THE SKIN IRRITATION RISK OF CHILI FRUIT NANO GEL ON EXPERIMENTAL RABBIT SKIN

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SUMMARY

Chili is not only a popular spice in food preparation but is also used as a traditional medicine. In particular, when used topically, the active ingredient called capsaicin in chili peppers helps treat bone and joint pain. ***Objectives****: The study aims to evaluate the local irritation on the healthy skin of rabbits of a nano gel product containing chili fruit extract provided by the Institute for Applied Research of Traditional Medicine.* ***Methods****: The study was conducted according to the guidelines of the Vietnam Ministry of Health, ISO 10993-10, and OECD on skin irritation assessment for topical products. Three healthy white New Zealand rabbits were used. Animals were applied gauze which was soaked in nano gel containing chili fruit extract at a dose of 0.5 grams on a healthy skin area 2.5 x 2.5 cm for 4 hours. We evaluated the irritation index including erythema and edema on the skin at 24, 48, and 72 hours after gauze removal.* ***Results****: Three rabbits were still healthy, and living normally, there were no signs of erythema and edema on the rabbit's skin, and the irritation indexes were 0 during 72 hours of continuous monitoring.* ***Conclusion****: The nano gel containing chili fruit extract applied topically does not irritate healthy rabbit skin.*

Keywords*: nano gel, chili pepper, capsaicin, skin irritation*

1. INTRODUCTION

Chili has been an indispensable spice in food preparation daily for Asian and Latin Americans. In Vietnam, chili is a spice vegetable with high economic value, which is grown mainly in the Central and Southern provinces and expanded to some Red River Delta provinces such as Vinh Phuc in recent years. The scientific name of the chili plant is *Capsicum annuum* L. or *Capsicum frutescens* L., belonging to the Solanaceae family [1]. According to traditional medicine, chili peppers have a spicy and hot taste with the effect of dispelling cold, strengthening the spleen, eliminating food, reducing pain, and treating cancer... Therefore, chili has been used to treat stomach aches due to cold, poor digestion, joint pain, snake bites...[1]. Chili peppers contain abundant vitamins and mineral salts such as vitamin C, vitamin A, vitamin B1, B6, vitamin K, calcium, magnesium, folate, potassium, thiamin, iron, copper... [2,3]. The alkaloid capsaicin has been identified as the main component related to chili's spicy taste and some of its pharmacological effects. Capsaicin stimulates the brain to produce endorphin, an endogenous morphine, which is used as a pain reliever, especially for chronic arthritis and cancer [4,5]. Recently, the active ingredient capsaicin has appeared in some commercial products

as an adjunct therapy in the treatment of bone and joint pain when used topically [4]. In addition, chili peppers also have other effects such as reducing lipid accumulation, losing weight, and preventing cancer due to their antioxidant and anti-inflammatory effects...[1,5]. Although chili has a potential therapy, there has not been much research to develop products from chili. The ingredient capsaicin that creates chili's spicy taste can also cause skin-mucous irritation and burning. To reduce the irritation of chili, many researchers have created products with nano-coated films to control the drug release process as well as prevent direct contact of the drug with the skin, thereby reducing irritation skin reaction [6,7,8].

For drugs used topically on the skin, assessing the risk for skin irritation is necessary before testing for pharmacological effects. Therefore, to supply the scientific pieces of evidence before testing the pharmacological effects, the study was conducted to evaluate the skin irritation risk of chili nano gel on experimental rabbit skin.

2. MATERIALS AND METHODS

2.1. Materials

The research product is chili fruit nano gel that reached the basic standard provided by the Institute for Applied Research of Traditional Medicine.

2.2. Experimental animals

The study was conducted on three adult healthy New Zealand white rabbits, at 9-10 weeks of age, weighing 2.0 - 2.2 kg, without skin diseases. Rabbits were housed separately and raised in experimental conditions for 5 days to acclimatize before the research with room temperature of 25 – 30^0C, and humidity of 60 - 70%. Rabbits were supplied with standard food for research animals and ad libitum water.

2.3. Skin irritation testing

Research on the skin irritation of chili nano gel was conducted according to the guidelines of the OECD [9], Vietnam Ministry of Health [10], and ISO 10993-10 [11] on skin irritation assessment for topical products.

Preparation of animals: About 24 hours before the commencement of the investigation, all test animals had hair thoroughly removed using electric clippers from the dorsal area of their trunks without abrading the skin, with a dimension of about 10 × 15 cm. Based only on appearance, only rabbits with a healthy, intact epidermis were selected for the experiment.

Dosage preparation: Undiluted chili nano gel was applied directly in a single dose (0.5 grams) to the skin of experimental rabbits. The surrounding skin areas were applied with distilled water to serve as a control.

Test material application: 0.5 grams of the undiluted test gel formulation was applied to the 2.5 × 2.5 cm area while the distilled water was applied to the surrounding skin on the day of treatment. Each application site was covered with 6.25 cm^2 (2.5 × 2.5 cm) of cotton gauze. The test patches were affixed using non-irritating adhesive tape, and the entire trunk was covered with a cotton bandage. The test gel formulation came into contact with the skin for four hours. Following the contact period, the protective covering and patches were removed, the treated region was cleaned with distilled water and patted dry with tissue paper, and the local skin reactions were noted. Each animal's erythema and edema irritation ratings at all measurement intervals after patch removal were tabulated and displayed. To evaluate the test item's potential for irritation, each animal's mean score was calculated separately during three scoring periods (24, 48, and 72 hours after patch removal) for erythema/eschar grades and edema grades.

Interpretation of findings: After four hours of post-experiment patch removal and 72 hr, the principal irritating index of the test gel formulation was obtained by adding

up the erythema and edema scores for all three test rabbits, dividing the result by the number of observations. The Primary Irritation Index (PII) is a metric that measures how irritancy is divided into several categories as shown in Table 2.

Table 1. Evaluation of skin reaction

Erythema and eschar formation	**Score**
- No erythema at all	0
- Very light erythema that can be barely seen with the naked eye	1
- Defined erythema	2
- Kind of severe erythema	3
- Severe erythema and light eschar	4
Total possible erythema score	*4*
Edema formation	
- No oedema at all	0
- Very light edema that can be barely seen with the naked eye	1
- Light edema that distinguishes the marginal area due to swelling	2
- Normal edema (about 1 mm of swollen edema)	3
- Severe edema (it swells up more than 1 mm and extends out of the exposed area)	4
Total possible edema score	*4*

If there were any lesions on the skin, the rabbits were observed for up to 14 days to evaluate the reversibility of the lesions. During the observation period, if the rabbits showed any signs of severe pain at any time, the experiment was discontinued.

Table 2. Rating table of skin irritation toxicity

Rating	**Primary Irritation Index (PII)**
Non irritant	0.0 – 0.5
Light irritant	0.6 – 2.0
Moderate irritant	2.1 – 5.0
Strong irritant	5.1 – 8.0

2.4. Statistical analysis

The data were collected, cleaned, and statistically analyzed by Microsoft Excel 2010 software.

3. RESULTS

During the trial, no death or aberrant clinical symptoms were noticed in any of the animals.

At 24, 48, and 72 hr following exposure, the skin response was scored (after removal of the dressing, gauze patches, and test item).

Table 3. Skin reaction score of chili nano gel at various time intervals (3 treatment sites)

Reaction	**24hrs**		**48hrs**		**72hrs**	
	Control	**Nano gel**	**Control**	**Nano gel**	**Control**	**Nano gel**
Erythema	0	0	0	0	0	0
Edema	0	0	0	0	0	0

The findings of a skin tolerance test are displayed in Table 3. After using the chili fruit nano gel, the rabbits' skin edema and irritation symptoms were not observed. After the test substance was removed from each rabbit, their erythema and edema ratings were "0" at all points during the observation.

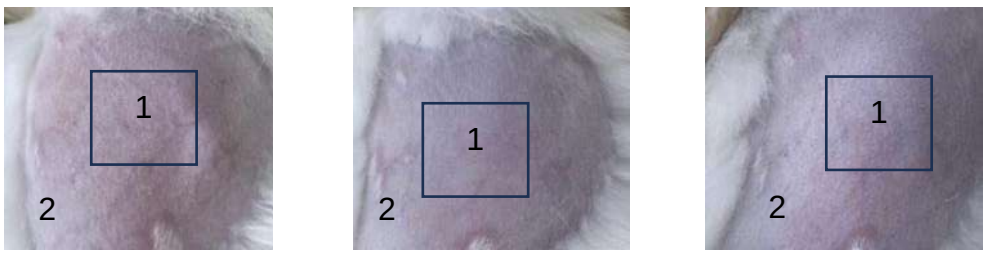
The Primary Irritation Index score for the skin was zero (Table 4).

Table 4. Primary Irritation Index (PII) on rabbit's skin

Animal identification number	Control	Nano gel
01	0	0
02	0	0
03	0	0
Total	**0**	**0**

Macroscopic images of rabbit skin at 72 hours following treatment did not differ between the chili nano gel applied area and the distilled water applied area.

The treated skin was intact; with no erythema, discoloration, inflammation, or corrosion compared to the untreated site (Figure 1).



Rabbit 1 ***Rabbit 2*** ***Rabbit 3***

Figure 1. Photographs of rabbit skin at 72 hours after removal of nano gel formulation

1: Applied with chili nano gel; 2: Applied with distilled water

4. DISCUSSION

Skin irritation assessment studies are generally recommended to be performed on skin that is prone to skin irritation. Rabbit skin is thinner and more sensitive than that of other rodents, and is therefore chosen in studies evaluating the skin irritation potential of an agent [9,10,11]. However, anatomically, rabbit skin has a thinner epidermis and is more susceptible to irritation than human skin. Therefore, if a drug is tested on rabbit skin and does not cause irritation, it is almost certain that the same result will be seen on human skin. To rule out the possibility of skin irritation on rabbits due to factors other than the reagent, we performed the same steps of applying gauze soaked in distilled water and bandaging on the adjacent healthy skin for comparison. The scoring assessment requires two investigators to work independently to eliminate subjective factors affecting the research results.

Erythema is a reddening of the skin or mucous membranes that disappears when pressed and reappears when released. This is one of the main manifestations of skin irritation caused by chemicals [12]. The substance applied to the skin's surface penetrated the stratum corneum and destroyed the underlying cell layers, which is the cause. Damaged keratinocytes release inflammatory mediators that act on the dermis, especially the stromal and vascular endothelium, causing vasodilation and increased vascular permeability, increasing blood flow (congestion), and causing erythema [12].

The study results did not record any erythema or scaling reactions in all experimental rabbits at 24 hours, 48 hours, and 72 hours after stopping exposure to the test gel (Table 3). The skin scales are formed to prevent the progression of erythema and appear in severe erythema reactions, so it is reasonable that no eschar was recorded.

Edema is defined as swelling relative to the surrounding skin and is likewise a primary manifestation of skin irritation [12]. Vasodilation and increased vascular permeability increase fluid leakage into body tissues and cause edema. The data in Table 3 also show that there were no other changes (changes in skin color, blisters, vesicles, dry skin...) on the rabbit skin in the skin areas where the samples were placed during 3 days of observation.

Thus, during the study period, all rabbits were normal, alert, and responded quickly to stimulation. The rabbit skin areas in contact with the test gel or distilled water did not show any signs of erythema, scaling, or edema at the time points before applying the test preparation, and after removing the gauze soaked with chili nano gel 24, 48, and 72 hours. The Primary Irritation Index of the test gel was determined to be 0 and corresponded to the classification of insignificant skin irritation.

Although chili has been used in folk medicine to treat many different diseases such as bone and joint pain, cancer, digestive disorders, snake bites, etc., chili has a spicy taste and often causes severe irritation and a burning sensation in the digestive tract as well as at the topical application [1]. A test by Winek CL et al. (1982) evaluating the toxicity of a chili sauce on animals showed that chili can cause mild irritation to the skin of New Zealand rabbits but moderate to severe irritation to the eyes [13]. The main active ingredient in chili that also creates the spicy taste of chili is capsaicin. Currently, there are some products with low capsaicin concentration including creams, gels, topical medications (0.025, 0.075, and 0.1%), and high capsaicin concentration patches (8%) have been developed to relieve pain such as bone and joint pain, neuropathic pain [4,14]. Products with high capsaicin concentrations (≥ 0.075% capsaicin) may be associated with a burning sensation, leading to severe skin irritation and patient noncompliance. This undesirable effect is because capsaicin is rapidly absorbed in the epidermis and binds to TRPV1 (transient receptor potential vanilloid subfamily member 1) receptors present on keratinocytes, simultaneously, the compound poorly penetrates the dermis, leading to capsaicin accumulation on the surface of the epidermis and causing skin irritation [15].

Recently, nanotechnology has been a strategy to reduce the irritation and allergy of active ingredients after application to the skin, by controlling the drug release and avoiding direct contact between the drug and the skin [6,7,8]. Studies have shown that the use of lipid nanoparticles carrying capsaicin is safe, without skin irritation, and local and systemic allergic manifestations. Anantaworasakul P et al. (2020) demonstrated that the lipid nanocarrier structure was more effective in encapsulating, and releasing capsaicin in vitro, and the permeability property enhanced the ability of capsaicin to be delivered into the dermis layer of the skin [6]. *In vitro*, skin irritation experiments showed that nanoparticles containing chili extract (high concentration of capsaicin 0.25%) minimized irritation compared to conventional chili extract. This was explained that lipid nanoparticles have a small structure and easy solubility in lipids, so it increased the penetration of capsaicin into the dermis, thereby minimizing irritation on the skin epidermis [6]. Other studies on lipid nanoparticles carrying capsaicin also showed similar results in reducing skin

irritation compared to normal capsaicin extract [7,8].

Evaluating the skin irritation of a topical product is a prerequisite step before assessing the efficacy of the drug. Using treatment products with inappropriate irritancy not only causes pain and discomfort to the patient but can also cause further damage. Rabbit skin is more sensitive than human skin. With the results of this study, it can be concluded that using chili nano gel will have almost no skin irritation risk in humans.

5. CONCLUSION

Evaluation of skin irritation of chili fruit nano gel on intact skin of experimental rabbits showed that the test gel did not cause erythema scabbing, or edema reaction after 24 hours, 48 hours, and 72 hours of gel removal. The primary irritation index was 0, corresponding to the classification of insignificant skin irritation, proving that chili fruit nano gel used topically was safe and did not cause skin irritation in experimental rabbits.

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