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Assessment of Tissue Inflammation, Bone Formation and Necrosis of Embelia Ribes Hydrogel Medicament Compared with Dexamethasone: An Animal Study

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KEYWORDS

Embelia ribes, hydrogel, intracanal medicament, inflammation, necrosis, bone regeneration, animal study

ABSTRACT

Introduction: Embelia ribes Burm. f., commonly known as false black pepper, has demonstrated diverse pharmacological activities. Recent advances in nanomedicine have enabled the incorporation of plant-based silver nanoparticles into hydrogel systems for potential intracanal applications.

Objective: This study evaluated and compared tissue inflammation, bone formation, and necrosis following implantation of an E. ribes-based hydrogel and dexamethasone in Wistar rats.

Methods: An E. ribes hydrogel was formulated using seed-mediated silver nanoparticles (3%), hyaluronic acid (3%), and hydroxyethyl cellulose (3%) in a 1:1:1 ratio. Eighteen Wistar rats were divided into three groups (n = 6 each): Group 1 – Calcium hydroxide, Group 2 – E. ribes hydrogel, and Group 3 – Dexamethasone. Cavities were prepared in the mandibular bone and filled with the respective medicaments. At 7, 14, and 28 days, tissues were harvested for histopathological evaluation of inflammation, bone formation, and necrosis. Statistical analysis was performed using Mann–Whitney U, Cochran's Q test, and kappa statistics for interobserver reliability.

Results: Histological sections revealed early granulation tissue and mild inflammation in the E. ribes hydrogel group, with progressive and organized bone formation by Day 28. In contrast, dexamethasone showed delayed bone remodelling with residual necrosis. Calcium hydroxide demonstrated healing but at a slower rate compared with E. ribes. Although no statistically significant differences were observed ($p > 0.05$), trends indicated reduced inflammation and enhanced bone regeneration with the hydrogel. By Day 28, no necrosis was detected in the E. ribes or calcium hydroxide groups, while mild necrosis persisted with dexamethasone.

Conclusions: The E. ribes-AgNP hydrogel demonstrated favourable biocompatibility, reduced inflammation, and enhanced bone regeneration compared to dexamethasone, supporting its potential as a safe intracanal medicament.

1. Introduction

Apical periodontitis (AP) is an inflammatory disease caused by a polymicrobial infection of the root canal. It is typically caused by bacterial invasion and infection resulting from factors such as dental caries, trauma, or incomplete endodontic treatment [1]. The use of suitable Material during the root canal therapy plays a pivotal role in the success of the treatment.[2]

As per studies, apical periodontitis can lead to bone resorption and the formation of apical lesions. The destruction and inflammation of the periapical tissues caused by the root canal infection are mainly due to the percolation of bacteria and their toxins.[3] Due to the poor understanding of the role of mechanical and chemical debridement, the intracanal drug should have strong antimicrobial and biocompatible.

Success in endodontic treatment was originally based on the triad of debridement, thorough infection control, and obturation of the root canal system, with each aspect equally important. In the current scenario, a successful root canal is based on broader principles.[4]Walton remarked that "Intracanal medicaments have traditionally gone hand-in-glove with endodontics (Torabi Nejad, M. and Walton, R.E). They are generally considered to be an integral part of treatment and important to the success of root canal therapy.[5]

The conventional medicament used is calcium hydroxide due to its superior properties. It has been shown to have peri

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radicular healing (Sjogren et al, 1990). Sjogren in 1991 stated that Ca (OH)2, applied for 7 days, eliminated bacteria in canal systems even up to 5 weeks later. Of the total, only 0.17% of calcium hydroxide dissolves to form Ca++ and OH, and it requires at least 1 day to exert full effect; hence, Ca (OH)2 is a slow-acting antiseptic (Ba-Hattab, R et al 2016).[6,7]. Bystrom et al have advocated that calcium hydroxide should be present in the canal for at least one week to be effective. Triple Antibiotic paste overtime has shown to have detrimental effects on the apical stem cells over a period of prolonged usage. Use of Intracanal steroids in a study by Maged demonstrated significant reduction of mean pain score rapidly compared to the placebo drug owing to its anti-inflammatory action. Further use of steroids showing no adverse side effects and recurrences of pain. Leder mix paste, triamcinolone and demeclocycline showed significant healing and more remaining tooth structure when used as medicament[8]

However, to weaker dentinal tubules and growing resistance to the conventional intracanal medicament, herbal medications have risen with nano-application [9]. The current study uses Embelia ribes seed-mediated silver nanoparticle based Intracanal medicament, incorporating hyaluronic acid and methyl propyl as a carrier. Embelia ribes is known for its active ethanolic ingredient embelin. Embelin is a photoactive constituent in seeds containing an array of medicinal properties.

The present animal study compares the inflammation, osteoinduction or bone formation and the tissue necrosis response comparing herbal medicament and dexamethasone over a period of four weeks.

2. Objectives

The objective of this study was to formulate and investigate an Embelia ribes–based hydrogel incorporated with biosynthesized silver nanoparticles as a potential root canal medicament. The research aimed to evaluate its biocompatibility, anti-inflammatory response, bone formation potential, and possible necrotic changes **in** comparison with the standard anti-inflammatory agent, dexamethasone. To achieve this, an in vivo experimental study was conducted on male Wistar rats**,** which served as the animal model for assessing the tissue response to the medicament. This study sought to determine whether the Embelia ribes hydrogel could offer a safe, effective, and natural alternative to conventional synthetic medicaments in endodontic practice.

3. Methods

The study was conducted with approval from the Institutional Committee for Animal Research (BRULAC/SDCH/SIMATS/IAEC/07-2024/13).

Sample Size Calculation

The total sample size was calculated to be 18 (n= 3), with an allocation ratio of 1:1, as determined by G*Power 3.1.2 software (SPSS version 0.17; SPSS Inc., Chicago, IL, USA) with alpha error left at 5% and statistical power of 95% using the means from a previous study evaluating the biocompatibility of materials by subcutaneous implantation in mice.

To study the inflammatory response and osteoinduction properties of the material, the medicament was placed in the cavity troughed in the mandibular bone. This was to analyse the impact of medicament when used in periapical lesions with loss of bone. Twenty-seven, six weeks old Wistar Rats weighing 250-300gms were included in the study. The rats were randomly divided into three experimental groups, with two rats groups in each.

Group 1: Calcium Hydroxide (16 mg Calcium Hydroxide / 1 ml of Distilled water)

Group 2: Embelia ribes mediated Intracanal medicament

Group 3: Dexamethasone (1 ml of 4 mg/ml)

The animals were anesthetized using an intraperitoneal injection of ketamine hydrochloride (47.5 mg/kg) and xylazine (10 mg/kg). Following anaesthesia, the dorsal region of each rat was shaved and disinfected with povidone-iodine solution (Betadine, Win Medicare Pvt. Ltd.). A surgical incision was made along the midline, parallel to the mandibular border, to access the periapical region. A bone trough measuring approximately 1 × 1 mm was prepared in the periapical area using carbide burs under continuous saline irrigation. The medicament corresponding to the designated experimental group was placed into the prepared site. The incision was then closed using black braided silk sutures, followed by disinfection with povidone-iodine solution.[10]

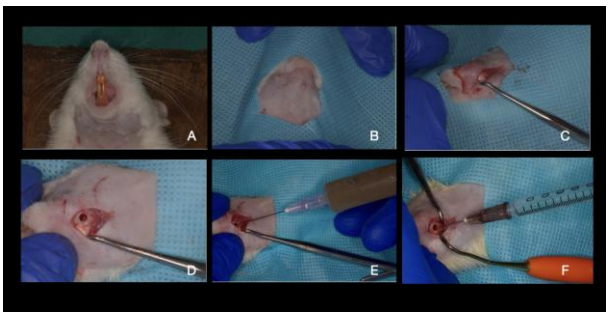

Fig 1 A: Anaesthetized male Wistar rats Shaved suprascapular region before incision and reflection in the vicinity of the lower jaw Troughed space in the mandible of Wistar rats for placement of the respective medicament. E: Placement of Embelia ribes mediated smart hydrogel in the mandible of the Wistar rats. F: Placement of Dexamethasone in the mandible of the troughed region

Journal of Chemical Health Risks
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Postoperatively, the animals were housed separately according to their respective groups, maintained in isolation, and provided with a balanced diet. At predetermined time intervals of 7, 14, and 28 days, the animals were euthanized using an overdose of the anaesthetic agent. Tissue samples from the medicament application site, as well as adjacent normal tissue, were collected and fixed in 10% buffered formalin for 48 hours before histopathological examination.

Table 1: Number of Wistar Rats in each group and their time of sacrifice

Evaluation period after medication placement	Calcium Hydroxide (16 mg Calcium Hydroxide/1 ml of Distilled water)	Embelia ribes mediated Intracanal medicament (n =3)	Dexamethasone (1 ml of 4 mg /ml)
7 days	2	2	2
14 days	2	2	2
28 days	2	2	2

Inference : From each rat tissue, two sections were taken for histopathologic analysis. 2 pathologists assessed all the slides and gave the scoring. On the given day intervals, the samples were sent for histopathological evaluation.

Histopathological Evaluation

Mandibular bone samples were harvested on days 7, 14 and 28 days following the implantation of the respective materials. The collected specimens were fixed in 10% buffered formalin and subsequently decalcified using formic acid. After decalcification, the tissues were processed and embedded in paraffin wax. Sections were cut at a thickness of 5 µm using a microtome and stained with Haematoxylin and Eosin (H&E) for histopathological evaluation.

The inflammatory tissue response was evaluated based on standardized grading criteria: Grade 0 indicated no inflammation, Grade 1 represented mild inflammation, Grade 2 moderate inflammation, and Grade 3 severe inflammation. The results were recorded and subjected to statistical analysis to assess the biocompatibility of the applied medicaments.

Statistical analysis

The statistical analysis for animal study was carried out using SPSS software version 23.0. Since the data was an ordinal variable, non-parametric tests were carried out for analysis. Mann-Whitney U test was done to compare the H and E scores between the test and the control group.[10,11].Cochran's Q test was carried out to compare the inflammation, vascularity and fibrosis scores within the groups from day 7, day 14 and day 28.Dunn's post hoc test was done for pair-wise comparison within the study groups. Interrater reliability using kappa statistics was done to evaluate the an agreement between pathologists 1 and 2 in assessing the H and E scores. A p-value <0.05 was considered significant in the above tests.

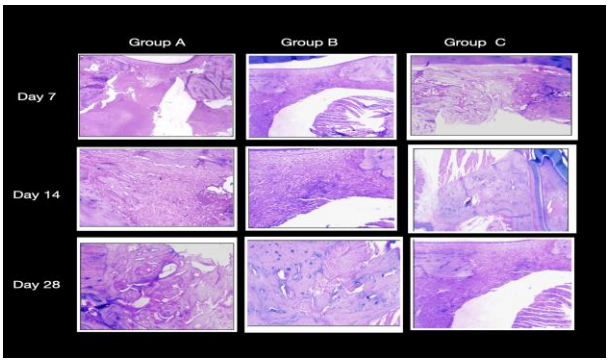

Fig 2:Histopathological Assessment : Group A :Dexamethasone Group B : Embelia ribes mediated hydrogel Group C : Calcium Hydroxide at an periodic observation of Day 7, Day 14 and Day 28.

4. Results

Histological evaluation of decalcified jawbone sections using H&E staining revealed distinct differences in the healing response between the dexamethasone-treated groups (Group A) and the Embelia ribes hydrogel-treated groups (Group B) over time. On Day 7, Group A exhibited a defect filled with granulation tissue, loose connective tissue, extravasated RBCs, necrotic debris, and a dense inflammatory infiltrate, with no evidence of new bone formation. Peripheral bone resorption and osteoclast activity were noted.

In contrast, Group B (E. ribes hydrogel) demonstrated granulation tissue with fewer inflammatory cells and early signs of bone remodeling, evidenced by both bone formation and resorption at the periphery of the defect. By Day 15, Group A (dexamethasone) showed mild inflammatory infiltration, and the presence of haphazardly deposited new bone interspersed within granulation tissue, along with resting and reversal lines, suggesting ongoing bone formation in the defect without necrosis.

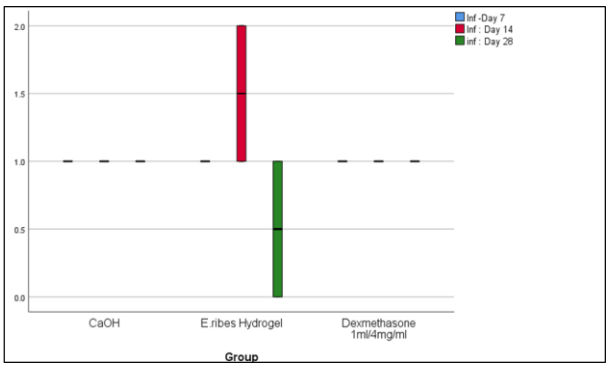

Fig 3: Box Whisker plot representing the median and interquartile range scores of inflammations with outliers among the study groups

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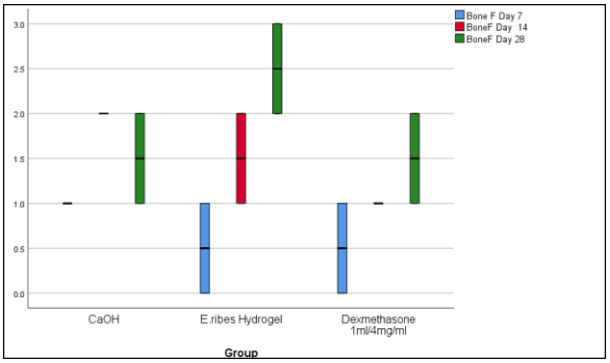

Fig 4 : Box Whisker plot representing the median and interquartile range scores of bone formation with outliers among the study groups

Notably, Group B (E. ribes hydrogel) displayed more organized bone remodeling with mild inflammatory infiltrate, mature bone formation, and distinct resting and reversal lines, also with no evidence of necrosis. These findings suggest that Embelia Ribes hydrogel promotes a more favorable and organized bone healing response compared to dexamethasone, particularly evident by Day 15 and day 28.

Table 2 : Comparison of the distribution of necrosis among the study groups

Group	Necrosis	N (%)					
		Day 7	p-value	Day 14	p-value	Day 28	p-value
$CaOH_2$	Present	0 (0)	0.667	1 (50)	0.472	0 (0)	0.301
	Absent	2 (100)		1 (50)		2 (100)	
E.ribes Hydrogel	Present	1 (50)		1 (50)		0 (0)	
	Absent	1 (50)		1 (50)		2 (100)	
Dexamethasone (1ml/4mg/ml)	Present	1 (50)		0 (0)		1 (50)	
	Absent	1 (50)		2 (100)		1 (50)	

Inference: There is no significant difference in distribution of necrosis between the groups at day 7, 14, and day 28 ($p>0.05$). However, E.ribes Hydrogel and $CaOH_2$ exhibits no necrosis at day 28.

5. Discussion

The current study evaluated the inflammatory response, bone formation, and necrosis following the implantation of an Embelia ribes-based hydrogel in comparison with dexamethasone and calcium hydroxide in Wistar rats. Histopathological findings revealed that the E. ribes hydrogel promoted early and organized bone formation with reduced inflammatory infiltration, whereas dexamethasone showed delayed bone remodeling and persistent necrosis [12,13]. These outcomes suggest that the plant-based hydrogel supports a more favorable biological healing response than the corticosteroid group.[14]

Although statistical significance was not achieved, likely due to the small sample size, the consistent trend toward reduced inflammation and enhanced bone formation with E. ribes is biologically relevant.[15] Previous reports have highlighted the limitations of calcium hydroxide, such as incomplete removal and delayed antimicrobial action, while intracanal steroids, though effective in pain reduction, may compromise periapical tissue healing with prolonged use.[16] In contrast, the phytoconstituents of E. ribes, particularly embelin, combined with silver nanoparticle incorporation, may provide anti-inflammatory and Oste inductive effects, aligning with earlier findings on the therapeutic benefits of plant-derived nanomedicines.[17,18]

The absence of necrosis in both the E. ribes and calcium hydroxide groups by Day 28 reinforces their safety and compatibility with host tissues.[19] Importantly, the organized bone remodeling observed in the E. ribes hydrogel group indicates a potential advantage in supporting periapical healing, particularly in cases of apical periodontitis where inflammation and bone loss are major concerns.[20] While further validation in larger cohorts and clinical settings is required, the present results support the role of herbal nanomedicine as a promising adjunct in endodontic therapy[21].

Conclusion

This suggests better biocompatibility and reduced cytotoxicity of the plant-based and calcium hydroxide medicaments compared to corticosteroid treatment. Although statistical significance was not achieved, likely due to small sample sizes and limited study duration, the collective trends indicate that the Embelia ribes-AgNP hydrogel supports favorable biological outcomes in terms of inflammation resolution, bone regeneration, and tissue compatibility, supporting its potential as a safe and effective intracanal medicament.

Clinical Relevance

This study suggests that an Embelia ribes-AgNP hydrogel could offer a biocompatible and osteoinductive alternative to conventional intracanal medicaments, potentially improving clinical outcomes in root canal therapy by promoting faster and more organized periapical healing.

Limitations and Future Scope

The present study was limited by its small sample size and short observation period, which may have restricted the detection of statistically significant differences. Additionally, only histopathological parameters were evaluated without molecular or microbiological correlation. Future studies with larger cohorts, longer follow-up, and advanced analyses are needed to confirm the osteoinductive and anti-inflammatory potential of *E. ribes* hydrogel and to translate these findings into clinical endodontic applications

Journal of Chemical Health Risks

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JCHR (2025) 15(5), 1922-1926 | ISSN:2251-6727

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Conflicts of Interest

The author declares no conflict of Interest.

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