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Original Article
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In Vitro Assays for the Assessment of Safety and Toxicity in Pharmacopuncture Derived from Animal

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Objectives: Among the various treatment methods involving the use of natural substances, pharmacopuncture using animal venom is a relatively new form of acupuncture that has been developed in South Korea and is gaining popularity worldwide. Pharmacopuncture with animal venom is widely used in clinical practice; therefore, ensuring its procedural safety is crucial. This study aimed to evaluate the safety and toxicity of pharmacopuncture using animal venom.
Methods: In October 2021, nine samples of animal venom-derived pharmacopuncture products were randomly collected from four External Herbal Dispensaries (EHDs). These samples underwent sterility and microbial limit testing to ensure they were free from microbial contamination. Toxicity tests were conducted using three different cell lines to evaluate cytotoxic effects.
Results: The sterility and microbial limit tests showed no microbial growth in any of the pharmacopuncture samples. However, the toxicity tests revealed that bee venom exhibited strong cytotoxicity. Furthermore, samples containing Bovis Calculus, Fel Ursi, and Moschus also demonstrated varying degrees of cytotoxic effects.
Conclusion: This study is the first to analyze the safety and toxicity of animal venom-derived pharmacopuncture products, providing evidence for its procedural safety. Although the samples analyzed were limited to four EHDs, these findings highlight the importance of further research on the safety and toxicity of pharmacopuncture to ensure its clinical application is both effective and safe.

Keywords: pharmacopuncture, animal venoms, safety assessment

INTRODUCTION

Natural products have historically been valuable sources of molecules with therapeutic potential and have been used for thousands of years to treat various diseases [1]. In South Korea, they are used in various forms, including herbal decoctions, pills/capsules, extracts, pharmacopuncture, and aromatherapies. Among these, pharmacopuncture, a relatively new form of acupuncture in which herbal extracts are injected into acupuncture points using a syringe, was developed in South Korea and has gained popularity in other regions worldwide [2]. Pharmacopuncture has shown therapeutic effects in various conditions, such as pain relief, rheumatoid arthritis, osteoarthritis, musculoskeletal disorders, chronic pelvic pain, heart rate variability, type 2 diabetes, and cancer, and its efficacy has been experimentally proven [2, 3]. In South Korea, pharmacopuncture has been widely adopted in Korean medicine clinics since the 1990s and is frequently performed on both inpatients and outpatients [3].

In Korean medicine clinics, animal venom is primarily used in pharmacopuncture, a technique that involves injecting toxins into acupuncture points [4]. Animal venoms are complex mixtures of peptides and proteins, with hundreds of components found in a single venom that vary depending on the species [5,

6]. Owing to their therapeutic potential, animal venoms are used in treatments for musculoskeletal disorders, infectious diseases, autoimmune diseases, cancer [7], hypertension [8], and gynecological conditions [9]. The most wellknown and researched animal venoms are those of bees, snakes, scorpions, and spiders [5]. Among these, bee venom acupuncture (BVA) involves injecting purified and diluted bee venom into acupuncture points. Bee venom (BV) contains various bioactive components including melittin, adolapamin, apamin, and mast cell-degranulating peptides [7]. BV has been reported to have immunoactivity, as well as antiinflammatory, analgesic, antipyretic, and antispasmodic effects, which make it suitable for various types of pain management [10]. According to a survey conducted on the usage of animal venom pharmacopuncture in domestic medical institutions, bee, snake, and toad venoms accounted for 99.06% of the total, with 779,931 preparations manufactured, and BV being the most commonly used remedy [4]. However, BV can trigger a life-threatening allergic reaction known as anaphylaxis. To mitigate this risk, sweet bee venom (SBV) is being developed as a safer alternative by removing substances that can induce anaphylaxis [11, 12].

In South Korea, a pharmacopuncture kit is considered a pharmaceutical product, requiring stringent management for patient safety because of its immediate absorption into the body through injection without passing through the digestive system [13]. Pharmacopuncture products are manufactured in External Herbal Dispensaries (EHDs) based on prescriptions from Traditional Korean Medicine (TKM) practitioners. EHDs are equipped with facilities for preparation support, storage, and drainage, in accordance with legal requirements [14, 15]. However, the types of facilities and equipment in EHDs vary, and sterilization facilities are not legally mandatory. Therefore, some EHDs have facilities that comply with aseptic manufacturing standards, whereas others do not [15]. To ensure the safety of pharmacopuncture in patients, the Ministry of Health and Welfare introduced an evaluation and certification system for EHDs in 2018 [3]. Currently, under the evaluation and certification system for EHDs implemented by the Ministry of Health and Welfare, pharmacopuncture EHDs (P-EHDs) are managed according to high standards aligned with the Korean Good Manufacturing Practices (KGMP). In the second certification cycle, safety-related criteria, including fire prevention, were added to enhance the existing standards [16-18]. However, certification does not guarantee complete safety, and there is no legal requirement to use pharmacopuncture products from officially certified institutions, leaving unresolved safety concerns. Currently, there are five officially certified P-EHD institutions in Korea (Namsangcheon Clinic, Girin Clinic, Anjung Clinic, Jaseng Hospital of Korean Medicine, and Jahwang Hospital of Korean Medicine); however, it is estimated that there are several uncertified institutions as well. To date, no studies have randomly collected pharmacopuncture products from clinical settings to evaluate their safety and toxicity. In light of this, we aimed to verify whether the safety of pharmacopuncture products from certified institutions can be trusted and to determine whether products from uncertified institutions could also be safely used. To achieve this, we randomly collected pharmacopuncture products from three certified P-EHDs (As of 2021, Namsangcheon Clinic, Girin Clinic, Jaseng Hospital of Korean Medicine) and one uncertified institution to conduct microbial limit and toxicity tests.

MATERIALS AND METHODS

1. Preparation of samples

The domestic pharmacopuncture products tested in this study were randomly obtained from four EHDs in October 2021. Nine animal-derived pharmacopuncture products were collected (Table 1). The samples were obtained aseptically in an unused state from each EHD and packaged in vials as per the nature of pharmacopuncture. All previous labels were removed, and the products were relabeled by the research team with symbols to conceal the manufacturer or product name. External organizations were commissioned to conduct the required testing. The

Table 1. Naming of the pharmacopuncture

Manufacturer	Composition	Re-naming
Jaseng	Bee venom (removing phospholipase A2 and histamine)	A
Girin	Sweet bee venom (melittin 0.1 mg/mL)	B
Girin	Bufonis Venenum	C
Namsangcheon	Calculus Bovis, Fel Uris, Moschus and other herbal medicine	D
Namsangcheon	Hominis placenta, Calculus Bovis, Fel Uris, Moschus	E
AJ	Scolopendrid	F
AJ	Calculus Bovis, Fel Uris, Moschus	G
AJ	Calculus Bovis, Fel Uris	H
AJ	Sweet bee venom	I

pharmacopuncture products were securely packaged to prevent breakage, placed in iceboxes with ice packs to maintain the cold chain, and delivered to each testing site via courier. The tests were conducted twice, with the first commissioned to Organization A and the second to Organization B. Additionally, Organization B conducted a pharmacopuncture toxicity assessment.

2. Phase 1 test - Organization A - sterility test

To verify the sterility of the collected pharmacopuncture products, an initial sterility test was conducted at Organization A. Prior to the test, the culture medium, cleaning solution (Fluid A) with LOT No. F1HB22316/expiry date: June 2022; canister set (LOT No. F1JB13909A /expiry date: July 31, 2023), and specimen containers were sterilized using a disinfectant. The culture media used were Fluid Thioglycollate Medium (FTM) (LOT No. F1HB15336/expiry date: June 2022) and Tryptic Soy Broth (TSB) (LOT No. F1HB15350/expiry date: June 2022). FTM is primarily used for culturing anaerobic bacteria but can also be used to detect aerobic bacteria, whereas TSB is suitable for culturing fungi and aerobic bacteria [19]. First, a canister was attached to an Equinox pump (Steritest Equinox pump, JS-Q3-Q-011), and the cleaning solution was filtered through a membrane filter. One side of the tube was sealed, filled with TSB, and reopened to seal the opposite tube, which was then filled with FTM for the negative control test. In this test, a canister was attached to the Equinox pump and the cleaning solution was filtered through a membrane filter. Using the Equinox pump, the vial was filtered, one side of the tube was sealed, and TSB was filled. After reopening the sealed tube, the opposite tube was sealed and filled with FTM. The canister containing the culture medium was removed, and the test information was recorded. FTM was cultured at 30℃-35℃ in an aerobic incubator (JS-Q3-Q-025), while TSB was cultured at 20℃-25℃ in an anaerobic and fungal incubator (JS-Q3-Q-026) for 14 days.

3. Phase 2 test - Organization B - microbial limit test

The microbial limit test is used to quantify colonies of the contaminating microorganisms. Therefore, the suitability test for the microbial limit test evaluates whether the inoculated microorganisms are recovered within a two-fold range after inoculating specific standard microorganisms, as specified in the pharmacopoeia, with no more than 100 colony-forming units [20].

1) Microbiological examination of non-sterile products: total viable aerobic count

This test quantitatively measures mesophilic bacteria and molds that can proliferate under aerobic conditions. The primary purpose of this test is to determine whether a substance or preparation is compliant with an established specification for microbiological quality [21]. All media and sample containers were disinfected using a disinfectant solution before use. To confirm test conditions, a 10-fold dilution was performed with normal saline instead of the test solution, and a negative control test was performed. The suitability of the counting method for detecting microbial growth in the presence of a product was verified. The results showed that the colony count in the test sample was between ½ and 2 times that in the control sample, indicating the suitability of the measurement method. The samples were tested using the pour-plate method. The test sample suspension was added to the prepared test and control solutions (without the test sample), and 0.1 mL of the prepared test solution was spread onto two different agar plates (soybean–casein digest agar medium and Sabouraud glucose agar medium). These plates were incubated at temperatures ranging from 25℃ to 37℃ for 1 to 2 days to observe microbial growth.

2) Microbiological examination of non-sterile products: tests for specified microorganisms

This test determines whether specific microorganisms are absent or present in limited quantities under specific conditions. The primary purpose of this test is to assess whether a substance or preparation conforms to established microbiological quality specifications [21]. All media and sample containers were disinfected with a disinfectant solution before use. To confirm the test conditions, a negative control test was conducted by inoculating TSB with 1 mL of physiological saline instead of the sample. The suitability of the counting method for detecting colonies in the presence of the product was assessed. No red or white clusters were formed, confirming the suitability of the counting method. Thereafter, samples were tested for the presence of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella*. Test samples were added to the prepared sample and control solutions (without samples), and 0.1 mL of the prepared sample solution was spread onto four different agar plates (MacConkey agar, XLD agar, Mannitol salt agar, and Cetrimide agar). The plates were incubated at temperatures ranging from 37℃ to 44℃ for 1 to 2 days to detect microbial growth.

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4. Organization B – pharmacopuncture toxicity test

The NCI-H292, Raw264.7, and BV-2 cell lines used in this experiment were obtained from the Korean Cell Line Bank. The cells were cultured in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin (100 U/mL). After acclimating the cells at 37℃ in an incubator maintained at a 5% CO_2 atmosphere, they were subcultured for further use. To measure cytotoxicity using the MTT assay, NCI-H292, Raw264.7, and BV-2 cells were seeded (0.18 mL) into 96-well plates at a density of 1×10^4 cells/well, followed by the addition of drug samples (0.02 mL). The cells were then incubated at 37℃ with 5% CO_2 for 24 h. After incubation, 0.02 mL of a 5-mg/mL MTT solution was added to each well, and the plates were incubated for an additional 4 h. Following incubation, the culture medium was removed, and 0.15 mL of DMSO was added to each well, followed by incubation for 15 min at room temperature (20℃-25℃). Subsequently, absorbance was measured at 540 nm using an ELISA reader. Cytotoxicity was determined by comparing the absorbance of the sample groups to the control groups, with lower absorbance indicating higher cell toxicity.

RESULTS

1. Phase 1 test - Organization A - sterility test

The test results confirmed that microbial growth was not observed in either the TSB or FTM media, indicating that the investigated specimens were sterile (Fig. 1).

2. Phase 2 test - Organization B - microbial limit test

Microbial limit testing of the samples, targeting aerobic microbes, yeasts, molds, *S. aureus*, *P. aeruginosa*, *E. coli*, and *Salmonella* revealed no microbial growth in any of the media. Therefore, these samples were confirmed to be sterile based on the lack of microbial contamination (Figs. 2, 3).

3. Organization B – pharmacopuncture toxicity test

Excluding the mast cell line, in two other cell lines, products C (Bufonis Venenum) and F (Scolopendrid) generally showed higher cell survival rates compared to other products, indicating lower toxicity. Coversely, pharmacopuncture products D and E, both containing Bovis Calculus, Fel Ursi, and Moschus, exhibited lower survival rates across all three cell types, indicating higher toxicity. Among the bee venom products, A and B exhibited low cell survival rates, indicating strong toxicity,

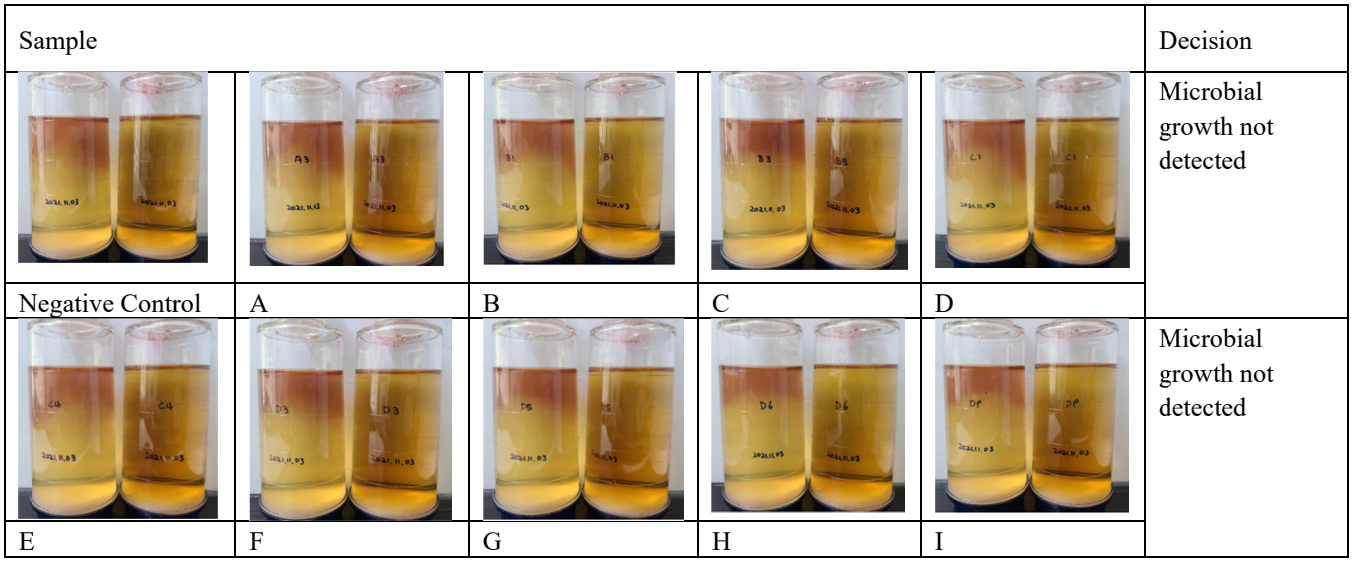
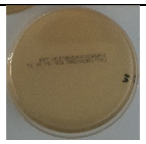
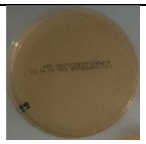


Figure 1. Microbial growth from pharmacopuncture products (A-I) at the sterility test. The sterility test was conducted to ensure the absence of microbial growth in these products, crucial for their safe clinical application. The results confirmed no microbial growth, indicating adherence to sterility standards in the manufacturing process.

Category		SET 1(2021.11.18)					Result Decision
Total aerobic microbial count							Not detected
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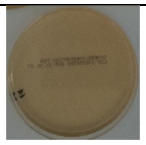
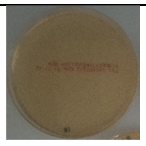
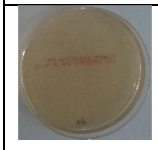
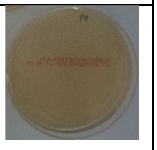
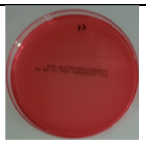
							
		E	**F**	**G**	**H**	**I**	
Total yeasts and molds count							Not detected
		Negative Control	**A**	**B**	**C**	**D**	
							
		E	**F**	**G**	**H**	**I**	
Tests for Specified Micro-organisms	*Staphylococcus aureus*						Not detected
		Negative Control	**A**	**B**	**C**	**D**	
							
		E	**F**	**G**	**H**	**I**	

Figure 2. Microbial growth from pharmacopuncture products (A-I) at the primary microbial limit test. This figure illustrates the successful compliance of all tested pharmacopuncture samples with microbial quality standards. The primary microbial limit test assessed the total aerobic microbial count, yeasts, molds, and specific microorganisms, with all samples showing no detectable microbial growth.

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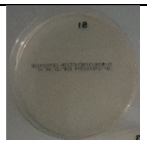
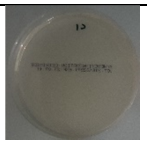
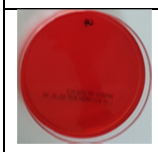
	Pseudomonas aeruginosa						Not detected
		Negative Control	**A**	**B**	**C**	**D**	
							
		E	**F**	**G**	**H**	**I**	
	Escherichia coli						Not detected
		Negative Control	**A**	**B**	**C**	**D**	
							
		E	**F**	**G**	**H**	**I**	
	Salmonella						Not detected
		Negative Control	**A**	**B**	**C**	**D**	
							
		E	**F**	**G**	**H**	**I**	

Figure 2. Continued.

whereas product I exhibited a high cell survival rate, indicating an absence of toxicity (Fig. 4).

DISCUSSION

In Korea, the safety of pharmacopuncture has been a topic of ongoing discussion. As pharmacopuncture involves inject-

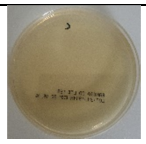
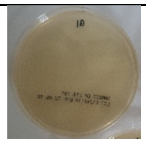
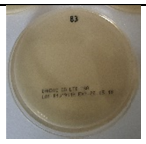
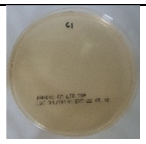
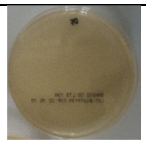
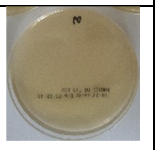
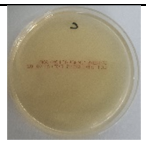
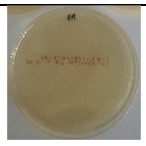
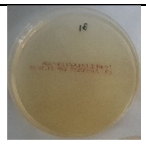
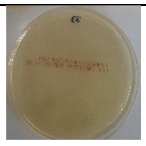
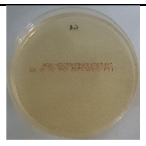
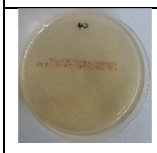
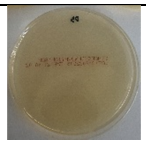
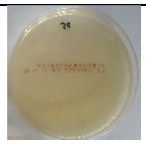
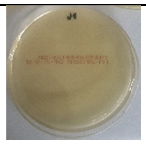
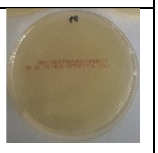
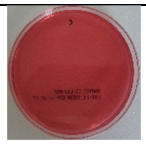
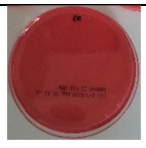
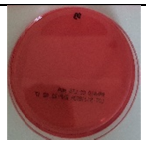
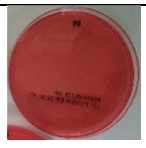
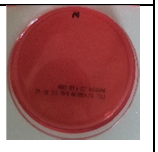
Category		SET 2(2021.11.24)					Result Decision
Total aerobic microbial count							Not detected
		Negative Control	**A**	**B**	**C**	**D**	
							
		E	**F**	**G**	**H**	**I**	
Total yeasts and molds count							Not detected
		Negative Control	**A**	**B**	**C**	**D**	
							
		E	**F**	**G**	**H**	**I**	
Tests for Specified Micro-organisms	*Staphylococcus aureus*						Not detected
		Negative Control	**A**	**B**	**C**	**D**	
							
		E	**F**	**G**	**H**	**I**	

Figure 3. Microbial growth from pharmacopuncture products (A-I) at the secondary microbial limit test. This figure demonstrates the absence of microbial growth in the samples, including tests for total aerobic microbial count, yeasts, molds, and specific harmful microorganisms, underlining the products' microbiological safety.

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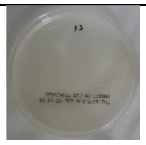
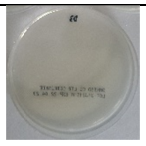
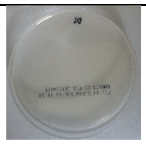
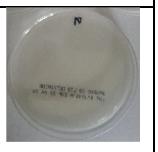
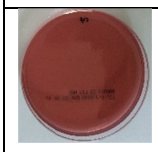
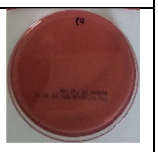
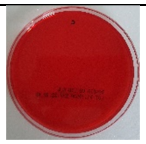
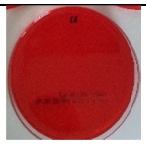
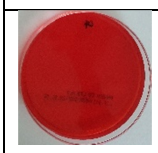
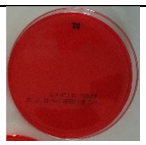
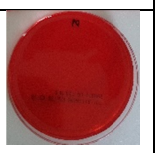
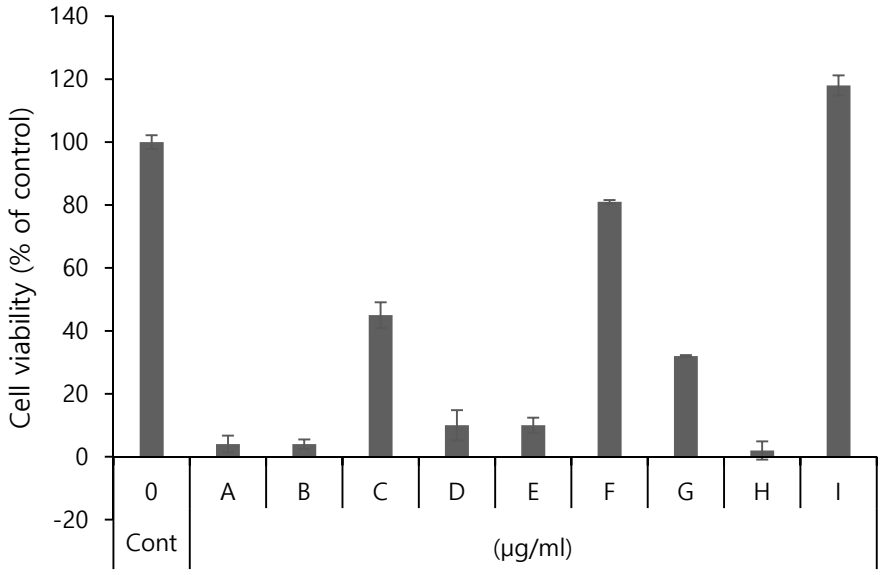
	Pseudomonas aeruginosa						Not detected
		Negative Control	**A**	**B**	**C**	**D**	
							
		E	**F**	**G**	**H**	**I**	
	Escherichia coli						Not detected
		Negative Control	**A**	**B**	**C**	**D**	
							
		E	**F**	**G**	**H**	**I**	
	Salmonella						Not detected
		Negative Control	**A**	**B**	**C**	**D**	
							
		E	**F**	**G**	**H**	**I**	

Figure 3. Continued.

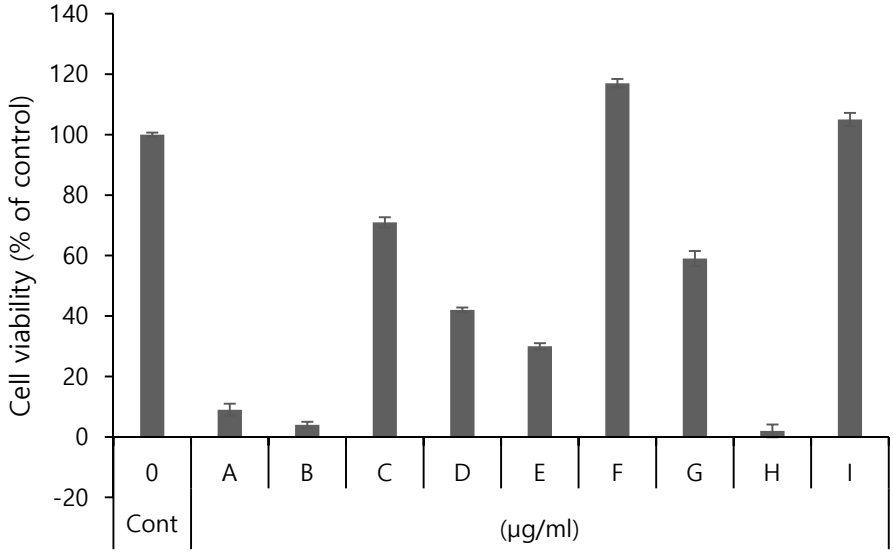
ing medicinal agents directly into the circulatory system, these agents must be manufactured in strictly sterile facilities to ensure patient safety [19]. Pharmacopuncture products vary in composition and extraction methods depending on the herbal ingredients used. EHDs are instrumental in producing various herbal ingredients, especially herbal medicines, which are challenging to prepare individually in traditional Korean medicine clinics. Consequently, almost all pharmacopuncture products in Korea are manufactured by EHDs and supplied to traditional medicine clinics [3, 14]. Before 2018, there was no certification

Cell viability: NCI-H292 (Mucinous epithelial cancer cell line)



0	A	B	C	D	E	F	G	H	I
100	4	4	45	10	10	81	32	2	118
2.2	0.4	0.4	3.6	1.4	1.1	4.1	2.4	0.6	3.2

Cell viability: Raw264.7 (Macrophage cell line)



0	A	B	C	D	E	F	G	H	I
100	9	4	71	42	30	117	59	2	105
0.7	1.2	0.5	1.5	5.8	3.5	1.7	3.6	0.7	2.2

Figure 4. Cell viability assessment of pharmacopuncture products (A-I) on three different cell lines: NCI-H292, Raw264.7, and BV-2. This figure displays the differential impact of each pharmacopuncture product on cell viability, indicating variations in toxicity among the products tested.

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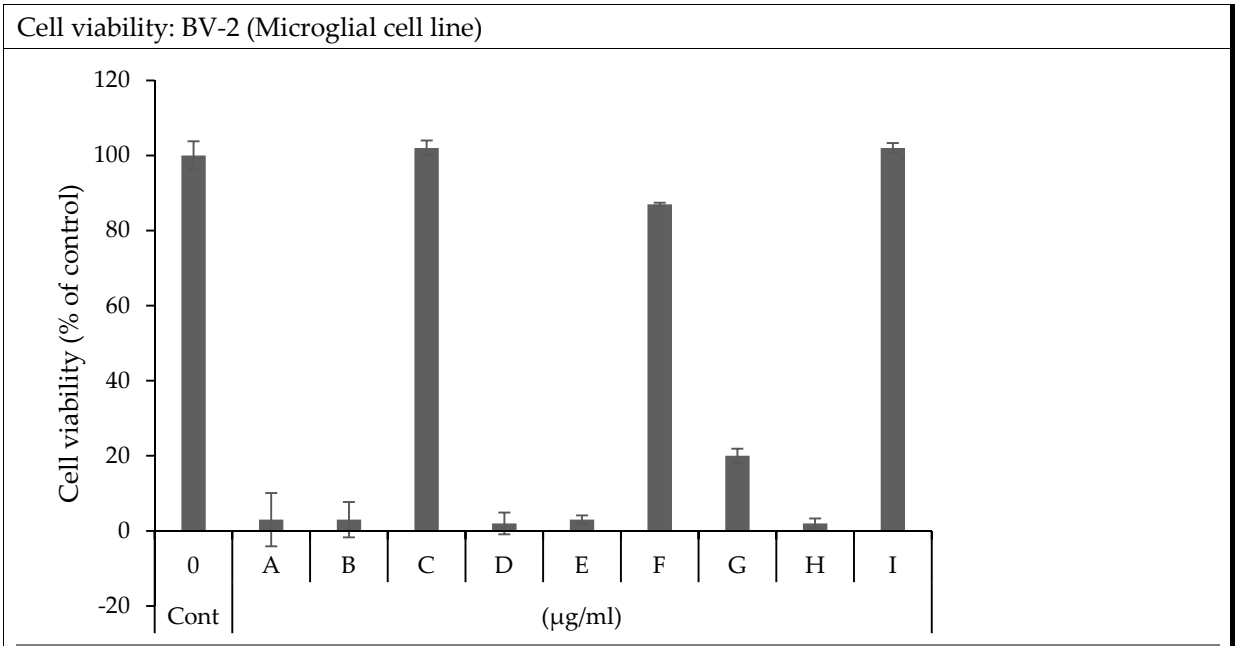

0	A	B	C	D	E	F	G	H	I
100	3	3	102	2	3	87	20	2	102
3.8	0.8	0.3	7	0.3	0.6	2	1.1	0.4	1.3

Figure 4. Continued.

system to verify whether EHDs maintain sterile manufacturing conditions. To address this, in September 2018, the Ministry of Health and Welfare introduced the "External Herbal Dispensaries Certification System," which evaluates not only the facilities and operation of herbal dispensaries but also the entire process from raw material sourcing to storage, preparation, packaging, and delivery. This certification system ensures the safety of herbal medicines including pharmacopuncture products [16-18]. The certification process differentiates between the preparation of herbal medicines and pharmacopuncture products, with the pharmacopuncture preparation standards encompassing 165 criteria that reflect the KGMP standards. However, compliance with these standards is voluntary, and there are no penalties for EHDs that opt not to obtain certification [3].

Sterility tests of the investigated animal-derived pharmacopuncture product samples showed no bacterial growth. Similarly, microbial limit testing (total viable count and specific microbial tests) detected no bacteria in any of these products, suggesting that they were manufactured under sterile conditions [20]. However, as only some pharmacopuncture products from certain EHDs were collected and analyzed, further comprehensive analysis with a larger and more diverse sample size is necessary.

The toxicity test results revealed that pharmacopuncture products containing BV, Bovis Calculus, Fel Ursi, and Moschus showed significantly lower cell survival rates in three different cell lines (NCI-H292, Raw264.7, and BV2 cells) compared to other pharmacopuncture samples. Among these, animal-derived pharmacopuncture products, especially those using BV, are widely used in Korea [4]. BV pharmacopuncture is known for its excellent pain relief effects; however, it is also associated with side effects, such as skin reactions and anaphylaxis, owing to its venomous nature [21, 22]. In fact, in a study involving an anonymous online survey of 500 respondents who had received pharmacopuncture within the past year, side effects were reported more frequently in the bee venom pharmacopuncture (BV-PA) group (16.7%) than in the non-BV-PA group (11.6%). Although no significant difference was observed in the severity of mild symptoms between the two groups, there was a significant difference in the severity of severe symptoms [23]. Therefore, many pharmacopuncture products, especially those containing BV, are produced without key allergenic components such as phospholipase A2 [24, 25]. However, the lack of allergenic ingredients does not always indicate nontoxicity.

It is reasonable to expect toxicity from animal-derived toxins, as these toxins have evolved to harm other organisms as part of their defense mechanisms [26]. For instance, experiments on mice revealed that botulinum toxin can have toxic effects on the liver and kidneys if the crucial toxin component is not removed during the manufacturing of the injection [23]. In addition, differences in BV toxicity have been linked to differences in dilution ratios or manufacturing methods used by individual companies [27].

Pharmacopuncture products containing Bovis Calculus, Fel Ursi, and Moschus also resulted in low cell survival rates. In contrast, a previous single-dose toxicity experiment in mice involving these substances showed no signs of acute toxicity [28]. However, the cell toxicity observed in this study highlights a potential discrepancy that warrants further large-scale investigations to verify this incongruity. Based on the observed cell toxicity, we hypothesize that Bovis Calculus, Fel Ursi, and Moschus may contain toxic components. For Bovis Calculus, the active ingredients include bile acids, bilirubin, and some inorganic salts [29]. The chemical composition of Bovis Calculus can vary depending on its type, and previous studies have suggested that Calculus bovis sativus (CBS) is relatively safer than Calculus bovis artifactus (CBA) because of its lower bilirubin content, suggesting that bilirubin in Bovis Calculus may be responsible for its toxicity [30]. When unconjugated bilirubin levels rise without binding to albumin, it can become toxic, induce neurotoxicity, interact with neurons, cause axonal damage, and damage mitochondria, leading to neuronal cell death [31]. Furthermore, both natural Calculus bovis (NCB) and CBS exhibited low toxicity in acute toxicity studies in mice. However, there are research findings indicating acute toxicity when NCB and CBS are injected intraperitoneally, suggesting the need for further toxicity studies on bilirubin [30].

Fel Ursi contains various chemical components, including bile, of which ursodeoxycholic acid (UDCA) is the main active ingredient [32]. UDCA is an FDA-approved medication for dissolving cholesterol gallstones, and has been proven safe for use in primary biliary cirrhosis (PBC) [33]. However, studies on its toxicity and side effects have revealed that UDCA may exhibit specific molecular toxicity, potentially harming cells and tissues at the molecular level. These toxic effects include the inhibition of DNA repair, coenzyme A activity, cyclic AMP function, p53 protein activity, and protein synthesis, as well as promotion of cell transformation and the induction of DNA strand breakage. Furthermore, it should be noted that there is very little difference between the recommended and toxic doses of UDCA; therefore, a slight increase in the dose could potentially have adverse effects [34].

Toxicity research on Moschus using zebrafish embryo development models revealed its potential to cause abnormal development of muscle and heart tissues. At concentrations of 80 and 100 μmol/L, 100% embryo mortality was observed 96 h after fertilization [35]. Furthermore, Moschus was found to induce the expression of CYP1A2 and CYP3A4 enzymes in human liver cells and exhibited significant liver toxicity in mice when administered at dosages exceeded 50 mg/kg [36].

Scolopendrid pharmacopuncture did not exhibit high toxicity, as indicated by high cell survival rates. As scolopendrids contain substances such as histamines and hemolytic proteins that can be toxic to humans and animals, in Korea, the head, legs, and tail are removed before extracting therapeutic substances to avoid these toxic substances [37]. Scolopendrids can inject venom through a pair of legs with claws. The venom injected through the claws is a complex mixture that includes peptides, enzymes, and other small molecules [6]. The low toxicity observed in this study may be attributed to the use of organs with less toxic substances for extraction. In addition, the LD50 (lethal dose for 50% of the tested subjects) of scolopendrid extract in mice following intravenous injection was 235 mg/kg, and the estimated approximate lethal dose for oral administration was 0.156 g/kg, which is equivalent to 0.75 g/kg in humans. This suggests a safety rating of grade 1, indicating that a dose reduction from 1/10 to 1/100 is required for safe use [38]. Accordingly, it is possible that the scolopendrid pharmacopuncture product investigated in this study was diluted to acceptably safe concentrations, resulting in low toxicity. Nonetheless, additional research is required to address these postulations.

The safety of pharmacopuncture, which requires sterility owing to its direct injection into the bloodstream, has been confirmed through aseptic testing and microbial limit testing. Although toxicity tests showed low survival rates for BV, Bovis Calculus, Fel Ursi, and Moschus, these products can be safely used when administered at appropriate dosages that do not exceed the lethal dose verified through animal experiments. However, this study investigated only a few pharmacopuncture produced by four EHDs. Therefore, future studies should aim to conduct a more comprehensive survey including various pharmacopuncture products from diverse institutions, to provide a broader assessment of their safety.

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CONCLUSIONS

The results of sterility, microbial limit, and toxicity tests conducted on a total of nine samples were as follows:

In the sterility test, no microbial growth was observed in either TSB or FTM media, confirming that the samples were sterile.

In the microbial limit test, no microbial colony proliferation was observed on any of the media targeting aerobic microbes, yeasts, molds, *S. aureus*, *P. aeruginosa*, *E. coli*, or *Salmonella*, further confirming the sterility of these samples.

In the toxicity test, BV toxicity was evident in all the three cell types. In addition, toxic properties were observed in pharmacopuncture preparations containing Bovis Calculus, Fel Ursi, and Moschus.

This study confirmed the sterility of pharmacopuncture products; however, toxicity was detected in some products, likely owing to the characteristics of the raw materials used. Given the potential risks associated with injecting these substances directly into the human body, their use should be limited to trained professionals with appropriate expertise. In addition, various *in vivo*/*in vitro* studies should be conducted to evaluate the clinical effectiveness and safety of herbal acupuncture, and a system should be established to systematically collect and monitor the side effects that occur, ensuring improved safety management for patients.

AUTHORS' CONTRIBUTIONS

Y.S. and H.J.; Conceptualization, Y.S. and H.J.; methodology, Y.S. and H.J.; software, Y.S. and H.J.; validation, Y.S. and H.J.; formal analysis, Y.S. and H.J.; investigation, Y.S. and H.J.; resources, Y.S. and H.J.; data curation, Y.S. and H.J.; writing—original draft preparation, K.-H.K.; writing—review and editing, K.-H.K.; visualization, K.-H.K. and S.-G.K; supervision, S.-G.K.; project administration, S.-G.K; funding acquisition. All authors have read and agreed to the published version of the manuscript.

DATA AVAILABILITY

The data will be made available upon reasonable request.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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