

## ASSESSMENT OF DERMAL IRRITATION OF LAO NHA QUE COCONUT OIL IN EXPERIMENTAL ANIMALS

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### ABSTRACT

Lao Nha Que virgin coconut oil (LNQ VCO) is a natural product intended to treat some skin diseases such as burns, atopic dermatitis, and xerosis.... To provide information on its local irritation, this study was carried out to evaluate the dermal irritation of LNQ VCO. 0.5 ml of VCO was applied on the skin in one side of the spine while 0.5 ml of distilled water was applied on the other side. After 4-hour exposure period, the residual LNQ VCO was removed and the skin reactions for erythema and oedema was described and scored at 1 hour, 24 hours, 48 hours and 72 hours after removing the patches. The results showed that the erythema appeared on the LNQ VCO-applied skin in two out of three rabbits and recovered up to 72h. The primary irritation index was calculated to categorize the skin irritation of LNQ VCO. The primary irritation index of LNQ VCO was 0.78. Thus, LNQ VCO might cause slight irritation on the rabbit's skin.

**Keywords:** virgin coconut oil, rabbits, dermal irritation.

### I. INTRODUCTION

Natural product application in treatment has a history of thousands of years and includes many products derived from plants. They have been used both for internal and external treatment [1]. Many diseases are treated through the skin route. At present, 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, 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. Currently, with the advance of pharmaceutical technology, natural product dermal preparations are commonly used in the form of oils, ointments, patches, and gels [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].

Lao Nha Que virgin coconut oil (LNQ VCO) is a natural product from abundant raw materials available in our country. Traditionally, the coconut oil is used to moisturize and treat skin infections. The effect of the coconut oil in some skin diseases has been successfully demonstrated in some previous studies [4-8]. Thus, LNQ VCO is intended to be used to treat several skin conditions such as burns, atopic dermatitis, and xerosis... Prior to conducting the research in humans, the product's safety and efficacy needs 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.

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**Date of receipt:** 26/2/2024

**Date of scientific judgment:** 25/3/2024

**Reviewed date:** 1/4/2024

The aim of this study was to investigate ability to cause irritation to the skin of LNQ VCO in experimental animals.

**II. MATERIALS AND METHODS**

**2.1. Investigational product**

Lao Nha Que coconut oil (LNQ CCO) is a product of Vi Dieu Nam Company Ltd. The product’s ingredient is virgin coconut oil (VCO) (100% of coconut oil).

**2.2. Experimental Animals**

Adult *New Zealand White* rabbits weighed  $2.0 \pm 0.2$  g were used as the study subjects. They were acclimatized for 7 days prior to the research and maintained in specific standard conditions for animals throughout the study period in the Laboratory of the Department of Pharmacology - Hanoi Medical University.

**2.3. Methods**

The experiment was carried out following the guidelines of OECD and ISO [9],[10] in three rabbits and included three periods:

- *Preparation period:*

24 hours before the experiment, rabbits were shaved to remove the fur in both sides of the spine for application and observation

of the test sites (aproximately 10 cm x 15 cm). The skin of rabbits was examined carefully to confirm that only animals with healthy, intact skin were used for the test.

- *Exposure period:*

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

- *Obeservation period:*

The skin reaction for erythema and oedema 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 was any lessions on the skin, the rabbits were observed up to 14 days to evaluate the reversibility of the lessions. During the observation peroid, if the rabbits showed any signs of severe pain at any time, the experiment was terminated.

*Table 1. Scoring of erythema and oedema [9],[10]*

<b>Erythema</b>	<b>Score</b>
-No erythema	0
-Very slight (barely perceptible)	1
-Well defined	2
-Moderate to severe	3
-Severe	4
<b>Oedema</b>	
-No oedema	0
-Very slight (barely perceptible)	1
-Well defined (edges of area raised)	2
-Moderate to severe (edges of area raised ~ 1 mm)	3
-Severe (edges of area raised more than 1 mm and extending outside the application area)	4

- *Primary irritation index calculation:*

After scoring the rabbits, all erythema scores and oedema scores at 24h, 48h and 72h were totalled separately for each test sample and blank for each rabbit. The primary irritation score for a rabbit was calculated by dividing the sum of all the scores by 6 (two test/observation sites, three

time points). To obtain the primary irritation index (PII) for the investigational product, all the primary irritation scores of the individual rabbits were added and divided by three.

The PII was compared with the categories of irritation response in Table 2.

**Table 2. Response categories according to PII [10]**

Response category	Mean PII
Negligible	0 – 0.4
Slight	0.5 – 1.9
Moderate	2 – 4.9
Severe	5– 8

### III. RESULTS

**Table 3. Scoring of erythema and oedema in experimental rabbits**

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

T: LNQ VCO - applied area

C: Control area

As shown in Table 3:

- On the control skin area of all rabbits (applied distilled water), no erythema, edema or any other abnormal signs was observed at all time points.

- On the tested skin area (applied LNQ VCO)

+ 1<sup>st</sup> rabbit: Erythema was well defined at 1h, 24h, 48h but it was no longer observed at 72h after removing VCO from the skin. No oedema was observed at all time points.

+ 2<sup>nd</sup> rabbit: Erythema was very slight (barely perceptible) at 24h, well defined at 48h and no longer observed at 72h after removing VCO from the skin. No oedema was observed at all time points.

+ 3<sup>rd</sup> rabbit: No erythema or oedema was observed at all time points.

There were no other abnormal signs other than erythema and edema observed in all rabbits.

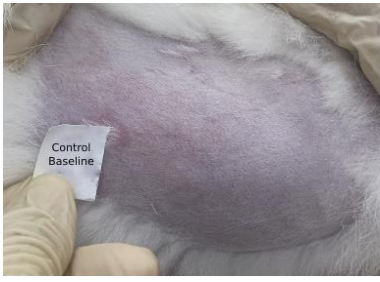









**Table 4. PII of LNQ VCO**

Rabbit	PII
1	1.33
2	1.0
3	0

The PII of LNQ VCO was calculated based on results shown in Table 4:

$$PII = (1.33 + 1.0 + 0)/3 = 0.78$$

According to the categories of irritation response in Table 2, LNQ VCO caused slight irritation in the rabbit’s skin.

Time points	Control	LNQ VCO
1	 <p>Control Baseline</p>	 <p>LNQ VCO Baseline</p>
1h	 <p>Control 1h</p>	 <p>LNQ VCO 1h</p> <p>Well-defined erythema</p>
24h	 <p>Control 24h</p>	 <p>LNQ VCO 24h</p> <p>Well-defined erythema</p>
48h	 <p>Control 48h</p>	 <p>LNQ VCO 48h</p> <p>Well-defined erythema</p>
72h	 <p>Control 72h</p>	 <p>LNQ VCO 72h</p>

**Figure 1.** Images of the dermal irritation on LNQ VCO applied skin in rabbits

#### IV. DISCUSSION

The dermal irritation test is a necessary test for a product intended to apply on skin. The principle of a dermal irritation test is that the investigational product is applied to the skin of an experimental animal and untreated skin areas of the animal serve 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].

The dermal irritation test of LNQ VCO was carried out according to standard guidelines of OECD and ISO [9],[10]. After applying LNQ VCO to the rabbit's skin, slight to well-defined erythema was observed in 2 out of 3 rabbits and the sign recovered before 72h. No oedema or any other abnormal signs observed in all rabbits. The primary irritation index was calculated and our results showed that LNQ VCO caused slight irritation on the rabbit's skin. A slight irritant on rabbit skin might cause no or mild irritation in humans because the rabbit's skin is more sensitive than the human skin. Thus, our results suggested a low skin irritation potential of LNQ VCO when used in patients.

Traditionally, coconut oil is used to moisturize and treat skin infections. The earlier studies have demonstrated several biological activities of coconut oil such as antimicrobial, analgesic, antipyretic, and antiinflammatory properties *in vivo* [4],[5]. The efficacy of VCO in wound healing,

atopic dermatitis and xerosis have also been demonstrated by other authors [6],[7],[8]. Therefore, the intended use of LNQ VCO in the treatment of several skin conditions such as burns, atopic dermatitis, and xerosis has a scientific basis. In the treatment of these diseases, if the patients use the products which can cause moderate to severe skin irritation, the skin lesions can be aggravated. LNQ VCO might cause no or very mild irritation on human skin. Our results were constant with the previous studies carried out to evaluate the irritation of coconut oil and its ingredients in experimental animals. It was demonstrated that it produced no significant skin irritation in the animals [11].

#### V. CONCLUSION

Our results demonstrated that LNQ VCO caused slight irritation on the rabbit's skin. With the proven biological effects of coconut oil, it can be concluded that LNQ VCO is completely suitable to be developed as a product to treat the skin diseases.

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