Original Article

# Potential application of a combined extract of *Channa striata* and calcium hydroxide for inhibiting lymphocytes and interleukin-1β cells in the asymptomatic irreversible pulpitis of Wistar rats *(Rattus norvegicus)*

Juni Jekti Nugroho[1,2], Risnawati Taha[1], Aries Chandra Trilaksana[1,2], Wahyuni Suci Dwiandhany[1,2]

[1]Department of Conservative Dentistry, Faculty of Dentistry, Hasanuddin University, [2]Department of Conservative Dentistry, Hasanuddin University Dental Hospital, Makassar, South Sulawesi, Indonesia

## Abstract

**Aim:** This study aimed to evaluate the effectiveness of combining *Channa striata* extract with calcium hydroxide (Ca(OH)2) in reducing lymphocyte counts and interleukin (IL)-1β levels in inflamed pulp teeth.

**Materials and Methods:** A laboratory experimental setup with a posttest control group (CG) design was employed. Thirty-two rat incisor teeth were divided into four groups: a positive CG (100% Ca(OH)2), treatment 1 (25% *C. striata* extract + 75% Ca(OH)2), treatment 2 (50% *C. striata* extract + 50% Ca(OH)2), and treatment 3 (75% *C. striata* extract + 25% Ca(OH)2). These test materials were applied to Class 1 cavities extending to the pulp roof, with samples collected on days 1, 3, 5, and 7. Data were analyzed using analysis of variance and *post hoc* least significant difference tests.

**Results:** In all the groups, the highest levels of lymphocytes and IL-1β were observed on day 3, followed by a decline on days 5 and 7. The combination of *C. striata* extract with Ca(OH)2 significantly reduced inflammatory markers, particularly lymphocyte and IL-1β levels.

**Conclusion:** The extract of *C. striata* holds significant potential as an innovative therapeutic approach for managing inflamed dental pulp.

**Keywords:** Calcium hydroxide; *Channa striata*; dental pulp; inflammation; interleukin-1β; lymphocytes

## INTRODUCTION

The dental pulp is a distinctive connective tissue comprising vascular, lymphatic, and neural elements originating from neural crest cells.[1] This tissue is situated in the central cavity of the tooth, encased within a rigid wall of enamel and dentin.[2-4] Both dentin and pulp tissue are specialized connective tissues derived from mesoderm, collectively referred to as the dentin–pulp complex. This term underscores the close anatomical, developmental, and functional interrelationships between dentin and pulp tissues.[5-8] When exposed to irritants, pulp tissue can become inflamed, and if this inflammation is not timely and effectively treated, it can exacerbate, leading ultimately to pulp necrosis.[9-11]

**Address for correspondence:**
Dr. Juni Jekti Nugroho,
Department of Conservative Dentistry, Faculty of Dentistry, Hasanuddin University, Makassar 90425, South Sulawasi, Indonesia.
E-mail: jektijuni@unhas.ac.id

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The acute inflammatory phase in the pulp typically spans 1–4 days postinjury. This rapid response to injury or other foreign substances facilitates the delivery of leukocytes and plasma proteins to the injury site.[12-14] Lymphocytes, which are adaptive immune cells, play a pivotal role in this inflammatory process due to their specialized ability to recognize antigens.[15] Moreover, interleukin-1β (IL-1β), a key proinflammatory cytokine, is crucial in mounting the host's defense against infection and injury in the pulp.[15,16]

Calcium hydroxide (Ca(OH)2) remains the gold standard for conservative treatment of inflamed dental pulp, owing to its high pH of approximately 12.5–12.8 and its mechanism of action, which involves the release of calcium ions (Ca2+) and hydroxyl ions (OH-).[16-21] This process, however, may lead to the formation of a liquefaction necrosis zone.[1,21] Consequently, there is a pressing need to identify combination materials with effective anti-inflammatory properties that offer minimal side effects in reducing dental pulp inflammation.[7,17,22]

One promising natural anti-inflammatory agent for dental pulp treatment is the extract of the Haruan fish (*Channa striata*).[7,11,22] *C. striata* is abundant in albumin, which has regenerative properties, enhances the viability of inflamed odontoblast cells, increases the density of collagen fibers in the dental pulp, and decreases matrix metalloproteinase-1 expression.[11,22] Furthermore, this extract has been observed to effectively reduce the expression of polymorphonuclear leukocytes and IL-1α in the inflamed dental pulp of Wistar rats.[7] Yet, there is a paucity of research evaluating the impact of *C. striata* extract on the reduction of lymphocytes and IL-1β levels in pulp inflammation.

This study aims to bridge this gap by analyzing the efficacy of the combination of *C. striata* extract and Ca(OH)2 in reducing the number of lymphocytes and IL-1β levels in the inflamed dental pulp. The findings from this research could contribute to developing more effective and safer treatments for dental pulp inflammation, ultimately improving patient outcomes. Such insights are critical in advancing the field of conservative dentistry and addressing the limitations associated with current treatment modalities.

## MATERIALS AND METHODS

### Study design

This study used a laboratory experimental design with a posttest control group (CG) design. The Wistar rats were sampled using simple random sampling based on Federer's calculation formula. The materials utilized in this study included *C. striata* extract, acquired from the traditional market in Maros, South Sulawesi, and commercial Ca(OH)2, specifically Ultracal™ XS from Ultradent, US.

### Sample preparation

*C. striata* were selected alive to maintain the quality of the extract. The *C. striata* extraction method was carried out in Feed Chemistry Laboratory, Faculty of Animal Science, Hasanuddin University, by steaming, referring to our previous research with some modifications.[7,11] Before steaming, the fish were cleaned of scales and entrails removed, and then the meat was cut into small pieces with a cross-sectional thickness of ±1 cm and separated from the bones. The 100% *C. striata* extract was made with a ratio of *C. striata* and aquadest of 1:1, using a temperature of 60°C–70°C (medium heat) for 30 min. The *C. striata* extract was a light yellow liquid collected in a container and then frozen and evaporated using a freeze dryer.

### Animal experiment

The Wistar rats (*Rattus norvegicus*) utilized in this research were sourced from the Bandung Institute of Technology, consisting of male rats aged 12–16 weeks weighing 300–400 g. Animal experimentation was carried out in Hasanuddin University Educational Veterinary Clinic. All rats were acclimatized to their cages for 2 weeks, during which their feeding and cage maintenance were systematically managed. A total of 32 teeth from these Wistar rats were selected based on the specific criteria: male rats weighing 300–400 g, aged between 12 and 16 weeks, possessing front lower jaw teeth free from caries, and with healthy periodontal tissues. The exclusion criteria were unhealthy rats indicated by dull hair, hair loss, rats that died during the study period, carious teeth, loose teeth, and fractured teeth. The samples were allocated into four groups, each comprising eight rats. The first group (CG) was the positive control, using 100% Ca(OH)2. The second group (P1), treatment 1, utilized 25% *C. striata* extract and 75% Ca(OH)2. The third group (P2), treatment 2, employed a 50% *C. striata* extract and 50% Ca(OH)2 combination. Finally, the fourth group (P3), treatment 3, was treated with a formulation containing 75% *C. striata* extract and 25% Ca(OH)2.

The Wistar rats were anesthetized intramuscularly with 10% ketamine HCl. The dose used was 15 mg/kg birth weight and can be repeated with an additional 1/3 or 1/2 of the initial dose injected. The tooth's surface to be excavated was disinfected using a cotton pellet soaked in 70% alcohol. A Class 1 cavity was prepared on the lower jaw front tooth using a handpiece with a round diamond bur (size ¼) (Mani Inc., Japan) to a depth of 0.5 mm until almost reaching the roof of the pulp and tiny pinpoint bleeding. The cavity was then irrigated with a sterile saline solution and dried using a cotton pellet. The test material was applied to the cavity using a ball applicator with a dose of 0.5 mg. The cavity was dried using a cotton pellet and sealed using resin-modified glass ionomer cement (GC® Corporation, Japan). Light curing was performed for 40 s. The samples were then decapitated and observed on days 1, 3, 5, and 7.

### Histological evaluation

Histological evaluation was conducted in the Anatomical Pathology Laboratory, Hasanuddin University Hospital and Biomolecular Biochemistry Laboratory, Faculty of Medicine, Brawijaya University, Malang. Samples underwent hematoxylin and eosin (HE) staining to count lymphocytes and immunohistochemistry (IHC) staining to quantify IL-1β levels. They were initially rinsed with distilled water, fixed in 10% formalin, and decalcified using 10% ethylenediaminetetraacetic acid. Subsequently, the samples were rinsed again, dehydrated, embedded in paraffin, and sectioned. Staining with HE and IHC was performed before the sections were analyzed using a digital image capture pathology scanner (Aperio CS model, Leica Biosystems, Buffalo Grove, IL, USA) at magnifications of ×400 and ×1000.

### Statistical analysis

The data were processed using IBM SPSS Statistics for Windows, Version 25.0. (Armonk, NY: IBM Corp.) The analytical tests used were analysis of variance and *post hoc* least significant difference tests with a significance level of $P < 0.05$.

## RESULTS

The results from the experimental analysis, as detailed in Table 1, illustrated a progressive decline in the mean lymphocyte counts (μg/mL) across all treatment groups over the observation period, extending from day 1 to day 7. Table 1 shows that the CG group using 100% Ca(OH)2 started with a mean lymphocyte count of 9.25 ± 1.71 μg/mL on day 1 and decreased to 5.50 ± 1.29 μg/mL by day 7. This declining trend was consistently observed in the treatment groups, incorporating varying ratios of *C. striata* extract and Ca(OH)2.

The P1 group, which comprised 25% *C. striata* extract and 75% Ca(OH)2, began with a lymphocyte count of 7.00 ± 0.82 μg/mL and ended the period with 3.50 ± 1.29 μg/mL. Both P2 and P3, which included higher proportions of *C. striata* extract (50% and 75%, respectively), exhibited even more significant reductions in lymphocyte counts, starting from 4.75 ± 1.26 to 4.50 ± 1.29 μg/mL, respectively, and reducing to 2.50 ± 1.29 and 2.00 ± 0.82 μg/mL by day 7.

Statistical analysis indicated significant differences across the days of observation, with $P < 0.05$ confirming the efficacy of the treatments. The most pronounced effect was observed in P3, which exhibited the lowest lymphocyte counts throughout the study period. These findings suggested that higher proportions of *C. striata* extract in the combination treatment contribute to more effective anti-inflammatory action, as evidenced by the more significant reduction in lymphocyte counts compared to the group treated only with Ca(OH)2.

Table 2 compares IL-1β levels (μg/mL) between the various treatment groups on different observation days. Significant differences were observed in the levels of IL-1β between the CG and all treatment groups (P1, P2, and P3), with the lowest values consistently reported in the treatment groups. This trend largely persisted through days 3 and 5, with most treatment groups showing significant reductions in IL-1β compared to the control. However, by day 7, the statistical significance of these differences diminished for some groups. While P1 did not show significant differences from the control on day 7, P2 and P3 maintained significantly lower IL-1β levels compared to the CG at the end of the observation period. Notably, the efficacy of the *C. striata* extract in reducing IL-1β appears to be dose dependent, as higher concentrations of the extract (as seen in P3) consistently showed the most significant reduction in IL levels throughout the study.

**Table 1: Comparison of mean lymphocyte count (μg/mL) among treatment groups across observation days**

| Observation day | Groups | Mean±SD | *P* |
|---|---|---|---|
| Day 1 | CG | 9.25±1.71 | 0.000* |
| | P1 | 7.00±0.82 | |
| | P2 | 4.75±1.26 | |
| | P3 | 4.50±1.29 | |
| Day 3 | CG | 12.00±1.15 | 0.005* |
| | P1 | 7.50±1.29 | |
| | P2 | 5.75±0.50 | |
| | P3 | 4.75±0.96 | |
| Day 5 | CG | 8.00±2.16 | 0.001* |
| | P1 | 5.25±1.26 | |
| | P2 | 3.00±0.82 | |
| | P3 | 2.75±0.96 | |
| Day 7 | CG | 5.50±1.29 | 0.006* |
| | P1 | 3.50±1.29 | |
| | P2 | 2.50±1.29 | |
| | P3 | 2.00±0.82 | |

*ANOVA test, $P<0.05$ significant. SD: Standard deviation, CG: Positive control, PI: Treatment 1, P2: Treatment 2, P3: Treatment 3. ANOVA: Analysis of variance, CG: Control group, SD: Standard deviation

**Table 2: Comparative analysis of interleukin-1β (μg/mL) among treatment groups over the observation period**

| Observation day | Groups | Control (+) | Treatment 1 | Treatment 2 | Treatment 3 |
|---|---|---|---|---|---|
| Day 1 | CG | | 0.001* | 0.000* | 0.000* |
| | P1 | | | 0.001* | 0.000* |
| | P2 | | | | 0.763 |
| | P3 | | | | |
| Day 3 | CG | | 0.020* | 0.018* | 0.020* |
| | P1 | | | 0.017* | 0.019* |
| | P2 | | | | 0.155 |
| | P3 | | | | |
| Day 5 | CG | | 0.001* | 0.000* | 0.000* |
| | P1 | | | 0.191 | 0.109 |
| | P2 | | | | 0.735 |
| | P3 | | | | |
| Day 7 | CG | | 0.001* | 0.000* | 0.000* |
| | P1 | | | 0.384 | 0.096* |
| | P2 | | | | 0.384 |
| | P3 | | | | |

*LSD (*Post hoc*) test. $P<0.05$ significant. CG: Positive control, PI: Treatment 1, P2: Treatment 2, P3: Treatment 3. LSD: Least significant difference, CG: Control group

Figure 1 illustrates the mean lymphocyte counts across different treatment groups over the observation period. The data indicated an apparent reduction in lymphocyte counts in all treatment groups (P1, P2, and P3) compared to the CG from day 1 through day 7. On day 1, the CG group had the highest mean lymphocyte count of 9.25, while the treatment groups P1, P2, and P3 showed lower counts at 7.00, 4.75, and 4.50, respectively. By day 3, lymphocyte counts peaked in all groups, with the CG group reaching 12.00, while the treatment groups had values of 7.50 (P1), 5.75 (P2), and 4.75 (P3). From day 5 onward, a significant reduction in lymphocyte counts was observed. The CG's count decreased to 8.00, while P1, P2, and P3 had reduced counts of 5.25, 3.00, and 2.75, respectively. This downward trend continued until day 7, where the CG's count further decreased to 5.50, and the treatment groups' counts were 3.50 (P1), 2.50 (P2), and 2.00 (P3).

Figure 2 depicts the changes in IL-1β levels measured in μg/mL across the different treatment groups over 4 observation days. On day 1, the CG had the highest IL-1β count of 11.50, followed by reductions in the treatment groups: P1 with 8.00, P2 with 4.25, and P3 with 4.00. A similar trend continued on day 3, with the CG group peaking at 13.50, while the treatment groups showed progressive decreases, with P3 consistently recording the lowest levels of IL-1β.

As the observation continued to day 5 and day 7, the treatment groups further demonstrated a consistent decline in IL-1β levels. By day 7, all treatment groups exhibited significant reductions, especially P3, which recorded the lowest level of 2.00. This marked a substantial decline from the initial measurements on day 1, indicating the potent anti-inflammatory effect of the treatments.

## Histological observation

Figure 3 illustrates the histological analysis of lymphocyte infiltration in the inflamed dental pulp of Wistar rats following the application of a combination of *C. striata* extract and Ca(OH)2, as observed on days 1, 3, 5, and 7. On day 1 (H1), the CG exhibited a high density of lymphocytes, indicative of an acute inflammatory response. In contrast, the treatment groups showed a noticeable reduction in lymphocyte presence, with the lowest levels observed in the P3 group, which had the highest concentration of *C. striata* extract. By day 3 (H3), lymphocyte infiltration remained substantial in the CG but began to diminish in the treatment groups. The trend of decreasing lymphocyte counts continued through days 5 (H5) and 7 (H7), with the treatment groups, particularly P3, demonstrating significantly fewer lymphocytes than the control.

Figure 4 displays the IHC staining results for IL-1β in inflamed dental pulp tissues of Wistar rats treated with varying combinations of *C. striata* extract and Ca(OH)2 across several observation days (days 1, 3, 5, and 7). In the CG, the expression of IL-1β was consistently high throughout the study period, showing a marked presence in the tissue. Treatment groups (P1, P2, and P3) demonstrated a significant reduction in IL-1β expression as the concentration of *C. striata* extract increased. By day 7, the IL-1β levels in P3, which had the highest concentration of *C. striata* extract, were noticeably lower than those in the CG, illustrating a profound reduction.

The high magnification views (×1000) clearly visualized the specific areas with marked reductions in IL-1β staining, particularly in the treatment groups. This observation was particularly evident on days 5 and 7, indicating the effectiveness of *C. striata* extract in modulating the inflammatory response in dental pulp tissues.

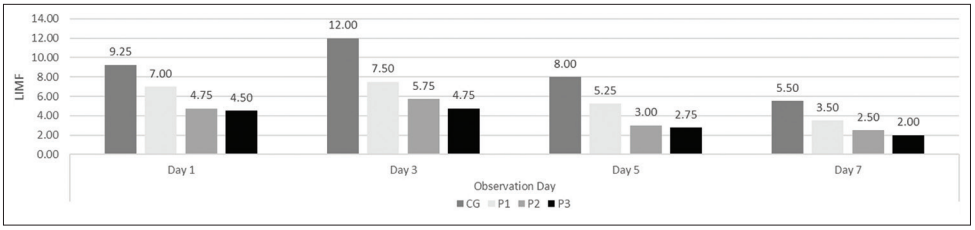


**Figure 1:** Mean lymphocyte counts over time for different treatment groups. LIMF: Lymphocyte, CG: Control group

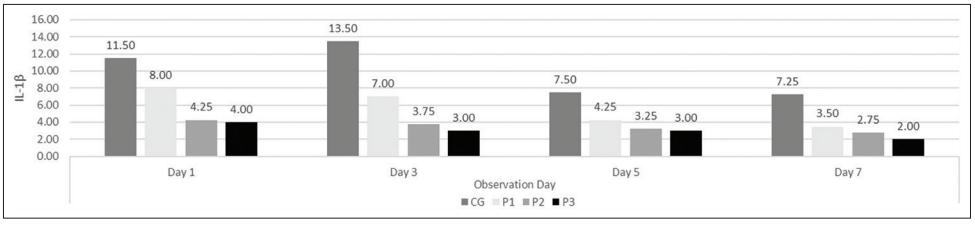


**Figure 2:** Mean interleukin-1β levels over time for different treatment groups. IL: Interleukin, CG: Control group

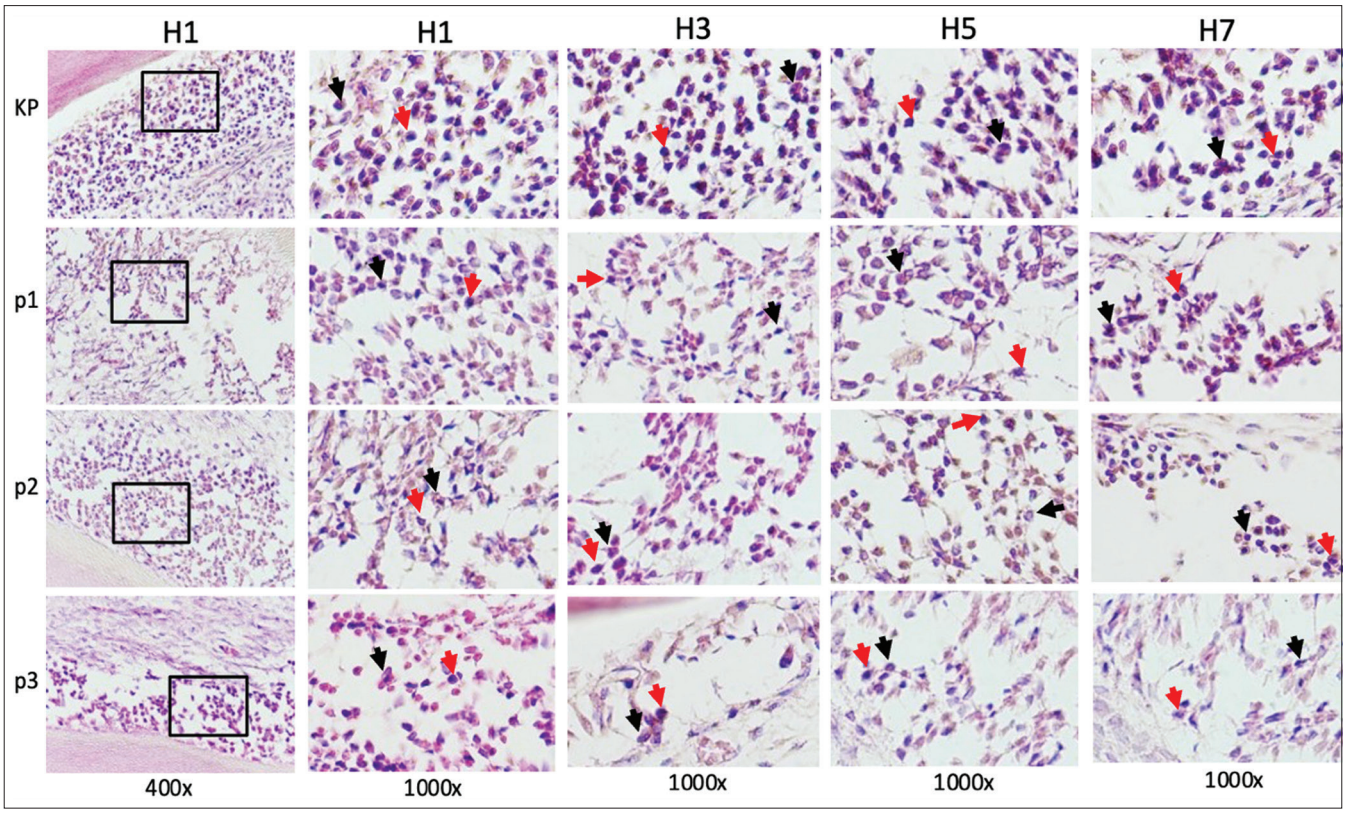


**Figure 3:** Histological analysis of lymphocyte infiltration (red arrows) in the inflamed dental pulp of Wistar rats treated with *Channa striata* extract and Ca(OH)2 on days 1, 3, 5, and 7 (visualized through H and E, ×400 and ×1000). Day 1 (H1), Day 3 (H3), Day 5 (H5), Day 7 (H7), Positive control (KP), treatment 1 (p1), treatment 2 (p2), treatment 3 (p3). Neutrofil (black arrow)

## DISCUSSION

This study analyzed the alterations in lymphocyte count and IL-1β levels within the inflamed dental pulp of Wistar rats subjected to a combination treatment of *C. striata* extract and Ca(OH)2. Following application of this combination, lymphocyte count exhibited a decline on days 3, 5, and 7, with the highest count observed on day 3. This finding aligns with previous studies by Siswanto *et al.*, which suggested that the number of lymphocytes increases in chronic inflammation because lymphocytes migrate to the wound area on day 1. The number will peak on days 3–6 and then decrease by day 7.[23] Lymphocytes function within the body's immune system, contributing to humoral and cellular responses during chronic inflammation.[14,16,17] In addition, lymphocytes also play a crucial role in releasing lymphokines, which significantly influence the inflammatory process by affecting the aggregation and chemotaxis of macrophages in the wound-healing process.[24]

This study observed a higher count of lymphocytes in the group administered with 75% *C. striata* extract and 25% Ca(OH)2 compared to the group treated solely with Ca(OH)2. These findings align with those of Tanumihardja *et al*., who demonstrated increased viability of odontoblast cell lines upon adding *C. striata* extracts compared to the Ca(OH)2 alone.[22] This correlation can be attributed to albumin in *C. striata* extract, a significant constituent known for its regenerative properties in damaged tissues or cells. Albumin facilitates the regulation of prostaglandin synthesis, which is crucial for the inflammatory phase and wound healing induction. Prostaglandins, as tissue by-products during inflammation, play a pivotal role in activating the macrophage system, thereby enhancing phagocytosis of foreign objects in the wound area.[24,25]

In addition to having a high albumin content, *C. striata* extract also contains unsaturated fatty acids that can suppress the inflammatory process. Unsaturated fatty acids can inhibit the cyclooxygenase enzyme, thereby reducing the synthesis of prostaglandins. Furthermore, *C. striata* extract also has antibacterial activity. This can reduce bacterial contamination that hinders wound healing.[26] In the present study, we observed a clear dose–response relationship, with higher concentrations of *C. striata* extract resulting in more pronounced anti-inflammatory effects. These findings underscore the potential of *C. striata* extracts as a natural alternative to traditional anti-inflammatory treatments, offering superior outcomes in managing inflammation in the dental pulp.

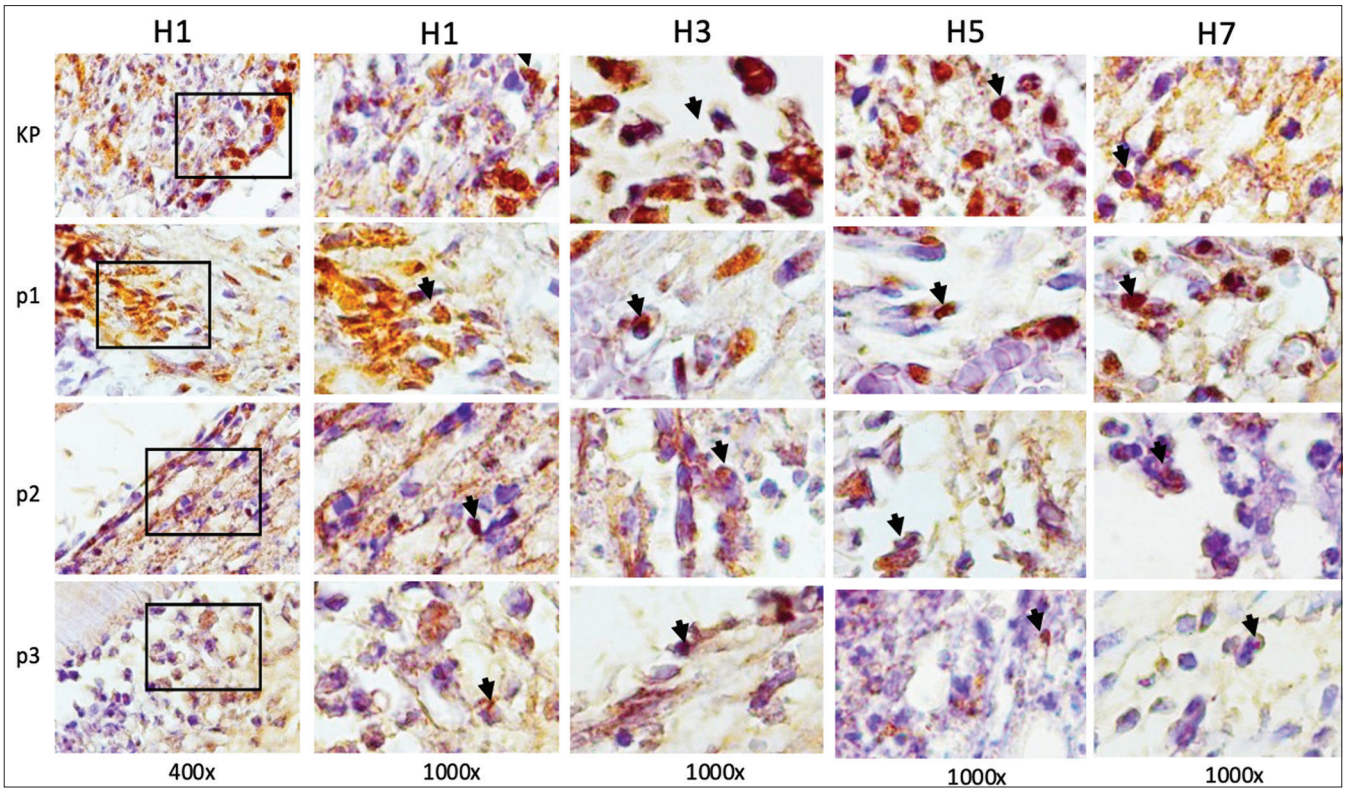


**Figure 4:** Histological analysis of interleukin-1β (black arrow) in the dental pulp after treatment with *Channa striata* extract and Ca(OH)2 (visualized through immunohistochemistry staining at ×400 and ×1000). Day 1 (H1), Day 3 (H3), Day 5 (H5), Day 7 (H7), Positive control (KP), treatment 1 (p1), treatment 2 (p2), treatment 3 (p3)

## CONCLUSION

The application of *C. striata* extract resulted in substantial decreases in inflammatory markers, specifically lymphocytes and IL-1β. Utilizing higher doses of this extract in combination with Ca(OH)2 shows great potential for enhancing clinical results and constitutes a notable progression in treating dental pulp inflammation. Nonetheless, additional research and clinical trials are essential to confirm and refine this promising treatment approach.

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Nil.

### Conflicts of interest
There are no conflicts of interest.

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