Original article

Single Intravenous-dose Toxicity of Water-soluble Carthami-flos Pharmacopuncture (WCF) in Rats

Da-jung Jung, Yoo-min Choi, Seok-hee Kim, Jong-uk Kim, Tae-han Yook*

Department of Acupuncture & Moxibustion Medicine, Woosuk University Hospital of Korean Medicine, Jeonju, Korea

Abstract

Objectives: This study was performed to analyze the toxicity and to find the lethal dose of the test substance Water-soluble Carthami-flos pharmacopuncture (WCF) when used as a single intravenous-dose in 6-week-old, male and female Sprague-Dawley rats.

Methods: The experiment was conducted at Biotoxtech according to Good Laboratory Practices. 20 female and 20 male Spague-Dawley rats were divided into 4 groups of 5 female and 5 male animals per group. The rats in the three experimental groups received single intravenous injections with 0.125-mL, 0.25-mL and 0.5-mL/animal doses of WCF, Groups 2, 3, and 4, respectively, and the control group, Group 1, received a single intravenous injection with a 0.5-mL dose of normal saline. Clinical signs were observed and body weight measurements were carried out for 14 days following the injections. At the end of the observation period, hematology, clinical chemistry, histopathological tests and necropsy were performed on the injected parts.

Results: No deaths occurred in any of the groups. Also, no significant changes in body weight, hematological parameters or clinical chemistry test results between the control group and the experimental groups were observed. Visual inspection after necropsy showed no abnormalities. Histopathological tests on the injected parts showed no significant differences, except for Group 1 females; however, the result was spontaneous generation and had no toxicological meaning because it was not dose-dependent. Therefore, this study showed that WCF had no effect on the injected parts in terms of clinical signs, body weight, hematology, clinical chemistry, and necropsy.

Conclusion: As a result of single intravenous-dose tests of the test substance WCF in 4 groups of rats, the lethal dose for both males and females exceeded 0.5 mL/animal. Therefore, WCF is a relatively safe pharmacopuncture that can be used for treatment, but further studies should be performed.

1. Introduction

Pharmacopuncture is a new form of acupuncture treatment combining acupuncture and herbal medicine. With acupuncture being based on the meridian theory and herbal medicine being based on Qi and flavor theory, pharmacopuncture utilizes both the meridian and the Qi and flavor theories. Pharmacopuncture is a unique treatment of traditional Korean medicine and uses needling and herbal medicine based on Qi and flavor theories and acupoint selection based on meridian theories [1].

*Corresponding Author

Tae-han Yook. Department of Acupuncture & Moxibustion Medicine, Woosuk University Hospital of Korean Medicine, 46 Eoeun-ro, Wansan-gu, Jeonju, Jeonbuk 560-833, Korea.
Tel: +82-63-220-8625 Fax: +82-63-220-8400
E-mail: nasiss@naver.com

Carthamus tinctorious L. (Carthami-flos) is an annual safflower belonging to the family *Compositae. Carthami semen* is the seed of the safflower and is composed of 20% — 30% fatty oil (acids), whose main constituents are glycerides, linoleic acid, and oleic acid, as well as serotonin, serotonin conjugate, and serotobenin. Carthami-flos contains safflower-yellow cathamin, which reportedly extends blood vessels, excites the uterus, and reduces blood cholesterol. In Korean medicine, Carthami-flos has warmness, which can affect detoxification, pain relief and blood circulation [2]. Carthami-flos pharmacopuncture (CF) is made by extracting and refining oil from *Carthami semen.* Protein, fat, ash content, and vitamins are the main constituents of *Carthami semen. Carthami semen* is pressed, and sludge is precipitated and then filtered in a dark room. The oil is then bottled and charged with nitrogen gas.

Many studies have demonstrated the effects of CF. Yoon *et al.* [3] investigated pain-relief effects in degenerative knee arthritis patients. Lee *et al.* [4] reported anti-pain effects in an arthritis model for experimental animals. Cho *et al.* [5] suggested that CF could reduce back pain according to the Oswestry Disability Index.

Water soluble Carthami-flos herbal acupuncture (WCF) is prepared by homogenizing CF, water for injection (WFI), and an emulsifier [6]. WCF is widely used nowadays, and Lee *et al.* [6] studied the single intramuscular-dose toxicity of WCF in Sprague-Dawley (SD) Rats. Therefore, this study was performed to analyze the toxicity of WCF under single intravenous-dose in 6-week-old SD rats and to verify the stability of WCF.

2. Materials and Methods

2.1. Materials

The WCF was provided by the Korean Pharmacopuncture Institute (KPI). WCF was developed both to increase the treatment effects by increasing the absorptivity and to decrease the side effects by reducing the residue of existing CF. WCF is prepared in three steps: In step 1, CF, WFI, and a primary emulsifier are mixed together and homogenized for 30 minutes by using a high-speed homogenizer. The emulsifier is added in step 2 and homogenized at 30 — 80℃. In step 3, the temperature is reduced, and the mixture is homogenized to yield WCF [6] (Fig. 1).

24 male and 24 female SD rats were purchased from ORIENTBIO Inc. The 24 male and the 24 female rats were 5 weeks old with body weights of 117.5 — 129.3 g, and 104.2 — 128.2 g, respectively. The laboratory conditions were as follows a temperature of 21.0 — 22.8℃, a relative humidity of 42.9% — 59.3%, 10 - 15 ventilations/hour, a lighting cycle of 12 hours/day, and an illuminance of 150 — 300 Lux.
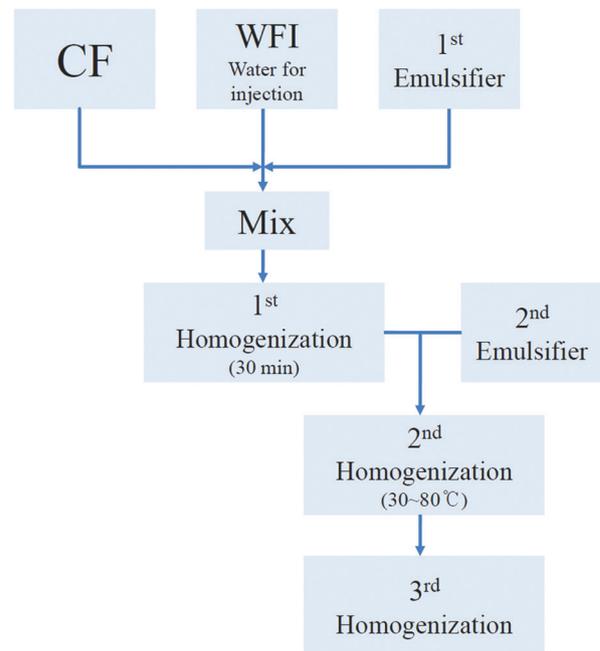

Figure 1 Flow diagram showing the manufacturing process of Water soluble Carthami-flos herbal acupuncture.

The rats were supplied with food (Teklad Certified Irradiated Global 18% Protein Rodent Diet 2918 C) and clean water (tap water of Cheongju filtered with a water sterilizer and irradiated with ultraviolet rays). After 7 days of acclimatization, the weights of the male rats were 184.1 — 203.8 g, and those of the female rats were 144.8 — 169.9 g. The 6-week-old SD rats were grouped by using the criteria of their weights being close to the mean weight. A total of 20 male rats and 20 female rats were distributed into 4 groups with 5 mice of each sex per group. The remaining animals were excluded from the test.

2.2. Methods

No deaths were observed as a result of single intravenous-dose in the male or the female rats during preliminary test (Biotoxtech Study No.: B13481P). Based on those results, 0.5 mL/animal was selected as the high dose (Group 4: G4), and 0.25 and 0.125 mL/animal as the mid-dose and the low dose (Group 3: G3 and Group 2: G2, respectively). Normal saline solution, 0.5 mL/animal, was used for the control group (Group 1: G1) (Table 1).

CF was prepared in a sterile laboratory at the KPI. After the seeds had been cleaned, ground and peeled, they were screw pressed on a prepared mat. Rough unwanted residue was removed, and natural precipitation was induced. The extract from a 3-step filter was packaged with nitrogen [7].

Injection was performed using a disposable syringe (1 mL, 26 G). In G2, 0.125 mL of WCF was injected through

the tail vein. In G3, 0.25 mL of WCF was injected through the tail vein as in G2. In G4 and G1, 0.5 mL of WCF and normal saline was injected through the tail vein, respectively. An intravenous dose was selected because WCF should be clinically applied to veins and muscles. This study was conducted under the approval of the Institutional Animal Ethics Committee.

2.3. Statistics

Body weights, hematology, and clinical chemistry test results were tested using SAS (version 9.3, SAS Institute Inc., U.S.A). The Bartlett test was performed for homoscedasticity (significance level: 0.05). In the case of homoscedasticity, a one-way analysis of variance (ANOVA) was performed to yield significance (significance level: 0.05), and multiple testing of Dunnett's t-test was carried out (significance level: 0.05 on both sides and 0.01). For heteroscedasticity, significance (significance level: 0.05) was checked by using the Kruskal-Wallis test.

3. Results

No mortalities occurred in any group (Table 2). There-fore, computation of the LD_{50} was impossible; however, a single intravenous dose of WCF at 0.5 mL/animal caused no mortalities. In addition, on the day of injection (day 0), clinical symptoms (type of toxicity symptom, time of expression, time of recovery, etc.) and fatalities were observed at 30 minutes, 1, 2, 4 and 6 hours after injection. From day 1 to day 14, clinical symptoms were observed once a day. As a result of observations until day 14 after injection of WCF, no abnormalities were found in any of the experimental rats (Table 3). Body weight was measured on the day of injection (before injection) and on days 3, 7 and 14. All groups showed continued increase in body weight after selection of the experimental groups. No significant differences existed among the experimental groups (Table 4).

All rats had been fasting for more than 18 hours before necropsy. Blood was collected from the abdominal aorta on day 15 after applying anesthesia with isoflurane. The hematology test was done by using a blood corpuscle analyzer (ADVIA 120, SIEMENS, Germany) after placing about 1 mL of the collected blood in a tube containing ethylenediaminetetraacetic acid (EDTA). Erythrocyte count (RBC), hemoglobin (HBG), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH),

Table 1 Grouping of the rats

Group	Injection (mL)	Number of animals (serial number)	
		Male	Female
G1: Control group	saline 0.5	5 (1101 — 1105)	5 (2101 — 2105)
G2: low-dose group	0.125	5 (1201 — 1205)	5 (2201 — 2205)
G3: mid-dose group	0.25	5 (1301 — 1305)	5 (2301 — 2305)
G4: high-dose group	0.5	5 (1401 — 1405)	5 (2401 — 2405)

Table 2 Mortality

Group	Dose of WCF (mL)	Sex	No. of animals	Mortality (dead/tested)
G1	0	Male	5	0/5
		Female	5	0/5
G2	0.125	Male	5	0/5
		Female	5	0/5
G3	0.25	Male	5	0/5
		Female	5	0/5
G4	0.5	Male	5	0/5
		Female	5	0/5

Table 3 Clinical signs from day 0 to day 14

Group	Dose of WCF (mL)	Sex	No. of animals	Clinical sign
G1	0	Male	5	NOA*
		Female	5	NOA
G2	0.125	Male	5	NOA
		Female	5	NOA
G3	0.25	Male	5	NOA
		Female	5	NOA
G4	0.5	Male	5	NOA
		Female	5	NOA

*NOA: No observable abnormality.

Table 4 Mean body weights

Group	Dose of WCF (mL)			Days after dosing				Gain (g)
				0	3	7	14	0~14
G1	0	Male	Mean	194.2	221.6	263.3	326.6	132.5
			S.D.	6.8	5.4	6.7	7.6	6.6
		Female	Mean	154.6	166.5	183.9	211.2	56.6
			S.D.	5.1	3.3	4.4	9.1	7.1
G2	0.125	Male	Mean	192.9	222.6	264.5	336.9	144.0
			S.D.	3.7	6.1	8.9	13.5	11.9
		Female	Mean	154.6	165.3	181.2	203.0	48.4
			S.D.	7.8	10.1	12.3	18.7	17.6
G3	0.25	Male	Mean	196.3	227.5	274.4	349.2	152.9
			S.D.	7.8	9.5	12.4	16.4	10.8
		Female	Mean	155.0	164.7	180.5	206.3	51.3
			S.D.	8.6	7.5	11.2	15.1	7.8
G4	0.5	Male	Mean	193.7	221.0	261.4	331.3	137.6
			S.D.	4.2	7.6	8.4	12.3	9.7
		Female	Mean	154.9	169.1	184.0	210.2	55.3
			S.D.	8.9	7.2	8.2	9.0	8.4

Table 5 Mean hematology parameters

Group	Dose of WCF (mL)	Sex		RBC (× 10^6 cells/ μL)	HGB (g/dL)	HCT (%)	RBC Indices			PLT (× 10^3 cells/ μL)	Reti (%)
							MCV (fL)	MCH (pg)	MCHC (g/dL)		
G1	0	M	Mean	7.23	15.1	42.1	58.4	20.9	35.8	1066	4.42
			S.D.	0.46	0.5	1.8	1.8	0.9	0.4	54	0.88
		F	Mean	7.73	15.3	41.7	54.0	19.9	36.7	1112	2.44
			S.D.	0.24	0.4	0.9	1.3	0.5	0.3	89	0.16
G2	0.125	M	Mean	7.28	15.0	42.0	57.7	20.7	35.8	1107	5.16
			S.D.	0.19	0.2	0.8	1.0	0.4	0.4	200	0.39
		F	Mean	7.79	15.4	42.1	54.1	19.8	36.5	1073	2.26
			S.D.	0.39	0.5	1.1	1.9	0.7	0.3	74	0.50
G3	0.25	M	Mean	6.87	14.7	41.4	60.2	21.4	35.5	1072	4.83
			S.D.	0.19	0.5	1.1	2.3	1.0	0.7	135	0.39
		F	Mean	7.61	15.9	42.9	56.5	20.9	36.9	1100	2.43
			S.D.	0.22	0.3	1.2	2.2	0.7	0.4	162	0.48
G4	0.5	M	Mean	7.18	15.0	41.8	58.2	20.8	35.8	1161	4.78
			S.D.	0.32	0.4	1.1	1.5	0.6	0.3	96	0.55
		F	Mean	7.58	15.4	41.8	55.2	20.3	36.9	1104	2.27
			S.D.	0.31	0.4	1.4	2.0	0.7	0.4	112	0.43

(Continued)

Group	Dose of WCF (mL)	Sex		WBC (× 10³ cells/μl)	NEU	LYM	MONO	EOS	BASO	PT (sec)	APTT (sec)
G1	0	M	Mean	9.19	15.2	81.3	2.0	0.5	0.1	17.7	15.9
			S.D.	2.34	7.0	7.2	0.5	0.2	0.1	0.9	1.2
		F	Mean	5.63	14.0	81.8	1.9	1.2	0.1	18.3	15.4
			S.D.	2.19	2.6	2.7	0.5	0.4	0.1	0.4	0.7
G2	0.125	M	Mean	8.91	11.4	84.7	1.9	0.5	0.2	16.5	14.1
			S.D.	1.58	2.5	2.6	0.2	0.3	0.0	0.5	2.7
		F	Mean	6.27	9.6	86.4	2.0	1.0	0.2	18.2	15.5
			S.D.	1.18	4.2	5.0	1.1	0.3	0.1	0.4	1.1
G3	0.25	M	Mean	11.30	11.3	85.2	1.8	0.3	0.3*	17.3	15.6
			S.D.	2.18	5.4	5.8	0.2	0.1	0.1	0.7	1.3
		F	Mean	9.30	11.4	85.1	1.4	0.8	0.2	18.5	15.7
			S.D.	2.87	5.5	5.7	0.3	0.2	0.0	0.5	1.5
G4	0.5	M	Mean	7.58	10.8	85.8	2.0	0.5	0.1	17.2	15.0
			S.D.	2.18	0.9	1.3	0.4	0.3	0.0	1.0	2.9
		F	Mean	7.10	11.1	85.5	1.6	0.9	0.2	18.2	16.0
			S.D.	1.87	3.7	4.1	0.4	0.4	0.1	1.1	0.8

Significantly different from control by Dunnett's *t*-test: *P < 0.01; S.D., standard deviation; N, number of animals; RBC, red blood cell; HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular cell volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular cell hemoglobin concentration; WBC, white blood cell; PLT, platelet; PT, prothrombin time; APTT, active partial thromboplastin time; NEU, neutrophils; LYM, lymphocytes; MONO, monocytes; EOS, Eosinophils; BASO, basophils; Reti, reticulocytes.

Table 6 Mean clinical chemistry

Group	Dose of WCF (mL)	Sex		ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)	Glu (mL/dL)	BUN (mg/dL)	Crea (mg/dL)	T-Bili (mg/dL)	T-Chol (mg/dL)
G1	0	M	Mean	28.0	93.4	829.0	0.29	127	11.4	0.37	0.03	86
			S.D.	2.3	18.4	122.8	0.10	21	1.6	0.03	0.01	17
		F	Mean	25.6	111.9	496.3	0.69	103	13.0	0.41	0.03	84
			S.D.	6.6	7.8	104.2	0.25	5	2.0	0.04	0.02	11
G2	0.125	M	Mean	27.4	96.3	870.4	0.30	125	12.0	0.36	0.04	96
			S.D.	2.0	20.8	202.2	0.16	12	2.4	0.02	0.02	16
		F	Mean	24.2	97.3	556.7	0.59	112	14.7	0.42	0.03	81
			S.D.	2.4	9.1	109.0	0.11	6	2.6	0.03	0.01	12
G3	0.25	M	Mean	29.3	100.5	969.0	0.57†	123	12.2	0.36	0.03	76
			S.D.	4.2	21.2	91.2	0.05	16	1.4	0.01	0.02	14
		F	Mean	24.6	94.8	534.9	0.68	113	12.3	0.40	0.03	78
			S.D.	4.1	21.0	177.4	0.13	5	1.4	0.04	0.02	19

(Continued)

Group	Dose of WCF (mL)	Sex		ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)	Glu (mL/dL)	BUN (mg/dL)	Crea (mg/dL)	T-Bili (mg/dL)	T-Chol (mg/dL)
G4	0.5	M	Mean	28.8	94.4	897.0	0.39	132	12.4	0.39	0.02	81
			S.D.	3.7	29.2	177.9	0.08	18	1.6	0.02	0.02	13
		F	Mean	25.5	97.6	518.5	0.50	105	13.2	0.41	0.04	78
			S.D.	3.2	12.4	105.2	0.09	8	2.0	0.03	0.02	14

Group	Dose of WCF (mL)	Sex		TG (mg/dL)	TP (g/dL)	Alb (g/dL)	A/G ratio	P (mg/dL)	Ca (mg/dL)	Na (mmol/L)	K (mmol/L)	Cl (mmol/L)
G1	0	M	Mean	44	5.4	2.3	0.76	8.71	10.2	140	4.5	103
			S.D.	28	0.3	0.1	0.04	0.44	0.4	1	0.2	1
		F	Mean	22	5.7	2.6	0.83	7.53	10.2	138	4.8	105
			S.D.	10	0.2	0.1	0.06	0.32	0.1	1	0.3	1
G2	0.125	M	Mean	60	5.4	2.3	0.77	8.92	10.2	139*	4.7	102
			S.D.	17	0.2	0.0	0.05	0.35	0.3	1	0.4	1
		F	Mean	16	5.7	2.6	0.82	7.64	10.1	139	4.5	105
			S.D.	9	0.1	0.1	0.05	0.46	0.4	1	0.3	2
G3	0.25	M	Mean	45	5.4	2.3	0.75	9.11	10.3	139	4.6	102
			S.D.	20	0.2	0.1	0.04	0.52	0.3	0	0.2	1
		F	Mean	17	5.5	2.5	0.82	7.26	10.1	138	4.4	105
			S.D.	6	0.3	0.1	0.02	0.32	0.2	2	0.3	2
G4	0.5	M	Mean	37	5.3	2.3	0.75	9.00	10.3	140	4.8	103
			S.D.	9	0.2	0.1	0.09	0.75	0.3	1	0.3	3
		F	Mean	20	5.5	2.5	0.84	7.70	10.1	138	4.5	105
			S.D.	11	0.3	0.2	0.05	0.17	0.3	1	0.5	1

Significantly different from control by Dunnett's t-test: *$P < 0.05$, †$P < 0.01$; S.D., standard deviation; N, number of animals; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma glutamyltranspeptidase; ALP, alkaline phosphatase; Glu, glucose; BUN, blood urea nitrogen; Crea, creatinine; T-Bili, total bilirubin; T-Chol, total cholesterol; TG, triglycerides; TP, total protein; Alb, albumin.

mean corpuscular hemoglobin concentration (MCHC), platelet (PLT), leucocyte count (WBC), WBC differential count (neutrophils, lymphocytes, monocytes, eosinophils, basophils) and Reti (reticulocytes) were measured. For the coagulation test, about 2 mL of the collected blood was placed in a tube containing 3.2% sodium citrate and was centrifuged for 10 minutes at 3,000 rpm before collecting the blood plasma. Measurement was done using a coagulation time analyzer (Coapresta 2000, SEKISUI, Japan). The prothrombin time (PT) and the activated partial thromboplastin time (APTT) were measured. The hematology test showed no significant differences between the normal saline group and any of the WCF groups, except that the BASO of G3 males showed statistically significant chang-es with $P < 0.01$; however, the result had no toxicological meaning because it was not dose-dependent (Table 5).

For the clinical chemistry test, the unused blood remaining after the hematology test was centrifuged for 10 minutes at 3,000 rpm before blood serum was collected. A clinical chemistry analyzer (7180, HITACHI, Japan) and an electrolyte analyzer (AVL9181, Roche, Germany) were used to measure alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyl transpeptidase (GGT), blood urea nitrogen (BUN), creatinine (Crea), total bilirubin (T-Bili), total protein (TP), albumin (Alb), A/G ratio, total cholesterol (T-chol), triglycerides (TG), phosphorus (P), glucose (Glu), calcium (Ca), chloride (Cl), sodium (Na) and potassium

Table 7 Necropsy findings

Group	G1		G2		G3		G4	
Dose of WCF (mL)	0		0.125		0.25		0.5	
Sex	M	F	M	F	M	F	M	F
No. of animals	5	5	5	5	5	5	5	5
Day of sacrifice	15	15	15	15	15	15	15	15
Unremarkable findings	5	5	5	5	5	5	5	5

External surface and all organs in the body cavity were unremarkable.

Table 8 Histopathological findings

Group	G1		G2		G3		G4	
Dose of WCF (mL)	0		0.125		0.25		0.5	
Sex	M	F	M	F	M	F	M	F
No. of animals	5	5	5	5	5	5	5	5
Day of sacrifice	15	15	15	15	15	15	15	15
Injection site remarkable findings	0	1*	0	0	0	0	0	0

*Cell infiltration, inflammatory, focal: mild grade.

(K). The clinical chemistry test showed no changes caused by the test substance in animals of either sex in any group, except that the Na of G2 males and the GGT of G3 males showed statistically significant changes with $P < 0.05$, $P < 0.01$, respectively; however, the result had no toxicological meaning because it was not dose-dependent (Table 6).

Visual inspection of all body organs and tissues was performed on all animals after necropsy. Body organs and tissues were extracted and fixed in 10% neutral buffered formalin, and histopathological observations were carried out. Visual inspection after necropsy showed no visual abnormalities in any of the male or the female animals in any experimental group (Table 7). In addition, histopathological tests on the injected parts showed no effect due to the test substance in any animals, except G1 females; however, the result was due to spontaneous generation and had no toxicological meaning because it was not dose-dependent (Table 8).

4. Discussion

Pharmacopuncture can produce the effects of both acupuncture and herbal medicine. Also, a synergistic effect would be expected because pharmacopuncture does not pass through the digestive system [8]. The treatment area of pharmacopuncture is the common acupoints, tender points, or responsive points that are relevant to other disorders. In the procedure, two different types of pharmacopuncture are used: aromatic and oil-based pharmacopunctures. Oil-based pharmacopunture is used for consumptive diseases or degenerative diseases, and the extracts of *Cervi cornu parvum* (CC), *Juglans sinensis dode* (JSD), and CF are used. Aromatic pharmacopuncture is used for inflammatory diseases or pain, and the extracts of *Fel ursi*, *Bezoar bovis*, and *moschus* are used [9].

Oil-based pharmacopuncture is used for kinetic disorders caused by excessive usage causing a deficiency of body lubrication, for consumptive disorder induced by lopsided fire and dryness from improper ascending water and descending fire, and for disorders in the elderly such as arthritis, arteriosclerosis, and hypertension. CC, JSD, and CF, where oil is the main component, are commonly used. CF is used to treat vertebral disorders (lumbar pain in the elderly, coccyalgia, and fractures), joint disorders (degenerative arthritis, sprains, and tennis elbow), soft tissue damage (frozen shoulder, plantar fasciitis, and sequela of sprain), contusions, menstrual irregularities, deficient-type constipation, and muscle induration [9]. CF can be used not only for pain relief [10] but also for a significant increase in regional cerebral blood flow [11]. Oh *et al.* [12] and An *et al.* [13] reported an anti-cancer effect. Woo *et al.* [14] studied the anti-oxidative effect. Also, the safety of CF has been demonstrated, and its side effects have been presented in several studies [6, 15, 16].

Oil-based pharmacopunctures such as CC, JSD, and CF, which are made with alcohol extraction or compression, require more attention than *Eight-principles* pharmacopuncture (EPP), which uses a distillation method. The most frequent side effects come from overdosed injection, erroneous procedure, and spoiled pharmacopuncture. Also, a possibility exists that an over-dosage of oil-based pharmacopuncture may progress into allergic reactions. Even though such reactions are not common, they still need to be addressed because the cause is not the production or the management of the pharmacopuncture, but the ordinary process of treatment, and a chance exists that the usage of oil-based pharmacopuncture may result in continuous medical malpractice [9].

WCF was newly developed to provide strong effects and to reduce side effects. WCF is prepared by homogenizing a mixture of CF, WFI, and an emulsifier and increases the treatment efficacy while maintaining the characteristics of existing CF [6]. In this study, an intravenous-dose toxicity test of WCF was performed to evaluate the stability of WCF, which had been manufactured by the KPI according

to Good Laboratory Practices. No mortalities occurred in any group. Also, no abnormalities in the clinical symptoms were observed in any of the four groups. No significant body weight differences were observed in any group. Hematologically, a change in the BASO of the G3 male group was observed, but that change had no clinical or toxicological meaning. The clinical chemistry test showed changes in the Na of the G2 male group and the GGT of the G3 male group, but those changes had no clinical or toxicological meaning. A clinical chemistry study with a greater number of subjects and for a longer period is deemed necessary to examine the long-term and/or the high-dose effects of WCF. Visual inspection after necropsy showed no visual abnormalities in the male and the female animals in any experimental group. Histopathological tests on the injected parts showed a cell infiltration in the G1 female group; however, the result was due to spontaneous generation and had no toxicological meaning.

WCF is expected to be widely used. Toxicity, dose, and side effects must be studied because pharmacopuncture inserts foreign substances into the human body. In this study, single intravenous-dose toxicity tests were performed on rats to verify the safety of WCF and to identify its side effects. The result of single intravenous-doses of WCF in the 4 groups of rats showed that the approximate lethal dose for both male and female rats exceeded 0.5 mL. Therefore, WCF is a relatively safe pharmacopuncture that can be used for treatment, but further studies should be performed in the future. Also, the toxicity and the safety of WCF for use on humans must be investigated.

5. Conclusion

In this study, intravenous injection of WCF did not cause any problems. As none of the test subjects was affected by injections of up to 0.5 mL of WCF, levels over 0.5 mL may have a detrimental effect. If the exact lethal dose is to be established, further studies are needed. Finally, the results clearly show that WCF dosing up to 0.5 mL is safe.

Acknowledgements

Conflict of interest

The authors declare that there are no conflict of interest.

References

1. Korean Pharmacopuncture Institute. Pharmacopuncturology: principles and clinical applications. Seoul: Elsevier Korea LLC; 2012. Chapter 1, Definition and History; p. 3. Chaper 3, Types of Pharmacopuncture; p. 10. Chaper 8, Eight Principle Pharmacopuncture; p. 128-9, 144-5.

2. Yook TH, Song BY, Sin MS, Kim BH, Park JJ, Yun JH. [Effects on the local thermal change following herbal acupuncture on D.I.T.I.]. The Acupuncture. 2000;17(3):57-68. Korean.

3. Yoon MY, Cho NG, Hur TY, Cho EH, Lee OJ, Kim KS. [Clinical study on effect of carthmi - flos herbal acupuncture therapy on osteoarthritis in knee joint]. The Acupuncture. 2002;19(2):189-200. Korean.

4. Lee HT, Lee HI. [Effect of cathami flos aqua-acupuncture on arthritis induced by injecting freund's complete adjuvant]. The Journal of East-West Medicine. 1998;23(3):6-20. Korean.

5. Cho TS, Son IS, Kwon HY, Youn HM, Jang KJ, Song CH, *et al*. [The effect of CF herbal acupuncture by oswestry disability index]. J Pharmacopunct. 2001;4(3):15-20. Korean.

6. Lee HG, Kim SC, Jung DJ, Choi YM, Sin MS, Choi SW, *et al*. [Single intramuscular-dose toxicity of water soluble carthmi-flos herbal acupuncture (WCF) in sprague-dawley rats]. J Pharmacopunct. 2014;17(1):27-34. Korean.

7. Kang KS, Lee JS, Kwon GR. [Clinical studies on the cases of side effect of CF herbal acupuncture treatment]. J Pharmacopunct. 2001;4(2):65-71. Korean.

8. Joo HJ. [Research on pharmacopuncture]. Daejeon. Korea Institute of Oriental Medicine. 1995;(5):193-210. Korean.

9. Korean Pharmacopuncture Institute. Pharmacopuncturology: principles and clinical applications. Seoul: Elsevier Korea LLC; 2012. Chapter 7, Meridian field pharmacopuncture; p. 112-4, 124.

10. Choi SW, Park PB, Oh SJ. [A case report of carpal tunnel syndrome with raynaud's phenomenon treated by bee venom and carthami flos pharmacopuncture]. J Pharmacopunct. 2009;12(1):103-8. Korean.

11. Kim SW, Jeong HW, Wei TS, Cho MR, Yun YC. [Experimental effects of aqua-acupuncture of carthami flos on the regional cerebral blood flow and mean arterial blood pressure in rats]. The Acupuncture. 2004;21(2):167-81. Korean.

12. Oh CS, Lee H, Yim YK, Seong NK. [Influence on the anti-cancer and immune response improvement of herbal-acupuncture with carthami flos infusion

solution into chung-wan (CV12)]. The Acupuncture. 2004;21(5):205-18. Korean.

13. An CS, Kwon KR, Lee SG. [The study on acute and sub-acute toxicity and sarcoma-180 anti-cancer effects of carthami tinctor-fructus herbal-acupuncture (CF)]. J Pharmacopunct. 2002;5(1):7-26. Korean.

14. Woo DS, Lee KM, Lee BH, Lim SC, Jung TY, Seo JC. [The scavenging effect on nitric oxide in carthami flos herbal-acupunture solution]. J Pharmacopunct. 2007;10(2):25-30. Korean.

15. Lee JY, Kang HM, Lim CS, Kwon KR. [Report on the side-effects of pharmacopuncture lubricants (CF, JsD)]. J Pharmacopunct. 2007;10(3):137-42. Korean.

16. Kim BW, Kwon KR. [The side effect of lubricants pharmacopuncture in rat tissues]. 2010;13(1):87-92. Korean.