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Original article

Single Dose Toxicity of Chukyu (spine-healing) Pharmacopuncture Injection in the Muscle of Rats

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Key Words
Chukyu (spine-healing) pharmacopuncture, toxicity test

Abstract

Objectives: This study was performed to analyze the single dose toxicity of Chukyu (spine-healing) pharmacopuncture.

Methods: All experiments were conducted at the Biotoxtech, an institution authorized to perform non-clinical studies under the regulations of Good Laboratory Practice (GLP) regulations. Sprague-Dawley rats were chosen for the pilot study. Doses of Chukyu (spine-healing) pharmacopuncture, 0.1, 0.5 and 1.0 mL, were administered to the experimental groups, and a dose of normal saline solution, 1.0 mL, was administered to the control group. This study was conducted under the approval of the Institutional Animal Ethic Committee.

Results: No deaths or abnormalities occurred in any of the four groups. No significant changes in weight, hematological parameters or clinical chemistry between the control group and the experimental groups were observed. To check for abnormalities in organs and tissues, we used microscopy to examine representative histological sections of each specified organ; the results showed no significant differences in any of the organs or tissues except in one case, where interstitial infiltrating macrophages were found in one female rat in the 0.5-mL/animal experimental group.

Conclusion: The above findings suggest that treatment with Chukyu (spine-healing) pharmacopuncture is relatively safe. Further studies on this subject are needed to yield more concrete evidence.

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1. Introduction

Pharmacopuncture therapy is a new acupuncture therapy based on herbal medicine, acupuncture & moxibustion medicine, and meridian theory [1]. Through a single procedure, it can achieve both the effects of acupuncture and herbal medicine. As the pharmacopuncture does not pass through the digestive system, it works fast and it has effects that oral administration does not have [2]. Highly effective herbs are selected, depending on the disease. Pharmacopuncture fluid is extracted from these selected herbs and injected into the meridian points or sore spots [3].

The constituents of the Chukyu (spine-healing) phar-

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This paper meets the requirements of KS X ISO 9706, ISO 9706-1994 and ANSI/NISO Z39.48-1992 (Permanence of Paper).

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macopuncture are *Cervi Parvum Cornu* (deer antlers), *Moschus* (musk), *Angelicae Gigantis Radix*, *Cnidii Rhizoma*, *Ostericii Radix*, *Angelicae Pubescentis Radix*, *Astragali Radix*, and *Scolopendra*. These were extracted at low temperature and low pressure in an aseptic room at the Korean Pharmacopuncture Institute. In the name 'Chukyu (脊俞)', 'Chuk' means spine, and 'yu' means healing, 'Chukyu' means spinal healing. Actually, Chukyu (spine-healing) pharmacopuncture is known clinically to have effects on lumbago and skelalgia [4].

Several studies on the relation between the toxicity and the composition of Chukyu pharmacopuncture have been conducted. Koo et al. conducted a philological study on the toxicity of *Cnidii Rhizoma* [5], Roh *et al*. conducted a philological study on the toxicity and side effects of *Angelicae Sinensis Radix* [6] and Park *et al*. conducted a philological study on the toxicity of *Angelicae Pubescentis Radix* [7]. Choi et al. reported that in toxicological tests, *Astragali Radix* showed no toxicity in rats [8] and Byun *et al*. reported that in an analysis by using microculture tetrazolium (MTT) method, the *Cervi Parvum Cornu* pharmacopuncture was not able to cause cell cytotoxicity in the hepatocytes of rats [9]. Lim *et al*. conducted a safety study of scolopendrid pharmacopuncture and showed no abnormal findings after scolopendrid pharmacopuncture treatment [10]. Nevertheless, objective single-dose toxicity testing of Chukyu pharmacopuncture, which is complex combination of herbs, has not been conducted yet.

The current research trend for single-dose toxicity testing of extracts is to study acute and subacute toxicity through Good Laboratory Practice (GLP) regulations. All the experiments for this research were conducted at Biotoxtech, a non-clinical studies authorized institution, under the GLP. This study was performed to analyze the single-dose toxicity and the lethal dose of Chukyu pharmacopuncture in rats.

2. Materials and Methods

The Chukyu pharmacopuncture was prepared in a sterile room at the Korean Pharmacopuncture Institute (K-GMP). After the mixing process with pure water, the pH was controlled to between 7.0 and 7.5. NaCl was added to make a 0.9% isotonic solution. The completed extract was stored in a refrigerator (2.1-6.6℃).

The animals used in this study were 6-week-old Sprague-Dawley rats. The reason Sprague-Dawley rats were chosen is that they have been widely used in safety test in the field of medicine, so the results can be easily compared with many other data bases. The mean weights of the rats were 179.8-198.5 g and 143.8-173.1 g, respectively, for the male and the female rats at the time of injection. For all animals, a visual inspection was conducted; all animals were weighed using a CP3202S system (Sartorius, Germany). During 7 days of acclimatization, the general symptoms of the rats were observed once a day. The weights of the rats were recorded on the last day of acclimatization. No abnormalities were found.

The temperature of the lab was 20.0-22.8℃, and the humidity was 48.5-65.9%. Enough food (Teklad Certified Irradiated Global 18% Protein Rodent Diet 2918C) and UV-filtered water were provided.

Groupings were done after 7 days of acclimatization. Animals were selected if their weights were close to the mean weight. In total, 20 male rats and 20 female rats were selected. The animals were randomly distributed into 4 groups (5 mice per group) as shown in, Table 1.

In clinical applications, the usual dose for Chukyu pharmacopuncture is 1.0 mL per treatment. No death occurred in the pilot test in which 1.0 mL of Chukyu pharmacopuncture was injected into each male and female rat. In this study 1.0 mL/animal was set as a high-dose, and 0.5 mL/animal and 0.1 mL/animal were set as mid and low doses, respectively. In the control group, 1.0 mL of normal saline solution was administered. A single dose, 0.1 and 0.5 mL/animal, was injected into the left thigh muscle of the rats in the low and mid-dose groups, respectively, and 0.5 mL of Chukyu pharmacopuncture was injected into each thigh muscles of the rats in the high-dose groups, for a total of 1.0 mL/animal, by disposable syringes. This study was conducted under the approval of the Institutional Animal

Table 1 Groups of animals

Group	Chuckyu injection (mL/animal)	Number of animals (serial number)	
		Male	Female
G1 control group	0	5 (1101-1105)	5 (2101-2105)
G2 low-dose group	0.1	5 (1201-1205)	5 (2201-2205)
G3 mid-dose group	0.5	5 (1301-1305)	5 (2301-2305)
G4 high-dose group	1.0	5 (1401-1405)	5 (2401-2405)

Ethic Committee of Biotoxtech Co., Ltd..

From the 1st day to 14th day after treatment, the general symptoms were examined once a day. On the day of dosing (day 0), the general symptoms (side effects, revealing time, recovery time, etc.), as well as mortality, were examined at 30 minutes and at 1, 2, 3, and 4 hours after injection. The weights were measured immediately before treatment and at 3, 7 and 14 days after treatment. After fasting for more than 18 hours, the rats were anesthetized by using *isoflurane*. Blood samples were taken from the abdominal aorta on the day of necropsy (15 days after injection). A 1-mL blood sample was analyzed by using an automatic hematology analyzer (ADVIA 120, SIEMEMS, Germany). A 2.0-mL blood sample was centrifuged for the blood coagulation test (3,000 rpm, 10 minutes). The results were measured by using an Automated Coagulation Analyzer (Coapresta 2000, SEKISUI, Japan). The blood taken from the abdominal aorta was used in the blood biochemical test. The results were measured by using an Automatic Analyzer (7180, Hitachi, Japan) and Electrolyte Analyzer (AVL9181, Roche, Germany). After observations had been terminated, the organs and tissues of all surviving animals were visually inspected and microscopically examined.

The weight, hematologic examination and blood chemical test results from the experiments were analyzed by using SAS (Statistical Analysis System, version 9.2,9.3, SAS Institute Inc., USA). A Bartlett test was conducted to evaluate the homogeneity of the variance and the significance. The one-way ANOVA test was conducted when the homogeneity of the variance was recognized, and the Kruskal-Wallis test was conducted post-hoc.

3. Results

In this study, no deaths or abnormalities occurred in any of the groups (Table 2, 3). In addition, no changes in weight were observed in any of the groups (Table 4). Finally, no meaningful changes in the hematological examination, blood chemical test or necropsy were noted (Tables 5, 6, 7). On histopathological examination, interstitial infiltrating macrophages were found in one female rat in the 0.5-mL/animal group, but no significant changes in the brain, lungs, liver, kidney and spinal cord related to the injections were found in the other groups (Table 8).

4. Discussion

Chukyu pharmacopuncture is used widely in clinics. Until now, there has been only one clinical review on the effects of Chukyu pharmacopuncture [4]. On the other hand, there have been many studies on the component herbs of this pharmacopuncture. *Cervi Parvum Cornu* which is a component of Chukyu pharmacopuncture has anti-arthritis effects, as well as analgesic effects [11, 12]. *Moschus* has been reported to impact the immune response [13]. *Angelicae Gigantis Radix*, *Cnidii Rhizoma*, *Angelicae Pubescentis Radix*, and *Astragali Radix* are known to have effects on arthritis in rats. [14-17]. In addition, *Scolopendra*, through its anti-inflammatory activity, has been reported to have therapeutic effects on patients with herniated intervertebral discs of the lumbar spine or cauda equine syndrome [18-20]. Thus, Chukyu pharmacopuncture and its component herbs have been reported to have many effects on several disorders. Although it is used in clinics, safety studies on Chukyu pharmacopuncture are insufficient, so more safety studies are needed. The toxicity test is an important data base and is essential for evaluating the safety of test substances in medications [21].

Table 2 Mortality

Group	Dose (mL/animal)	Mortality (dead / tested)	
		Male	Female
G1	0	0% (0 / 5)[a]	0% (0 / 5)
G2	0.1	0% (0 / 5)	0% (0 / 5)
G3	0.5	0% (0 / 5)	0% (0 / 5)
G4	1.0	0% (0 / 5)	0% (0 / 5)

Table 3 Clinical signs

Group	Dose (mL/animal)	Sex	Number of animals	Clinical signs
G1	0	Male	5	NAD*
		Female	5	NAD
G2	0.1	Male	5	NAD
		Female	5	NAD
G3	0.5	Male	5	NAD
		Female	5	NAD
G4	1.0	Male	5	NAD
		Female	5	NAD

*NAD: no abnormalities detected

Table 4 Body weights in grams

Group	Dose (mL/animal)	Sex		Days after administration			
				0	3	7	14
G1	0	Male	Mean	189.9	213.7	248.9	295.8
			S.D.	6.0	7.1	8.8	17.5
			N	5	5	5	5
		Female	Mean	156.6	168.9	183.8	206.7
			S.D.	9.7	12.2	15.0	18.8
			N	5	5	5	5
G2	0.1	Male	Mean	190.9	214.5	250.4	306.8
			S.D.	4.3	7.9	8.8	8.2
			N	5	5	5	5
		Female	Mean	158.1	169.6	185.2	209.2
			S.D.	11.2	8.0	8.7	10.9
			N	5	5	5	5
G3	0.5	Male	Mean	188.4	215.4	251.3	302.9
			S.D.	4.0	6.6	7.2	8.5
			N	5	5	5	5
		Female	Mean	157.0	169.3	184.0	202.9
			S.D.	10.7	9.4	12.9	12.8
			N	5	5	5	5
G4	1.0	Male	Mean	190.1	214.9	252.0	304.6
			S.D.	8.4	11.7	14.8	17.6
			N	5	5	5	5
		Female	Mean	160.5	171.1	189.3	215.5
			S.D.	11.5	13.9	20.7	23.5
			N	5	5	5	5

N: Number of animals, S.D.: Standard deviation

Table 5 Mean hematology parameters

Group	Dose (mL/animal)	Sex		RBC ($x10^3$ cells/$\mu\ell$)	HGB (g/dL)	HCT (%)	RBC Indices			PLT ($x10^3$ cells/$\mu\ell$)	Reti (%)
							MCV (fL)	MCH (pg)	MCHC (g/dL)		
G1	0	Male	Mean	7.33	15.2	45.5	62.1	20.7	33.4	1230	4.6
			S.D.	0.33	0.6	1.9	1.7	0.3	0.6	88	0.8
			N	5	5	5	5	5	5	5	5
		Female	Mean	7.41	15.3	44.5	60.1	20.7	34.5	1527	2.7
			S.D.	0.29	0.5	1.3	1.3	0.5	0.3	788	0.6
			N	5	5	5	5	5	5	5	5

G2	0.1	Male	Mean	7.27	14.8	44.1	60.7	20.4	33.7	1310	4.7
			S.D.	0.25	0.3	1.1	1.8	0.6	0.2	183	0.8
			N	5	5	5	5	5	5	5	5
		Female	Mean	7.45	15.6	45.0	60.5	20.9	34.6	1124	3.1
			S.D.	0.43	0.8	2.4	1.0	0.5	0.4	112	0.4
			N	5	5	5	5	5	5	5	5
G3	0.5	Male	Mean	7.24	14.8	44.0	60.9	20.4	33.4	1339	5.0
			S.D.	0.36	0.4	1.0	1.6	0.5	0.2	129	0.7
			N	5	5	5	5	5	5	5	5
		Female	Mean	7.37	15.3	44.5	60.4	20.8	34.5	1207	3.2
			S.D.	0.45	0.8	2.1	1.5	0.4	0.4	131	0.3
			N	5	5	5	5	5	5	5	5
G4	1.0	Male	Mean	7.04	14.3	42.9	61.0	20.4	33.4	1339	5.0
			S.D.	0.31	0.5	1.4	1.2	0.7	0.5	136	0.7
			N	5	5	5	5	5	5	5	5
		Female	Mean	7.57	15.5	45.1	59.6	20.5	34.4	1234	2.8
			S.D.	0.39	0.7	2.0	1.1	0.3	0.4	180	0.3
			N	5	5	5	5	5	5	5	5

Group	Dose (mL/animal)	Sex		WBC ($x10^3$ cells/μL)	WBC differential count (%)					PT (sec)	APTT (sec)
					NEU	LYM	MONO	EOS	BASO		
G1	0	Male	Mean	9.19	18.0	77.4	2.7	0.6	0.2	17.3	13.6
			S.D.	3.16	2.3	2.8	0.6	0.3	0.1	0.6	1.2
			N	5	5	5	5	5	5	5	5
		Female	Mean	5.88	13.6	82.6	1.6	0.9	0.2	18.0	13.3
			S.D.	2.05	4.8	4.7	0.3	0.1	0.2	0.9	1.9
			N	5	5	5	5	5	5	5	5
G2	0.1	Male	Mean	8.68	14.0	81.5	1.9	0.5	0.2	17.6	13.6
			S.D.	2.44	2.8	3.6	0.6	0.1	0.1	0.6	0.8
			N	5	5	5	5	5	5	5	5
		Female	Mean	6.26	12.5	83.9	1.5	0.9	0.2	17.7	13.9
			S.D.	1.05	5.2	5.3	0.4	0.2	0.0	0.4	1.8
			N	5	5	5	5	5	5	5	5
G3	0.5	Male	Mean	9.49	15.8	80.4	2.1	0.4	0.2	16.6	13.7
			S.D.	3.11	1.7	2.1	0.8	0.2	0.1	1.3	0.5
			N	5	5	5	5	5	5	5	5
		Female	Mean	4.88	16.1	80.1	1.9	0.9	0.2	17.6	13.0
			S.D.	2.05	5.5	5.4	0.8	0.5	0.1	0.5	1.0
			N	5	5	5	5	5	5	5	5

G4	1.0	Male	Mean	8.34	14.5	82.0	1.8	0.5	0.2	17.9	13.8
			S.D.	3.11	1.7	2.1	0.8	0.2	0.1	1.3	0.5
			N	5	5	5	5	5	5	5	5
		Female	Mean	5.25	14.8	81.3	1.9	0.9	0.2	17.8	12.9
			S.D.	0.80	3.6	3.7	0.3	0.2	0.1	0.6	1.5
			N	5	5	5	5	5	5	5	5

N: Number of animals, S.D.: Standard deviation

Table 6 Mean clinical chemistry

Group	Dose (mL/animal)	Sex		ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)	Glu (mg/dL)	BUN (mg/dL)	Crea (mg/dL)	T-Bili (mg/dL)	T-Chol (mg/dL)
G1	0	Male	Mean	32.0	84.4	956.7	0.36	124	11.4	0.38	0.03	65
			S.D.	2.3	8.5	233.2	0.11	23	1.3	0.03	0.01	5
			N	5	5	5	5	5	5	5	5	5
		Female	Mean	22.7	77.7	525.0	0.94	114	12.1	0.39	0.03	89
			S.D.	2.9	10.0	176.9	0.89	9	1.4	0.03	0.01	16
			N	5	5	5	5	5	5	5	5	5
G2	0.1	Male	Mean	31.8	89.2	999.1	0.47	116	10.5	0.37	0.03	59
			S.D.	6.4	14.3	152.0	0.16	6	1.6	0.02	0.01	10
			N	5	5	5	5	5	5	5	5	5
		Female	Mean	27.3	91.9	603.2	0.71	117	12.6	0.41	0.02	91
			S.D.	4.9	21.5	119.0	0.19	12	0.6	0.03	0.01	15
			N	5	5	5	5	5	5	5	5	5
G3	0.5	Male	Mean	29.3	84.9	806.5	0.37	125	11.2	0.38	0.04	72
			S.D.	4.5	18.8	189.6	0.18	6	1.3	0.01	0.01	11
			N	5	5	5	5	5	5	5	5	5
		Female	Mean	24.0	76.7	511.0	0.58	116	13.5	0.41	0.02	77
			S.D.	2.7	9.9	60.5	0.22	8	1.4	0.02	0.01	23
			N	5	5	5	5	5	5	5	5	5
G4	1.0	Male	Mean	29.0	79.7	882.8	0.47	123	12.3	0.39	0.03	64
			S.D.	4.2	4.1	292.1	0.27	12	1.7	0.04	0.02	8
			N	5	5	5	5	5	5	5	5	5
		Female	Mean	23.5	77.5	593.8	0.60	125	11.8	0.40	0.01	87
			S.D.	1.9	12.7	151.8	0.27	16	2.0	0.04	0.00	19
			N	5	5	5	5	5	5	5	5	5

Group	Dose (mL/animal)	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)
			Mean	39	5.3	2.3	0.77	8.31	10.0	141	4.7	101
		Male	S.D.	25	0.2	0.2	0.05	0.48	0.2	1	0.2	1

G1	0	Female	N	5	5	5	5	5	5	5	5	5
			Mean	18	5.7	2.6	0.86	7.55	10.0	140	4.7	103
			S.D.	8	0.2	0.1	0.05	0.66	0.2	2	0.3	1
G2	0.1	Male	N	5	5	5	5	5	5	5	5	5
			Mean	41	5.4	2.3	0.75	8.41	10.0	140	4.6	101
			S.D.	19	0.1	0.1	0.03	0.24	0.3	1	0.3	2
		Female	N	5	5	5	5	5	5	5	5	5
			Mean	14	5.8	2.7	0.85	7.30	10.2	140	5.1	104
			S.D.	5	0.3	0.3	0.08	0.56	0.4	1	0.3	2
G3	0.5	Male	N	5	5	5	5	5	5	5	5	5
			Mean	53	5.4	2.3	0.75	8.81	10.0	139	4.5	101
			S.D.	8	0.2	0.1	0.04	0.15	0.2	2	0.3	1
		Female	N	5	5	5	5	5	5	5	5	5
			Mean	11	5.7	2.7	0.89	7.38	10.2	140	5.1	104
			S.D.	5	0.5	0.4	0.09	0.76	0.5	2	0.2	2
G4	1.0	Male	N	5	5	5	5	5	5	5	5	5
			Mean	43	5.3	2.3	0.77	8.18	10.0	139	4.6	102
			S.D.	20	0.2	0.1	0.02	0.60	0.3	1	0.3	2
		Female	N	5	5	5	5	5	5	5	5	5
			Mean	19	5.5	2.5	0.83	7.76	10.3	139	4.7	103
			S.D.	6	0.3	0.1	0.02	0.35	0.2	1	0.3	1
			N	5	5	5	5	5	5	5	5	5

N: Number of animals, S.D.: Standard deviation

Table 7 Mean clinical chemistry

Findings	Group							
	G1 (0 mL/animal)		G2 (0 mL/animal)		G3 (0.5 mL/animal)		G4 (1.0 mL/animal)	
	Male	Female	Male	Female	Male	Female	Male	Female
Number of rats examined	5	5	5	5	5	5	5	5
Unremarkable findings	5	5	5	5	5	5	5	5

Table 8 Histopathological findings

Findings	Group							
	G1 (0 mL/animal)		G2 (0 mL/animal)		G3 (0.5 mL/animal)		G4 (1.0 mL/animal)	
	Male	Female	Male	Female	Male	Female	Male	Female
Number of rats examined	5	5	5	5	5	5	5	5
Remarkable findings (Infiltration, macrophages, interstitial)	0	0	0	0	0	±1	0	0

Grade- ±: minimal

This study was performed to provide objective safety data for Chukyu pharmacopuncture. Doses of 0.1, 0.5, 1.0 mL of Chukyu pharmacopuncture were administered to the experimental groups, and a 1.0-mL dose of normal saline solution were administered to the control group. In all four groups, no deaths occurred, and no abnormalities were found. No significant changes in the clinical signs, weights, hematologic examination results and blood chemical test results were noted between the control group and the experimental groups. In necropsy for checking for abnormalities in organs and tissue, no significant histopathological findings were noted except for one case where interstitial infiltrating macrophages were found in one female rat in the 0.5-mL/animal group.

To assess the toxicity of Chukyu pharmacopuncture, we need to study its acute and chronic side effects and its relations with capacity reaction more. Animal testing is the most fundamental and basic way to perform safety assessments [22]. The Korea Food & Drug Administration has testing protocol guidelines for the study of toxicity, and all experiments should be conducted following Good Laboratory Practice (GLP) regulations [23]. In this study, a 1.0-mL dose of Chukyu pharmacopuncture caused no considerable side effects in either male or female rats, which indicates that this dose is safe to use and does not cause severe histological abnormalities. Further studies on the subject should be conducted to yield more concrete evidence to support this finding.

5. Conclusion

The results showed that administration of 1.0-mL/animal Chukyu pharmacopuncture did not cause any changes in weight or in the results of hematological, blood chemical, and necropsy examinations. It also did not result in any mortality, which indicates that Chukyu pharmacopuncture administration can be used as a safe treatment.

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