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Safety assessment of female sexual hygiene product containing cannabidiol in new zealand white rabbit and clinical trial

Atiwut Kamudhamas [a,b], Sombat Muengtaweepongsa [c], Sitthiphon Bunman [c,d], Winit Longlalerng [e], Nakarin Sivapornpan [f], Siwapol Thitayarasa [b], Chuntida Kamalashiran [g], Pratya Phetkate [g,*]

[a] Department of Obstetrics and Gynecology, Faculty of Medicine, Thammasat University, Pathum Thani, Thailand
[b] Sexual Health Center, Thammasat University Hospital, Pathum Thani, Thailand
[c] Center of Excellence in Stroke, Faculty of Medicine, Thammasat University, Pathum Thani, Thailand
[d] Department of Community Medicine and Family Medicine, Faculty of Medicine, Thammasat University, Pathum Thani, Thailand
[e] Department of Coronary Artery Disease, Thammasat University Hospital, Pathum Thani, Thailand
[f] Division of Dermatology, Department of Medicine, Thammasat University Hospital, Pathum Thani, Thailand
[g] Department of Integrative Medicine, Chulabhorn International College of Medicine, Thammasat University, Pathum Thani, Thailand

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ABSTRACT

Cannabis is an herb known for its significant pharmacological effects. This study evaluated the safety of a female sexual hygiene product containing cannabidiol on the skin of experimental animals and humans. Irritation symptoms were tested using three female New Zealand white rabbits. In human trials, irritation and sensitivity symptoms were assessed in 30 healthy female volunteers. The results from the animal study indicated that body weight and clinical symptoms remained normal, with only slight irritation noted in the primary irritation index of the skin (PII = 0.6). In human trials, transepidermal water loss was observed during the induction phase but decreased over time. No abnormalities were found during the induction phase; however, irritation occurred during the challenge phase, which resolved after 8 h. It was concluded that the rinse-off product caused slight irritation when applied for more than 4 h in animals and 24 h in humans, which differs from its intended use of immediate rinse-off, where no irritation was observed.

1. Introduction

The rising use of female sexual hygiene products raises concerns about disrupting the natural pH balance and microbiota, potentially causing infections [1]. Prevalence data indicates that bacterial vaginosis affects around 23–29 % of women of sexually active age worldwide, highlighting the importance of safe and effective hygiene practices [2]. Female sexual hygiene products play an important role in the daily cleansing routines of many women [3]. While many of these products promise cleanliness and odor control, some inadvertently alter the crucial pH balance and microbiota, which protect against infections [4,5]. Notably, although extensive literature addresses the internal vaginal environment, studies focusing on the external vulva and the effects of intimate hygiene practices on it are sparse. Hence, educating female and health care professionals about the importance of female sexual hygiene and its associated risks remains paramount. Gentle vulvar cleansing is recommended for promoting optimal vulvovaginal health. The risks of internal douching have redirected attention to external feminine washes [6,7], particularly those fortified with lactic acid and boosting an acidic pH. Such formulations bolster skin equilibrium and potentially serve as beneficial supplementary treatments for those grappling with vaginal infections or antibiotic usage [8]. Proper vulvar cleansing is also advised for females troubled by odorous discharges, and routine use of feminine washes might diminish bacterial vaginosis recurrence probabilities [9,10]. Adhering to clinical guidelines, females are counseled to employ pH-balanced, hypoallergenic agents for daily vulvar cleaning. The

Abbreviations: ANDAs, Abbreviated new drug applications; CBD, cannabidiol; H, hour; PII, Primary Irritation Index; PIS, Primary Irritation score; RTECS, the Registry of Toxic Effects of Chemical Substances; TEWL, Transepidermal water loss.

* Correspondence to: Department of Integrative Medicine, Chulabhorn International College of Medicine, Thammasat University, 99 Moo.18 (Rangsit Campus), Paholyothin Rd, Khlong Nueng, Khlong Luang, Pathum Thani 12120, Thailand.
E-mail address: pratya@tu.ac.th (P. Phetkate).

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formulation of these external washes needs to be careful; they must offer gentle cleansing without disturbing the natural flora, especially in settings where females employ them frequently [3,11,12].

Assessment of the risk of irritation and sensitization in new cleansing products before market launching is a systematic testing approach for the development of scientifically sound products, evaluating adverse effects and allergies [13]. Particularly, feminine hygiene products used for external genital cleanliness should have an appropriate pH level (ranging between 3.8 and 5.0), which is moderately acidic, to avoid disrupting the ecosystem or compromising the healthy vaginal immune barrier environment [14–16].

In Thailand, the surge in advertisements for female sexual hygiene products is evident. Such promotions are believed to be molding the hygiene habits of Thai women. Recently, the spotlight has shifted to cannabidiol (CBD), a primary non-psychoactive phytocannabinoid in *Cannabis sativa* L., revered for its anti-inflammatory and antioxidant properties [17,18]. The chemistry and pharmacology of CBD, as well as various molecular targets, have been extensively studied [19,20]. The endocannabinoid system, an emerging area of research, features endogenous lipid-based retrograde neurotransmitters, which, upon binding to cannabinoid receptors, can modulate various cellular responses [21,22]. This system's profound influence spans from the central nervous system to skin homeostasis and barrier function [23–25]. Even though an application of CBD to topical health products is plausible, there is limited clinical evidence of its efficacy for skin disorders and the safety test [26]. The researchers have developed a feminine sexual hygiene product containing CBD from cannabis extract, designed for use on the external genital area. This is a mild, pH-friendly cleanser that aids in protecting the skin from dryness [27]. Consequently, this study aims to assess the safety of the product in both animal and human trials.

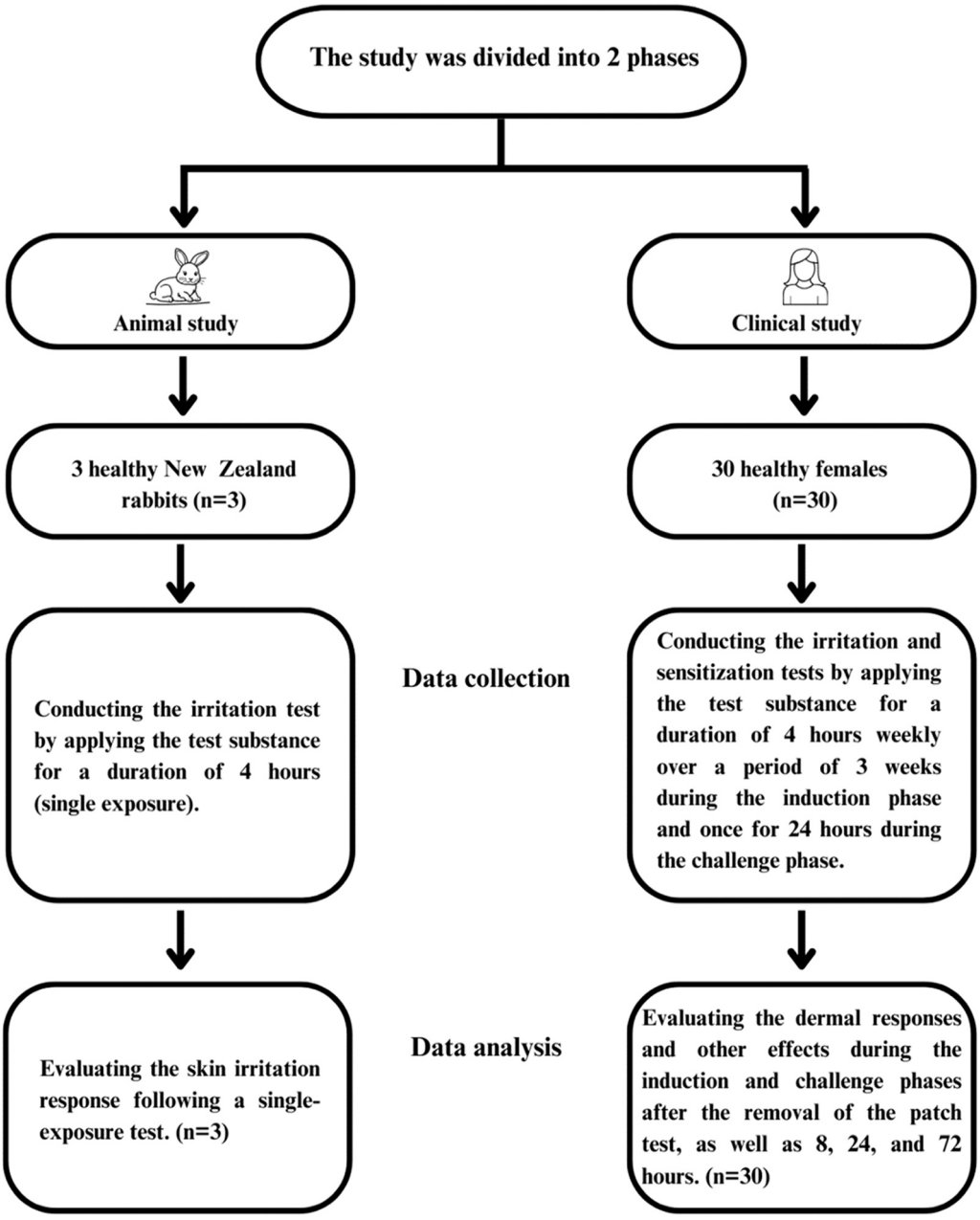

Fig. 1. Study flowchart.

2. Materials and methods

2.1. Study design

This study was conducted in two phases. The first phase involved assessing the safety of a female sexual hygiene product containing CBD derived from cannabis extract in animals. The animal experiments were carried out at the National Laboratory Animal Center over a period of 15 days in June 2022. The second phase focused on human trials, which were conducted at the Sexual Health Clinic of Thammasat Hospital in Pathum Thani, Thailand, from March to May 2023 (Fig. 1).

2.2. Animal

The New Zealand white rabbit, is the preferred animal for testing dermal irritation, as indicated by the extensive data on this species in the Registry of Toxic Effects of Chemical Substances (RTECS). Rabbits have been utilized to produce the majority (85 %) of the existing data found in publicly accessible literature [28].

In this study, female New Zealand white rabbits were selected, with a body weight ranging from 2757 to 2987 g and ages between 10 and 12 weeks. These animals were procured from the Animal Production Office of the National Laboratory Animal Center. Each rabbit was housed separately in a stainless-steel cage and provided with a standard diet of 8RD65/65 Lot no. G5, supplied by Perfect Companion Group Co., Ltd. The nutritional content of the diet was analyzed by the laboratory of Perfect Companion Co., Ltd., and microbial contamination levels were monitored by the laboratory of the Quality Control Office. The animal facility maintained a temperature between 20.5 and 22.6°C, a humidity level between 46.0 % and 52.2 %, and a 12:12-h light/dark cycle throughout the experiment. The animals underwent an acclimatization period of eight days prior to the application of the test substances. The study received approval from the National Laboratory Animal Center in Nakhon Pathom, Thailand.

2.3. Skin irritation test in New Zealand white rabbits

Following the ISO 10993–23 guidelines from the International Organization for Standardization (2021) [28], we evaluated the potential in skin irritation of a cannabis extract-based sexual hygiene product using New Zealand white rabbits. Prior to testing, the rabbits' back fur was clipped to expose a 10 cm×15 cm area on either side of the spine, ensuring healthy, intact skin. For the primary test, a gauze soaked in 0.5 ml of the product was applied to each rabbit's side, with a saline-soaked gauze as a control. These were covered with 2.5 cm×2.5 cm non-occlusive dressings and bandaged. After exposure for 4 h, the sites were cleaned and marked, with observations made under natural or full-spectrum lighting. Monitoring the animals encompassed daily metrics like body weight, dietary intake, and health, while skin reactions were scored at specific intervals, including erythema and edema evaluations. The evaluator for the experimental animals was a veterinarian from the National Laboratory Animal Center in Nakhon Pathom, Thailand.

Erythema and edema formation were assessed according to the following irritation scores: no erythema or edema (score 0), very slight erythema or edema (score 1), well-defined erythema or edema (score 2), moderate erythema or edema (score 3), and severe erythema or edema (scores 4 for severe erythema and 5 for severe edema) [29]. For this single-exposure test, the primary irritation index (PII) was derived from cumulative erythema and edema grades at 24, 48, and 72-h post-exposure intervals. Scores for each sample and control were compiled, and the overall PII was determined by averaging the scores across all animals. The mean scores were then compared to predefined irritation response categories: 0–0.4 indicating a negligible response, 0.5–1.9 indicating a slight response, 2.0–4.9 indicating a moderate response, and 5.0–8.0 indicating a severe response [16]. This comparison facilitated the final documentation of the product's irritation potential.

2.4. Participants and sample size

Thirty female participants, aged between 18 and 50 years, were enrolled in this open-label clinical trial. All participants were in good health, not pregnant or breastfeeding, and had no chronic diseases involving skin abnormalities or irregularities associated with the immune system. Participants had no history of skin cancer, had not taken any immunosuppressants or oral antihistamines, had no scars, tattoos, open wounds, or sunburns on their skin, and were not allergic to herbs or cannabis extracts. During the study period, the participants were prohibited from taking any other medications or supplements. The study was conducted with written informed consent from all participants, and they could withdraw at any time.

2.5. Transepidermal water loss test in humans

Transepidermal water loss (TEWL) represents the predominant objective metric employed to evaluate the barrier functionality of the skin, quantifying the amount of condensed water that permeates through a designated area of the stratum corneum to the skin's surface within a specific time interval [30]. For the TEWL evaluation, a patch test was performed on the upper arm, the same as the skin sensitization test, and remained adhered for 4 h with weekly applications for 3 weeks. Before applying the tests, the skin in the designated areas was cleaned and completely dried. Separate applications were made on three different areas of the upper arm for each participant. The evaluation comprised three tests: a negative control using pure white petrolatum, a positive control using 0.5 % sodium lauryl sulfate, and a sample test using the female sexual hygiene product containing 0.1 % CBD. The Tewameter® TM 300 (Courage + Khazaka Electronic, Cologne, Germany) was used to measure TEWL, with readings taken immediately post-patch removal and 4 and 8 h later. Before starting, externally calibrated loggers documented research room conditions, and compared the results to those of an external temperature sensor from another system. Adhering to cosmetic science guidelines for TEWL, optimal conditions were established, areas with excessive hair were avoided, designated regions were consistently marked, and the probe was applied without pressure, ensuring residual condensation vapor was minimized post-measurement [30,31].

2.6. Skin sensitization test on humans

The potential for skin sensitization of a cannabis extract-based sexual hygiene product for females was evaluated following the assessing the irritation and sensitization potential of transdermal and topical delivery systems for ANDAs, guidance for industry [32].

The upper arm was chosen as the testing site for this skin sensitization test due to its standard use in patch testing for skin irritation and sensitization, supported by various studies [33,34]. Research has shown it is commonly used to assess the irritant properties of feminine products and moisturizers [35]. Additionally, studies have demonstrated that while the back is more sensitive to irritant challenges, the upper arm remains a reliable and convenient site for such tests [36,37]. Therefore, we selected the upper arm for testing due to its convenience and accessibility, allowing for easy application and monitoring of the product without compromising the privacy and comfort of the participants. Evaluating irritation directly on the genital area would be invasive and sensitive, posing ethical and practical challenges.

During the induction phase, approximately 20 μl of the product was applied to the intact skin of the upper arm of volunteers using an occlusive patch (finn chamber 8 mm (50 mm^2)) for a duration of 4 h, every other day for three weeks. Following this, participants underwent a two-week rest phase before the challenge phase, in which they were

patched again for 24 h on a previously untreated area of skin. The evaluator for this test was the dermatologist. For assessment, the dermal response score and other effects score [32] (Table 1) were employed to evaluate the results immediately after the removal of the patch test, as well as 8, 24, and 72 h post-removal, during both the induction and challenge phases.

2.7. Statistical analysis

In the animal study, data on irritation were presented as visual scores based on the erythema and edema grading system, and PII was calculated. Whereas human study, the data were analyzed using the Stata software version 17 for the Windows operating system. The results have been presented in the form of descriptive statistics. The results, such as age, weight, height, BMI, and TEWL, have been presented as percentages of the mean and standard deviation, while the history of underlying diseases, smoking, alcohol consumption, food allergies, and drug allergies have been presented as frequency and percentage. The efficiency of the product with TEWL was analyzed by one-way ANOVA with Tukey's post hoc test. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. The results of animal study

3.1.1. General clinical observation

All the animals were clinically normal throughout the study. The consumption of food and water was regular. Animals gained weight as normal (Table 2).

3.1.2. Skin observation

In the single exposure test, after animals were exposed to the test substance, their skin was scored at 24, 48, and 72 h (Table 3). Some of animals showed signs of skin irritation (erythema and edema) higher than control (normal saline). Additionally, the redness and swelling were visible to the naked eye. All sites were graded, and the grading scores were used to calculate the primary irritation index (PII) by dividing the total irritation score by the number of observations. The PII was found to be 0.6, indicating that the formulation caused a slight response (Table 3, Fig. 2).

Table 1
Measurement scale for skin sensitization assessment.

Dermal response score	Irritation score	Reaction	Irritation score
Skin appearance		Observation	
• No evidence of irritation	0	• No other effects or only a slightly glazed appearance	A (0)
• Minimal erythema that is barely perceptible	1	• Markedly glazed appearance	B (1)
• Definite erythema that is readily visible and minimal edema or minimal popular response	2	• Glazing with peeling and cracking	C (2)
		• Glazing with fissures	F (3)
• Erythema and papules	3		
• Definite edema	4	• Film of dried serous exudates covering all or part of the TDS site	G (3)
• Erythema, edema, and papules	5		
• Vesicular eruption	6		
• Strong reaction spreading beyond the application site	7	• Small petechial erosions and/or scabs	H (3)

Table 2
Individual body weight and clinical observation of single-exposure test.

Animal No.	Body weight (g)					Clinical observation
	Prior of exposure		After exposure			
	Quarantine day	Exposure day	24 h	48 h	72 h	
1	2757	2860	2894	2920	2937	Clinically normal
2	2987	3390	3375	3433	3454	Clinically normal
3	2768	2990	3012	3051	3086	Clinically normal

3.2. The results of human study

3.2.1. Baseline characteristics of participants

The baseline demographic characteristics of participants were presented in Table 4. All 30 participants had the body mass index (BMI) within the normal range, possess no underlying disease, and no smoking and alcohol consumption. Additionally, they have no history of drug allergies, food allergies, or sensitivities to other personal care products.

3.2.2. The evaluation results of TEWL

After adhering for 4 h with three tests on separate applications on three different areas for each participant, the experimental findings indicated that immediately following the removal of the patch test (0 h), there were statistically significant differences in the percentage changes of the TEWL values among the three groups. The positive control group exhibited the highest percentage change, followed sequentially by the product test group and the negative control group. At 4 and 8 h post-removal of the test patch (4 h, 8 h), the percentage changes of TEWL values in the positive control group were statistically significantly higher than those in the negative control group and the product test group. However, there was no statistically significant difference between the percentage changes of TEWL values for the negative control and the product test groups. Furthermore, upon examining the intra-group differences, it was observed that the percentage changes of TEWL values significantly decreased from the time of patch test removal (0 h) up to 8 h post-removal (Fig. 3).

3.2.3. The assessment results of the irritation and sensitization tests

The evaluation results of skin sensitization for female sexual hygiene product containing CBD from cannabis extract can be divided into two testing phases: the induction phase (the test substance was applied for 4 h) and the challenge phase (the test substance was applied for 24 h). During the induction phase, there was no observed skin irritation or redness at the test site across all evaluated time intervals. In the challenge phase, no immediate irritation was observed right after the removal of the patch in 76.67 % of the participants. This percentage increased to 90 % after 8 h. Meanwhile, minimal erythema that was barely perceptible was observed in 23.33 % of the participants immediately after the removal of the patch test and decreased to 10 % at 8 h post-removal. Any irritation symptoms normalized within 24 h post-removal. Other responses in both the induction and challenge phases showed no other effects in 100 % of the volunteers across all evaluated time intervals (Table 5, Fig. 4).

4. Discussion

Proper female hygiene is critical for women's intimate health, as the external genital area serves as the primary defense in protecting the genital tract from infection [3]. Therefore, developing female sexual hygiene products is crucial [12]. These products must undergo rigorous assessment for the risk of irritation and sensitization before being brought to market [13]. This research aims to investigate the safety of

Table 3
Skin irritation response observation of the single-exposure test.

Rabbit No.	Reaction	Test site			Total	Control site			Total	Individual Primary Irritation score (PIS)	Primary Irritation Index (PII)
		24 h	48 h	72 h		24 h	48 h	72 h			
1	Erythema	0/0	0/0	0/0	0	0/0	0/0	0/0	0	0	
	Edema	0/0	0/0	0/0		0/0	0/0	0/0			
2	Erythema	1/1	0/1	0/1	4	0/0	0/0	0/0	0	0.7	0.6
	Edema	0/0	0/0	0/0		0/0	0/0	0/0			
3	Erythema	1/1	0/1	0/1	6	0/0	0/0	0/0	0	1	
	Edema	1/1	0/0	0/0		0/0	0/0	0/0			

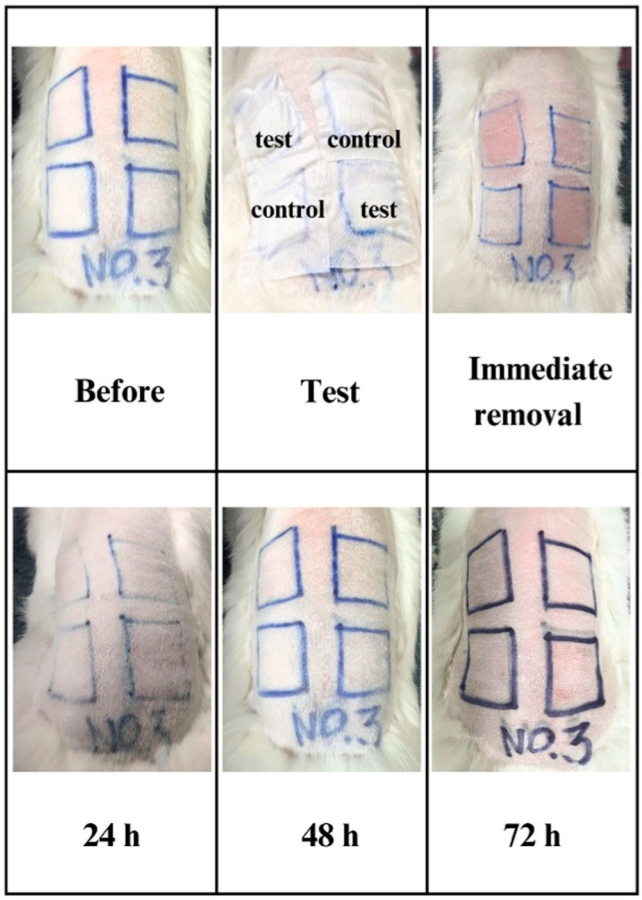

Fig. 2. Skin irritation test on rabbits.

female sexual hygiene product containing CBD from cannabis extract in both animal and human trials. In the animal study, three female rabbits were used to assess skin irritation. The resulting PII of the test substance was 0.6, following the testing standards outlined in Biological Evaluation of Medical Devices – Part 23: Test for Irritation 2021 (Standardization 10993–23) [28]. A PII value between 0.5 and 1.9 is categorized as slight irritation [16]. Clinical symptoms and weight loss in the test animals serve as indicators of the product's toxicity. If there is toxicity or disruption in nutrient absorption, it might be reflected in weight reduction [38]. However, this study found no abnormal clinical symptoms or any weight changes in the test animals. Following animal testing, research on human volunteers further confirms the risk of hypersensitivity reactions associated with the use of this product. This study adhered to the FDA guidelines: 'Assessing the irritation and sensitization potential of transdermal and topical delivery systems for ANDAs; October 2018,' which involves two phases of testing: the induction phase (exposure for 4 h) and the challenge phase (exposure for 24 h). The upper arm was selected as the testing site because it is

Table 4
Characteristics of participants.

Baseline characteristics	mean ± sd	n (%)
Age (years)	37.9 ± 7.78	
Weight (kg)	59.58 ± 9.20	
Height (cm)	158.47 ± 5.15	
BMI (kg/m^2)	22.67 ± 3.12	
Underlying diseases		
No		30 (100.00)
Yes		0 (0.00)
Smoking and alcohol consumption		
No		30 (100.00)
Yes		0 (0.00)
Drug allergies		
No		30 (100.00)
Yes		0 (0.00)
Food allergies		
No		30 (100.00)
Yes		0 (0.00)
Cleansing product allergies		
No		30 (100.00)
Yes		0 (0.00)

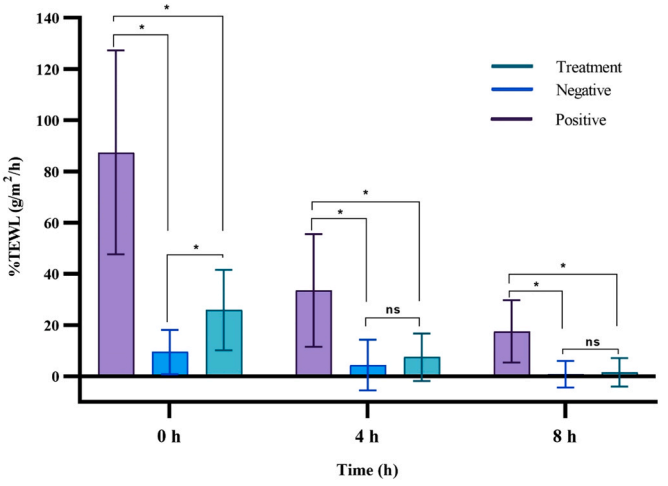

Fig. 3. The percentage change of the TEWL values among the three groups. One-way ANOVA followed by Tukey's post-test, ns = not significant, and *$p < 0.001$.

commonly used in patch tests for skin irritation and sensitization. The upper arm differs from the external genital area in several key aspects, such as skin sensitivity, pH levels, and microbiota [39,40]. However, we prolonged the adhesion of the product to the upper arm skin for 4 and 24 h. This duration was chosen to ensure that if there was any potential for skin irritation, it would likely manifest within this timeframe, providing a preliminary indication of the product's safety. The results showed that only 23 % exhibited minimal erythema, 10 % showed erythema, and minimal edema or minimal popular response, which resolved within 8–24 h during the challenge phase. Notably, in

Table 5
Assessment results of the irritation and sensitization tests.

Parameters	Induction phase				Challenge phase			
	0 h	8 h	24 h	72 h	0 h	8 h	24 h	72 h
Dermal response								
• No evidence of irritation; n(%)	0	0	0	0	23 (76.67)	27 (90.00)	30 (100.00)	30 (100.00)
• Minimal erythema that is barely perceptible; n(%)	0	0	0	0	7 (23.33)	3 (10.00)	0	0
• Definite erythema that is readily visible and minimal edema or minimal popular response; n(%)	0	0	0	0	0	0	0	0
• Erythema and papules; n(%)	0	0	0	0	0	0	0	0
• Definite edema; n(%)	0	0	0	0	0	0	0	0
• Erythema, edema, and papules; n(%)	0	0	0	0	0	0	0	0
• Vesicular eruption; n(%)	0	0	0	0	0	0	0	0
• Strong reaction spreading beyond the application site; n(%)	0	0	0	0	0	0	0	0
Other effects								
• Slightly glazed appearance; n(%)	0	0	0	0	0	0	0	0
• Markedly glazed appearance; n(%)	0	0	0	0	0	0	0	0
• Glazing with peeling and cracking; n(%)	0	0	0	0	0	0	0	0
• Glazing with fissures; n(%)	0	0	0	0	0	0	0	0
• Film of dried serous exudates covering all or part of the application site; n(%)	0	0	0	0	0	0	0	0
• Small petechial erosions and/or scabs; n(%)	0	0	0	0	0	0	0	0

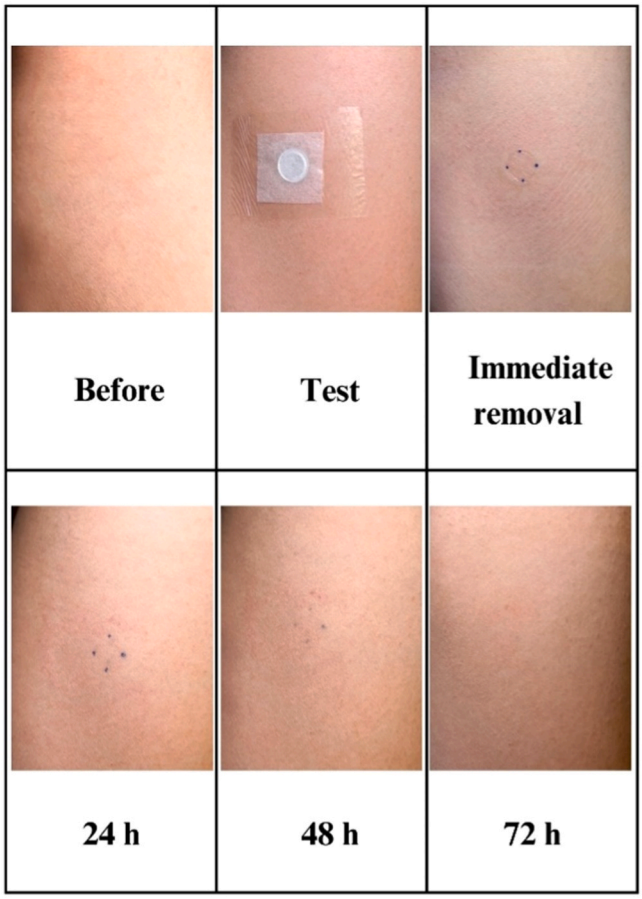

Fig. 4. Skin irritation and sensitization tests on humans.

real-world scenarios, these products are typically used for 1–2 minutes and rinsed off immediately, in contrast to the 24-h exposure in the testing. As a result, slight irritation occurred, but no allergic reactions were observed [16]. Regarding TEWL, an initial increase in water loss was noted after exposure to the test substance. However, as time progressed, water loss decreased continuously. This is because the product is a cleanser containing surfactants, which aid in cleansing and contribute to stratum corneum hydration [41]. Furthermore, skin toxicity reports related to cannabinoid usage primarily stem from inhalation or ingestion rather than the local effects of transdermal applications [42–44]. Additionally, the aqueous layers beneath the stratum corneum act as a barrier to hydrophobic cannabinoid diffusion, making significant systemic absorption unlikely [45]. Previous studies have reported the potential benefits of CBD in various external use products, such as soaps, shampoos, and others, for addressing specific skin issues, including dryness, inflammation, and allergic contact dermatitis [46,47]. CBD ointment was found to be safe and clinically effective in improving the quality of life for patients with certain skin conditions [48]. Other research also demonstrated the anti-inflammatory properties of CBD in animal models of contact dermatitis. [49]. Moreover, Studies indicated that transdermal administration of CBD results in minimal systemic absorption, thereby reducing the risk of systemic side effects. For instance, a study on dogs showed that transdermal delivery of a low-THC cannabis extract led to measurable but relatively low serum concentrations of CBD, suggesting limited systemic exposure through the dermal route [50]. Similarly, a study on healthy adults found that transdermal application of CBD maintained consistent plasma levels without significant psychoactive effects, demonstrating the safety and low systemic absorption of this administration method [51]. The pharmacokinetic data further support that while CBD does enter systemic circulation, the levels are not sufficient to cause significant systemic effects [51]. Studies also highlight that topical and transdermal CBD applications are considered safe due to minimal systemic absorption, with no observed psychiatric side effects, unlike oral administration, which may cause nausea, vomiting, and headaches [52,53]. Therefore, female sexual hygiene product containing CBD from cannabis extract is safe to use as a cleansing product for the skin.

4.1. Limitations

The study conducted a safety assessment of a female sexual hygiene product containing CBD, tested on New Zealand white rabbits and human participants. A limitation of this research lies in the disparity between the experimental conditions and actual product use in daily life. Specifically, the area of application and duration of exposure in the study did not match typical usage patterns. For example, the product was applied for extended periods in the study, whereas it is intended for immediate rinse-off in everyday use. This discrepancy may have influenced the findings, particularly concerning the irritation levels observed. Further research should evaluate the safety of the product under conditions that closely mimic its real-world application. This includes testing the product on appropriate body areas for cleansing and

adhering to realistic usage durations. Such studies will provide a more accurate assessment of the product's safety profile during typical use.

5. Conclusions

The research indicated that when the CBD from cannabis extract was developed into a female sexual hygiene product, it exhibited minor skin irritation when applied for prolonged durations. Specifically, in animal study, the product caused slight irritation when applied for over 4 h, and in human trial, irritation was observed when applied for more than 24 h. Nevertheless, when used as directed with an immediate rinse-off, no irritation was evident.

Ethical approval

The animal study was conducted in accordance with the guidelines provided by the International Organization for Standardization's ISO 10993–23: Biological evaluation of medical devices 2021. The protocol received approval from the National Laboratory Animal Center Anmima Care and Use Committee (NLAC-AUC) at Mahidol University, Nakhon Pathom, Thailand, with approval number RA2022–17. For the human study, approval was granted by the Ethics Committee of the Faculty of Medicine, Thammasat University, with the project number MTU-EC-OB-1–182/65, 229/2565. Additionally, the trial has been registered with the Thai Clinical Trials Registry under the identifier TCTR20230330011.

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CRediT authorship contribution statement

Pratya Phetkate: Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Atiwut Kamudhamas:** Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **Sombat Muengtaweepongsa:** Supervision, Methodology, Conceptualization. **Sitthiphon Bunman:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Winit Longlalerng:** Formal analysis, Data curation, Conceptualization. **Nakarin Sivapornpan:** Methodology, Investigation, Data curation. **Siwapol Thitayarasa:** Resources, Methodology, Conceptualization. **Chuntida Kamalashiran:** Investigation, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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