Propolis and Commonly Used Intracanal Irrigants. Comparative Evaluation of Inflammatory Potential

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Aim: *The present study evaluated the inflammatory/ irritant potential of propolis in comparison with commonly used intracanal irrigants such as chlorhexidine and calcium hydroxide, with normal saline solution as control using an animal (Wistar rats) model.* ***Method****: 2% Evans blue was intravenously injected into the lateral caudal vein. 0.1 ml each of the test solutions was intradermally injected into the experimental sites designed on their shaved backs. The animals were then sacrificed after 1½ and 3 hours respectively. Each piece of skin containing the injected solution was excised, immersed in 4ml formamide and incubated at 45°C for 72 hours. After filtration with glass wool, optical density(OD) was measured using a spectro¬photometer and analyzed statistically.* ***Results****: At 620 nm irrespective of time, the mean optical density with Calcium Hydroxide was found to be maximum (0.197±0.095) while that with DMSO Propolis was found to be minimum (0.070±0.016). Both at 90 min and 180 min, the mean optical density with Calcium Hydroxide was found to be maximum.* ***Conclusions****: On short term evaluation, maximum inflammation was seen with calcium hydroxide followed by chlorhexidine and DMSO extract of propolis. Minimum inflammation was seen with sterile physiologic saline. With progress of time, maximum inflammation was seen with calcium hydroxide followed by chlorhexidine and DMSO extract of propolis which was non-significant.*

Keywords: *propolis, intracanal irrigants, inflammatory potential, optical density*

INTRODUCTION

The first objective of an ideal biomechanical preparation is achieved by a double-pronged attack i.e. skillful instrumentation coupled with liberal irrigation. This aims to eliminate most of the bacterial contaminants of the canal as well as the necrotic debris and dentin.[1] The pulp chambers and root canals of untreated teeth that need endodontic treatment are filled with gelatinous masses of necrotic pulp remnants and tissue fluid contaminated with numerous bacteria. Instruments thrust into these canals are likely to force such noxious materials through the apical foramen with resulting periradicular inflammation and/or infection. Therefore, the canals are irrigated with a solution capable of disinfecting them and dissolving organic matter before and at frequent intervals during instrumentation. In addition to the debriding action, irrigation serves the purpose of facilitating instrumentation by lubricating canal walls and by floating out dentinal fillings.

A wide variety of irrigating agents are available. It is recommended that the practitioner understands the potential advantages and disadvantages of the agent to be used. Ironically speaking, the only concern while irrigating the infected root canals is: Antimicrobial nature of the medicament. What majority of pediatric dentists overlook while using an endodontic irrigant is its inherent irritant/ inflammatory potential, which must be a factor of equally grave concern as its antimicrobial action. This fact is highlighted during the course of studying propolis in the second part of our research trial.

One such agent from holistic medicine is currently being incorporated into dentistry: Bee Propolis, a resin secreted by the same to cover their hives. Bretz *et al* (1998) determined the antimicrobial and healing potential of propolis and calcium hydroxide on direct dental pulp exposures in rats and concluded that propolis maintained a relatively low microbial cell population as well as in stimulated reparative dentin formation with less inflammation.[2]

Furthermore, Öztürk *et al* conducted a study to compare the anti-inflammatory effect of propolis and corticosteroids where Propolis showed significant anti-inflammatory effects on Endotoxin-Induced Uveitis (EIU) in rabbits.[3] Da Silva *et al* evaluated the irritant potential of propolis, *Casearia sylvestris* and Otosporin using Wistar rats.[4] Propolis was the least irritant solution and was considered apt for use during endodontic procedures as an irrigant.

Henceforth, the objective of our present study was to evaluate the inflammatory/ irritant potential of 4% dimethyl sulfoxide extract

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Table 1. Intergroup Comparison

S.No.	Comparison	"t"	"p"
1.	A vs B	-2.080	0.042
2.	A vs C	-6.994	<0.001
3.	A vs D	0.586	0.560
4.	B vs C	-1.731	0.089
5.	B vs D	2.179	0.033
6.	C vs D	7.223	<0.001

of propolis in comparison with commonly used intracanal irrigants like 2% chlorhexidine and 4% calcium hydroxide with normal saline solution as control using an animal (Wistar rats) model. This was done using the physicochemical method for quantification of the enhanced vascular permeability (Evans blue test).[5]

MATERIALS AND METHOD

The study was carried out in The Department of Toxicology, Central Drug Research Institute, Lucknow, Uttar Pradesh, India after gaining approval from Institutional Animal Ethics Committee (IAEC) (68 / 09 / Toxi / IAEC 27-04-2009).

The sample population consisted of thirty wistar rats (*Rattus norvegicus*), irrespective of sex, weighing approximately 275 grams + 20% of mean weight. The temperature of the experimental animal room was maintained at 22°C (+3°C) with a relative humidity of 30% - 70%. Artificial lighting, with the sequence of 12 hrs light and 12 hrs dark was followed. A concentrational laboratory diet was administered with an unlimited supply of water. Prior to the experiment, animals were housed in these conditions for 6–8 days to become acclimatized.

The rats were first anesthetized using i/m Ketamine (Aneket, Mumbai, India) (0.2-0.4 mg/kg body weight). Their backs were shaved and four experimental sites were designated for an intradermal injection of each test solution. Their tails were washed and dried in order to facilitate the injection of 2% Evans blue administered intravenously in the lateral caudal vein/ tail vein.

Immediately after this, 0.1 ml of each test solution namely Sterile Physiologic Saline (Group A), 2% Chlorhexidine (Group B), 4% Calcium Hydroxide (Group C) and 4% Dimethyl Sulfoxide (DMSO) extract of Propolis (Group D), was injected intradermally into the experimental sites following a rotational system for ease of recognition of experimental sites (Figure 1).

Subsequently fifteen wistar rats sacrificed at 1½ hour interval and 15 were sacrificed at 3 hour interval. Evaluation of the inflammatory exudate was performed after 1½ and 3 hour interval. For the same, dorsal skin was first dissected and skin lesions were excised out. Each piece of skin containing the lesion was cut into small pieces and added to 4ml of formamide. The formamide containing the tissue was then homogenized using a tissue homogenizer. This was followed by incubation of the solutions for 72 hrs at 45°C, ensuring maximal extraction of the Evans Blue Dye. After incubation at the specified temperature and time, the homogenate was filtered using Glass Wool to remove all particulate matter. The clear fluid thus obtained was subjected to a Spectrophotometer (Bio-tek®) for measurement of Optical Density at 620 nm wavelength.

This method as proposed by Udaka *et al* has often been employed in an attempt to quantify the irritant potential of several substances injected intradermally (or inoculated *in vivo*) and also to evaluate the effectiveness of anti-inflammatory drugs.[5] We used the tissue concentration of Evans blue (EB) as a marker for plasma extravasation. After the dye is injected intravenously, complete and tight binding of the dye to serum albumin occurs leading to the formation of a protein-bound dye complex which gets extravasated at the site of irritation/inflammation. Complete extraction of this complex by formamide has well been validated in the past; thus quantifying and demonstrating altered vascular permeability.[4,5] This method analyzes the plasma exudate produced after an increase in vascular permeability that can be inferred by means of spectrophotometric measurement of Evans blue dye at 620 nm wavelength.

The statistical analysis was done using SPSS (Statistical Package for Social Sciences) Version 15.0 statistical Analysis Software (SPSS Inc., Chicago, IL, USA).

RESULTS

At 620 nm irrespective of time, the mean optical density of Group C was found to be maximum (0.197±0.095) while that of Group D was found to be minimum (0.070±0.016).

Analysis of variance revealed statistically significant differences amongst the groups (F=11.694; p<0.001).

Intergroup comparison (Table 1) revealed a statistically significant difference between the groups when Group A was compared with Group C (p<0.001) and Group B (p=0.042). A statistically significant difference in mean OD was seen between Group B and Group D (p=0.033) and also between Group C with Group D.

Thus the findings reveal that at 620 nm wavelength irrespective of time taken, the mean OD of Group C was maximum while that of Group D was minimum, demarcated by statistically significant intergroup differences.

At 1½ hour interval, the mean optical density of Group C was found to be maximum (0.206±0.085) while that of Group A was found to be minimum (0.064±0.018) (Table 2, Figure 1). On the basis of observations made the following order of increase in optical density was seen:

Table 2. Comparison of Optical Density in different groups at 1 ½ hour interval

Group	N	Mean	SD	SE	95% Confidence Interval for Mean		Min	Max
					Lower Bound	Upper Bound		
A	15	0.064	0.018	0.005	0.055	0.074	0.032	0.101
B	15	0.118	0.066	0.017	0.081	0.154	0.049	0.303
C	15	0.206	0.085	0.022	0.159	0.253	0.055	0.341
D	15	0.065	0.015	0.004	0.057	0.074	0.045	0.088
Total	60	0.113	0.079	0.010	0.093	0.134	0.032	0.341

Table 3. Analysis of Variance of Optical Density in different groups

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.198	3	0.066	21.723	<0.001
Within Groups	0.170	56	0.003		
Total	0.369	59			

Group A ~ Group D < Group B < Group C

Analysis of variance revealed statistically significant differences amongst the groups (F=21.723; p<0.001) (Table 3).

After 3 hours, the mean optical density of Group C was found to be maximum (0.188±0.105) while that of Group D was found to be minimum (0.075±0.015) (Table 4, Figure 2). On the basis of observations made the following order of increase in optical density was seen:

Group A ~ Group B ~ Group D < Group C

Analysis of variance revealed statistically significant differences amongst the groups (F=2.908; p=0.042). (Table 5).

At 620 nm wavelength, a significant increase in mean OD was seen after 3 hours as compared to 1½ hours in Group A. No significant difference in OD was seen at different time intervals in all the three other groups. In all the groups except Group C mean OD after 3 hours was higher as compared to that at 1 ½ hour interval yet the difference was significant statistically only for Group A. In Group C, the mean value after 3 hours was lower as compared to that at 1½ hour interval (Table 6, Figure 3).

DISCUSSION

The second part of the Propolis study was concerned with the evaluation of inflammatory/irritant potential (corresponding directly to optical density) of the four irrigants being used. The evaluation was done as a change in optical density of the affected animal tissue at 620 nm at 1 ½ and 3 hour intervals. The combined results for both time intervals showed the mean value to be maximum for calcium hydroxide followed by chlorhexidine, normal saline and propolis groups respectively.

The results for 1½ hour interval and after 3 hours independently showed variable nature. At 1½ hour interval, the mean inflammatory activity of calcium hydroxide was significantly higher as compared to other groups. On the other hand, after 3 hours, a significant increase in inflammatory activity was observed with calcium hydroxide.

Propolis, a natural product, has been studied widely regarding its wound healing, pain relieving and anti-inflammatory activity. As a matter of fact, propolis is a natural product and has no absolute contraindication.[6,7] However, one of the biggest hurdle in use of propolis as a regular medication is lack of standardization of propolis collection areas, the ways to perform this and solvents used to extract in order to obtain better results. Dobrowolski *et al* have also demonstrated that propolis had an anti-inflammatory effect against acute and chronic models of inflammation.[8] However, Hay *et al* described a case report of a patient who had acute oral mucositis with ulceration as a result of using propolis-containing lozenges; highlighting that allergic reaction to even biogenic or natural products can so occur and must not be neglected by the clinician.[9]

Mirzoeva *et al* proposed the possible mechanism of the therapeutic action of propolis on inflammation *in vivo*.[10] In the present study too Propolis showed significantly lower inflammatory activity as compared to both chlorhexidine and calcium hydroxide. Similar observations were also made by Burdock *et al* [11] and Borrellia *et al*.[12] However, in the study of Bretz *et al* both calcium hydroxide and propolis extract showed equivalent inflammatory activity.[2]

Tanomaru *et al* showed chlorhexidine to have a higher inflammatory activity as compared to calcium hydroxide in an *in vitro* animal model.[13] However, in the current study, at 1 ½ hour interval the results were just the reverse. However, the observations made by Jeansonne *et al,* [14] White *et al* [15] and Leonardo *et al* 1999[16] were that 2% chlorhexidine solution has a wide spectrum of antibacterial effect as well as prolonged residual effect, suggesting its use as an irrigating solution in infected root canals. Furthermore, the use of 2% chlorhexidine has been demonstrated as being irritating to directly exposed pulpal tissue, but not if the pulp has been extirpated and when used as a periodontal irrigant did not cause obvious toxic effects on gingival tissue.[17,18]

Conversely, calcium hydroxide has been reported to have a detrimental effect on periodontal tissues when used as an intracanal medicament during routine endodontic therapy. Blomlof *et al* observed that calcium hydroxide could negatively influence marginal soft tissue healing and suggested the completion of endodontic therapy prior to the removal of cementum as might occur during endodontic-periodontal combined therapy.[19] Contrary to these findings, Holland *et al* observed that periodontal healing associated with infected root canals filled with calcium hydroxide was not hindered 6 months after experimental periodontal surgical injury in dogs.[20]

In the present study, calcium hydroxide showed the maximum inflammatory activity. It would be appropriate to mention here that the inflammatory activity in present study was evaluated only

Table 4. Comparison of Optical Density in different groups at 620 nm after 3 hours

Group	N	Mean	SD	SE	95% Confidence Interval for Mean		Min	Max
					Lower Bound	Upper Bound		
A	15	0.082	0.021	0.005	0.070	0.093	0.056	0.112
B	15	0.155	0.227	0.059	0.029	0.281	0.056	0.970
C	15	0.188	0.105	0.027	0.129	0.246	0.076	0.497
D	15	0.075	0.015	0.004	0.067	0.084	0.047	0.099
Total	60	0.125	0.132	0.017	0.091	0.159	0.047	0.970

Table 5. Analysis of Variance of Optical Density in different groups

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	0.138	3	0.046	2.908	0.042
Within Groups	0.888	56	0.016		
Total	1.026	59			

up to a period of 3 hours whereas in earlier studies these were evaluated for a longer duration of time. In our study we used the spectrophotometric evaluation method which seemed to provide better and accurate results even in shorter duration of time. Calcium hydroxide owing to its strong alkaline nature causes irritation in the adjoining tissue area and hence results in a stronger inflammatory activity as compared to other medicaments being used in the study. The findings during the first part of our study highlighted the utility of propolis as an effective antimicrobial and after demonstration of its least inflammatory/irritant potential during the second part; it can be proposed to be used as an effective intracanal irrigant during endodontic treatment especially in pediatric dental patients. However, considering the variability in its performance related to its different compositions and geographical differences of origin, an organized study to standardize its use is highly recommended.

CONCLUSIONS

On short term evaluation, maximum inflammation was seen with calcium hydroxide followed by chlorhexidine and DMSO extract of propolis. Minimum inflammation was seen with sterile physiologic saline. With progress of time, maximum inflammation was seen with calcium hydroxide followed by chlorhexidine and DMSO extract of propolis which was non-significant.

Henceforth, we can now quite aptly highlight that during endodontic procedures in pediatric dental patients, a fact that is much disregarded or overlooked is the spread of inflammation, which is quite rapid in children. While using an irrigant for desire of disinfecting a root canal, a practitioner often neglects its inherent nature to induce certain degree of inflammation itself which can further lead to worse situations. This implies that an irrigant of choice should not just be the one with optimum antimicrobial properties but also that which induces a minimal degree of periradicular inflammation.

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Table 6. Comparison of Optical Density in different groups at different time intervals at 620 nm wavelength

S.No.	Group	1 ½ hour (n=15)		3 hour (n=15)		"t"	"p"
		Mean	SD	Mean	SD		
1.	A	0.065	0.018	0.082	0.021	-2.458	0.020
2.	B	0.118	0.066	0.155	0.227	-0.615	0.543
3.	C	0.206	0.085	0.188	0.105	0.511	0.613
4.	D	0.065	0.015	0.075	0.015	-1.729	0.095