Short Communication

Biocompatibility and anti-inflammatory effects of Thai cannabidiol-containing topical anesthetic gel prototype in a dermal irritation test

Pasutha Thunyakitpisal[1,2#] Supattarayan Thongjit[1#]
Apitchaya Pruksametanan[1] Wijit Banlunara[3,4*]

Abstract

The objective of this study is to investigate and compare the effect of cannabidiol (CBD)-containing and non-CBD topical anesthetic gel prototypes on skin irritation in rabbits at 24, 48, and 72 h post-application. Topical anesthetic gel prototypes containing 0.1% CBD and without CBD were developed. Six rabbits (*Oryctolagus cuniculus*) were used for skin irritation testing according to ISO 10993-23: 2021. The animals were randomly and equally divided into two groups (CBD and non-CBD gel groups). The gels were applied to the animals' skin at the dorsal area of the flank with good contact for 4 h. Distilled water was used as the control. After exposure, skin reactions (erythema and oedema scores), the primary irritation score, and the primary irritation index were evaluated immediately and at 24, 48, and 72 h post-application. Data were analyzed using the SPSS program with a paired t-test ($P < 0.05$). Both the CBD and non-CBD groups exhibited mild skin irritation. However, the CBD group showed a lower primary irritation index, particularly at the 72-h time point. In conclusion, the CBD-containing topical anesthetic prototype has mild skin irritation. CBD may help alleviate the irritating effects of topical local anesthetics.

Keywords: cannabidiol, dermal irritation test, rabbits, topical anesthetic gel

[1]*Institute of Dentistry, Suranaree University of Technology, Nakhon Ratchasima 30000, Thailand*
[2]*Oral Health Center, Suranaree University of Technology Hospital, Nakhon Ratchasima 30000, Thailand*
[3]*Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand*
[4]*Center of Excellence in Materials and Bio-Interfaces, Chulalongkorn University, Bangkok 10330, Thailand*
[#]*Equal distribution for this work*
* ***Correspondence:*** *wijit.k@chula.ac.th* (W. Banlunara)
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Introduction

Oral ulcer and oral mucositis are common oral inflammatory lesions in chemoradiation therapy for head and neck cancer patients, causing discomfort and severe pain (Razmara and Khayamzadeh, 2019). These unfavorable effects would interfere with daily activities, e.g., swallowing, drinking, chewing, and toothbrushing, resulting in poor quality of life. Conventional treatment interventions: topical analgesic, antimicrobial, and anti-inflammatory agents have been introduced (da Cruz Campos *et al*., 2014; Ahmad *et al*., 2019; Ono *et al*., 2024). Currently, a topical corticosteroid supplemented with local anesthetic is generally used as the treatment choice due to its anti-inflammatory and immunosuppressive effects. However, the possible adverse effects from steroid therapy and the potential for secondary infection from long-term use increase the risk of intraoral fungal infection and adrenal suppression (George and Balan, 2018). Herbal medicines have been interesting as alternative medicines with a suitable usage form (da Cruz Campos *et al*., 2014).

Cannabidiol (CBD) is extracted primarily from the Cannabis sativa (hemp) plant. CBD has become a focus of medical research due to its anti-inflammatory, immunosuppression, antioxidant, and analgesic properties without psychological effects (Burstein, 2015; Atalay, 2019; Milando and Friedman, 2019; Robaina Cabrera *et al*., 2021). The 300 mg/day of CBD has been considered a low oral dose for safety and efficiency in adults (Arnold *et al*., 2023). Therefore, CBD could be a promising substance to protect against the development of oral mucositis (; Cuba *et al*., 2017; Cuba *et al*., 2020; Li *et al*., 2022; Liu *et al*., 2022). With optimal concentration, CBD may be an alternative chemical agent instead of steroid. Our group has developed the topical anesthetic gel with 0.1% (w/w) CBD. The rheology properties of prototypes were tested. The skin irritation test (ISO 10993-23; 2021) was investigated. The purpose of this study was to evaluate the inflammatory effect of CBD-containing topical anesthetic gel and the non-CBD topical anesthetic gel on the skin over 72 h observation. The data obtained from this study would provide initial information on the possible application, dosages, benefits, and safety of the CBD-containing topical anesthetic gel for future applications.

Materials and Methods

Chemicals: Lidocaine hydrochloride and hydroxypropyl cellulose were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Tetracaine hydrochloride was obtained from Tokyo Chemical Industry Co., Ltd, Tokyo, Japan. The 10% (w/v) CBD oil was obtained from Chao Phya Abhaibhubejhr Hospital, Prachin Buri, Thailand. All reagents used in this experiment were analytical and food grade.

The 0.1% CBD contained 10% lidocaine and 2% tetracaine hydrochloride topical anesthetic gel preparation: For the topical anesthetic gel (non-CBD gel), the lidocaine hydrochloride and tetracaine hydrochloride were dissolved in distilled water. Then the solution was mixed with hydroxypropyl cellulose to generate a topical anesthetic gel prototype. The 0.1% CBD-containing topical anesthetic gel was generated by adding 10% (w/v) CBD into the lidocaine and tetracaine solution. The solution was then mixed with hydroxypropyl cellulose to create the 0.1% CBD-containing topical anesthetic gel prototype.

Animal: A pilot experiment was performed with the minimum number of animals required. Six 6-month-old New Zealand white rabbits (*Oryctolagus cuniculus*) were obtained from the National Laboratory Animal Center, Mahidol University, Nakon Pathom, Thailand. In the acclimatization period, the rabbits were given access to food and water *ad libitum* and maintained at 20±3°C with a 12 h light/12 h dark cycle for 12 days and during the course of the study.

Skin irritation test: The experiment protocol was approved by the Research Ethics Committee in animal, Naresuan University, Phitsanuklong, Thailand (NU-S6 70501-01). The procedures were performed according to ISO 10993-2 (2021) with minor modifications. Briefly, 24 h before the experiment, fur was removed by closely clipping at the dorsal area of the flank of the animal (20x15 cm). The rabbits were equally randomly divided into 2 groups: CBD-containing and non-CBD topical anesthetic gel (n=3/group). The experimental skin area was divided into 4 zones: upper left, upper right, lower left, and lower right. For randomization, a block assignment was designed in which letters "A" and "B" were the upper left and lower right, and the upper right and lower left, respectively. The operator randomly picked up the letter and experimented. A 0.5 g of topical anesthetic gel was applied to the design zone, while distilled water was put on the other zones as a negative control. The examination area was covered with a sterile gauze patch and wrapped with a semi-occlusive, elastic bandage. After 4 h of exposure, the materials were removed, and the exposed skin was gently washed with sterile normal saline. The skin reaction (erythema and oedema) was evaluated at 24, 48, and 72 h post-operation. All procedures were performed by the same investigator with a blind method.

Skin reactions were graded by erythema and edema (Table 1) from a 0–4 grading scale of each according to ISO10993-23, 2021. For erythema formation: 0–no erythema; 1–very slight erythema, barely perceptible; 2–well-defined erythema; 3–moderate to severe erythema; and 4–severe erythema; beef redness to slight eschar formation. For edema: 0–no edema; 1–very slight edema, barely perceptible; 2–slight edema (edges of area well defined by raising); 3–moderate edema (raised about 1 mm); and 4 – severe edema (raised more than 1 mm and extending beyond the area of exposure). The primary irritation (PI) score was measured from the difference between the sum of the average scores for erythema and edema at 24, 48, and 72 h of each topical anesthetic prototype or saline-treated sites of each rabbit. The primary irritation index (PII) was measured as the arithmetic mean of the PI score of the three animals and then evaluated following

the cumulative irritation index categories in rabbit (Table 1).

Statistical analysis: The data were collected and presented as the means. A comparison between the mean values of PI of the CBD and free CBD containing topical anesthetic groups was made using an independent t-test. Statistical significance was considered at $P < 0.05$.

Table 1 Criteria of Erythema and Oedema Formation Score (A) and Category of cumulative irritation index in rabbit (B) followed ISO 10993-23.

(A) Criteria of Erythema and Oedema Formation Score

Erythema and Eschar Formation	**Score**
No Erythema	0
Very Slight Erythema; Barely Perceptible	1
Well-Defined Erythema	2
Moderate to Severe Erythema	3
Severe Erythema; Beef Redness to Eschar Formation Preventing Grading of Erythema	4
Oedema Formation	**Score**
No Oedema	0
Very Slight Oedema; Barely Perceptible	1
Slight 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 the Area of Exposure	4
Maximum Possible	8

(B) Category of cumulative irritation index in rabbit (ISO 10993-23)

Mean score of PII	**Response category**
0–0.4	No irritation
0.5–1.9	Slight irritation
2.9–4.9	Moderate irritation
5–8	Severe irritation

Results and Discussion

Visual appearance and pH measurements of non-CBD and CBD-containing topical anesthetic gels: The non-CBD topical anesthetic gel exhibited a clear, viscous consistency. The CBD-containing gel appeared homogenous, viscous, and yellowish (Fig. 1). The pH of the non-CBD gel and CBD-containing topical anesthetic gel were 5.0±0.5 and 5.7±0.4, respectively.

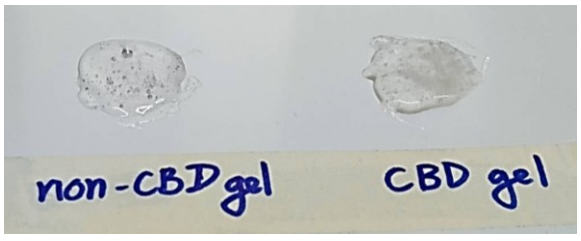

Figure 1 The general appearance of non-CBD and CBD-containing topical anesthetic gels. The non-CBD topical anesthetic gel demonstrated a homogeneous, clear, and viscous consistency, while the CBD-containing gel showed a homogeneous, viscous, and yellowish appearance.

The effect of a CBD-containing topical anesthetic gel prototype on skin animals: All animals recovered well and showed no signs of illness from the test materials. The rabbits demonstrated a normal amount of food and drink intake and an increase in body weight within the following 72 h observation (data not shown).

In the CBD group, the animals demonstrated a very slight erythema (score 1) at 24 h post-operation. No edema was detected at all observations (score 0). The 2 of 3 animals continued to have very slight erythema (score 1) at 48 and 72 h post-operation (Fig. 2). The Individual primary irritation score (PI) of CBD-treated group was presented in Table 2. The primary irritation index (PII) of CBD-treated group was 0.72 (Table 2).

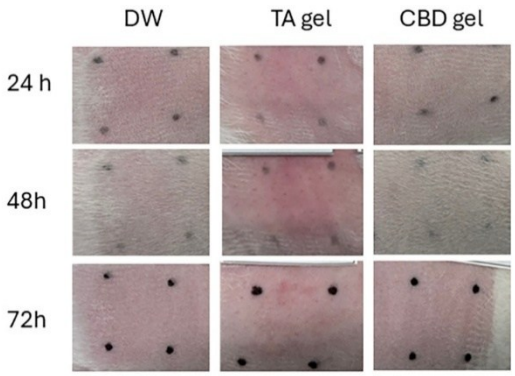

Figure 2 The representative skin reaction score of animal no. 2 shows score 1 of erythema at 24, 48, and 72 h with score 1 of oedema formation at 48 h of TA group. The score 1 of CBD group shows at 24 hr. (DW= distilled water as control, TA = Non-CBD topical anesthetic gel; CBD = CBD-containing topical anesthetic gel prototype).

The effect of a non-CBD topical anesthetic gel prototype on skin animals: All rabbits revealed mild erythema (score 1) at 24 h post-operation. Animals no. 2 and 3 continue to have this very slight redness at 48 and 72 h observation. Rabbit no. 2 demonstrated very slight erythema at 24 and 48 h post-operation, and a well-defined erythema (score 2) at 72 h post-operation (Fig. 2). The Individual PI of the topical anesthetic gel group was presented in Table 2. PII of the non-CBD topical anesthetic gel prototype group was 1.11 (Table 2).

Table 2 Show the primary irritation score (PI) and primary irritation index (PII) of CBD treated group (A) and non-CBD treated group (B).

(A)

Animal no.	Experimental groups and location		Skin reaction score						PI[1] of each experimental group	PI[1] of each animal	PII[2]
			24 h		48 h		72 h				
			erythema	edema	erythema	edema	erythema	edema			
1	CBD gel	UR	1	0	1	0	1	0	1	1	0.72
		LL	1	0	1	0	1	0			
	distilled	UL	0	0	0	0	0	0	0		
	water	LR	0	0	0	0	0	0			
2	CBD gel	UL	0	0	0	0	0	0	0.17	0.17	
		LR	1	0	0	0	0	0			
	distilled	UR	0	0	0	0	0	0	0		
	water	LL	0	0	0	0	0	0			
3	CBD gel	UR	1	0	1	0	1	0	1	1	
		LL	1	0	1	0	1	0			
	distilled	UL	0	0	0	0	0	0	0		
	water	LR	0	0	0	0	0	0			

(B)

Animal no.	Experimental groups and location		Skin reaction score						PI[1] of each experimental group	PI[1] of each animal	PII[2]
			24 h		48 h		72 h				
			erythema	edema	erythema	edema	erythema	edema			
1	TA gel	UR	1	0	1	0	1	0	1	1	1.11
		LL	1	0	1	0	1	0			
	distilled	UL	0	0	0	0	0	0	0		
	water	LR	0	0	0	0	0	0			
2	TA gel	UL	1	0	1	1	1	0	1.17	1.17	
		LR	1	0	1	0	1	0			
	distilled	UR	0	0	0	0	0	0	0		
	water	LL	0	0	0	0	0	0			
3	TA gel	UR	1	0	1	0	0	0	1.17	1.17	
		LL	1	0	1	0	2	1			
	distilled	UL	0	0	0	0	0	0	0		
	water	LR	0	0	0	0	0	0			

PI = Primary irritation score; PII = Primary irritation index
UR = upper right; UL = upper left; LL = lower left; LR = lower right
TA = Non-CBD topical anesthetic gel; CBD = CBD-containing topical anesthetic gel prototype

The cumulative irritation index revealed the mild irritation effect of both CBD-containing and non-CBD topical anesthetic gels. There was no significant difference between PI score of CBD-containing topical anesthetic gel and non-CBD topical anesthetic gel ($P > 0.05$).

In this study, our group has developed a topical anesthetic gel prototype containing 10% lidocaine hydrochloride and 2% tetracaine hydrochloride (non-CBD topical anesthetic), and 0.1% (%w/w) CBD topical anesthetic (CBD-containing topical anesthetic). Our data demonstrated that mixture of lidocaine and tetracaine hydrochloride caused mild irritation on the dermal skin of the rabbit up to 72 h observation. The CBD alleviates the local inflammatory effect (redness and edema) of this topical anesthetic formula. This finding corresponds with the other reports about the anti-inflammatory effect of CBD *in vitro* and *in vivo* (Burstein, 2015; Pisanti *et al.*, 2017; Petrosino *et al.*, 2018; Milando and Friedman, 2019; Lowin *et al.*,2020; Jiang *et al.*, 2022; Mazzantini *et al.*, 2024).

It should be noted that the difference in pH between the test substances and the skin of the rabbit could be relevant to the observed mild irritation. As the normal pH of rabbit skin is approximately 6.7, the non-CBD gel may have been slightly acidic for this animal skin, which potentially contributes to the irritation than that of the CBD-containing gel (Draize, 1942; Proksch, 2018). However, considering that the normal human skin pH ranges from 4.1 to 5.8, the gels are likely to be more compatible and may pose less risk of irritation in humans (Segger *et al.*, 2008; Proksch, 2018).

Although the intracellular signal transduction pathway of CBD on anti-inflammation has been reported, the underlying mechanism of the anti-inflammatory effect of CBD is still unclear. CBD could bind to both cannabinoid receptors CB_1 and CB_2, selectively CB_2, or CB_1-CB_2 heteroreceptor complexes (Navarro *et al.*, 2018; Seltzer *et al.*, 2020; Ma *et al.*, 2021; O'Brien, 2022). Moreover, CBD could also bind to PPARγ, JAK/STAT, GPR3/6/12/18/55, TRPV1/2, adenosine A2A receptor, TRPA1, 5-hydroxytryptamine receptor, and mitochondrial proteins (Burstein, 2015; Atalay *et al.*, 2019; Jastrzab *et al.*, 2019; Peyravian *et al.*, 2022). CBD reduced the release of inflammatory mediators such as TNF-α, IL-1β, nitric oxide, and inducible nitric oxide synthase, which stimulate the underlying intracellular signaling pathways, including nuclear factor kappa-B (NF-κB), mitogen-activated protein kinase (MAPK), and Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathways (Sunda and Arowolo, 2020; Ma *et al.*, 2021; Kongkadee *et al.*, 2022; Kim *et al.*, 2024). However, the precise mechanisms of CBD on anti-inflammation still need more investigation.

Based on our preliminary data, the 0.1% CBD-containing topical anesthetic gel reduced the

inflammatory effect at 72 h. observations compared with non-CBD topical anesthetic gel.

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