Randomized, Double-Blinded, Split-Submental Study Comparing the Efficacy of the Hemp Seed Extract Cream to Minimize Risk of Postinflammatory Hyperpigmentation after Radiofrequency Microneedling Device.

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Abstract

Post-inflammatory hyperpigmentation (PIH) is a form of hypermelanosis that occurs as a result of several factors, such as inflammation dermatoses. The utilization of fractional radiofrequency microneedling (FRM) for skin resurfacing and subdermal collagen remodeling has noted transient side effects, including PIH. Hemp seed extract contains both tetrahydrocannabinol (THC) and cannabidiol (CBD), which have been found to have anti-inflammatory effects. The objective of the study was to evaluate the risk of post-inflammatory hyperpigmentation after fractional radiofrequency microneedling at the submental compared between the hemp seed extract cream and the placebo cream. Eighteen people, with an average age of 51.61 ± 13.74 SD and an age range of 25–66 years, Fitzpatrick skin type (FST) II–V, received radiofrequency microneedling at the submental area. The study was conducted just once. On the submental area, the 24-coated pin fractional tip with a 3 mm depth had been applied. Every participant has an RF energy level of 15 millijoules. For seven consecutive days following treatment, each participant received a random assignment to apply HSE cream on one side of the submental area and a placebo cream on the other. In each follow-up, two dermatologists who were blinded to the evaluation process graded the intensity of PIH in the photos taken before and after therapy at 0, 4, and 12 weeks. The change in skin melanin index was ascertained by the use of Antera 3D analysis. Four individuals had prolonged hyperpigmentation. Topical 1% HSE cream applied for a short period following FRM is still not a substitute post-treatment therapy for PIH prophylaxis. All eighteen individuals finished the course of treatment.

Keywords*: *Micro-needling Radiofrequency, Hemp Seed Extract, CBD, Cannabidiol, After-laser Care

1. Introduction

The utilization of fractional radiofrequency microneedling (FRM) has experienced an increase in popularity due to its efficacy in treating many dermatological concerns, such as skin resurfacing, subdermal collagen remodeling, face telangiectasia, wrinkles, acne scars, hyperpigmentation, and skin tightness. Temporary side effects, specifically post-inflammatory hyperpigmentation (PIH), were observed despite the widespread acceptance of FRM as a therapeutic modality.

PIH is a frequently reported issue that results from various skin illnesses and treatment options. This acquired pigmentation may have been influenced by several skin diseases that were previously evident. Skin problems such as acne and eczema, as well as external factors like microdermabrasion and other electromagnetic therapies (such as ultrasound, radiofrequency, lasers, light-emitting diodes, visible light, and FRM), are commonly associated with the development of PIH. The hemp seed extract (HSE) comprises two significant biochemical constituents, namely THC and CBD. CBD, an abbreviation for cannabidiol, is a non-psychoactive compound found in the Cannabis sativa (hemp) plant. CBD has shown significant therapeutic potential in various ailments, including immunological, neurological, carcinogenic, cardiovascular, dermatological, and inflammatory conditions, as supported by substantial research. CBD functions by modifying the immune system and decreasing immunological reactions to diminish inflammation and subsequent cytokine production (Farinin B et al., 2020).

Consequently, the primary objective of this research is to assess the effectiveness of a topical cream derived from hemp seeds in reducing PIH, specifically in the submental region, among Asians individuals. The submental region will be treated with fractional radiofrequency microneedling once. The outcomes of all

participants will undergo objective and subjective evaluations from pre-treatment to three months post-treatment. The authors anticipate that the participants will adhere to their regular daily routines.

Some studies compare CBD to dexamethasone for anti-inflammatory effects. Results indicate CBD and dexamethasone have similar anti-inflammatory effects, mostly via suppressing MAPK and NF-κB signaling pathways but through distinct intracellular mechanisms (Wang et al., 2022). A randomized, split-face trial applying short-term use of topical corticosteroids following ablative fractional resurfacing (fractional CO_2) found that the use of topical clobetasol propionate 0.05% ointment in the following days of ablative fractional resurfacing is linked to a reduction in the incidence of PIH (40% on the steroid side and 70% on the placebo side) (Cheyasak et al., 2015).

2. Objectives

1) To evaluate the efficacy of short-term application of HSE cream on the incidence of PIH after using FRM at the submental area compared with placebo cream in Thai.
2) To evaluate the side effects and adverse events of short-term use of HSE cream.

3. Materials and Methods

Eighteen participants with FST II-V were treated in the Dermatology Department of Benchakitti Park Hospital, Bangkok, Thailand, in July 2023. The study protocol was approved by the Human Research Ethics Committee of Thammasat University (MTU-EC-OO-0-175/65).

3.1 Inclusion criteria

1) Participants are 20 years old and older.
2) Fitzpatrick's skin type II-V
3) Participants want to be treated in FRM at the submental area.

3.2 Exclusion criteria

1) A patient using progesterone or estrogen, or who is becoming pregnant or breastfeeding
2) A Patient with smoking, skin infection, inflamed acne, or photosensitive disease
3) A Patient receiving concurrent care for the affected skin region
4) A Patient with a history of keloid scarring
5) A Patient who took isotretinoin
6) A Patient who, in the three months prior, had filler injections or ablative or nonablative laser skin resurfacing procedures
7) A Patient with precancerous or cancerous skin or thyroid disease
8) A Patient with mental illnesses or personality problems that may have an impact on their ability to complete self-assessment forms or adhere to follow-up instructions
9) A Patient with any neurological system disorders (such as myasthenia gravis)
10) A Patient with a pacemaker or internal defibrillator insertion

3.3 Sample size

The sample size was calculated using testing for one population proportion (superiority or non-inferiority).

$$n = \frac{(z_{1-\alpha}+z_{1-\beta})^2 p(1-p)}{(\epsilon-\delta)^2}$$
$$\epsilon = p - p_0$$

-Z = 1.96 (for a 95 percent confidence level)
p0 = 0.75 (reference value)
p = 0.40 (proportion)
δ = 0 (margin)
α = 0.05
® = 0.1 (the authors assumed that the acceptable margin of error for the proportion being estimated is not more than 10%). Reference: Cheyasak, N., Manuskiatti, W., Maneeprasopchoke, P., & Wanitphakdeedecha, R. (2015). Topical corticosteroids minimize the risk of postinflammatory hyperpigmentation after ablative fractional CO2 laser resurfacing in Asians. *Acta Dermato-venereologica*, *95*(2), 201–205. https://doi.org/10.2340/00015555-1899

N = 17
The drop-out subjects = 5%
As a result, the sample size of the present study is 18

3.4 Study design

A double-blind, split-submental prospective clinical trial.

3.5 Equipment

A radiofrequency microneedling device with four coated pin tips was utilized. (Fractora^{R}; Invasix, Irvine, CA, USA).

Parameters for treatment in this study:

RF energy levels:	15 millijoules/pin
Pin length:	3000μ
Configuration:	6x4 coated tips
Ablation depth (mm):	3

Hemp seed extracted cream (HSE)

Hemp seed extracted cream: The hemp seed cream in this study is composed of water, propylene glycol, disodium EDTA, propylene glycol, allantoin, IPP, mineral oil, tween 20, carbomer 940, acetyl alcohol, lipomulse luxe, 1% hemp seed oil, triethanolamine (TEA), and phenoxyethanol.

Placebo cream

Placebo cream: Placebo cream in this study is composed of water, carbomer 940, allantoin, IPP, disodium EDTA, lipomulse luxe, mineral oil, tween 20, acetyl alcohol, triethanolamine (TEA), and phenoxyethanol.

3.6 Study protocol

Those who were qualified and willing to take part in the study were informed of its goals, supplies, procedures, and possible side effects. Physicians conducted a physical examination and took a history before enrollment, in accordance with the inclusion criteria. For each participant, baseline and post-treatment Antera3DTM images and standardized 2D photographic documentation were acquired three times: first at baseline (prior to treatment), second one month after treatment, and third visit (three months post-treatment) with the same camera settings, lighting, and patient positioning. The digital photographs were taken by a SonyA7II (Sony, Japan) with a facial photo fixture, and the Antera3DTM was used to obtain the skin melanin index. The patient and the camera were guaranteed a collection distance and set angles by the fixture. The imaging station allowed for the examination of subjects in controlled lighting and offered fixed camera angles for the frontal areas over the entire profile. Additionally, similar setting positions on the camera guaranteed even illumination of the submental area. About 45 minutes before ~~to~~ the treatment, topical EMLA cream (2.5% lidocaine and 2.5% prilocaine) was used to anesthetize the submental area. 70% alcohol and a mild soap solution were used to clean the treated region. A 24-coated pin tip was used for microneedling fractional radiofrequency treatment of submental regions. The stated area was covered by a 20% overlapping pass that included the treatments. Every participant received the same manners. One microneedling RF procedure was carried out. Both participants and physicians noted adverse effects. For a faster increase in healing processes, the volunteers had to abstain from using steroids or anti-inflammatory medications. Following each procedure, the patients were randomized to apply 0.5 FTU of hemp seed extract cream to one side and placebo cream to the other twice daily for seven consecutive days. To prevent contamination, the subjects were asked to apply a placebo cream medially to the lateral and HSE cream using two different cotton-tipped applicators. Following the seven-day course of treatments, the subjects were permitted to use only non-branded, specific sunscreen and the same basic emollients throughout the duration of the study.

3.7 Result evaluations

3.7.1 Subjective evaluation

3.7.1.1 Without knowing when the photos were taken, two dermatologists not participating in the research reviewed participant photos taken before and after each visit's therapy on a computer screen. On a five-severity scale, the degree of change was evaluated:

No PIH defined as non

1-25% darkening	defined as minimal
26-50% darkening	defined as mild
51-75% darkening	defined as moderate
76-100% darkening	defined as severe

3.7.1.2 Following every follow-up appointment, the participants and the therapist evaluated the adverse effects and made a record of them. Erythema, edema, crusting, pain, duration of crust until complete removal, pigmentation changes, infection, and scarring were among the side effects.

3.7.2 Objective evaluation

Antera 3D was utilized to evaluate an alteration in the submental region's skin melanin index. Each participant's change in the same area was compared using the index, which was recorded as a number.

3.8 Statistical analysis

Data will be analyzed using Stata statistical software version 15.0 (StataCorp, College Station, Tx, USA). The baseline characteristic and outcome measurement are described using means (standard deviation) for continuous variables and numbers (%) for categorical variables. The pair t-test was carried out to compare the clinical change scores at baseline and 12-week follow-up visits for each treatment regimen. To assess the risk of PIH between two post-treatment regimens, the Chi-square test was used. P-values less than 0.05 are going to be considered statistically significant. The variations in the means of pigmentation evaluated with the Antera3D (baseline, 4 and 12 weeks after FRM treatment) were tested using repeated measures analyses of variance and multivariate analysis. A probability value of less than 5% was considered statistically significant in the two-sided statistical tests.

4. Results and Discussion

4.1 Clinical results

Eighteen participants, thirteen females and five males with FST II-V, were enrolled in the study, and completed all sessions and follow-up after treatment. All participants's ages were in the range of 25 to 66 years old; the average was 51 ± years.

4.1.2 Subjective results

At 1 month after treatment, the intensity of the side treated with placebo cream had no significant difference (p = 0.717) in the incidence of PIH (PIH incidence of 33.3%) following FRM compared to the side treated with HSE cream (PIH incidence of 27.8%). The intensity of PIH was mostly rated as minimal in 27.8% (5/18) and 33.3% (6/18) of patients with HSE cream treatment, and placebo cream treatment, respectively. Compared with D0, the incidence and intensity of PIH of HSE and placebo within each group showed statistically significant differences, p-values were 0.025 and 0.014, respectively.

At 3 months after treatment, the intensity of the side treated with placebo cream had no significant difference (p = 0.7) in the incidence of PIH (PIH incidence of 27.8%) following FRM compared to the side treated with HSE cream (PIH incidence of 22.2%). The intensity of PIH was mostly rated as minimal in 22.2% (4/18) and 27.8% (5/18) of patients with HSE cream treatment and placebo cream treatment, respectively. Compared with D0, the incidence and intensity of PIH of HSE and placebo within each group showed statistically significant differences; p-values were 0.046 and 0.025, respectively. The subjective assessment of the severity of PIH is shown in Table 1. The clinical changes before and after treatment at each follow-up visit are presented in Figure 1.

Table 1: The subjective assessment severity of PIH

		Severity		
		HSE	Placebo	p-value (b)
PIH		n	n	
D0	none	18	18	1
	minimal	0	0	
	mild	0	0	
D30	none	13	12	0.717
	minimal	5	6	
	mild	0	0	
p-value (a)		0.025*	0.014*	

D90	none	14	13	0.7
	minimal	4	5	
	mild	0	0	
p-value (a)		0.046*	0.025*	

* P-value <0.05 was considered statistically significant.
P-value(a) = comparison within the group
P-value(b) = comparison between HSE and Placebo

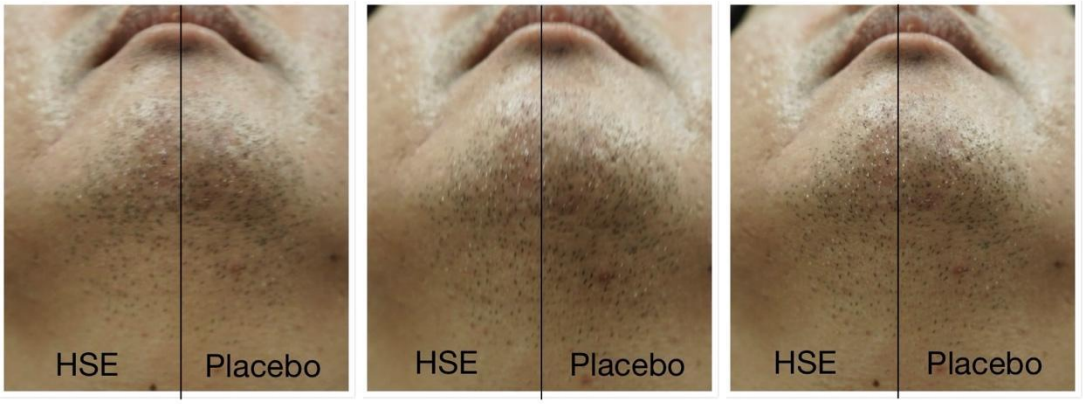

Figure 1: The photographs compare clinical before, 1month follow-up, and 3 months follow-up.

4.1.2 Adverse effects and complications

The recovery process was evaluated by using the questionnaire to evaluate the duration of the side effects, as shown in Table 2. The HSE cream-treated sides were associated with a shorter duration of postoperative pain (p = 0.02) compared with the placebo cream-treated side, which was statistically significant. There were no significant differences in the duration of edema, erythema, or crust between the two postoperative regimens.

Table 2: The duration of side effects

	HSE	**Placebo**	**Mean difference (95% CI)**	***p*-value**
Edema (day)	2.06 ± 2.01	1.67 ± 1.37	0.39 (-0.13, 0.9)	0.13
Erythema (day)	2.61 ± 1.94	2.33 ± 1.57	0.28 (-0.26, 0.81)	0.288
Crust (day)	6.11 ± 8.34	4.94 ± 5.34	1.17 (-0.45, 2.79)	0.147
Pain (hr)	3.61 ± 2.2	4.17 ± 2.48	-0.56 (-1.01, -0.1)	0.02*

* P-value <0.05 was considered statistically significant.

4.1.3 Objective results

The Antera 3D camera was used to evaluate the skin melanin index at in the submental area. The means of the melanin index in each follow-up visit did not show a significant decrease in the average of the HSE cream, treated side compared to placebo cream as shown in Table 3 and Figure 2.

Table 3: Objective melanin index assessment using Antera3D analysis.

Melanin index	**HSE**	**Placebo**	**Mean difference (95%CI)**	***p*-value**
D0	0.41 ± 0.06	0.39 ± 0.1	0.02 (-0.03, 0.07)	0.356
D30	0.4 ± 0.1	0.43 ± 0.07	-0.03 (-0.09, 0.04)	0.365
D90	0.41 ± 0.05	0.41 ± 0.05	0 (-0.01, 0.01)	0.878

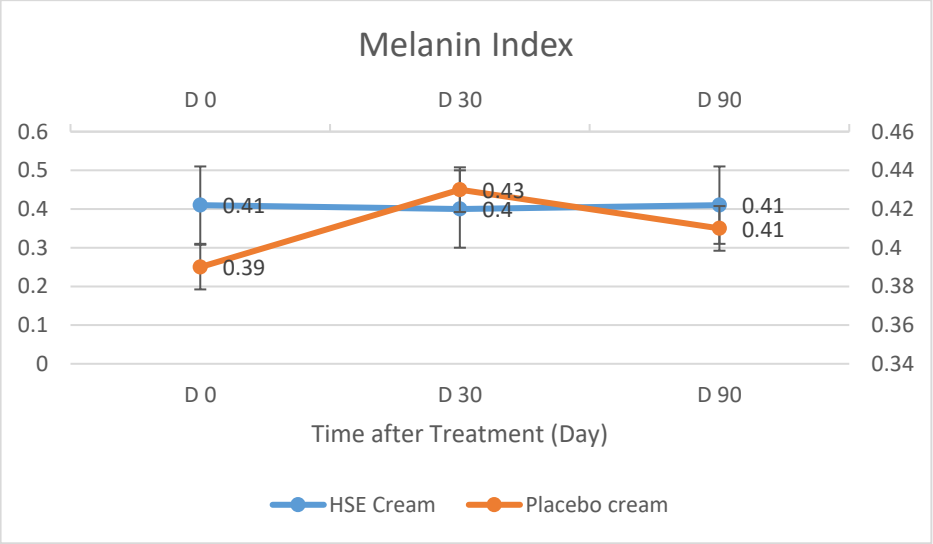

Figure 2 Melanin index by Antera3D comparing before, 1 month follow-up, and 3 months follow-up.

4.2 Discussion

The use of a FRM device has proven to be an effective method for addressing several dermatological problems, including the treatment of acne scars, striae, skin rejuvenation, wrinkles, and facial telangiectasia. While FRM has been universally accepted as a treatment, certain temporary side effects, like PIH. The prevention of PIH resulting from treatments with energy-based devices involves the implementation of photoprotection measures and the administration of corticosteroids for anti-inflammation after FRM. HSE is a bioactive compound known for its anti-inflammatory and immunomodulatory properties by inhibiting the immune response mediated by B-cells and attenuating the activity of T-cells, decreasing interleukin-17 (IL-17A), interferon-gamma (IFN-γ), nitric oxide (NO), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α).

This study shows that applying topical 1% HSE cream contained an anti-inflammatory effect similar to dexamethasone compared with placebo cream after treating the submental area with insulated 24-coated pin tips. FRM have no significant difference in the duration of crusting, edema, erythema, and incidence of PIH at 1 and 3 months after the treatment between HSE cream-treated sites and placebo-treated sites in the previous study, and the incident rate of PIH in the previous literature review after FRM is approximately 16%, but our study shows that the PIH rate of HSE cream is 22.2% and the placebo cream is 27.8% without a statistical difference.

The higher incident rate of PIH than in the previous study may be caused by the irritant contact dermatitis from anesthetic cream, the difference between the skin type of the patient, the difference of the machine itself, the treated area of the microneedling, and aftercare after FRM (photoprotection). Using antera3D analysis, it was measured to show that the melanin index of the HSE cream side was lower than the placebo cream side, but there was no statistical difference both 1 and 3 months after treatment. Only 1% of HSE cream may not be the proper concentration for the anti-inflammation effect to reduce PIH after FRM. However, the patient self-evaluation found that the duration of the pain on the HSE cream side was shorter than the placebo cream, which was statistically significant, suggesting that the HSE cream contained cannabinoids that activated G protein coupled CB1 and CB2 cannabinoid receptors to cause pain. CB1 receptors are highly expressed in the body's pain-processing structures in the peripheral and central nervous systems, as well as in non-neural cells. On the other hand, CB2 receptors are mainly found in cells belonging to the innate and adaptive immune systems. CB2 receptors inhibit neurotransmitters and neuropeptides from the presynaptic nerve ending, modify postsynaptic neuron enthusiasm, activate the descending inhibiting pain pathway, and lessen neuron inflammatory conditions.

The clinical change may not be significant because the mean pain durations of HSE and placebo were 3.61 and 4.17 hours, respectively, which is a small difference. The clinical use of HSE cream in the future for decreased pain duration after treatment may still need further research studies to be an alternative treatment for analgesic cream aftercare.

However, the study had some limitations the follow-up visit should have been set at 24-72 hours after treatment to exclude irritant contact dermatitis from anesthetic cream the sample size should have a greater number of participants and the proper concentration of HSE cream to perform the anti-inflammation effect. Further study is needed to be conducted on a larger sample size, use different concentrations of HSE cream, or set up more follow-up visits after the treatment to maximize the benefit of preventing PIH and minimize the risk of its adverse effects.

5. Conclusion

Short-term application of topical 1% HSE cream after FRM is still not an alternative post-treatment therapy for the prevention of PIH, yet it provides the patient with an analgesic effect.

6. Acknowledgements

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