POTENTIAL SKIN IRRITATION OF TAY SON TAM KIET HERBAL SPRAY IN RABBITS

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Tay Son Tam Kiet herbal spray (TSTK) is a traditional external preparation composed of several medicinal herbs and minerals, including Rhizoma Rhei, Flos Carthami, Ramulus Cinnamomi, Natrium Sulfuricum, Gummi resina Olibanum, Acacia catechu, Rhizoma Bletillae striatae, Sanguis Draconis, Aloe vera, Radix Astragali membranacei, and Radix Paeoniae lactiflorae with the intended use to relieve musculoskeletal pain, swelling, and bruising. To provide preclinical safety information, this study aimed to evaluate its dermal irritation potential in experimental animals. In this study, 0.5 ml of TSTK was applied to one side of the dorsal skin of rabbits, while 0.5 ml of distilled water was applied on the opposite side as a normal control. Both sites were covered with gauze and fixed for 4 hours. The erythema and edema were scored at 1h, 24h, 48h and 72h post-removal. Results showed that no erythema, edema, or other skin lesions were observed in all rabbits at any time point. According to the Organisation for Economic Co-operation and Development (OECD) Test Guideline 404 and the International Organization for Standardization (ISO) classifications, the TSTK herbal spray did not cause skin irritation in rabbits. These results support its safety for further development and clinical application.

Keywords: Skin irritation, Tay Son Tam Kiet, herbal spray, rabbits.

I. INTRODUCTION

Musculoskeletal diseases represent one of the most common causes of non-fatal disability worldwide, affecting more than 1.63 billion people worldwide and ranking as a leading contributor to reduced quality of life and work capacity.[1-3] Heavy work overload, traffic accidents, and incorrect posture in daily activities further increase the incidence. The global burden is immense, with conditions ranging from chronic disorders such as osteoarthritis and rheumatoid arthritis to acute trauma such as sprains, strains, and contusions.[3] If not treated promptly and properly, it can cause disability or affect work productivity and quality of life. This highlights musculoskeletal diseases as both a public health and socioeconomic challenge.

The clinical symptoms of acute musculoskeletal trauma are diverse but typically include pain, swelling, tenderness, and functional limitation. In more severe cases, patients may present with hematomas, joint instability, or dislocation. Chronic musculoskeletal diseases may evolve into degenerative changes that exacerbate disability over time.[2,3] Today, many treatments rely on pharmacological therapeutics such as NSAIDs, corticosteroid injections, and non-pharmacological approaches including physical therapy, exercise, orthotic devices.[4] However, long-term use of medicines, that have systemic effects is often limited by adverse effects, including gastrointestinal bleeding, hepatotoxicity, and renal impairment.[5,6] Therefore, there is an increasing interest in

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Received: 25/11/2025
Accepted: 24/12/2025

traditional medicine, particularly topical herbal formulations, which are considered safer due to localized action.

Traditional medicine, including herbal plants and minerals, has long been employed for trauma management, particularly in Asia. Topical formulations containing herbal extracts are widely used to relieve pain, reduce swelling, and promote tissue repair.[7] Tay Son Tam Kiet (TSTK) is a Vietnamese traditional preparation formulated as an alcohol-based spray, containing the extracts of *Rhizoma Rhei, Flos Carthami, Ramulus Cinnamomi, Natrium Sulfuricum, Gummi resina Olibanum, Acacia catechu, Rhizoma Bletillae striatae, Sanguis Draconis, Aloe vera, Radix Astragali membranacei,* and *Radix Paeoniae lactiflorae*. The combination of medicinal materials was based on folk experience from traditional remedies used for the treatment of injuries. The product is indicated for relieving pain, bruising, swelling, sprains, and dislocations, thereby supporting the recovery of soft-tissue injuries. As a topical agent, its dermal safety must be confirmed through standardized testing, as herbal compounds may still cause erythema, edema, or sensitization in sensitive individuals.

Although the constituents of Tay Son Tam Kiet herbal spray are of natural origin and have long been employed in traditional medicine, evaluation of product safety particularly skin irritation potential remains mandatory in accordance with international pharmaceutical safety standards. According to the Organisation for Economic Co-operation and Development (OECD) guidelines and ISO 10993-10, topical products must undergo skin irritation assessment before widespread use.[8,9]

As the Vietnamese pharmaceutical industry advances toward international standards of quality and safety, conducting preclinical studies to evaluate the safety of pharmaceutical products is very important. This is particularly relevant for complex herbal and mineral formulations such as Tay Son Tam Kiet herbal spray, where the assessment of skin irritation potential is necessary to ensure safety. Therefore, this study was conducted because of the practical requirement to verify product safety and conformity with international standards. The objective of this study is to evaluate the potential skin irritation of Tay Son Tam Kiet spray in rabbits.

II. SUBJECTS AND METHODS

1. Plant material

The preparation of the TSTK Herbal Spray was conducted by the Binh Dinh Pharmaceutical and Medical Equipment Joint Stock Company, Vietnam under Good Manufacturing Practice (GMP) standards. Traditional medicinal materials were qualified based on the Vietnamese Pharmacopeia 5th edition (Vietnamese Ministry of Health 2017). The spray formulation contains, per 100 mL of solution, extracts of *Rhizoma Rhei (3,75g), Flos Carthami (3,75g), Ramulus Cinnamomi (3,75g), Natrium Sulfuricum (1,87g), Gummi resina Olibanum (1,87g), Acacia catechu (1,87g), Rhizoma Bletillae striatae (1,87g), Sanguis Draconis (1,87g), Aloe vera (1,87g), Radix Astragali membranacei (1,87g),* and *Radix Paeoniae lactiflorae (1,87g)*.

2. Chemicals and Equipments

Sterile bandage and gauze cut into square pieces, 2.5 cm × 2.5 cm; 1 mL syringe; medical adhesive tape; rabbit restraining table and fixation straps; magnifying glass.

3. Experimental animals

New Zealand White rabbits, averaging 2.0 - 2.2 kg in body weight, were housed individually in separate cages and maintained under

standard conditions. Animals were provided free access to a standard diet and water. Before the start of the experiment, all rabbits were acclimatized to the laboratory environment for 7 days at the Department of Pharmacology, Hanoi Medical University.

4. Methods

The skin irritation potential of Tay Son Tam Kiet herbal spray was evaluated in accordance with the OECD and ISO 10993-10 guidelines for assessment of dermal irritation of topical products.[8,9]

The study was performed in three *New Zealand White* rabbits with clean and healthy skin. Twenty-four hours before product administration, an area approximately 10 x 15 cm^2 on both sides of the spine was carefully shaved. Three rabbits with normal, intact skin without any lesions were included in the study.

For each rabbit, a 2.5 x 2.5 cm site was selected on each side and treated as follows: one side of the spine was applied with 0.5 mL of Tay Son Tam Kiet herbal spray and on the other side, with 0.5 mL of distilled water. The administration region was wrapped with gauze and adhesive with a non-stimulating tape, ensuring the bandage was not excessively tight, and was maintained for 4 hours. After 4 hours, all dressings were removed and the application sites were gently cleansed with clean water to remove residual test material.

All animals were observed to evaluate for erythema and edema at 1, 24, 48, and 72 hours after removal of the test product. In cases where lesions were observed, rabbits were monitored for up to 14 days to assess reversibility. Observation was discontinued once complete recovery was confirmed.

Skin reactions were evaluated based on erythema and edema according to the scoring system described in previous studies.[8,9] For erythema, the scores ranged from 0 to 4, in which: 0 indicated no erythema; 1 indicated very slight erythema (barely perceptible); 2 indicated well-defined erythema; 3 indicated moderate to severe erythema; and 4 indicated severe erythema. The maximum possible erythema score was 4. For edema, the scoring system also ranged from 0 to 4: 0 indicated no edema; 1 indicated very slight edema (barely perceptible); 2 indicated well-defined edema with raised edges; 3 indicated moderate to severe edema with edges raised approximately 1 mm; and 4 indicated severe edema, with edges raised more than 1 mm and extending beyond the application area. The maximum possible edema score was 4.

The primary irritation index (P.I.I.) was calculated based on the degree of erythema and edema at 24 hours, 48 hours and 72 hours. Skin irritation was determined according to PII. A PII value from 0 to 0.4 indicates a negligible level of irritation. Values between 0.5 and 1.9 correspond to a slight irritation. When the PII ranges from 2 to 4.9, the irritation is considered moderate. Finally, a PII of 5 to 8 indicates a severe level of skin irritation.[8,9]

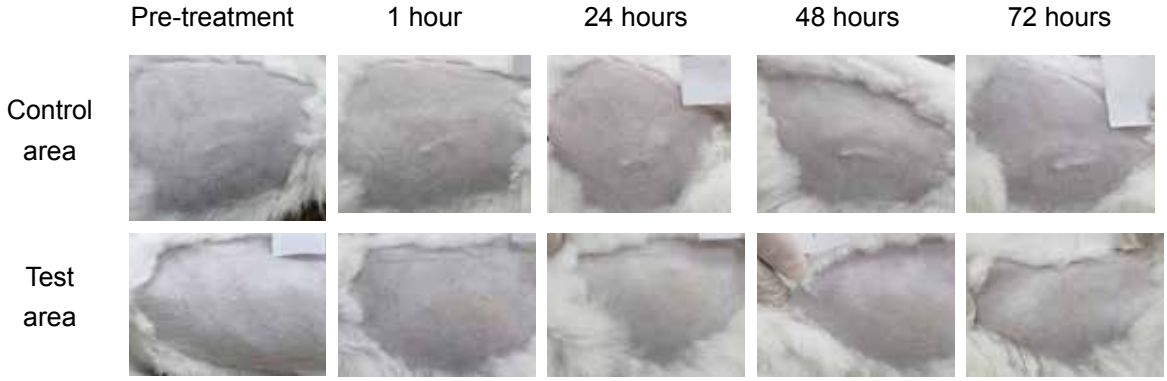
III. RESULTS

The results of the dermal irritation study of Tay Son Tam Kiet herbal spray are summarized in Table 1. No clinical sign, change in food consumption or body weight gain was observed in all rabbits. No dermal response, including erythema or edema, was found on either the side treated with Tay Son Tam Kiet herbal spray or the side applied with water at all time points. The PII was calculated to be 0 in Tay Son Tam Kiet herbal spray - applied area at 24 hours, 48 hours, and 72 hours post-removal.

Table 1. Evaluation of erythema and edema in Tay Son Tam Kiet spray-applied rabbits and Response categories according to PII

Rabbit	Erythema								Edema								PII	Category
	1h		24h		48h		72h		1h		24h		48h		72h			
	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	Negligible
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Negligible
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Negligible
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Negligible

T: Tay Son Tam Kiet herbal spray-applied area C: control area



Figue 1. Images of Tay Son Tam Kiet spray-applied skin in rabbits

IV. DISCUSSION

The evaluation of the potential skin irritation caused by Tay Son Tam Kiet (TSTK) medicated alcohol liniment was necessary in the context of the widespread use of traditional herbal products in the management of musculoskeletal disorders and soft tissue injuries. The alcohol-based liniment dosage form is widely applied due to its convenience, rapid onset of effect, and high safety profile when administered topically. The alcohol liniment dosage form offers several advantages. Ethanol not only acts as a solvent and preservative for herbal active constituents but also enhances skin permeability, thereby facilitating rapid transdermal absorption of bioactive compounds.[10,11] Upon topical administration, the product exerts direct effects on the injured area, without the systemic adverse events commonly associated with oral nonsteroidal anti-inflammatory drugs (NSAIDs), such as gastrointestinal irritation, ulceration, and hepatic or renal impairment.[6,12] In addition, this dosage form is convenient, portable, and suitable for immediate application after injury, making it especially valuable for athletes or workers with a high risk of trauma.

However, to ensure scientific rigor and compliance with international standards, all topical preparations must undergo evaluation of skin irritation potential in accordance with OECD and ISO 10993-10.[8,9] This served as the rationale for conducting the present study, which aimed to provide objective evidence regarding the safety of this product prior to its broader clinical application.

The results of the experiment in *New Zealand White* rabbits demonstrated that no erythema or edema was observed at any of the recorded time points (1 hour, 24 hours, 48 hours, and 72 hours) following topical application. The calculated primary irritation index (PII) was 0, corresponding to the category "non-irritant." This finding indicates that TSTK medicated alcohol liniment exhibits high dermatological safety, with no adverse local reactions. A review of the existing literature indicates that data on the dermal toxicity of the individual components of this product remain limited. Such results are important, as safety is the primary consideration for any preparation intended for topical use.

The formulation of TSTK liniment contains multiple herbal and mineral ingredients with documented pharmacological activities in analgesia, anti-inflammation, circulation improvement, and wound healing. Several constituents in the formulation have been shown to inhibit spinal pain transmission or the release of pain mediators such as prostaglandins and leukotrienes, thereby attenuating inflammatory pain.[13-15] In addition, polyphenols from *Cinnamomum cassia Presl*, paeoniflorin from *Paeonia lactiflora* exhibit multi-target actions that reduce serum NO, TNF-α, and PGE2, while downregulating the expression of iNOS, COX-2, and NF-κB, critical regulators of the inflammatory.[16-18] Other herbs such as *Bletilla striata*, *Carthamus tinctorius*, and *Daemonorops draco Blue* contribute hemostatic, circulation-promoting, and anti-edematous effects, further enhancing tissue recovery and the resolution of hematoma following trauma.[19-21] The synergistic interplay among these diverse mechanisms highlights the distinctive therapeutic potential of multi-component traditional formulations compared with single-compound synthetic agents.

The available evidence suggests that TSTK spray may have potential as a supportive option for musculoskeletal disorders and soft tissue injuries, with a generally favorable safety profile. However, further studies, including repeated-dose toxicity and pharmacodynamic evaluations in validated experimental models, are needed to confirm its therapeutic value and long-term safety.

V. CONCLUSION

The results of this study indicate that no erythema, edema, or other skin lesion was detected in any of the rabbits at any time point. According to the OECD and ISO classification criteria, TSTK herbal spray is considered non-irritating to the skin in rabbits.

ACKNOWLEDGEMENT

We would like to thank Hanoi Medical University for providing the facilities and support for the successful execution of this study. The authors declare that there are no conflict of interest related to this work.

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