Evaluation of tissue inflammatory response of four intracanal medicament – An animal study

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Abstract
Aim: The aim of the study was to evaluate the tissue inflammatory response of origanum vulgarae, omeprazole, triple antibiotic paste (TAP), and calcium hydroxide in an animal model.

Materials and Methods: Seventy Five male Wistar rats were randomly divided into four experimental groups and one control group: Group 1: control \((n=15)\), Group 2: 0.25 mg origanum vulgarae/1 ml of cellulose \((n=15)\), Group 3: 2 mg omeprazole/1 ml of distilled water \((n=15)\), and Group 4: 1 mg TAP/1 ml of distilled water \((n=15)\), Group 5: 16 mg calcium hydroxide/1 ml of distilled water \((n=15)\). A trough was made in the periapical bone and the medicament of the respective groups was placed. After the 7th, 14th, 28th days, the animals were euthanized and tissue specimen was prepared for histological examination.

Results: The results were analyzed statistically. On the 7th and 14th days, all the experimental groups showed severe inflammatory response with no statistical significance, whereas on the 28th day, the inflammatory response was graded based on the mean value, in which omeprazole showed moderate inflammatory cells followed by TAP. Mild inflammatory response was seen in calcium hydroxide and origanum vulgarae, showing no statistical significance.

Conclusion: Within the limitation of the study, severe inflammatory response was reported on the 7th and 14th days in all experimental groups, whereas on 28th day, there was a moderate inflammatory response seen in omeprazole, followed by TAP. Calcium hydroxide and origanum vulgarae showed a mild inflammatory response.

Keywords: Calcium hydroxide; omeprazole; origanum vulgarae; triple antibiotic paste

INTRODUCTION
The success of the root canal treatment is not achievable only by chemomechanical preparation alone in case of inflammatory periapical lesion. Ideally, such a situation warrants the need of an intracanal medicament. Although there are criteria required for an ideal intracanal medicament, there are few important properties that a medicament should possess the antimicrobial property and should be biocompatible.\(^{[1]}\)

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Due to its superior properties, calcium hydroxide has been conventionally used as an intracanal medicament. When the intracanal medicament has been introduced into the canal and comes into the contact with the periapical tissues, there are possibilities to induce periapical inflammation.\(^{[2]}\) This might be due to the commercial incorporation of certain substances that affects the biocompatibility.

Triple antibiotic paste (TAP) used for the elimination of bacteria was first described by Hoshino et al. in 1996. Since then, several reports showed the ability of TAP in removing microbiota from the root canal system. In the later years, Ruparel et al.\(^{[3]}\) reported TAP to have a detrimental effect on the survival of stem cells of the apical papilla. Keeping this as a standpoint, TAP has been included in the present study to evaluate the tissue inflammatory response.

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In routine endodontic practice, mostly, the above two agents have been used as an intracanal medicament. The use of omeprazole, a proton-pump inhibitor, is very limited to be used as a potential medicament during root canal treatment.\textsuperscript{4,5} It inhibits neutrophil migration and the formation of reactive oxygen species. It also has the ability to inhibit the resorptive process.

Due to the increase in the incidence of antimicrobial resistance, the use of herbal agents as an intracanal medicament has come into play in endodontics. There is no research on the usage of origanum vulgare leaf in endodontics. This compound possesses antimicrobial and antioxidant property. Apart from that, our molecular study (unpublished) reported that it can inhibit osteoclastogenesis.

Till date, there are no reports on the inflammatory tissue response of omeprazole and origanum vulgare. Therefore, the authors of the present study decided to evaluate the tissue response to assess the potential ability to use as an intracanal medicament.

**MATERIALS AND METHODS**

The study was conducted at Saveetha Dental College, BRULAC, Chennai, after obtaining approval from the Institutional Ethical Committee for Animal Research (BRULAC/SIMATS/IAEC/07-2019/030). Seventy Five male Wistar rats aged 8 weeks weighing 200 ± 15 g were included in the study. The rats were randomly divided into one control group and four experimental groups.

- **Group 1:** Control ($n = 15$)
- **Group 2:** 0.25 mg origanum vulgare/1 ml of cellulose ($n = 15$)
- **Group 3:** 2 mg omeprazole/1 ml of distilled water ($n = 15$)
- **Group 4:** 1 mg TAP/1 ml of distilled water ($n = 15$)
- **Group 5:** 16 mg calcium hydroxide/1 ml of distilled water ($n = 15$).

The rats were anesthetized with intraperitoneal injection of ketamine hydrochloride (47.5 mg/kg) and xylazine (10 mg/kg). Using povidone-iodine solution (Betadine, Win Medicare Pvt., Ltd), the back of the animal was disinfected after it being shaved. The incision was made in the midline, parallel to the border of the mandible, and the periapical region was accessed. A trough measuring $1 \times 1$ mm was made in the periapical bone using carbide burs under continuous saline irrigation. Medicament of the respective groups was placed into the trough made and the edges of the skin were stitched with surgical nylon sutures and disinfection was again done using povidone-iodine solution. After the experimental stage, the animals were kept in cages according to the group and placed in isolation and were maintained on a balanced diet.

At the time interval of 7, 14, and 28 days, the animals were euthanized with anesthetic overdose. The tissue surrounding the placement of the medicament was sectioned and also the normal tissue was stored in 10% buffered formalin which was kept for 48 h for histopathological examination.

Based on the scoring criteria, the tissue inflammatory response was graded as mild, moderate, and severe: Grade 0: no inflammatory response, Grade 1: mild inflammation, Grade 2: moderate inflammation, and Grade 3: severe inflammation. The gradings were recorded and the results were statistically analyzed.

**Histopathological examination**

Figure 1 shows the inflammatory response of different intracanal medicaments at three different time periods under $\times 40$ magnification. The overall grading analysis for inflammation at different time periods is represented in Graph 1.

**Statistical analysis**

The collected data were analyzed using IBM. SPSS statistics software 23.0 Version (IBM SPSS Predictive Analytics Community, Armonk, New York). Descriptive statistics mean and standard deviation (SD) were computed for continuous variables. To find the significant difference in the multivariate analysis, the Kruskal–Walli’s test followed...
RESULTS

Descriptive statistics with mean and SD was performed for all the groups. A comparison was done between groups at different time periods. All the experimental groups showed severe inflammatory responses on the 7th and 14th days. There was no statistical significance. On the 28th day, there was a statistically significant difference between Group I and III \( (P = 0.033) \). Furthermore, there was a statistically significant difference between Group III and V \( (P = 0.026) \).

Within the group, comparison was made on the 7th, 14th, and 28th days. The overall result was statistically significant difference \( (P = 0.005) \). Wilcoxon signed-rank test was used to assess the significance within the group. The overall result showed a statistically significant difference \( (P \leq 0.01) \).

It was found that on the 7th and 14th days, there was no statistically significant difference in inflammatory response. On the 28th day, the inflammatory response was graded based on the mean value in which omeprazole showed moderate inflammatory cells followed by TAP. Calcium hydroxide and origanum vulgare showed a mild inflammatory response.

DISCUSSION

In a routine endodontic practice, depending on the clinical condition and several other influencing factors, the decision has to be taken whether to perform single or multivisit root canal treatment. It is not reliable to depend on chemo-mechanical preparation and activation devices alone to achieve a three-dimensional disinfection of the root canal system. Emphasize should be made on the role of intracanal medicament.

Calcium hydroxide and TAP have been commonly employed as an intracanal medicament depending on the clinical scenario. This study was conducted to evaluate the inflammatory response origanum vulgare and omeprazole in a rat model. The concentration of origanum vulgare used in this study was based on the results of our pilot study after performing its antimicrobial efficiency. Based on the antimicrobial reports of the previous study, the concentration of other medicaments was chosen.\(^{[4,6]}\)

The most reliable test to assess the biocompatibility of any medicament includes the in vivo implantation of material.\(^{[7]}\) In the present study, the medicament was not placed inside the polyethylene tubes, it was placed directly in contact with the periapical tissues. Due to similar responses in genetic patterns, Wistar rats were employed in the present study.\(^{[8]}\) On the 7th, 14th, and 28th days, the tissue response was evaluated for histological response. In the present study, a qualified examiner interpreted the inflammatory tissue response based on the presence of macrophage, plasma cells, and other markers of inflammation.\(^{[9]}\)

Origanum vulgare (Lamiaceae family) is an endemic plant found in India, the Southern part of Iran and the Mediterranean region, which has been used traditionally for the antiseptic purpose. Monoterpene hydrocarbon and oxygenated monoterpenes are compounds present in Origanum vulgare. The major constituent of the origanum vulgare is carvacrol (41%). Oxygenated monoterpenes carvacrol causes inhibition of Adenosine Triphosphatase (ATPase) activity and increase the nonselective permeability of bacterial cell membrane. It causes inhibition of the microbial colonization, thereby making the microbes more sensitive.\(^{[10]}\) Apart from antibacterial property, carvacrol has also shown to be a strong antioxidant.\(^{[11,12]}\) High monoterpenic hydrocarbons, such as \( \alpha \)-pinene and linalool, which were found in oregano essential oil in the current study, have been extensively proven as strong antimicrobial substances.\(^{[13]}\)

The results of this study report that the tissue inflammatory response of origanum vulgare on the 14th and 28th days was much reduced when compared with TAP and omeprazole. On comparing calcium hydroxide with origanum vulgare, the results showed that there was no statistically difference in inflammatory response on the 14th and 28th days. As this is the first study on the usage of origanum vulgare in an animal model, there is no previous report to compare the tissue inflammatory response.

Gomes-Filho et al.\(^{[14]}\) evaluated the tissue response of rats to calcium hydroxide and TAP, both medicaments demonstrated moderate inflammation after 7–15 days, similar to the control group. This inflammation subsided after 30 days and calcium hydroxide and TAP were reported to be biocompatible.

In the previous study,\(^{[15]}\) biocompatibility of calcium hydroxide and TAP was evaluated at different concentrations.
It was found that calcium hydroxide to be more cytotoxic at 1 and 10 mg/mL when compared to TAP. The results of this study were contradictory to the result of the present study. Furthermore, several other studies reported calcium hydroxide to exert cytotoxic effects on different types of fibroblast cells.[16-19] Literature search revealed that cytotoxicity of calcium hydroxide is dependent on the concentration and also based on the vehicle used.

The results of the present study showed calcium hydroxide to produce the least inflammatory response on the 14th and 28th days compared to TAP and omeprazole. However, there was no statistically significant difference between origanum vulgare and calcium hydroxide. The inflammatory response induced by calcium hydroxide differs with the use of different vehicles.[20] Previous studies show controversial reports on the usage of propylene glycol as it produced an increased inflammatory response.[21-23] Other studies reported the use of different vehicles which showed no increase in inflammatory cells.[24,25] In the present study, distilled water was used as a vehicle for calcium hydroxide. This can also be a reason for reduced inflammatory cells in calcium hydroxide.

Wagner et al.[26] were the first person to suggest the use of omeprazole as an antibacterial agent against Enterococcus faecalis. Their study concluded omeprazole to be superior to calcium hydroxide. The present study reports that tissue inflammatory response of omeprazole was found to be higher than TAP, calcium hydroxide, and origanum vulgare. The results of origanum vulgarae and omeprazole cannot be compared with previous studies as no reports are existing.

In the present study, all the tested medicaments showed a severe inflammatory response on the 7th day. This might be due to aggression suffered during the surgical process. A material is said to be biocompatible if only there is a significant decrease in inflammatory cells with time.[26]

Clinical implication
From the present study, it was evident that origanum vulgarae and calcium hydroxide can be used as an intracanal medicament as the inflammatory response was decreasing over a period of time. Further clinical trials are required to evaluate the healing efficiency of origanum vulgarae and its retrievable ability as an intracanal medicament.

CONCLUSION
Within the limitation of the study, it can be concluded that severe inflammatory response was reported on the 7th and 14th days in all experimental groups, whereas on the 28th day, there was a moderate inflammatory response seen in omeprazole, followed by TAP. Calcium hydroxide and origanum vulgarae showed a mild inflammatory response.

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Conflicts of interest
There are no conflicts of interest.

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