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# Analysis of the Analgesic Effects of Polyherbal Topical Formulations Using Hot Plate Test In Mice

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**Abstract:** ***Background:*** *Natural phytoconstituents have gained increasing attention for their analgesic and anti-inflammatory activities. Bioactive compounds such as curcumin, capsaicin, berberine, mangiferin, and piperine possess significant pharmacological properties and may provide safer alternatives for pain management.*

***Objective:*** *The present study aimed to evaluate the analgesic activity of topical formulations containing combinations of selected phytoconstituents using the hot plate model in mice.*

***Methods:*** *Analgesic activity was assessed using the hot plate method described by Nathan B. Eddy and Dorothy Leimbach. Swiss albino mice (25–30 g) were divided into groups (n = 4) and treated with control, test formulations (F2, R5, T4), and a standard marketed formulation. Latency time for paw licking or jumping was recorded at 0, 30, 60, 120 and 150 minutes following topical administration. Percentage pain threshold inhibition was calculated and statistical analysis was performed using GraphPad Prism.*

***Results:*** *All tested formulations significantly increased the latency time compared with the control group. Among the formulations, R5 (Curcumin + Capsaicin + Piperine) demonstrated the highest analgesic activity with maximum pain inhibition of 84.98%, which was comparable to the standard formulation.*

***Conclusion:*** *The results indicate that polyherbal formulations containing bioactive phytoconstituents possess significant analgesic activity. The synergistic interaction among these compounds may contribute to enhanced pain-relieving effects.*

**Keywords:** *Analgesic activity, Polyherbal formulation, Curcumin, Capsaicin, Piperine, Hot plate test*

## 1. INTRODUCTION

Pain is one of the most common clinical symptoms associated with inflammation, injury, and pathological conditions. Despite the availability of several analgesic drugs, their prolonged use may lead to adverse effects such as gastrointestinal irritation, renal dysfunction, and cardiovascular complications. Consequently, there is increasing interest in exploring natural phytoconstituents with analgesic potential (1,2). Phytochemicals derived from medicinal plants have demonstrated significant pharmacological activities including anti-inflammatory, antioxidant, and analgesic effects. Polyherbal formulations containing combinations of bioactive compounds may

produce synergistic therapeutic effects, improving efficacy and reducing toxicity (3). Curcumin, a polyphenolic compound derived from Curcuma longa, exhibits potent anti-inflammatory and analgesic activity by inhibiting inflammatory mediators such as cyclooxygenase-2 (COX-2), tumor necrosis factor-α (TNF-α), and nuclear factor-κB pathways (4,5). Capsaicin, the active component of Capsicum species, produces analgesic effects by activating the transient receptor potential vanilloid-1 (TRPV1) receptor, resulting in desensitization of nociceptive neurons (6). Piperine, obtained from Piper nigrum, enhances the bioavailability of several drugs and phytochemicals and exhibits analgesic and anti-inflammatory properties (7). Berberine, an isoquinoline alkaloid present in plants such as Berberis species, has demonstrated anti-inflammatory, antioxidant, and analgesic effects through modulation of inflammatory signaling pathways (8). Mangiferin, a xanthone glycoside found in Mangifera indica, possesses strong antioxidant and anti-nociceptive activities (9). The hot plate test, developed by Nathan B. Eddy and Dorothy Leimbach, is widely used to evaluate centrally acting analgesics. The method measures latency to pain response following exposure to thermal stimulus and is considered a reliable model for assessing analgesic activity (10).

Therefore, the present study was undertaken to evaluate the analgesic potential of topical formulations containing combinations of these phytoconstituents using the hot plate method in mice.

## 2. Materials And Methods

### 2.1. Experimental Animals

Swiss albino mice weighing 25–30 g were used in the study. Animals were maintained under standard laboratory conditions with controlled temperature and humidity and a 12-hour light–dark cycle. Animals had free access to standard laboratory diet and water ad libitum. Twenty-four hours before the experiment, animals were allowed access only to water. The experimental protocol was approved by the Institutional Animal Ethics Committee of Rajaram bapu College of Pharmacy, Kasegaon. Approval number: RCP//P-2/2024. The institute is registered under the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

### 2.2. Composition of Polyherbal Formulations

***Table no1.*** *Composition*

| Formulation | Composition |
|---|---|
| F | Curcumin (1%) + Capsaicin (1%) + Berberine (1%) |
| T | Curcumin (1%) + Capsaicin (1%) + Mangiferin (1%) |
| R | Curcumin (1%) + Capsaicin (1%) + Piperine (1%) |

### 2.3. Hot Plate Test

The analgesic activity was evaluated using the hot plate method described by Eddy and Leimbach with slight modifications. The temperature of the hot plate was maintained at 55 ± 1°C. Each mouse was placed on the hot plate and the latency time for paw licking or jumping response was recorded as an indicator of pain perception. Baseline pain threshold was recorded before treatment. Test formulations and the standard marketed formulation were applied topically. Pain threshold was recorded at: 0 min, 30 min, 60 min, 120 min and 150 min. Percentage inhibition of pain threshold was calculated using the following equation: [Pain\ Threshold\ Inhibition (%) = \frac{(Pt - P0)}{P0} \times 100]

**Where:** P0 is Baseline pain threshold, Pt is Pain threshold at specific time interval.

### 2.4. Statistical Analysis

Statistical analysis was performed using GraphPad Prism. Results were expressed as

Mean ± SEM. Data were analyzed using Dunnett's multiple comparison test, with statistical significance considered at $p < 0.05$.

## 3. RESULTS

The hot plate test was employed to evaluate the central analgesic activity of the polyherbal topical formulations (F2, R5, and T4) in comparison to the control and standard drug groups. The latency time — defined as the time taken by the mice to respond to the thermal stimulus — was recorded at 0, 30, 60, 120, and 150 minutes post-administration, and the results are summarized in Table No. 2.

At baseline (0 min), all groups demonstrated comparable latency times ranging from 2.66 to 3.33 seconds, confirming uniformity among the groups prior to treatment. Following administration, a progressive and significant increase in latency time was observed across all test formulations compared to the control group, which showed a slight decline in latency over time (from 3.33 sec at 0 min to 1.75 sec at 120 min), suggesting a natural decrease in pain sensitivity without treatment. Among the test formulations, T4 demonstrated notably high latency values, reaching a peak of 14.08 seconds at 120 minutes, which was closely comparable to the standard drug (14.83 sec at 120 min). R5 also exhibited strong analgesic activity with a maximum latency of 14.00 seconds at 60 minutes, while F2 showed a moderate response with a peak of 9.00 seconds observed at 30, 120, and 150 minutes.

The standard drug consistently recorded the highest latency times throughout the study period, reaching 14.83 seconds at 120 minutes. All three formulations — particularly T4 and R5 — demonstrated latency times approaching those of the standard, indicating a significant and sustained analgesic effect. These findings suggest that the polyherbal formulations possess meaningful central pain inhibitory activity, as evidenced by their ability to delay the nociceptive response to thermal stimuli over the 150-minute observation period.

***Fig. 1.*** *Analgesic effect of Formulations.*

Effect of Formulations expressed as pain response in seconds induced by hot plate in mice (n = 5). The results are shown as mean ± SEM and a significant difference from the control group is shown as $P < 0.001$.

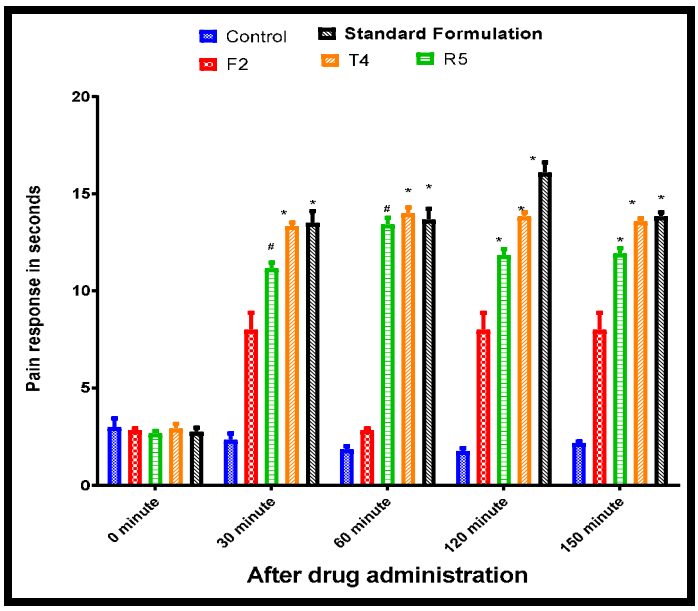
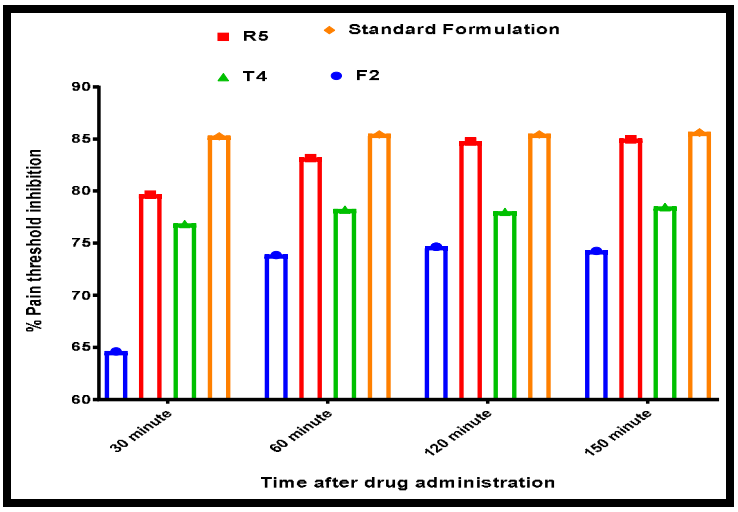



***Fig. 2.*** *Percentage Pain threshold inhibition of Formulations in hot plate test.*

Effect of Formulations expressed as the %inhibition threshold induced by hot plate in mice (n =5). The results are shown as average mean inhibition at that particular time point.




## 4. Discussion

Pain perception involves complex physiological pathways including peripheral nociceptor activation, spinal cord processing, and supraspinal modulation within the central nervous system (1,12). Experimental animal models such as the hot plate test are widely used to evaluate centrally mediated analgesic responses because the test specifically measures supraspinal reflexes triggered by thermal stimuli (10,13). In the present investigation, topical formulations containing combinations of phytoconstituents significantly increased latency time in mice compared with the control group, indicating elevation of the pain threshold. Among the tested formulations, R5 (Curcumin + Capsaicin + Piperine) demonstrated the highest analgesic activity. Curcumin exerts analgesic effects by inhibiting inflammatory mediators such as prostaglandins, COX-2, nitric oxide, and pro-inflammatory cytokines including TNF-α and interleukin-1β (4,5). Capsaicin produces analgesic effects primarily through activation of the TRPV1 receptor, which leads to desensitization of nociceptive neurons and depletion of neuropeptides such as substance P (6). Piperine enhances the bioavailability of various therapeutic compounds including curcumin by inhibiting hepatic and intestinal metabolizing enzymes and increasing gastrointestinal absorption (7). Therefore, the presence of piperine in the R5 formulation may potentiate the analgesic activity of curcumin and capsaicin. The formulation containing berberine also exhibited moderate analgesic activity. Berberine has been reported to inhibit inflammatory cytokines and oxidative stress pathways, contributing to its antinociceptive properties (8). Similarly, mangiferin possesses strong antioxidant and anti-inflammatory activities and has been shown to inhibit inflammatory mediators involved in pain pathways (9). The enhanced analgesic effect observed in the polyherbal formulations may therefore be attributed to synergistic interactions among multiple phytochemicals, which act on different pathways involved in pain perception.

## 5. Conclusion

The present study demonstrates that polyherbal topical formulations containing curcumin, capsaicin, berberine, mangiferin, and piperine exhibit significant analgesic activity in the hot plate model in mice.Among the tested formulations, R5 (Curcumin + Capsaicin + Piperine) showed the highest analgesic effect, comparable to the standard marketed formulation. These findings highlight the therapeutic potential of polyherbal topical formulations as alternative analgesic agents for pain management.

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