

Formulation and Evaluation of Anti-Inflammatory Emulgel Derived from *Nigella sativa*

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ABSTRACT

Inflammatory conditions require effective management to alleviate symptoms such as pain, swelling, and redness. This study focuses on the formulation and evaluation of an anti-inflammatory emulgel derived from *Nigella sativa* (Kalonji), known for its therapeutic properties. The formulation aimed to deliver the active compound thymoquinone transdermally, leveraging the emulgel system's advantages, including ease of application, enhanced skin penetration, and prolonged contact time.

The extraction of thymoquinone was performed using the Soxhlet apparatus with ethanol, achieving a percent yield of 23.68%. Phytochemical screening confirmed the presence of alkaloids, flavonoids, and saponins in the extract. The emulgel was formulated using Kalonji extract, Tween 80, glycerine, Carbopol 940, triethanolamine, and distilled water. Four formulations were prepared and evaluated based on stability, homogeneity, and physical properties. The most stable formulation, F4, demonstrated suitable viscosity (13,250–22,000 cPs), Spreadability (51.47 cm²), and pH (mean 6.06).

Drug content analysis confirmed uniform distribution of the extract within the emulgel. Diffusion studies using Franz diffusion cells indicated a maximum drug release of 78.47% within 1 hour. The protein denaturation assay showed significant anti-inflammatory potential, and the CAM assay confirmed the formulation's non-irritant nature.

This study concludes that the Kalonji-based anti-inflammatory emulgel is a promising candidate for transdermal application, offering a natural and effective approach to managing inflammation.

Keywords: *Nigella sativa*, Kalonji, Emulgel, Anti-inflammatory, Thymoquinone, Transdermal delivery

I. INTRODUCTION

Inflammation is a complex biological response to harmful stimuli such as pathogens,

damaged cells, or irritants. It is a critical part of the body's defence mechanism, aiming to eliminate the initial cause of cell injury, clear out necrotic cells, and initiate tissue repair(1). While acute inflammation is essential for healing, chronic inflammation is linked to the pathogenesis of various diseases, including arthritis, cardiovascular diseases, diabetes, and cancer(2,3).

Non-steroidal anti-inflammatory drugs (NSAIDs) remain a cornerstone in the treatment of inflammatory conditions. However, prolonged use of NSAIDs is associated with significant adverse effects, such as gastrointestinal complications, renal toxicity, and increased cardiovascular risks(4,5). These limitations necessitate the search for safer, more effective alternatives.

Herbal medicines are increasingly recognised for their anti-inflammatory potential, largely due to their multi-targeted mechanisms, lower side effect profiles, and cost-effectiveness. Among these, *Nigella sativa* (commonly known as Kalonji or black seed) has gained considerable attention. Numerous studies have reported that *Nigella sativa* and its major bioactive component, thymoquinone, possess potent anti-inflammatory, antioxidant, antimicrobial, and anticancer properties(6–8). Thymoquinone has been shown to modulate pro-inflammatory mediators such as cytokines, prostaglandins, and leukotrienes, contributing to its therapeutic efficacy(9).

Emulgel, a hybrid of emulsions and gels, offer an effective transdermal delivery system by combining the controlled drug release of emulsions with the favourable patient compliance and Spreadability of gels(10). This formulation enhances the skin penetration of hydrophobic drugs, making it particularly suitable for delivering thymoquinone.

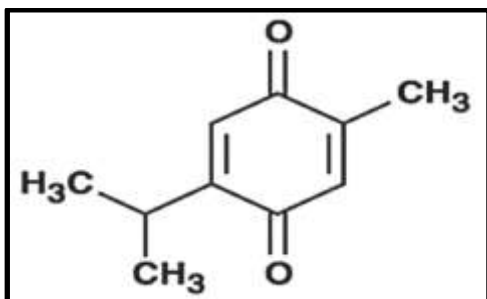
This study aims to formulate and evaluate an anti-inflammatory emulgel incorporating *Nigella sativa* extract to provide a natural, effective, and safe alternative for the management of inflammatory conditions.

Properties of thymoquinone

Thymoquinone, also known as 2-isopropyl-5-methyl-1,4-benzoquinone, is an essential component in volatile oil.

It is a substance having an aromatic ketone structure which can also be synthesised by thymol or carvacrol oxidation.

Due to its strong natural antioxidant properties, it has recently been preferred in the formulation of pharmaceutical, food, and cosmetic products rather than the synthetic antioxidants of butylated hydroxytoluene, butylated hydroxyanisole, and tertiary butyl hydroquinone.



The chemical structure of thymoquinone

Physical properties

Molecular Formula	C10H12O2
Molecular Weight	164.20 g/mol
State	Solid
Melting point	44 – 45 . C
Boiling Point	230 – 232 . C
Appearance	Golden brown to dark brown flaky crystals

Mechanism of anti-inflammatory action of kalonji oil extract

- In arachidonic pathway they inhibit enzyme cyclooxygenase and 5-lipoxygenase, thereby inhibiting the production of chemical mediators.
- They retain the anti-inflammatory effect by inhibiting various pro inflammatory transcription factors such as NF-KB/STAT3 by inducing several stimuli including cytokines.
- They have a suppressive effect on Nitric Oxide production. Nitric oxide has a pro-inflammatory activity and it is produced from

activated macrophages in the case of inflammation. (9)

Application of thymoquinone

- Antibacterial activity:-** TQ has demonstrated antimicrobial activity against various pathogens, including bacteria, viruses, and fungi. It can inhibit the growth and replication of harmful microorganisms, making it a potential natural alternative for treating infections and supporting immune health.
- Anticancer Properties:** Research indicates that TQ possesses anticancer properties by inducing apoptosis (cell death) in cancer cells, inhibiting tumour growth, and reducing angiogenesis (formation of new blood vessels that support tumour growth). These findings suggest a potential role for TQ in cancer prevention and treatment.
- Anti-inflammatory Effects:** TQ exhibits potent anti-inflammatory properties by inhibiting inflammatory mediators and signalling pathways. This makes it beneficial in conditions characterised by chronic inflammation, such as arthritis, asthma, and inflammatory bowel diseases. TQ's anti-inflammatory effects contribute to reducing tissue damage and promoting healing.
- Antioxidant activity:** TQ has demonstrated significant antioxidant properties, particularly in protecting against oxidative stress-induced cell damage, TQ administration resulted in improved survival rates, maintained mesenteric artery blood flow, and exhibited anti-inflammatory and antioxidative effects.



Kalonji

II. MATERIALS AND METHODS

Materials

- Kalonji extract (*Nigella sativa*)
- Tween 80 (emulsifying agent)
- Glycerine (solubilising agent)
- Carbopol 940 (thickening agent)
- Triethanolamine (neutralising agent)
- Distilled water (vehicle)
- Fertilised hens' eggs
- Phosphate buffer (pH 7.4)

Equipment and Software

- Soxhlet Extraction Apparatus (Borosil, India)
- Digital Weighing Balance (Eureka)
- UV-Visible Spectrophotometer (Peak Instruments C200S)
- pH Meter (Elico)
- Mechanical Stirrer (Remi Motors)
- Franz Diffusion Cell (Orchid Scientific)
- Brookfield Viscometer (DV2T)
- Microsoft Excel (data analysis)

Extraction of Thymoquinone

Nigella sativa seeds were finely ground and extracted using a Soxhlet apparatus with ethanol at 70% efficiency for six hours(11). The ethanolic extract was concentrated by rotary

evaporation, and the final yield was stored in amber bottles. The percent yield was calculated as:

$$\% \text{ Yield} = (\text{Weight of Extract Obtained} / \text{Weight of Raw Material}) \times 100$$

Phytochemical Screening

The ethanolic extract was tested for alkaloids (Mayer's reagent), flavonoids (Shinoda test), and saponins (foam test) using standard protocols(12).

Standard Curve Preparation

A stock solution of thymoquinone was prepared by dissolving 100 mg in 100 mL of ethanol to obtain 1000 µg/mL. From this, working standards ranging from 2–10 µg/mL were prepared by serial dilution. Absorbance was measured at 200–400 nm using a UV-visible spectrophotometer, and the wavelength of maximum absorbance (λ_{max}) for thymoquinone was determined to be 253 nm. This λ_{max} was then used to prepare a calibration curve for quantitative estimation. using a UV-visible spectrophotometer. A calibration curve was plotted, and the regression equation was used to determine the thymoquinone concentration in samples(13).The absorbance values obtained for different concentrations of the standard solution are presented in **Table 1**. The standard calibration curve was plotted using the data from Table 1, as illustrated in **Figure 1**.

Table 1: Concentration vs. Absorbance Data for Standard Curve Preparation

Concentration (µg/mL)	Absorbance ($\lambda = [\text{wavelength}] \text{ nm}$)
500	0.338
1000	0.613
1500	0.880
2000	1.148
2500	1.408

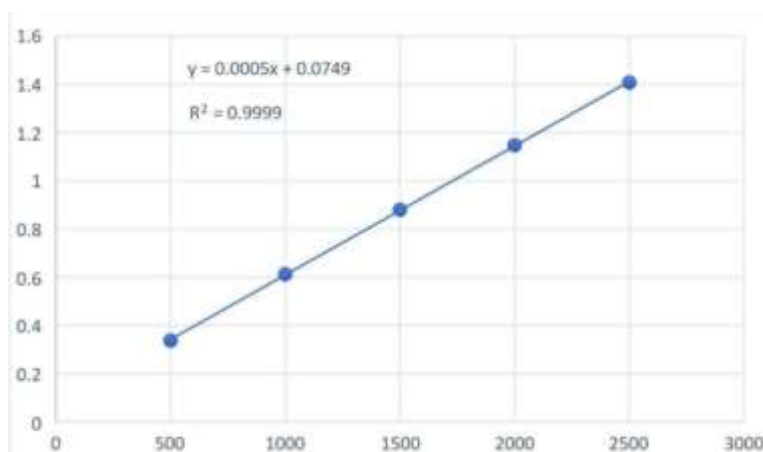


Figure 1. Standard Curve of Kalonji Seeds Extract: Absorbance vs. Concentration

Thymoquinone Estimation

The concentration of thymoquinone in the extract and formulations was determined using the standard curve generated above. Absorbance values were interpolated to calculate the actual concentration.

Dose Calculation

Dose calculation was performed using the interspecies conversion method based on Body

Surface Area (BSA). BSA is widely accepted as a reliable basis for dosage translation between animals and humans because it correlates closely with total body water, extracellular fluid volume, and metabolic activity. Animal doses can be converted into human-equivalent doses by using standard conversion factors derived by Paget and Barnes (1964) as given in **Figure 2**.

Absolute Dose	70 kg Human	12 kg Dog	4 kg Monkey	2 kg Cat	1.5 kg Rabbit	400 gm G. pig	200 gm Rat	20 gm Mouse
70 kg Human	1.0	0.32	0.16	0.076	0.07	0.031	0.018	0.0026
12 kg Dog	3.1	1.0	0.52	0.24	0.22	0.1	0.06	0.008
4 kg Monkey	6.1	1.9	1	0.45	0.42	0.19	0.11	0.016
2 kg Cat	13	4.1	2.2	1.0	0.92	0.41	0.23	0.03
1.5 kg Rabbit	14.2	4.5	2.4	1.08	1.0	0.44	0.25	0.04
400 gm G. pig	31.5	10.2	5.2	2.4	2.25	1.0	0.57	0.08
200 gm Rat	56.0	17.8	9.2	4.2	3.9	1.74	1.0	0.14
20 gm Mouse	387.9	124.2	64.1	29.7	27.8	12.25	7.0	1.0

Figure 2: Interspecies Dose Conversion Table Based on Body Surface Area (BSA) as Proposed by Paget and Barnes (1964)

The known effective anti-inflammatory dose of thymoquinone in mice is **3 mg/kg**. This animal dose was converted into an absolute dose for a standard mouse weighing 20 g, then translated into a human dose using the BSA conversion factor, and finally recalculated into a dose per kilogram of human body weight:

1. **Mouse dose** = 3 mg/kg
2. **Absolute dose for a 20 g mouse** = $(3 \text{ mg/kg} \times 20 \text{ g}) \div 1000 = 0.06 \text{ mg}$
3. **Human-equivalent absolute dose** (for a standard 70 kg adult) was calculated by multiplying the mouse absolute dose by the conversion factor from the Paget and Barnes table (conversion factor for mouse to human = 387.9): $0.06 \text{ mg} \times 387.9 = 23.27 \text{ mg}$
4. **Human-equivalent dose per kg** = $(23.27 \text{ mg} \div 70,000 \text{ mg}) \times 1000 = 0.33 \text{ mg/kg}$

Thus, the effective dose of thymoquinone for a 70 kg human was calculated as approximately **23.27 mg** or **0.33 mg/kg** per application.

FORMULATION DEVELOPMENT

Four emulgel formulations (F1–F4) were prepared by varying the concentrations of Kalonji extract, surfactant (Tween 80), and gelling agent (Carbopol 940). The general procedure involved the preparation of the emulsion phase and gel phase separately, followed by incorporation to form a stable emulgel.

Procedure:

1. **Gel base preparation:** Carbopol 940 was dispersed in distilled water and allowed to hydrate overnight. The pH was adjusted using triethanolamine to obtain a uniform gel base.
2. **Oil phase:** The ethanolic extract of Kalonji seeds
3. **Aqueous phase:** Distilled water was heated gently and used to dissolve any additional water-soluble components, and we added Glycerine and Tween 80 to it.
4. **Emulsion formation:** The oil and aqueous phases were mixed with continuous stirring using a mechanical stirrer until a stable emulsion formed.
5. **Incorporation into gel:** The emulsion was gradually added to the hydrated Carbopol gel

base with slow mechanical stirring to form the emulgel.

Table 2: Composition of Formulations:

Ingredients	F1	F2	F3	F4
Kalonji Extract (% v/v)	1	2	3	4
Carbopol 940 (% w/v)	1	1	1.5	2
Tween 80 (% v/v)	2	2	2.5	3
Glycerine (% v/v)	10	10	10	10
Triethanolamine (q.s)	To adjust pH	To adjust pH	To adjust pH	To adjust pH
Distilled Water (q.s)% v/v	100	100	100	100

Each formulation was evaluated, and F4 was selected based on appearance, Spreadability, and stability. The final emulsion was incorporated into the Carbopol gel base with neutralisation using triethanolamine.

EVALUATION

Each formulation was evaluated using standard protocols:

- **pH** using a digital pH meter
- **Viscosity** with Brookfield Viscometer (Spindle 3 at 100 rpm)
- **Spreadability** using glass slide method: $S = \frac{\pi x^2}{4}$ where S is Spreadability and x^2 is the square of the diameter.
- **Drug content** using UV absorbance at 254 nm against a standard curve.
- **Drug Content Uniformity:** 1 g of formulation was accurately weighed and dissolved in 100 mL of ethanol. The solution was sonicated for 30 minutes and filtered through Whatman filter paper. 1 mL of this solution was taken and diluted to 10 mL using ethanol. The absorbance of the solution was measured at 254 nm using a UV-visible spectrophotometer.

In Vitro Diffusion Study

The in vitro diffusion study was conducted using a Franz diffusion cell with parchment paper as the membrane(15). The receptor chamber was filled with 20 mL of phosphate buffer (pH 7.4), maintained at $37 \pm 0.5^\circ\text{C}$ and continuously stirred with a magnetic stirrer.

A quantity of **0.25 g of emulgel** was applied evenly onto the membrane in the donor compartment. At regular intervals of 10 minutes over a total period of 60 minutes, **1 mL samples were withdrawn** from the receptor compartment

and replaced with an equal volume of fresh phosphate buffer to maintain sink conditions. Each sample was filtered and analysed at **254 nm** using a UV-visible spectrophotometer. The cumulative drug release (%) was calculated using the standard curve of thymoquinone.

The diffusion study that we carried out was a Reverse Method.

Protein Denaturation Assay

In this, we evaluated the ability of Kalonji oil extract to inhibit the heat-induced denaturation of egg albumin, providing insights into their potential anti-inflammatory properties(16).

A 1% BSA solution was prepared in phosphate buffer (pH 6.4). 1 mL of formulation was added to 5 mL of BSA solution, incubated at 37°C for 20 min, and then heated at 70°C for 5 min. Absorbance was measured at 660 nm. % inhibition was calculated using:

$$\% \text{ Inhibition} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} \times 100$$

CAM Assay

Fertilised hen's eggs were incubated at 37.5°C for 7 days. A window was made to expose the CAM. 0.5 mL of emulgel was applied and observed for vascular changes such as haemorrhage, lysis, and coagulation after 5 min. The irritation score was calculated as per Luepke's method(17).

III. RESULT

All four formulations (F1–F4) were successfully developed and subjected to physicochemical and pharmacological evaluation. The outcomes are presented below:

Physicochemical Evaluation

The pH of all formulations ranged between 5.8 and 6.2, with F4 exhibiting a pH of

6.06 ± 0.2, indicating suitability for topical application. Viscosity measurements revealed values ranging from 13,250 cPs to 22,000 cPs, with F4 displaying the highest viscosity. Spreadability of the formulations was measured, and F4 recorded the greatest Spreadability value of 51.47 cm².

Drug Content and Uniformity

Drug content analysis across all batches confirmed uniform distribution of thymoquinone. F4 demonstrated the most consistent drug content. For the drug uniformity, the Mean drug% is **11.5**, and the standard deviation was found to be **0.88**.

Calculated Dose and Final Formulation Strength

Based on the dose calculation, the optimised emulgel formulation was designed to deliver a therapeutically effective dose of thymoquinone. For a standard topical application weighing **250 mg (0.25 g)**, the required amount of thymoquinone was approximately **23.27 mg**. To meet this target dose, the formulation was designed

to contain about **10% (w/w)** of Nigella sativa extract.

The calculation was as follows:

- **Required amount of extract per application (0.25 g) = 23.27 mg**
- Thus, the formulation contained approximately **9.44 g of extract per 100 g of emulgel**, rounded to **10% (w/w)** for practical preparation and labelling purposes.

In Vitro Diffusion Study

The diffusion profile of thymoquinone from the emulgel formulations was studied using a Franz diffusion cell. F4 showed the highest cumulative release of thymoquinone, with 78.47% release observed at 180 minutes, as shown in **Table 3**, indicating enhanced permeation characteristics. A graph of the cumulative percent released v/s Time was plotted.

$$\text{The equation used was } y = 0.0005x + 0.0749$$

$$r^2 = 0.9999$$

Where, y = Absorbance and x = Concentration

Table 3: Time-Dependent Absorbance and Percentage Drug Release in In Vitro Diffusion Study.

Time (hrs)	Absorbance	Amount of drug remaining to be released (mcg)	Amount of drug released (mcg)	%Drug release
1	0.828	15062	9938	39.75
2	0.530	9102	15898	63.60
3	0.344	5382	19618	78.47

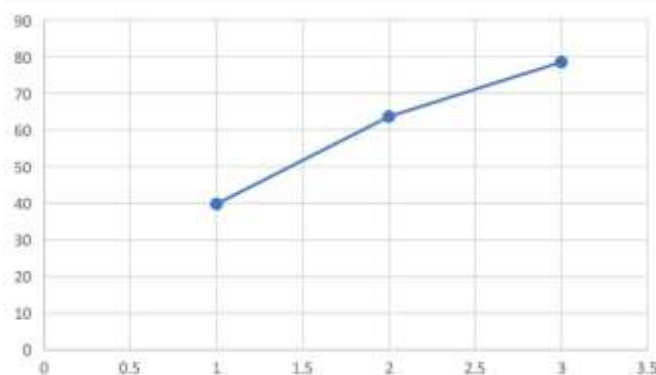


Figure 3: In Vitro Drug Release Profile Over Time

Figure 3. Graph showing the in vitro drug release profile over time. The x-axis represents time (in hours), and the y-axis shows the cumulative percentage of drug released (%).

Protein Denaturation Test

The anti-inflammatory activity of the formulation was evaluated using a protein denaturation assay. The results are presented in **Table 4** and illustrated in **Figure 4**. A concentration-dependent increase in % inhibition

was observed, indicating effective suppression of protein denaturation.

Table 4: Inhibition of Protein Denaturation by Formulation: % Inhibition at Various Concentrations

Concentration (mcg/ml)	% Inhibition
50	63.07
100	83.07
200	71.21
300	63.07
400	73.84

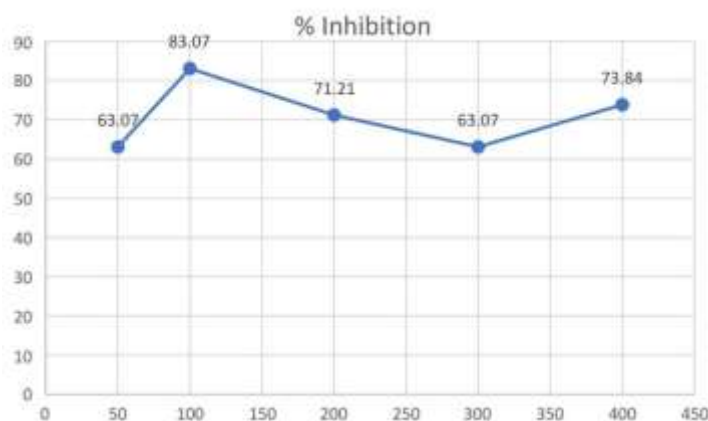


Figure 4: Inhibition of Protein Denaturation: Effect of Concentration on Anti-Inflammatory Activity

Figure 4. Graph showing the inhibitory effect of formulation on protein denaturation at varying concentrations. The x-axis represents concentration (mcg/mL), and the y-axis indicates % inhibition of protein denaturation, suggesting anti-inflammatory potential.

CAM Assay

The CAM assay was conducted to assess irritation potential. No signs of vascular damage, including haemorrhage, lysis, or coagulation, were observed; hence, it was proved that our formulation was non-irritant.

Our reading came to **0.5446**, which is within the range of non-irritant, as shown in **Figure 5**

MMTS*	Irritation classification
0.0–0.5	Non-irritative
0.6–2.5	Practically non-irritative
2.6–15.0	Minimally irritative
15.1–25.0	Mildly irritative
25.1–50.0	Moderately irritative
50.1–80.0	Severely irritative
80.1–100.0	Extremely irritative
100.1–110	Maximally irritative

*MMTS = Maximum Mean Total Score

Figure 5: Representative Images of CAM Assay Showing Irritation Limits

IV. DISCUSSION

The present study focused on the formulation and evaluation of a thymoquinone-based emulgel for transdermal anti-inflammatory therapy. The use of *Nigella sativa*, known for its broad-spectrum therapeutic potential, particularly its anti-inflammatory and antioxidant properties, served as the basis for developing a safe and effective topical formulation. Emulgel are particularly suited for hydrophobic drugs such as thymoquinone due to its superior permeation abilities and enhanced patient compliance.

The physicochemical evaluations demonstrated that all formulations fell within acceptable parameters, with F4 exhibiting optimal characteristics. A pH close to the skin's natural range (6.06) confirmed its compatibility and reduced the risk of dermal irritation. High viscosity and Spreadability values indicated ease of application and retention on the skin.

Drug content uniformity confirmed that thymoquinone was evenly distributed across the emulgel matrix, ensuring dose consistency in each application. This aligns with results from prior studies on herbal gel formulations using plant-based bioactives, where content uniformity was critical for therapeutic effectiveness.

The *in vitro* diffusion study revealed that the formulation facilitated the highest release of thymoquinone, reaching 78.47% within 180 minutes. The release profile supports the use of emulsifiers and penetration enhancers like Tween 80, which enhance the drug's ability to cross the skin barrier. These findings are consistent with Franz cell studies conducted on other lipophilic compounds formulated in emulgel.

The protein denaturation assay confirmed that the formulation possessed significant anti-inflammatory activity. Thymoquinone's mechanism of inhibiting protein denaturation supports its traditional use in inflammation management. Prior literature has demonstrated thymoquinone's efficacy in reducing inflammatory mediators like prostaglandins, which contributes to this observation.

The CAM assay showed that the formulation didn't cause irritation or vascular damage, reinforcing the dermatological safety of the selected ingredients. This test is a well-established predictive model for assessing mucosal and skin irritation potential.

Overall, the developed emulgel exhibited promising characteristics in terms of stability, drug delivery, and therapeutic activity. The integration

of *Nigella sativa* extract in a topical vehicle such as an emulgel holds potential for future clinical studies and therapeutic applications in inflammatory disorders.

V. CONCLUSION

A novel anti-inflammatory emulgel incorporating thymoquinone from *Nigella sativa* was successfully formulated. The optimised formulation showed favourable physicochemical characteristics, excellent Spreadability, sustained drug release, potent anti-inflammatory activity, and excellent dermal compatibility. This formulation may be considered a promising candidate for the topical treatment of inflammation with improved patient compliance and minimal side effects.

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