THE RISK OF SKIN IRRITATION OF CLARTÉ IN EXPERIMENTAL RABBITS

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SUMMARY

CLARTÉ is an external genital hygiene product, thus assessing skin irritation is an important test before commercializing the product for the community. ***Objectives:*** *The study evaluated the local irritation on healthy rabbit skin of CLARTÉ manufactured and supplied by VIHECO PHARMA., JSC.* ***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 shaved off the fur on the back. Animals were applied directly 0.5 mL of CLARTÉ solution on the back skin and covered the application sites with a 2,5 cm × 2,5 cm non-occlusive dressing for 4 hours. We evaluated the irritation index including erythema and edema on the skin at 1, 24, 48, and 72 hours after removing the patches.* ***Results:*** *Three rabbits were still healthy, and living normally. There was very slight or well-defined erythema and very slight edema on the rabbit's skin applied by CLARTÉ after the first 24-hour treatment. The irritation index of CLARTÉ was 0.44, corresponding to negligible skin irritation during 72 hours of continuous monitoring.* ***Conclusions:*** *CLARTÉ product applied topically caused negligible irritation on healthy rabbit skin.*

Keywords*: CLARTÉ', rabbit, skin irritation*

1. INTRODUCTION

Natural product application in treatment has a history of thousands of years and includes many plant-based products. They have been used both for internal and external treatment [1]. Many diseases are treated through the skin route. Presently, dermal natural products still have a mainly local effect [2]. In the previous time, due to the limitations of technology, medicine powders or crude herbal extracts were applied in formulations such as topical powders, and pastes for skin administration. Although it was hard to control the quality of those products, they were still widely utilized in some clinical cases because of their simple acquirement and convenient usage. Natural product dermal preparations are now widely utilized in the form of oils, ointments, patches, and gels thanks to advancements in pharmaceutical technology [1]. This route of administration is widely accepted by patients and physicians for its flexibility, first-pass hepatic metabolism avoidance, higher local efficacy, and fewer risks of systemic side effects [3].

CLARTÉ is manufactured and supplied by VIHECO Central Pharmaceutical Joint Stock Company. CLARTÉ is an external genital hygiene product that is used to gently clean the external genital area and help prevent bacteria and genital fungus. Its components are derived from natural extracts including Piper Betle extract, Rosa Damascena Flower extract, Aloe Vera extract, and Dimocarpus Longan extract; and combined with many other

chemical agents such as glycerin, PEG 400 (Polyethylene glycol 400), HEC (Hydroxyethylcellulose), CAB (Cocamidopropyl betaine), PEG 75 lanolin, saliguard EHGP… Some of its ingredients can easily cause skin irritation such as sodium lauryl sulfate [4], fragrances [5], and ethylhexylglycerin [6]. In addition, it also contains soothing ingredients to reduce skin irritation such as aloe vera [7], and panthenol [8,9]... Before conducting the research in humans, the product's safety and efficacy need to be proven in experimental animal models. In particular, the dermal irritation test in animals is one of the most important toxicity studies with a topical product. Thus, we conducted this study to investigate the skin irritation ability of CLARTÉ on healthy rabbit skin.

2. MATERIALS AND METHODS

2.1. CLARTÉ solution

CLARTÉ was an external genital hygiene product manufactured and supplied by VIHECO PHARMA., JSC. The product's ingredients included: Sodium lauryl sulfate, glycerin, and neo. Fer-Lactobacillus Ferment Lysate, PEG 400 (Polyethylene Glycol 400), HEC (Hydroxyethylcellulose), Sodium Chloride, CAB (Cocamidopropyl Betaine), PEG 75 Lanolin, Saliguard EHGP (Ethylhexylglycerin, Phenoxyethanol), Piper Betle Extract, Rosa Damascena Flower Extract, Tween 20, Aloe Vera Extract, Panthenol, Disodium EDTA, Menthol, Lactic acid, Dimocarpus Longan Extract, fragrance.

2.2. Experimental animals

Three adult healthy *New Zealand* White rabbits at 10 weeks of age, weighed 2.0 ± 0.2 kg, and had no skin diseases, were used in this study. They were acclimatized for 7 days before the research and maintained in specific standard conditions for animals throughout the study period in the Laboratory of the Department of Pharmacology, University of Medicine and Pharmacy, Vietnam National University.

2.3. Methods

The experiment was carried out following the guidelines of the Vietnam Ministry of Health [10], OECD [11], and ISO 10993-10 [12] for skin irritation assessment of topical products.

Animal preparation: 24 hours before the experiment, rabbits were shaved to remove the fur on both sides of the spine for application and observation of the test sites (approximately 10 × 15 cm). The skin of rabbits was examined carefully to confirm that only animals with healthy and intact skin were used for the test.

Exposure period: 0.5 ml of CLARTÉ was applied to an area (approximately 2.5 × 2.5 cm) of the skin on one side of the back while 0.5 ml of distilled water was applied on the other side. Each application site was covered by a gauze patch (approximately 2.5 × 2.5 cm) and wrapped with a semi-occlusive bandage for 4 hours. After the exposure period, the residual test product was removed and the skin was carefully dried without altering the existing response of the skin.

Observation and assessment: The skin reaction for erythema and edema was described and scored according to the scoring system in Table 1 at 1 hour, 24 hours, 48 hours, and 72 hours after removing the patches. 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 1. Scoring system for skin reaction

Erythema and eschar formation	**Score**
- No erythema	0
- Very slight erythema (barely perceptible)	1
- Well-defined erythema	2
- Moderate erythema	3
- Severe erythema (beet-redness) to eschar formation preventing grading of erythema	4
Oedema formation	
- No oedema	0
- Very slight oedema (barely perceptible)	1
- Well-defined oedema (edges of area well-defined by definite raising)	2
- Moderate oedema (raised approximately 1 mm)	3
- Severe oedema (raised more than 1 mm and extending beyond exposure area)	4
Maximal possible score for irritation	**8**
Other adverse changes at the skin sites shall be recorded and reported	

Rabbit skin reaction category: After scoring the rabbits, all erythema scores and edema scores at 24h, 48h, and 72h were separated for each test sample and control site for each rabbit. The Primary Irritation Index (PII) for a rabbit was calculated as the total erythema and edema scores divided by the number of observation times (two tests for each observation site, three-time points). Classification of the reaction on rabbit skin according to the Primary Irritation Index (PII) in Table 2.

Table 2. Rabbit skin reaction categories

Reaction category	**Primary Irritation Index (PII)**
Negligible	0 – 0.5
Slight	> 0.5 – 2.0
Moderate	> 2.0 – 5.0
Severe	> 5.0 – 8.0

2.4. Statistical analysis

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

3. RESULTS

Symptoms of erythema and edema were assessed on 3 rabbits at 1 hour, 24 hours, 48 hours, and 72 hours after removing the test sample applied to the skin and evaluating the score according to Table 1. The results are described in Tables 3 and 4.

Table 3. Erythema score in experimental rabbits

Rabbit	**1h**		**24h**		**48h**		**72h**	
	T	**C**	**T**	**C**	**T**	**C**	**T**	**C**
1	0	0	0	0	0	0	0	0
2	0	0	1	0	0	0	0	0
3	0	0	2	0	0	0	0	0

T: CLARTÉ - applied area; C: Control area

As shown in Table 3, on the control skin area of all rabbits (applied distilled water), no erythema and any other abnormal signs were observed at all time points: 1 hour, 24 hours, 48 hours, and 72 hours. On the tested skin area (applied CLARTÉ), no erythema sign was observed at all the time points in Rabbit 1st, but there

was a sign of very slight erythema in Rabbit 2nd and well-defined erythema in Rabbit 3rd at 24 hours after removing CLARTÉ. The erythema reactions on the skin of two rabbits disappeared completely after 48 hours of observation.

Table 4. Edema score in experimental rabbits

Rabbit	1h		24h		48h		72h	
	T	C	T	C	T	C	T	C
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	0	1	0	0	0	0	0

T: CLARTÉ - applied area; C: Control area

Data in Table 4 have shown that no edema or any other abnormal signs were observed at all time points on the control skin area of all rabbits (applied distilled water). On the tested skin area (applied CLARTÉ), no edema sign was observed at all the time points in Rabbit 1st and Rabbit 2nd, however, there was a sign of very slight edema in Rabbit 3rd at 24 hours after removing CLARTÉ. The edema reactions on the skin of Rabbit 3rd disappeared completely after 48 hours of observation.

Table 5. Primary Irritation Index (PII) on rabbit skin

Rabbit	C	T
1	0	0
2	0	0.33
3	0	1.0
Total	0	0.44

T: CLARTÉ - applied area; C: Control area

The average irritation score of CLARTÉ on rabbit skin ranged from 0-1.0 and the overall Primary Irritation Index (PII) of 3 rabbits was 0.44 which was in the range of 0-0.5 and belonged to the level of negligible skin irritation (Table 5).

The signs of erythema and edema in Rabbit 3rd can be observed at the time points of 24 hours after CLARTÉ removal in Figure 1.

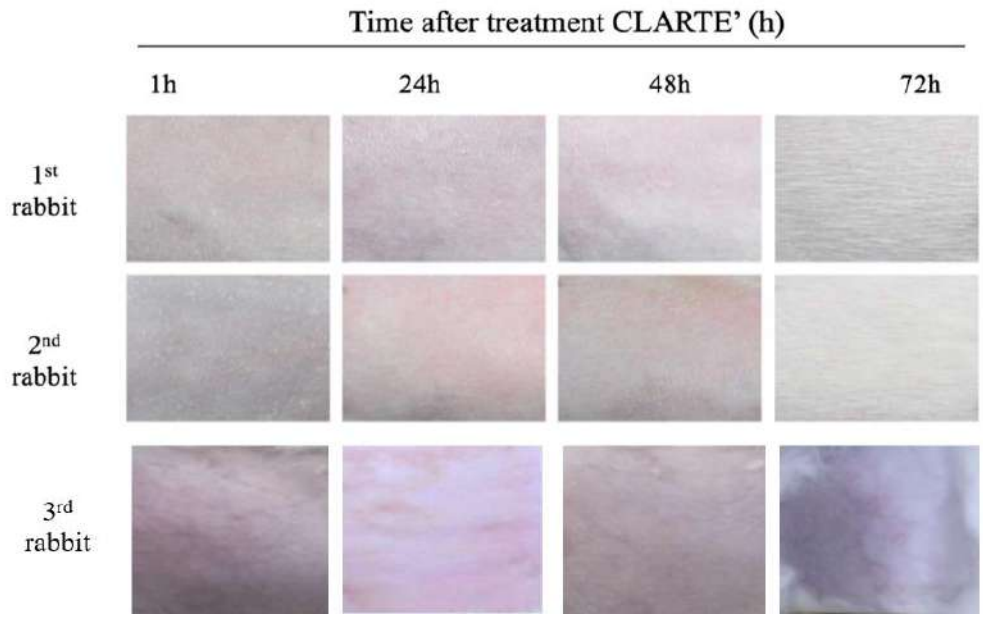

Figure 1. Images of the rabbit skin after applying CLARTÉ

In addition, observation of the rabbits' total heath during the follow-up period showed that there were no dead rabbits in the study. At the time points of the study, all rabbits were healthy, conscious, quick to respond to stimuli, and did not have digestive disorders (such as diarrhea, increased salivation, ...).

4. DISCUSSION

Assessment of irritation of pharmaceutical and cosmetic products with natural compounds is a significant step in the evaluation of their biocompatibility. The principle of a dermal irritation test is applying the test product to the skin of experimental animals and untreated skin sites of the animal as the control. The degree of irritation is observed and scored at specified intervals. If there is any sign of irritation observed, the study should be sufficient to evaluate the reversibility of the lesions [9]. In a dermal irritation test, the rabbit is the preferable experimental animal because the rabbit's skin is very sensitive to external factors and can easily detect the irritating potential of the investigational products [9,10]. However, the anatomical rabbit skin has a thinner epidermis and is more susceptible to irritation than human skin. Therefore, when the tested drug on the rabbit skin has no irritation, it is almost certainly similar to human skin. To limit the interference factors that affected the results, we carried out parallelly applying distilled water and bandage gauze on the healthy skin sites for comparison. During the study period, all rabbits were healthy and responded quickly to stimuli. The rabbit skin area exposed to the distilled water did not have erythema, scaling, or edema at 1, 24, 48, and 72 hours after the gauze removal.

Erythema is a phenomenon of red skin or mucous membranes that disappears if it is pressed and reappears if it is released. This is one of the main manifestations of skin irritation caused by chemicals [13]. The reason is that the test substance applied on the skin surface penetrates the stratum corneum and destroys the underlying skin layers. The damaged keratinocytes release inflammatory mediators on the dermis, especially the stromal and the vascular endothelium, causing vasodilation and increased vascular permeability, increasing blood flow and causing erythema [13]. The results showed that 2 out of 3 rabbits developed a very slight or well-defined erythema on the skin after the first 24 hours of applying CLARTÉ. However, the erythema completely disappeared after 48 hours of treatment. Scaling is formed to prevent the progression of erythema and appears in severe erythema (beet-redness), so it is reasonable to not record the eschar formation. The results were suitable to the skin irritation as a reversible skin injury since the repair mechanism was established immediately after the injury appearance [11]. This reversible manifestation indicated that the test drug is safe to a certain extent.

Edema is defined as swelling relative to the surrounding skin, which is also a major manifestation of skin irritation [13]. Vasodilation and increased vascular permeability increase fluid drainage into tissues and cause edema. The results showed that 1 out of 3 rabbits had very slight edema after 24 hours of treatment, but it completely disappeared after 48 hours of observation. The Primary Irritation Index (PII) of the CLARTÉ sample on 3 rabbits was 0.44, corresponding to a negligible skin irritation level according to the classification of rabbit skin irritation of the Vietnam Ministry of Health (Table 2) [10].

CLARTÉ contains some components that are able to skin irritation in clinical practice such as Sodium lauryl Sulfate [4] and ethylhexylglycerin [6]. To reduce skin irritation, many ingredients were added to the tested product such as

Aloe Vera, Panthenol…[7,8]. Various studies confirmed dexpanthenol's moisturizing and skin barrier-enhancing potential. It prevents skin irritation, stimulates skin regeneration, and promotes wound healing [8]. D-panthenol could improve the symptoms of skin irritation, such as dryness of the skin, roughness, scaling, pruritus, erythema, and erosion/fissures [9]. Research to evaluate the skin irritation of a topical product is a prerequisite before mentioning the effectiveness of the drug. Rabbit skin is more sensitive than human skin. With the results of this study, it can be confirmed that the use of CLARTÉ has almost negligible irritation on human skin.

5. CONCLUSIONS

The results of the evaluation of skin irritation of CLARTÉ on healthy rabbit skin showed that 2 out of 3 rabbits developed very slight or well-defined erythema on the skin after the first 24 hours of applying CLARTÉ. However, the erythema completely disappeared after 48 hours of treatment. 1 out of 3 rabbits had very slight edema after 24 hours of treatment, but it completely disappeared after 48 hours of observation. There were no cases of moderate to severe erythema, and no cases of well-defined to severe edema. The Primary Irritation Index (PII) of CLARTÉ on 3 rabbits was 0.44, corresponding to a negligible skin irritation level according to the classification of rabbit skin irritation.

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