J Acupunct Res 2020;37(3):167-172

Journal of Acupuncture Research

Journal homepage: http://www.e-jar.org

Original Article

Evaluation of the Single-Dose Toxicity of Capsaicin Pharmacopuncture in Rats

Ji Hye Hwang [1], Jaseung Ku [2], Chul Jung [3,*]

1 Department of Acupuncture and Moxibustion Medicine, College of Korean Medicine, Gachon University, Seongnam, Korea
2 Bogwang Korean Medical Clinic, Seoul, Korea
3 Namsangcheon Korean Medicine Clinic, Seoul, Korea

Article history:
Submitted: June 18, 2020
Revised: July 09, 2020
Accepted: July 20, 2020

Keywords:
acupuncture, capsaicin, pharmacopuncture, safety, toxicity

https://doi.org/10.13045/jar.2020.00185
pISSN 2586-288X eISSN 2586-2898

ABSTRACT

Background: This study aimed to assess the toxicity of capsaicin (CP) pharmacopuncture in an animal model.
Methods: The toxicity of a single-muscular dose of CP (45.45 mg/mL) was evaluated in 6-week-old male and female Sprague-Dawley rats. A total of 20 rats were assigned to 2 groups which were sex and weight matched. All rats acclimatized for 1 week before receiving 1.0 mL of CP (45.45 mg/mL) or normal saline solution (control) intramuscularly. The general condition and mortality of the animals were observed. The rats were sacrificed 2 weeks after CP was administered and histopathology was performed.
Results: No abnormal symptoms or deaths were observed, and there was no difference in body weights between the CP and control groups throughout the study. No significant differences in histopathology were observed between the groups.
Conclusion: No toxicological changes related to the administration of CP were observed. This study indicated that the safe dose of CP in Sprague-Dawley rats was 1.0 mL of CP (45.45 mg/mL) or less. Further studies are needed to confirm the safety of CP in the human body.

Introduction

The safety and efficacy of herbal medicine used in traditional Chinese medicine (TCM) and traditional Korean medicine (TKM) have been proven long-term through clinical experience, however, some studies have reported side effects and safety concerns of herbal medicines [1,2]. Therefore, it is very important to scientifically test herbal medicines for toxicity.

Pharmacopuncture is a new method of acupuncture involving the injection of herbal medicine (TCM and TKM), and is often used to provide rapid analgesia in clinical practice because it can deliver relief faster than oral administration [3,4].

Capsaicin (CP) pharmacopuncture is a medicine mainly containing red pepper based on V pharmacopuncture. The 9 herbs included in CP pharmacopuncture medicine are Capsicum annuum L., Moschus, Ursi Fel, Bovis Calculus, Scutellaria baicalensis, Phellodendron amurense, Pulsatilla koreana, Sophora tonkinensis, and Aucklandia lappa. V pharmacopuncture includes Moschus, Bovis Calculus and Ursi Fel, and has proven safety and efficacy through long-term clinical experience, toxicity evaluation, efficacy studies (such as anti-inflammatory and antioxidant effects), and clinical reports [5-9]. It was developed to treat neuropathic pain such as nerve entrapment and neurosensory abnormalities, myofascial pain, and hypertrophy by utilizing the capsaicin component contained in red pepper.

Capsaicin is a water-insoluble derivative of homovanillic acid [10] and the main active ingredient in capsicum fruits. It has been reported to be effective in treating inflammatory disorders (such as psoriasis and rheumatoid arthritis) [11], neuropathic pain (such as diabetic neuropathy), postherpetic neuralgia, and cluster headaches [12], urinary system diseases [13], obesity [14], vulvovaginal pain syndrome [15], cancer [16,17], and skin diseases [17,18].

Although CP pharmacopuncture is based on V pharmacopuncture, which has been verified for safety, there have been no systematic, scientific, clinical or experimental studies on the efficacy and

*Corresponding author. Chul Jung
Namsangcheon Korean Medicine Clinic, Seoul 06656, Korea
Email: jcnu2000@daum.net
ORCID: Ji Hye Hwang https://orcid.org/0000-0002-6304-1972, Jaseung Ku https://orcid.org/0000-0003-4365-5587, Chul Jung https://orcid.org/ 0000-0002-2522-5279

safety of CP, with the exception of 1 case report of patients with hypoesthesia of lower limb [19].

Whilst the safety of CP pharmacopuncture has been confirmed by clinical experience, before it is widely used in TKM clinics, further scientific verification of its efficacy and safety is needed. This begins with animal studies where the safety of CP pharmacopuncture can be investigated. In this study a single-dose of CP pharmacopuncture was delivered using intramuscular injection in Sprague-Dawley (SD) rats to test CP toxicity.

Material and Methods

Materials

CP consists of 9 herbs: Capsicum annuum L. (11 mg/mL), Moschus (0.1 mg/mL), Ursi Fel (0.075 mg/mL), Bovis Calculus (0.05 mg/mL), Scutellaria baicalensis (10 mg/mL), Phellodendron amurense (10 mg/mL), Pulsatilla koreana (10 mg/mL), Sophora tonkinensis (10 mg/mL), and Aucklandia lappa (5 mg/mL).

Moschus, Ursi Fel, and Bovis Calculus were extracted at 65°C using water and alcohol (v/v = 1:1) for 24 hours. The alcohol was removed using a vacuum pressure concentrator at 45°C. Other raw materials were distilled using a low-temperature vacuum extractor with purified water for injection. After mixing the extracted products, it was diluted with purified water and filtered sequentially using Whatman No. 2 filter paper, 5 μm, 0.45 μm, and 0.2 μm cellulose acetate filter. Salt was added, and the CP solution was pH titrated.

CP was manufactured as the finished product (45.45 mg/mL in a sealed vial) at an extramural facility, Namsangcheon Herbal Medicine Dispensary (Yongin, Korea) meeting Korean Good Manufacturing Practice standards. Normal saline solution (JW Pharmaceutical Co., Ltd., Korea) was used as a control in this study.

Experimental animals

For this study, 6-week-old male and female SDrats (Orient Bio Inc. Seongnam, Korea) were used. On the day the animals arrived in the animal facility, a visual inspection was performed and their body weights were recorded using an electronic scale (CP3202S, Sartorius, Gottingen, Germany). The body weights ranged between 185.9-204.9 g for the males and 137.0-163.9 g for the female rats.

During 1 week of acclimatization, the general condition of the rats, was checked daily, and all rats were judged to be normal. Rats were housed under controlled environmental conditions with an ambient temperature of 20.4-21.5°C, relative humidity of 46.3-64.6%, ventilation 10 - 15 times/hour, 12-hour/12-hour light/dark cycle, illuminance of 150-300 Lux, and free access to water and food (Teklad Certified Irradiated Global 18% Protein Rodent Diet 2918C, Envigo, Huntingdon, UK). After the acclimatization period, the rats were divided into 2 groups (control and experimental).

Animals in this study were treated according to the Korean National Institute of Health and the Korean Academy of Medical Sciences guidelines (Biotoxtech study number B18663) at Biotoxtech (Cheongwon, Korea) where Good Laboratory Practice regulations [20-22] were adhered to. Approval for this study was granted from the Institutional Animal Ethics Committee of Biotoxtech (180554).

Single-dose intramuscular toxicity test of CP in SD rats

The target tissue for CP was muscle, so the rats were injected intramuscularly. The anticipated clinical dose of CP was 0.1 to 1.0 mL according to preliminary testing (Biotoxtech study number B18663P1) where no mortality was observed after an intramuscular injection with 1.0 mL of CP. Based on the preliminary testing, the dose of CP was 1.0 mL (G2) and the same amount of saline solution was administered in the control group (G1; Table 1). There were 10 male and 10 female rats selected with a body weight closest to the average body weight. G1 and G2 were sex and body weight matched and injected intramuscularly with 0.5 mL into the left and right femur muscle.

On Day 0, the day of injection, the general condition of the rats were observed and types of toxic indications, toxicity expression, recovery times, and any deaths were recorded 30 minutes, and 1, 2, 4, and 6 hours after CP pharmacopuncture or saline injection. From Day 1 to Day 14, all rats were observed once daily for their general condition. All rats were weighed before injections on Day 0, and after injections on Days 3, 7, and 14.

After the observation period, all animals were euthanized by anesthetizing with CO_2 and exsanguinating through the abdominal aorta. A detailed visual inspection of organs and tissues of the whole body was performed. Following which the organs and tissues were removed, autopsied, and fixed in 10% neutral buffered formalin solution. For the injection site, fixed organs and tissues were subjected to general tissue processing such as cutting, dehydration and paraffin embedding, and tissue sections were prepared and sliced, followed by Hematoxylin and Eosin (H&E) staining. The remaining organs and tissues were preserved in 10% neutral buffered formalin solution. Histopathological examination was performed on organs and tissues. A local tolerance test was performed on the administration site of all rats.

Statistical analysis

Body weight analysis, before and after the single-dose intramuscular toxicity test was performed using SAS (Version 9.3, SAS Institute Inc., NC, USA). Equal variance was determined using the Folded-F test, and statistical significance ($p < 0.05$, $p < 0.01$) was determined using the Student's t test.

Table 1. Single-dose Intramuscular Toxicity Tests in SD Rats.

Group		Dose (mL/animal)	Dose amount (mL/animal)	No. of animals (subject no.)	
				Male	Female
G1	Control (normal saline)	0	1.0	5 (1,101–1105)	5 (2,101–2105)
G2	CP	1.0	1.0	5 (1,201–1205)	5 (2,201–2205)

CP, capsaicin containing pharmacopuncture medicine.

Results

Effects of CP pharmacopuncture on the general health, body weight and morbidity of the animals

During the observation period, no deaths, abnormalities, general symptoms of ill-health were observed in the 1.0 mL of CP-treated and control group of rats (Tables 2 and 3).

During the experiment, male rat weights in the CP and the control group changed from 194.7 ± 6.8 g (G1) and 192.8 ± 7.4 g (G2) to 316.9 ± 11.7 g (G1) and 314.2 ± 34.5 g (G2), respectively. In female rats the weights changed from 150.2 ± 10.7 g (G1) and

Table 2. Effects of CP Pharmacopuncture on Mortality in Single-dose Intramuscular Toxicity Tests in SD Rats.

Sex	Group/ dose (mL/animal)	No. of animals	Days after dosing															Mortality
			0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
Male	G1 0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
	G2 1.0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
Female	G1 0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
	G2 1.0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5

CP, capsaicin.

Table 3. Effects of CP Pharmacopuncture on Clinical Signs in Single-dose Intramuscular Toxicity Tests in SD Rats.

Sex	Group/ Dose (mL/animal)	No. of animals	Clinical sign	Days after dosing				
				0.5	1	2	4	6
Male	G1 0	5	NOA	5	5	5	5	5
	G2 1.0	5	NOA	5	5	5	5	5
Female	G1 0	5	NOA	5	5	5	5	5
	G2 1.0	5	NOA	5	5	5	5	5

Sex	Group/ dose (mL/animal)	No. of animals	Clinical sign	Days after dosing													
				1	2	3	4	5	6	7	8	9	10	11	12	13	14
Male	G1 0	5	NOA	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	G2 1.0	5	NOA	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Female	G1 0	5	NOA	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	G2 1.0	5	NOA	5	5	5	5	5	5	5	5	5	5	5	5	5	5

CP, capsaicin; NOA, no observable abnormality.

Table 4. Effects of CP Pharmacopuncture on Mean Body Eeight in Single-dose Intramuscular Toxicity Tests in SD Rats.

Sex	Group/ dose (mL/animal)		Days after dosing				Gain (g) 0–14
			0	3	7	14	
Male	G1 0	Mean	194.7	222.7	258.0	316.9	122.2
		SD	6.8	9.6	11.0	11.8	6.3
		N	5	5	5	5	5
	G2 1.0	Mean	192.8	218.7	254.2	314.2	121.4
		SD	7.4	12.4	19.2	34.5	28.7
		N	5	5	5	5	5
Female	G1 0	Mean	150.2	164.0	178.7	197.0	46.8
		SD	10.7	15.6	19.4	31.7	21.2
		N	5	5	5	5	5
	G2 0	Mean	151.1	168.3	186.6	211.4	60.2
		SD	6.9	9.0	9.4	12.3	5.8
		N	5	5	5	5	5

Statistical evaluation was performed on body weight using SAS (Version 9. 3, SAS Institute Inc., NC, USA). First, the equipotential was tested using the Folded-F test, and equal variance was observed, and then performed the Student's t test (p <0.05).
CP, capsaicin.

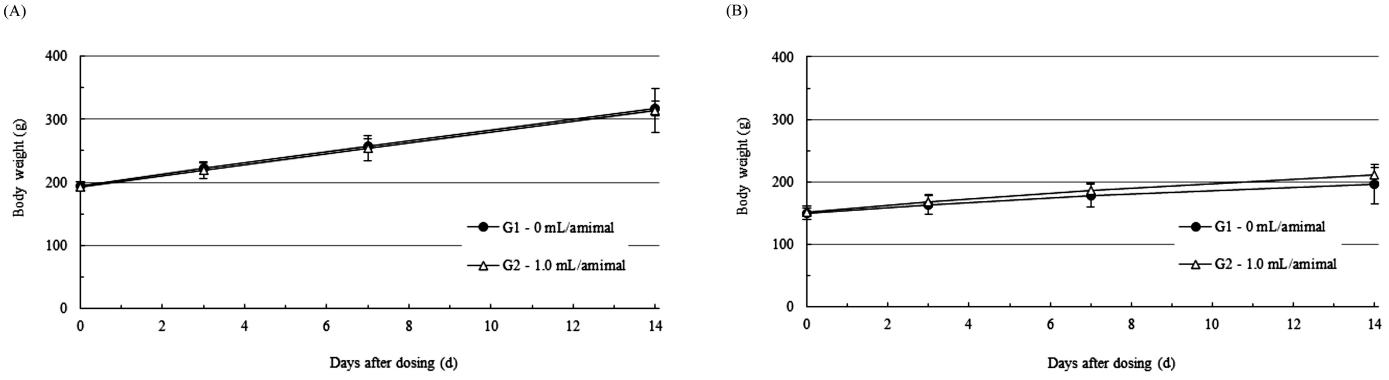

Fig. 1. Effects of CP pharmacopuncture on the body weights of male (A) and female (B) SD rats.
Values are expressed as mean ± SD (n = 5). Statistical evaluation was performed on body weight using the Student's t test (p < 0.05).
CP, capsaicin.

151.1 ± 6.9 g (G2) to 197.0 ± 31.7 g (G1) and 211.4 ± 12.3 g (G2), respectively. However no statistically significant changes in body weight were associated with CP pharmacopuncture (Fig. 1; Table 4).

Effects of the CP on visual autopsy findings

During the autopsy, no significant visual findings were observed in organs and tissues throughout the body of male and female rats in the the CP-treated or control groups.

Effects of the CP on histology of the injection sites

As a result of a local tolerance test on the femur muscle injection site of the male or female rats in the CP-treated and control groups, there were no histological changes related to the test substance, CP [1.0 mL of CP (45.45 mg/mL)], in this study.

Discussion

Pharmacopuncture has been widely applied, especially for musculoskeletal disorders in TKM [23]. A study of serious

adverse events (AEs) reported in the use of pharmacopuncture and acupuncture in musculoskeletal diseases indicated that the incidence of AEs associated with acupuncture and pharmacopuncture treatment was low, and in most cases were not severe [24]. Various herbal medicines delivered through pharmacopuncture have been used in clinical practice in TKM and many more medicines are being developed. In the case of newly developed pharmacopuncture, in addition to efficacy evaluation studies, toxicity evaluation will demonstrate the safety of the herbal medicine formulation and meet the requirements for clinical trial approval [20-22,25].

CP is a pharmacopuncture medicine developed for the treatment of musculoskeletal diseases. Although the safety of pharmacopuncture used in musculoskeletal disorders has been reported, it is necessary to provide scientific evidence of toxicity testing for CP pharmacopuncture. In this study a single-dose toxicity for CP (1.0 mL of CP at a concentration of 45.45 mg/mL), was performed in SD rats.

The single dose toxicity test study qualitatively and quantitatively determines toxic phenomena occurring within a short time after a single administration of the test substance in a single dose, to confirm a fixed safe dose which does not cause adverse events or is life threatening to the animal [26,27]. Due to the potential for the occurrence of gender-related differences in toxic symptoms per drug [28,29], 1 or more animals of both sexes should be evaluated for toxicity.

In this study, 10 male and 10 female SD rats were used. For 14 days after the administration of the CP pharmacopuncture or normal saline injections, general conditions such as types of toxic indications, toxicity expression and recovery times, any deaths, and body weight were observed. Following euthanasia, an autopsy and local examination of the administration site were performed. As a result, changes in general condition, weight, and histology due to a single dose intramuscular injection of CP, in male and female rats were not observed. In addition, changes in tissues and organs, mortality or abnormalities due to the injection of CP were not observed in any rats. There were no significant differences in body weight change during the observation period of 14 days after CP pharmacopuncture. Thus, CP pharmacopuncture in 1.0 mL (at a concentration of 45.45 mg/mL) seems to be safe for rats.

CP pharmacopuncture is administered at a dose in a volume of 1.0 mL or less in clinical practice of TKM, however, studies on toxicity and AEs have not been officially reported. This study is the first study on the toxicity of CP in an animal study, however, this study investigated toxicity with only a single dose over a short observation period of 14 days. Therefore, additional toxicology studies such as the micronucleus test and the mouse lymphoma assay, as well as further studies on long-term and multiple dose effects, will be required in order to ensure the safety of CP pharmacopuncture.

Conclusion

This study showed no differences in mortality or abnormal clinical signs, weight changes, necropsy and histopathological findings between test and control groups of rats demonstrating a safe and non-toxic dose of CP pharmacopuncture in SD rats was less than 1.0 mL of CP at a concentration of 45.45 mg/mL. Although the safety of CP pharmacopuncture was confirmed in this animal study, further studies are needed to confirm the safety of CP in the human body.

Conflicts of Interest

The authors have no conflicts of interest to declare.

Acknowledgments

This work was supported by from the National Research Foundation of Korea (NRF) grant funded by the Korean government (no.: NRF- 2017R1C1B5076224).

References

[1] Teschke R, Wolff A, Frenzel C, Schulze J. Review article: Herbal hepatotoxicity--an update on traditional Chinese medicine preparations. Aliment Pharmacol Ther 2014;40:32-50.
[2] Kim EJ, Chen Y, Huang JQ, Li KM, Razmovski -Naumovski V, Poon J et al. Evidence-based toxicity evaluation and scheduling of Chinese herbal medicines. J Ethnopharmacol 2013;146:40-61.
[3] Jung C, Jung JH, Lee MS. A clinical study of immune pharmacopuncturology. Chungnam (Korea): Kyungrak medical publishing co.; 2011. p. 127-133. [in Korean].
[4] Korean Acupuncture and Moxibustion Society. Acupuncture Medicine. Seoul (Korea): Hanmi Medical Publishing Co.; 2020. [in Korean].
[5] Kim HJ, Gwan R, Han JW, Jung C. Anti-oxidant and anti-inflammatory effects of V Yakchim. J Korea Immuno-Yakchim Soc 2013:2:1-8. [in Korean].
[6] Kim HJ. In Vitro Chromosome Aberration Test of V-YAKCHIM in Chinese Hamster Lung Cell. J Korea Immuno-Yakchim Soc 2013:2:19-27. [in Korean].
[7] Jung C. In vivo Micronucleus test of V-YAKCHIM in ICR Mice. J Korea Immuno-Yakchim Soc 2013:2:5-10. [in Korean].
[8] Lee MS, Jung C. Comparative Sutdies on the Biological Activity of V and A Yakchim. J Korea Immuno-Yakchim Soc 2015:4:37-45. [in Korean].
[9] Cho S, Jung C, Kim K, Ko S, Jung H, Park J. A Case Study of Acute Appendicitis Improved by Pharmacopuncture Treatment. J Intern Korean Med 2019;40:208-219. [in Korean].
[10] Papoiu AD, Yosipovitch G. Topical capsaicin. The fire of a 'hot' medicine is reignited. Expert Opin Pharmacother 2010;11:1359-1371.
[11] Singletary K. Red pepper: overview of potential health benefits. Nut Today 2011;46:33-47.
[12] Pawar SS, Bharude NV, Sonone SS, Deshmukh RS, Raut AK, Umarkar AR. Chilles as food, spice, and medicine: A perspective. Int J Pharm Biol Sci 2011;1:311-318.
[13] Silva C, Rio ME, Cruz F. Desensitization of bladder sensory fibers by intravesical resiniferatoxin, a capsaicin analog: long-term results for the treatment of detrusor hyperreflexia. Eur Urol 2000;38:444-452.
[14] Singletary K. Red pepper: overview of potential health benefits. Nut Today 2011;46:33-47.
[15] Steinberg AC, Oyama IA, Rejba AE, Kellogg-Spadt S, Whitmore KE. Capsaicin for the treatment of vulvar vestibulitis. Am J Obstet Gynecol 2005;192:1549-1553.
[16] Ito K, Nakazato T, Yamato K, Miyakawa Y Yamada T, Hozumi N et al. Induction of apoptosis in leukemic cells by homovanillic acid derivative, capsaicin, through oxidative stress: implication of phosphorylation of p53 at Ser-15 residue by reactive oxygen species. Cancer Res 2004;64:1071-1078.
[17] Hail Jr N, Lotan R. Examining the role of mitochondrial respiration in vanilloid-induced apoptosis. J Natl Cancer Inst 2001;94:1281-1292.
[18] Ständer S, Luger T, Metze D. Treatment of prurigo nodularis with topical capsaicin. J Am Acad Dermatol 2001;44:471-478.
[19] Chung YJ, Lee HJ, Lee YK, Lee JH, Gong HM, Jun SA et al. Case Report of Hypoesthesia of Lower Limb with Additional CP Pharmacopuncture. J Physiol Pathol Korean Med 2019;33:158-162. [in Korean].
[20] Korea Food and Drug Administration (KFDA) [Internet]. Good Laboratory Practice (GLP). KFDA; 2017. Available from: http://www.law.go.kr/admRulInfoP.do?admRulSeq=2100000086689.
[21] Organisation for Economic Co-operation and Development (OECD) [Internet]. OECD series on principles of good laboratory practice and compliance monitoring-ENV/MC/CHEM-98-17-. OECD; 1998. Available from: http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/mc/chem(98)17&doclanguage=en.
[22] Lee SH [Internet]. Drug Approval System of China. NIFDS. 2018. Available

from: http://www.nifds.go.kr/brd/m_95/view.do?seq=30941&srchFr=&srchTo=&srchWord=&srchTp=&itm_seq_1=0&itm_seq_2=0&multi_itm_seq=0&company_cd=&company_nm=&page=1.
[23] Lee YJ, Shin JS, Lee J, Kim MR, Park KB, Lee HD et al. Usage report of pharmacopuncture in musculoskeletal patients visiting Korean medicine hospitals and clinics in Korea. BMC Complement Altern Med 2016;16:292.
[24] Kim MR, Shin JS, Lee J, Lee YJ, Ahn YJ, Park KB et al. Safety of acupuncture and pharmacopuncture in 80,523 musculoskeletal disorder patients: A retrospective review of internal safety inspection and electronic medical records. Medicine 2016;95:e3635.
[25] Lee MY, Seo CS, Kim JY, Shin HK. Genotoxicity evaluation of Guibi-Tang extract using an in vitro bacterial reverse mutation assay, chromosome aberration assay, and in vivo micronucleus test. BMC Complement Altern Med 2014;14:215.
[26] Center for Drug Evaluation and Research [Intenret]. Guidance for industry: Single dose acute toxicity testing for pharmaceuticals. 1996. Available from: https://www.fda.gov/media/72288/download.
[27] Diallo A, Eklu-Gadegkeku K, Agbonon A, Aklikokou K, Creppy EE, Gbeassor M. Acute and sub-chronic (28-day) oral toxicity studies of hydroalcohol leaf extract of Ageratum conyzoides L (Asteraceae). Trop J Pharmaceut Res 2010;9:463-467.
[28] Sung H, Lee E. Single-dose intramuscular toxicity of Mahwangcheonoh pharmacopuncture in a rat model: Toxicity of Mahwangcheonoh pharmacopuncture in SD rats. J Pharmacopunct 2016;19:336-343.
[29] Miller MA. Gender-based differences in the toxicity of pharmaceuticals—the Food and Drug Administration's perspective. Int J Toxicol 2001;20:149-152.