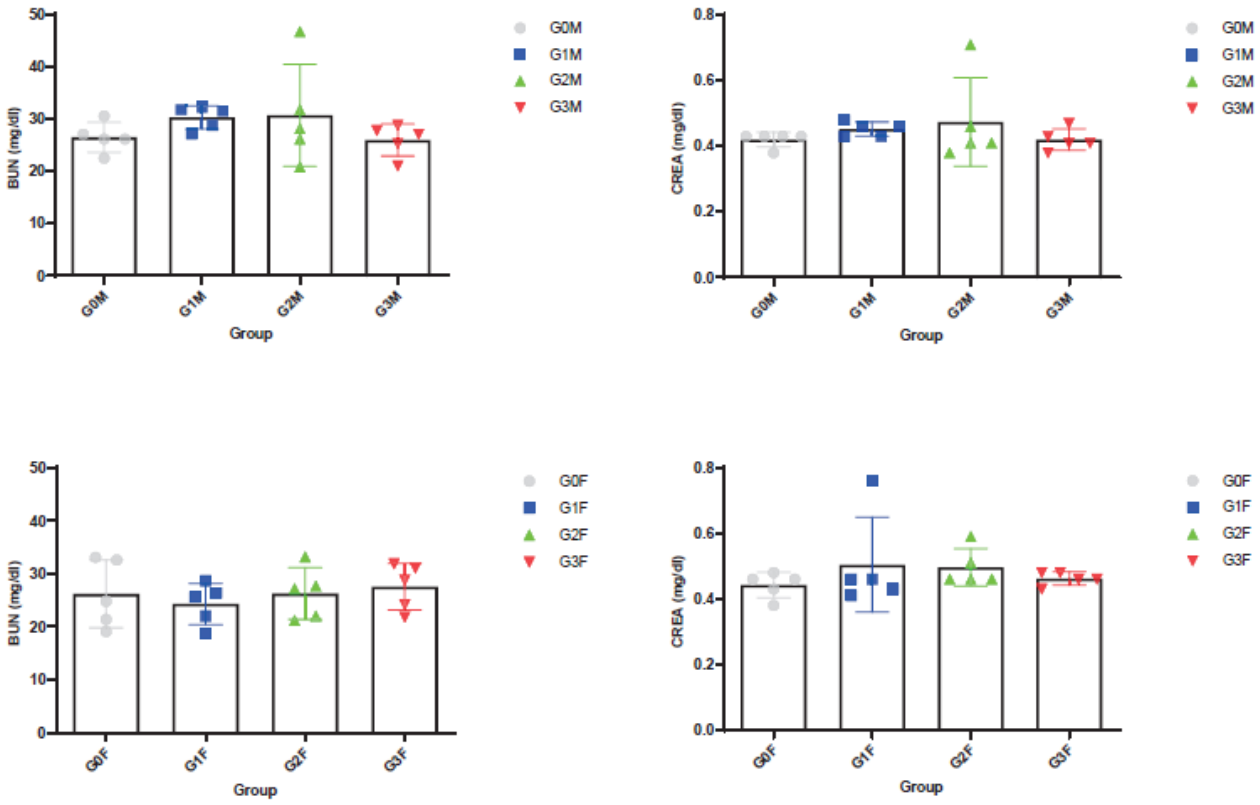

Figure 3: Serum concentrations of BUN and CREA after Cheongmokpye administration to evaluate kidney injury. After a single oral treatment for a 14-day observation period, serum levels of BUN and CREA were measured. Values are expressed as mean±S.D. of five mice. BUN, blood urea nitrogen; CREA, creatinine.

Table 5: Relative organ weights of male animals exposed to Cheongmokpye in the single-dose toxicity study.

Group	Relative organ weights (male)						
	Lung (%)	Heart (%)	Thymus (%)	Kidney (%)	Adrenal G (%)	Spleen (%)	Liver (%)
G0M	0.539±0.03	0.398±0.03	0.139±0.02	0.624±0.08	0.007±0.00	0.256±0.02	3.911±0.27
G1M	0.546±0.02	0.394±0.03	0.138±0.02	0.629±0.06	0.006±0.00	0.261±0.04	4.108±0.18
G2M	0.532±0.04	0.406±0.02	0.150±0.05	0.638±0.05	0.005±0.00	0.249±0.04	3.916±0.37
G3M	0.535±0.03	0.404±0.02	0.118±0.03	0.607±0.06	0.005±0.00	0.280±0.06	3.746±0.19
	Pancreas (%)	Brain (%)	LN (%)	Urinary B (%)	Testis (%)	Epididymis (%)	
G0M	0.449±0.04	1.220±0.06	0.013±0.00	0.080±0.01	0.307±0.01	0.114±0.02	
G1M	0.390±0.03 [a)]	1.277±0.07	0.015±0.00	0.085±0.01	0.312±0.03	0.107±0.02	
G2M	0.396±0.04	1.264±0.12	0.014±0.00	0.084±0.01	0.340±0.02	0.110±0.01	
G3M	0.378±0.03 [a)]	1.290±0.11	0.012±0.00	0.079±0.01	0.360±0.06	0.116±0.02	

The mice received a single administration of Cheongmokpye or distilled water. The samples were measured for relative organ weights (%=absolute organ weight / body weight) and values are expressed as means±SD in 5 mice. [a)], $p<0.05$; [b)], $p<0.01$; [c)], $p<0.005$.

Table 6: Relative organ weights of female animals exposed to Cheongmokpye in the single-dose toxicity study.

Group	Relative organ weights (female)						
	Lung (%)	Heart (%)	Thymus (%)	Kidney (%)	Adrenal G (%)	Spleen (%)	Liver (%)
G0F	0.622±0.05	0.420±0.02	0.254±0.06	0.594±0.10	0.012±0.00	0.424±0.06	3.751±0.20
G1F	0.610±0.05	0.414±0.02	0.230±0.03	0.545±0.04	0.014±0.00	0.345±0.10	3.748±0.21
G2F	0.604±0.06	0.417±0.02	0.201±0.04	0.529±0.03	0.013±0.00	0.374±0.03	4.003±0.26
G3F	0.571±0.08	0.411±0.03	0.253±0.04	0.493±0.06	0.013±0.00	0.314±0.07	3.705±0.33
	Pancreas (%)	Brain (%)	LN (%)	Urinary B (%)	Ovary (%)	Uterus (%)	
G0F	0.461±0.07	1.701±0.10	0.011±0.01	0.059±0.02	0.035±0.01	0.331±0.06	
G1F	0.492±0.08	1.682±0.20	0.006±0.00	0.055±0.01	0.044±0.01	0.500±0.32	
G2F	0.477±0.04	1.646±0.02	0.015±0.01	0.063±0.01	0.068±0.04	0.364±0.09	
G3F	0.465±0.12	1.636±0.14	0.016±0.00	0.052±0.01	0.035±0.02	0.272±0.05	

The mice received a single administration of Cheongmokpye or distilled water. The samples were measured for relative organ weights (%=absolute organ weight/body weight) and values are expressed as means±SD in 5 mice. [a], $p<0.05$; [b], $p<0.01$; [c], $p<0.005$.

Table 7: Changes in hematological parameters of male animals exposed to Cheongmokpye in the single-dose toxicity study.

Group	Relative organ weights (male)						
	WBC (10^3/uL)	Neu (%)	Lym (%)	Mon (%)	Eos (%)	Bas (%)	PLT (10^3/uL)
G0M	4.19±1.53	16.90±4.29	76.92±5.05	3.96±1.30	2.22±0.63	0.00±0.00	1426.0±139.9
G1M	6.34±0.79	18.34±2.60	75.32±2.27	3.52±0.44	2.82±0.62	0.00±0.00	1485.4±183.1
G2M	7.08±1.76	16.84±3.42	75.60±5.13	3.38±0.47	4.18±2.47	0.00±0.00	1325.4±396.8
G3M	6.29±1.58	19.98±3.01	72.44±4.31	4.46±1.31	3.10±1.12	0.00±0.00	1498.2±335.0
	RBC (10^6/uL)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	
G0M	7.53±0.49	12.88±0.72	39.98±1.91	53.18±1.56	17.14±0.45	32.24±0.24	
G1M	7.76±0.62	13.62±1.01	41.66±3.09	53.74±1.83	17.56±0.68	32.72±0.68	
G2M	9.18±0.49	15.38±1.01	47.34±2.94	51.52±0.90	16.74±0.40	32.50±0.35	
G3M	8.69±0.47	14.74±0.98	45.18±2.48	52.04±1.69	16.94±0.40	32.56±0.84	

The mice received a single administration of Cheongmokpye or distilled water. The samples were analyzed for hematological parameters (13 items) and values are expressed as means±SD in 5 mice. [a], $p<0.05$; [b], $p<0.01$; [c], $p<0.005$.

changes were not observed in G2, concentration-dependent changes were not observed and across-sex uniformity was not observed; therefore, we concluded that the changes in G1 and G3 were not drug-related toxicological changes.

The OECD guidelines recommend that the highest administered dose of test materials should be 5,000 mg/kg or equivalent to the maximum solubility and they also recommend that the dosage volume should be kept below 20 mL/kg in cases of acute toxicity in mice.[19] Because there are no available toxicological data following oral chemokine treatment in female and male mice, the highest dosage was 5,000 mg/kg in a volume of 10 mL, the limited dosage for rodents and the recommended oral dose volume in mice. In the present study, 2,500 and 1,250 mg/kg body weight were chosen for administration in the middle- and low-dose groups, respectively, as recommended by the OECD guidelines. Female and male vehicle control groups were included in this experiment. The test material was administered once by gastric gavage using distilled water as the vehicle.

Platycosides are the active ingredients of *Platycodon grandiflorum* and their structures contain a triterpenoid aglycone and two sugar chains. Platycodin saponins have a variety of key effects in biological systems, including anti-inflammatory, anti-allergy and anti-tumor activities. Platycodin saponins are among the saponins found in the roots of *Platycodon grandiflorum*.[20] Lancemaside A is another main ingredient in *Codonopsis lanceolata and* it has similar biological benefits. The saponins of *Codonopsis lanceolata* are called triterpenoid saponins and approximately 20 species, including lancemasides, codonolasides, eclalbasaponin and

Table 8: Changes in hematological parameters of female animals exposed to Cheongmokpye in the single-dose toxicity study.

Group	Relative organ weights (female)						
	WBC (10^3/uL)	Neu (%)	Lym (%)	Mon (%)	Eos (%)	Bas (%)	PLT (10^3/uL)
G0F	7.42±2.23	15.18±2.89	77.30±3.41	3.50±0.96	4.02±1.59	0.00±0.00	1138.8±208.6
G1F	5.94±1.97	14.64±2.42	78.36±4.15	2.88±0.82	4.12±2.34	0.00±0.00	1207.4±262.8
G2F	6.08±2.22	19.86±6.88	72.78 ±9.43	2.26±0.63	5.10±2.38	0.00±0.00	1312.8±107.6
G3F	5.88±2.26	17.16±2.94	77.28±4.59	1.82±0.33	3.74±1.80	0.00±0.00	1309.8±203.7
	RBC (10^6/uL)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	
G0F	8.76±0.41	15.06±0.33	46.08±0.98	52.66±2.18	17.22±0.45	32.76±0.62	
G1F	8.45±0.35	14.92±0.48	45.58±1.01	54.02±2.42	17.68±0.53	32.74±0.89	
G2F	8.59±0.43	14.70±0.81	44.94±3.04	52.32±1.90	17.10±0.34	32.76±1.07	
G3F	9.10±0.14	15.50±0.55	48.62±1.74	53.44±1.49	17.06±0.42	31.90±0.37	

The mice received a single administration of Cheongmokpye or distilled water. The samples were analyzed for hematological parameters (13 items) and values are expressed as means±SD in 5 mice. [a], $p<0.05$; [b], $p<0.01$; [c], $p<0.005$.

Table 9: No observed adverse effects level, NOAEL.

Items	NOAEL		
Animal	Lethal Dose	MTD	Targets and side effects
Male	> 5,000 mg/kg	> 5,000 mg/kg	No specific targets
Female			

echinocystic acid, have been reported. Among these, lancemaside A is the most abundant in *Codonopsis lanceolata radix*.[21]

The composition ratio showed that the Cheongmokpye variety used in this experiment contained twenty-three herbal extracts. The main components were *Platycodon grandifloras, Codonopsis lanceolata and Panax ginseng,* all of which have demonstrated immune enhancement and respiratory protection effects.[9-11] Furthermore, *Morus alba L.* and *Perilla frutescens (L.), Britt. Var. acute (Thunb.), Kudo and Lonicera japonica Thunberg* have been reported to enhance respiratory immunity and suppress cough.[12-14]

Platycosides have also been used as medicines and appear to protect organs via antioxidative effects. Additionally, *Codonopsis lanceolata* is utilized as an ingredient in food and traditional medicine.[21] Moreover, the dried root of *Codonopsis lanceolata* has been utilized to treat inflammatory respiratory diseases. Consequently, the two principal ingredients of Cheongmokpye are *Platycodon grandiflorum* and *Codonopsis lanceolata*, which are expected to have anti-inflammatory, anti-allergic and disease-preventive effects.

Our results revealed that a single oral dose of Cheongmokpye does not induce *in vivo* damage. No deaths or injuries were observed in mice treated with 5,000 mg/kg, thus establishing its safety. However, a detailed experimental analysis of its toxic components is essential to further support the use of Cheongmokpye. To overcome some of the limitations of this study, it will be necessary to evaluate the toxicity of repeated administration for longer than 28 days. Moreover, further studies will be necessary to identify the molecular mechanisms for toxicity and the effects of long-term exposure to Cheongmokpye.

CONCLUSION

A single oral administration of Cheongmokpye to ICR mice was well tolerated and had few negative effects. Based on these results, the no observed adverse effect level (NOAEL) is 5,000 mg/kg. Our findings show significant point for the development of health-related functional foods. this study offers considerable insights for research fields and industries alike. However, it should be kept in mind that further studies are needed to evaluate the toxicity of repeated administration over a period longer than 28 days.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

Do-Hyung Kim, Jin Won Park: Conceptualization, Writing - Original Draft. **Hyeong-Jun Park, Hye Won Kwak, Daegeun Kim and Yongkwan Kim:** Investigation. **Ho Park, Jae-Hwan Nam:** Methodology. **Wang-kwon Kim, Sang-In Park:** Conceptualization, Supervision. All authors have read and approved the manuscript.

ABBREVIATIONS

Adrenal G: Adrenal gland; **ALP:** Alkaline phosphate; **ALT:** Alanine aminotransferase; **AST:** Aspartate aminotransferase; **Bas:** Basophile; **BUN:** Blood urea nitrogen; **bw:** Body weight; **CMP:** Cheongmokpye; **CREA:** Creatinine; **DM:** Desquamation; **Eos:** Eosinophile; **HCT:** Hematocrit; **HGB:** Hemoglobin; **LD:** Lethal dose; **LN:** Lymph node; **Lym:** Lymphocytes; **MCH:** Mean corpuscular hemoglobin; **MCHC:** Mean corpuscular hemoglobin concentration; **MCV:** Mean corpuscular volume; **MFDS:** Ministry of food and drug safety; **Mon:** Monocytes; **MTD:** Maximum tolerated dose; **Neu:** Neutrophils; **NOAEL:** No observed adverse effect level; **OECD:** Organization of economic cooperation development; **PLT:** Platelets; **RBC:** Red blood cell; **rHP:** Red pulp lymphoid cell hyperplasia; **SD:** Standard deviation; **SMEs:** Small and medium-sized enterprises; **TIPA:** The Korea technology and information promotion agency for SMEs; **WBC:** White blood cell; **wDE:** White pulp lymphoid cell decreased.

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