Invivo Analgesic and Anti-Inflammatory Characterization of Transdermal Patch Containing Essential Oils

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Abstract
This study evaluates the in vivo anti-inflammatory and analgesic potential of transdermal patches formulated with herbal essential oils—Capsaicin, Methyl Salicylate, Menthol, and Thymol—using optimized polymeric matrices. Transdermal patches were prepared using HPMC K100, HPMC K15, and PEG as the base and tested using carrageenan-induced rat paw edema and tail flick tests. Patches demonstrated significant anti-inflammatory activity by reducing paw swelling and exhibited notable analgesic effects. The findings confirm the suitability of essential oil-based transdermal patches as promising alternatives for pain and inflammation management with improved patient compliance and sustained drug release.

Keywords: Transdermal patch, Essential oils, Capsaicin, Anti-inflammatory, Analgesic, Carrageenan-induced paw edema, Tail flick test.

Introduction

Pain and inflammation are common clinical symptoms managed using various pharmacological approaches, often limited by side effects and poor patient compliance. Essential oils like Capsaicin, Methyl Salicylate, Menthol, and Thymol possess well-documented analgesic and anti-inflammatory properties. However, their use is restricted due to volatility and localized irritation. Transdermal drug delivery systems (TDDS) offer a non-invasive, controlled-release approach that bypasses the gastrointestinal tract and minimizes systemic side effects. This study aims to formulate and evaluate transdermal patches incorporating these essential oils and assess their in vivo pharmacological efficacy. (Libby P et al., 2024)

Materials and method

Capsicum oleoresin, Wintergreen oil, Mentha oil, and Thymol oil were purchased from Indenta Research & Development Centre, Mumbai. HPMC 100M and HPMC 15M CR were purchased from Panacea Biotech. All the chemicals and reagents used were of analytical grade.

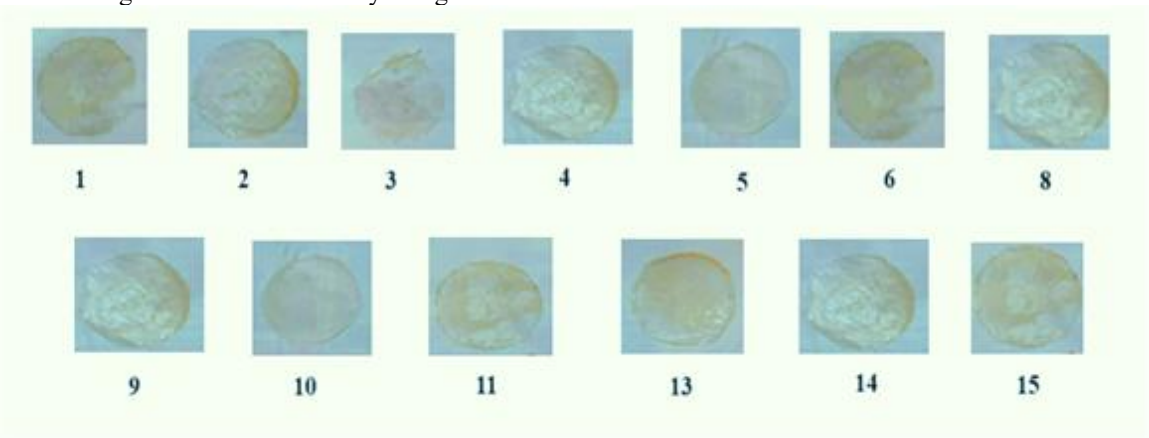

Figure 1: Essential oil patches (Formulation of batches by QbD Software)

In-vivo Analgesic and Anti-Inflammatory Activity

An albino male Wistar rat (220–320 g) of both sexes aged 6–8 weeks were used to investigate the analgesic and acute anti-inflammatory activities. The animals were housed, and placed in polypropylene boxes, held at room temperature.

They were given free access to water both before and throughout the experiment. The handling and euthanasia of animals were conducted in accordance with internationally recognized guidelines. Additionally, the Institutional Animal Ethical Committee of the School of Pharmacy approved all procedures.

Animal grouping and dosing
Six animals per study group were randomly assigned to one of three categories (negative control, positive control, and one group receiving herbal transdermal patches. In all models, the first group (negative control) received 10 ml/kg of distilled water whereas the second group (positive control) was given standard drug (20 mg/kg of morphine) for a hot plate method. Conversely, 150 mg/kg of aspirin for Hot plate method, and Volini gel at 10 mg/kg was administered for positive control group in carrageenan induced paw edema.

Hot plate method
In the hot plate method, the experimental animals were introduced into a metallic plate that would heat to a constant temperature of 55 °C +/- 1 °C. Paw biting, paw removal from the plate, and leaping or jumping out of plate were the main behavioral elements that were considered as a reaction time. After an overnight fast (12 h), animals were given the extract, vehicle or standard drug (morphine) as per grouping. Mice were individually placed on a hot plate with a cut-off time of 15 s to avoid damage to the animals' paws. The response time was measured by observing how long it took the mice to jump from the hot plate or lick their paws. Reaction times were measured to determine the percentage of elongation at 0, 30, 60, 90, and 120 min post administrations of each agent (Almeida Junior SD et al., 2019)
% elongation = (Latency test- Latency Control)/ Latency test * 100

Paw edema induced by carrageenan
In this model, 1 h before injecting carrageenan (1% w/v in normal saline, 0.05mL) into the dorsal area of the right hind paw of mice, the standard drug, the vehicle and various doses of plant extract were given. Thereafter the thickness of the paw was determined with a micrometer at 0, 1, 2, 3, and 4 h following the carrageenan administration. Finally, the width of the swelling in the paw with respect to the mice that took distilled water was calculated using equations below (Mansouri MT et al., 2015)

% edema inhibition = (PSC - PST)/ PEC *100
PSC= Paw swelling in Control
PST= Paw swelling in treatment groups

Results and discussion
In-vivo Analgesic and Anti-Inflammatory Activity
***In-vivo* Analgesic activity using Hot plate method**
When compared to the negative control at a 30 min, selected herbal transdermal patch exhibited considerable analgesic affect in hot plate method ($p < 0.001$) compared to negative control.
Regarding the percentage of elongation, the greatest effect was observed at 120 min, with values of 69.51%

1. Group I – Negative Control (Distilled Water)
- Reaction times decreased progressively from 3.67 s (0 min) to 1.83 s (120 min).
- This trend indicates no analgesic effect; the decrease over time may reflect natural variability or sensitization due to repeated testing.
- As expected, distilled water serves as a baseline for comparison, showing no influence on pain perception.

2. Group II – Positive Control (Aspirin)
- Reaction time increased from 2.67 s (baseline) to a maximum of 9.33 s at 90 and 120 min.
- The % elongation (increase in pain response latency) ranged from 78.64% (30 min) to 80.37% (120 min).
- This reflects the onset and sustained analgesic effect of aspirin, peaking around 90–120 minutes.
- It validates the methodology and confirms that the hot plate test is sensitive to analgesic activity.

3. Group III – Test Group (Herbal Transdermal Patch)
- Reaction time rose from 5.33 s (baseline) to 6.01 s (120 min), with a fluctuating pattern in between.
- % elongation was 65.9% (30 min), dipped to 42.85% (60 min), then increased again to 69.51% (120 min).
- These findings indicate the transdermal patch produced a moderate, time-dependent analgesic effect.
- The initial rise in latency suggests rapid onset, while the subsequent dip and recovery may reflect controlled drug release and systemic absorption dynamics.

***In-vivo* Anti-inflammatory activity**
Trandermal patch has been observed to exert its anti-inflammatory actions via controlling many inflammatory pathways in both acute and chronic inflammation. Table 2 shows the anti-inflammatory effect of the transdermal patch and Indomethacin in comparison. The results gives an important data that transdermal patch exhibited a statistically

significant reduction in rat paw edema volume in descending order with better % inhibition (63.81 %) at 24 h as compared to the control group. In comparison to the Volini Gel, it also exhibits a reduction in rat paw volume with higher % inhibition (65.57 %) but it was limited at 6 h and it was decreased thereafter upto 36.18 % at 24 with increased rat paw volume. in vivo anti-inflammatory activity results obtained from the Carrageenan-induced paw edema model, comparing the responses of three groups:

- Group I: Treated with distilled water (negative control)
- Group II: Treated with Volini gel (positive control)
- Group III: Treated with the herbal essential oil-based transdermal patch (test group)

Carrageenan-induced paw edema is a standard model to evaluate anti-inflammatory activity. It exhibits a biphasic response:

- Early phase (1–2 hours): Mediated by histamine, serotonin, and kinins.
- Late phase (3–6 hours): Involves prostaglandins and leukotrienes.

The reduction in paw volume is an indicator of anti-inflammatory efficacy.

Group I – Negative Control (Distilled Water)

- Paw volume gradually increased, peaking at 0.622 mL at 4 h, then decreased slightly by 24 h.
- No inhibition observed, as expected from a non-active treatment.
- This validates the model's sensitivity and confirms that the inflammation was successfully induced.

Group II – Volini Gel (Standard Anti-inflammatory)

- Showed moderate reduction in paw volume across all time points.
- Maximum inhibition at 24 h: 63.8%, with steady progression over time.
- Demonstrates sustained anti-inflammatory activity, particularly during the late phase of inflammation.
- Inhibition began as early as 1 h (5.6%), indicating fast onset of action.

Group III – Herbal Transdermal Patch

- Demonstrated superior early and mid-phase inhibition compared to Volini:
  - At 2 h, inhibition was 26.5% vs. 11.9% (Volini)
  - At 4 h, inhibition peaked at 59.5% vs. 32.3%
  - At 6 h, reached the highest inhibition: 65.6%
- Indicates strong suppression of both early and late inflammatory mediators.
- However, by 24 h, inhibition decreased to 36.2%, suggesting the effect may taper off over a full day unless re-applied or formulated with prolonged-release agents.

Table: 1 Invivo analgesic activity

Groups	0 min	30 min	% Elongation	60 min	% Elongation	90 min	% Elongation	120 min	% Elongation
GroupI	3.67 ± 0.21	2.67 ± 0.49	–	2.00 ± 0.25	–	2.00 ± 0.36	–	1.83 ± 0.32	–
GroupII	2.67 ± 0.28	5.33 ± 0.61	78.64	8.67 ± 1.38	62.47	9.33 ± 0.49	69.34	9.33± 0.48	80.37
GroupIII	5.33 ± 1.02	7.83 ± 0.60	65.9	3.50 ± 0.67	42.85	4.67 ± 0.67	57.16	6.01 ± 0.67	69.51

Notes: Each value represents mean ± S.E.M; *n* = 6 for each treatment

Table: 2 Invivo anti-inflammatory activity

Groups	Paw volume (Mean ± SEM)						% Inhibition					
	1 h	2 h	4 h	6 h	8 h	24 h	1 h	2 h	4 h	6 h	8 h	24 h
Group I	0.574 ± 0.020	0.588 ± 0.023	0.622 ± 0.028	0.610 ± 0.024	0.582 ± 0.039	0.514 ± 0.031	–	–	–	–	–	–
Group II	0.542 ± 0.060	0.518 ± 0.06	0.421 ± 0.028	0.405 ± 0.150	0.344 ± 0.028	0.186 ± 0.049	5.6	11.9	32.3	33.6	40.9	63.8
Group III	0.538 ± 0.007	0.432 ± 0.021	0.252 ± 0.005	0.210 ± 0.009	0.246 ± 0.013	0.328 ± 0.003	6.3	26.5	59.5	65.6	57.7	36.2

Notes: Each value represents mean ± S.E.M; *n* = 6 for each treatment

Conclusion

The in vivo evaluation of the herbal essential oil-based transdermal patch demonstrated significant analgesic and anti-inflammatory activities in experimental animal models.

In the hot plate analgesic test, the test group exhibited a time-dependent increase in reaction time, indicating an effective central analgesic action. The patch produced a notable 69.51% elongation in latency at 120 minutes, which, although slightly lower than the standard aspirin group (80.37%), reflects a substantial pain-relieving effect. The pattern of fluctuating response suggests a controlled drug release from the patch, maintaining analgesia over time.

In the carrageenan-induced paw edema model, the transdermal patch showed superior efficacy compared to the standard Volini gel, especially during the early and mid-phases of inflammation. Maximum inhibition of paw edema was 65.6% at 6 hours, highlighting the patch's ability to suppress both histamine- and prostaglandin-mediated inflammatory responses. Although the inhibitory effect declined to 36.2% at 24 hours, it still surpassed the standard treatment's efficacy at that point, which showed 63.8% inhibition but started declining afterward.

These findings suggest that the formulated herbal transdermal patch is effective in managing both acute pain and inflammation, likely through synergistic action of essential oils such as Capsaicin, Menthol, Methyl Salicylate, and Thymol. The patch offers the advantages of non-invasive delivery, sustained release, and enhanced patient compliance, making it a promising candidate for alternative pain and inflammation therapies.

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