A clinical efficacy of 30% ethenolic extract of Indian propolis and Recaldent™ in management of dentinal hypersensitivity: A comparative randomized clinical trial

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ABSTRACT

Objective: The aim of this study is to evaluate the efficacy of 30% ethenolic extract of Indian propolis compared with Recaldent™ (casein phosphopeptide-amorphous calcium phosphate) in reduction of dentinal hypersensitivity, a randomized, double-blind, split mouth, controlled clinical trial was conducted among the patients residing in Central Jail.

Materials and Methods: A sample of 73 teeth from 13 patients having at least three teeth with dentinal hypersensitivity (DH) were randomly allocated into three treatment groups: Group A: 30% ethenolic extract of Indian propolis, Group B: Recaldent™, Group C: Sterile water. Verbal rating scale was used to record the degree of hypersensitivity based on patient’s response to tactile and air blast stimuli. The baseline scores were obtained. Each intervention group received applications of their respective agents consecutively on 1st, 7th, 14th, and 21st day. After each application the scores were recorded.

Results: Both the 30% Indian propolis and Recaldent™ showed significant reduction in DH. Conclusion: Recaldent™ was found to be significantly better in reducing the DH compared to propolis and sterile water (P < 0.01).

Key words: Dentin hypersensitivity, Indian propolis, recaldent, split mouth, verbal rating scale

INTRODUCTION

Dentinal hypersensitivity (DH) is defined as “short, sharp pain arising from exposed dentin in response to thermal, tactile, osmotic, or other stimuli and not attributable to any other tooth defect.”[1-3] Dentin is the calcified tissue arranged in parallel tubes (dental tubules) surrounding the dental pulp and it primarily isolates blood vessels and nerves in the pulp from external elements.[1,2] Dentin exposure coupled with shrinkage of the gums or gingiva is known as gingival recession and is the site where DH occurs. Grinding of the teeth, tooth malformations, unilateral chewing, root scraping and planning, and missing teeth are among the causes of dentin exposure and gingival recession.[1-5]

Several theories exist to explain DH; however, the most accepted is the hydrodynamic theory. To date, analysis of most data supports a theory that these stimuli induce fluid flow within dental tubules, which triggers baroreceptors near the pulp, leading to pain.[6] This hydrodynamic theory of pain generation assumes an exposed dentin surface and patent tubules that allow fluid flow to reach the pulp where the baroreceptors reside.[7]

Estimates of DH prevalence vary. According to Kielbassa, 9-30% of the adult population suffers from
DH,[7] while Dowell, et al. estimated that 50% of the general population is affected and 100% of the patients with periodontal conditions have experienced DH at some time.[8]

Panoply of current diagnostic and treatment strategies for DH suggests considerable uncertainty among dental practitioners about how to manage this condition.[9] DH is generally diagnosed through patient interview and clinical examination, detecting an exaggerated pain response to minimal stimuli, even while brushing teeth. DH is classified as mild, moderate, or severe, based on intensity of pain.[3,10]

A variety of treatments have been tried to stop or minimize pain caused by DH. The most widespread treatments involve application of desensitizing agents and other materials such as toothpaste containing strontium salts or potassium salts, high-concentration fluoride varnishes, cyanoacrylate adhesives, and restorative materials on the affected area.[3] Despite the large number of published studies, however, there is still no consensus on which product constitutes the "gold standard" for DH treatment.[3,9]

A material GC Tooth Mousse based on the Recaldent™ is a unique complex containing amorphous calcium phosphate (ACP) and casein phosphopeptide (CPP), derived, from milk casein. The preparation is recommended in hard tissue re-mineralization as well as in DH reduction due to its ability to block opened dentinal tubules.[11,12]

At present, the commonly used desensitizing agents generally are favorable in short-time, while blow the mark in a long-term and hence the development of new desensitizing agents is needed.[5,13] The search for a natural desensitizing agent with long lasting effects has led to the observation that propolis can have promising effects on DH.[14]

Propolis is a naturally-occurring bee product and it was first used as a medicine by the Egyptians and use of it was continued by the Greeks and Romans. It is widely used in homeopathic and herbal practice as an antiseptic, anti-inflammatory, antimycotic, and bacteriostatic agent. The primary constituents of propolis are flavones, flavanones, and flavanols. It is also composed of resin (55%), essential oils and wax (30%) mixed with bee glue “the salivary secretions of bees” and pollen (5%) and other constituents (10%), which are amino acids, and minerals.[15]

There are many clinical applications of propolis in dentistry. To exemplify, a few are-relief from denture ulcerations and stomatitis, halitosis, mouth freshener, periodontal pocket/abscess, mouthwash, cervical, dentinal, and root caries sensitivity.[14-18] Some in vitro studies have successfully shown that, propolis has clinically significant effect on reduction of dentin permeability.[9] but to date, there have been very few studies done on desensitizing effect of propolis in vivo.[20]

Hence the aim of the present study is to evaluate the clinical efficacy of 30% ethenolic extract of Indian propolis compared with Recaldent™ in reduction of DH.

MATERIALS AND METHODS

Study design and settings
A randomized, double blind, split mouth, clinical trial was conducted among patients of Central Jail, Bhopal, who fulfilled with following selection criteria.

Selection criteria
Patient inclusion criteria for those fulfilling the preliminary screening
- Patients aged 18-40 years, residing in Central Jail Bhopal.
- Patients having at least 3 caries-free cervical lesions with DH.
- The loss of dentin should be less than 2 mm deep as per Tooth Wear Index (TWI) code ‘2’ and ‘3’ (defect up to 2 mm thick/loss of enamel and substantial loss of dentin, but not exposing pulp or secondary dentin).[21]
- Patients with adequate oral hygiene and only those who are willing to participate in the study.

Patient exclusion criteria
- Patients with history of any systemic illness and/or psychological diseases, and previous hospitalization.
- Teeth having dental caries, cracks or fractures in the cervical areas of the teeth.
- Teeth with TWI code 0 (no change of contour), 1 (minimal loss of contour) and 4 (defect more than 2 mm deep/pulp exposure, or exposure of secondary dentin).
- Teeth with any extensive or unsatisfactory restorations, prosthesis or orthodontic appliances involving the cervical areas.
- Patients with history of drug addictions.
- Patients using analgesic and/or anti-inflammatory drugs.
- Patients who failed to give consent.
Withdrawal criteria
Failure to complete follow-up after undergoing initial treatment and giving consent.

Method of collection of data

Ethical approval
The research protocol was approved by the Ethics Committee in People’s Dental Academy, Bhopal. Approval from the higher authorities of the Central Jail, Bhopal was also taken to conduct the trial.

Preliminary screening
A preliminary screening of 100 individuals was carried out at Central Jail premises, Bhopal. A pre-designed dental chart form for each patient was used to record cervical abrasion using TWI (TWI, Smith and Knight, 1984).[21] The TWI is usually assessed on visual examination and it evaluates all the tooth surfaces (i.e., cervical, buccal (labial), lingual, occlusal or incisal). In the present study only the cervical surfaces were evaluated for abrasion and were coded according to criteria for TWI. This modification was carried out to focus on the objectives of the study.

Patients scoring TWI code ‘2’ or ‘3’ and having at least three cervical lesions with DH were eligible to participate in the study. The purpose behind this selection was just to keep the trial conditions as similar as possible among all the study participants.

Informed consent
Participants were informed about the purpose and design of the investigation and signed an appropriate informed consent form.

Sample size
A sample consisted of 13 patients with 74 teeth with DH who had fulfilled the selection criteria. The size of sample was determined by a power calculation based on data from previous trials.[13,22] The power of the sample was 80% for the default significance level (alpha level) at 0.01.

Study duration
The experimental period was for 21 days.

Study procedure
The prospective investigation was randomized, double-blinded, split mouth, and negative controlled. The selection of the patients was based on the clinical examinations and the criteria described previously. Clinical diagnosis was performed by using uniform source of light, provided by a conventional operating dental light system, a mouth mirror, an explorer, and periodontal probe in the dental wing of Central Jail, Bhopal.

A registry of patients who met the selection criteria was created and a Clinical Report Form was prepared for each participant.

Randomization
All the teeth were divided into three treatment groups. A list of selected teeth was prepared and arranged in a sequence. One tooth was randomly selected from the list and remaining additional teeth were selected at evenly spaced interval of 3 units systematically until a desired sample of 25 was obtained for Group A. Similar procedure was employed to select the teeth for Group B and Group C and a desired sample of 25 and 24 were obtained respectively.

• Group A: 30% Indian propolis as a test group (n = 25), (Purchased from Hi-Tech Natural Products, India Ltd.)
• Group B: Recaldent™ (GC Tooth Mousse) is a CPP-ACP, as a positive control (n = 25), (Purchased from GC Corporation, India).
• Group C: Sterile distilled water as a negative control (n = 24).

Blinding
The patients and the examiner who evaluated the effectiveness (other than the operator), were not aware of the type of treatment corresponded to each tooth.

Application procedure
The desensitizing agents were applied by a trained and experienced operator, on days 1st, 7th, 14th and 21st as follows:
• Removal of debris and calculus, if any, around the affected teeth using hand scalers.
• Isolation of the teeth with cotton rolls.
• The tooth surfaces were dried with a cotton pellet and compressed air by using an air syringe for 15 s.
• Propolis extract and a placebo were applied directly on the DH site using a truncated needle and let dried for 60 s. Recaldent™ (CPP-ACP) was applied to the sensitive lesions as recommended by the manufacturer.
• Care was taken to ensure none of the product touched other zones of the oral mucosa.
• Excess was removed by using cotton pellets.

The patients were instructed not to rinse, eat or drink for 30 min after the treatment and avoid using any other professionally or self-applied desensitizing agent in the course of the investigation.
Effectiveness evaluation
The effectiveness evaluation was carried out by a calibrated examiner. The calibration of the examiner was carried out at the department of Public Health Dentistry in Peoples Dental Academy, Bhopal.

Examiner calibration
The examiner was trained and calibrated to record the sensitivity patterns on a group of 10 patients who were diagnosed with DH.

The intra examiner weighted kappa value was calculated using the baseline values for hypersensitivity and reexamining all the patients and was determined to be 0.73.

Each tooth received two stimuli:• Clinical probing (tactile stimulus) and• Air blast (thermal evaporative stimulus).

The probe stimulus was applied under slight manual pressure in the mesiodistal direction on the cervical area of the tooth. The test was repeated 3 times before recording the final score. Air blast was applied with an air syringe for 1-2 s at the distance of 1 cm of the tooth surface to avoid desiccating the dentin surface while the adjacent teeth were protected by the examiner finger.

The degree of hypersensitivity reported by the participant with each stimulus was determined according to the verbal rating scale (VRS) from 0 to 3, in which:
• 0 = No discomfort,
• 1 = Minimum discomfort,
• 2 = Mild discomfort, and
• 3 = Intense discomfort.

The values were collected before the intervention (baseline values) and after each application, on days 1st, 7th, 14th, and 21st respectively. The spilt mouth technique was used to obtain the standardized response from each patient for all three treatment groups.

Evaluation of success/failure
The final criteria for evaluation were:
• Rapid reduction in DH (after 1st and 2nd application),
• Overall reduction in DH (after 4th application) and
• No reduction in DH.

Safety evaluation
Two safety variables were evaluated: irritation and burning sensation in the mucosa next to the treatment site.

Irritation was evaluated on two levels.
• Level 1 = No change in color or texture, and
• Level 2 = change in color or texture.

For burning sensation, the patient was questioned and responses were classified as:
• Level 1 = no burning sensation at treatment site, and
• Level 2 = burning sensation at treatment site.

These evaluations were conducted during follow up visits on days 7th, 14th, and 21st.

Adverse events
Provisions were also made to record AEs, understood as any unfavorable medical event that occurred during the study, not necessarily attributable to the experimental treatment. Such events might include erythema or redness on any part of the skin, stinging, fever, or headache following products application in the mouth. Patients were instructed to go to the clinic immediately if these or any other AE appeared. Provisions were made to withdraw the participants reported with AE. In this study, no participant reported with AE.

Statistical analysis
All the data were entered into a personal computer in a Microsoft excel sheet and the statistical analysis was performed by using SPSS software version 19. The General linear model was used for comparing across the 3 types of treatments. The mean DH score between the three intervention groups at each time interval was compared using two-way analysis of variance (ANOVA). Wherever, ANOVA yielded significant results, Bonferroni test (post hoc) was used for multiple comparisons between three treatments. The comparison of mean scores in each intervention group between baseline and other time intervals was carried out using Friedman’s test. The level of statistical significance was set at 0.01.

RESULTS
A total of 13 male patients with a mean age of 37 years presenting 74 hypersensitive teeth were evaluated in the study. All the teeth were distributed into three treatment groups. Air blast and probing stimuli were used to record the degree of hypersensitivity at baseline and after each application for a period of 3 weeks using VRS.

For air blast and probing stimulus, 78% and 92% overall reduction in DH were observed in both 30% Indian propolis and Recaldent groups respectively. Rapid
reduction in DH was observed in both 30% Indian propolis (78%) and Recaldent™ (81%) groups. Majority of the teeth with DH in sterile water group did not show any change (65%) from the baseline [Table 1 and Figure 1].

The mean differences among all the three treatment groups were significant for air blast stimulus. The greater reduction in DH was seen in Recaldent™ group followed by 30% Indian propolis group [Table 2 and Figure 2].

The mean differences among two treatment groups were significant for probing stimulus. The greater reduction in DH was seen in Recaldent™ group followed by 30% Indian propolis group [Table 3 and Figure 3].

There was a significant reduction in DH for all the treatment groups after each application for air blast. While for probing stimulus, a significant reduction was observed in both Recaldent™ group and 30% Indian propolis group [Table 4].

Safety evaluation
- No burning sensation or irritation of mucosa was recorded during application of different test groups.

### Table 1: Overall percentage reduction in dentinal hypersensitivity for both air blast and probing stimulus

| Group                  | Rapid reduction (after 2nd application) N (%) | Overall reduction (after final application) N (%) | No reduction (after final application) N (%) |
|------------------------|-----------------------------------------------|-------------------------------------------------|---------------------------------------------|
| 30% Indian propolis   | 19.5 (78)                                     | 19.5 (78)                                       | 5.5 (22)                                   |
| Recaldent             | 19.5 (81.2)                                   | 22 (91.6)                                       | 2 (8.4)                                    |
| Sterile water         | 7 (29)                                        | 8.5 (35.4)                                      | 15.5 (64.5)                                |

### Table 2: Comparison of mean difference between different treatment groups for air blast stimulus

| Group (I)         | Group (H)         | Mean difference | Standard error | Significance (P value) |
|-------------------|-------------------|-----------------|----------------|------------------------|
| 30% Indian propolis | Recaldent         | 0.5110*         | 0.1491         | 0.005                  |
| Sterile water     | 0.4473            | 0.1491          | 0.015          |
| Recaldent         | 0.5110*           | 0.1491          | 0.005          |
| Sterile water     | 0.9583*           | 0.1506          | 0              |
| 30% Indian propolis | Recaldent         | 0.4473          | 0.1491          | 0.015                  |
| Sterile water     | 0.9583*           | 0.1506          | 0              |

*P<0.01, Group (I) and Group (H) are used for denoting two groups as interpreted by SPSS software

### Table 3: Comparison of mean difference between different treatment groups for probing stimulus

| Group (I)         | Group (H)         | Mean difference | Standard error | Significance (P value) |
|-------------------|-------------------|-----------------|----------------|------------------------|
| 30% Indian propolis | Recaldent         | 0.1337          | 0.1579         | 1                      |
| Sterile water     | 0.6515*           | 0.1596          | 0.001          |
| Recaldent         | 0.1337            | 0.1579          | 1              |
| Sterile water     | 0.7851*           | 0.1612          | 0              |
| 30% Indian propolis | Recaldent         | 0.6515*         | 0.1596          | 0.001                  |
| Sterile water     | 0.7851*           | 0.1612          | 0              |

*P<0.01, Group (I) and Group (H) are used for denoting two groups as interpreted by SPSS software

### Table 4: Differences in mean ranks in different groups at baseline and after each application for both air blast and probing stimulus

| Treatment group       | Stimulus applied | Baseline 1st application | 2nd application | 3rd application | 4th application | P value |
|-----------------------|------------------|--------------------------|-----------------|-----------------|----------------|---------|
| 30% Indian propolis   | A.B.             | 4.66                     | 2.96            | 2.44            | 2.34           | 2.6     | 0*      |
|                       | P.S.             | 4.3                      | 2.94            | 2.58            | 2.66           | 2.52    | 0*      |
| Recaldent             | A.B.             | 4.83                     | 3.19            | 2.65            | 2.17           | 2.17    | 0*      |
|                       | P.S.             | 4.4                      | 3.31            | 3.19            | 2.17           | 1.94    | 0*      |
| Sterile water         | A.B.             | 3.44                     | 3.29            | 3.38            | 2.19           | 2.71    | 0.002*  |
|                       | P.S.             | 3.39                     | 2.78            | 2.85            | 2.72           | 3.26    | 0.308   |

A.B.: Air blast stimulus, P.S.: Probing stimulus, *P<0.01
No adverse reactions occurred during the trial.

Similarly, no any other adverse reactions (AE) were recorded during the investigation period.

**DISCUSSION**

DH is a very common painful sensation, which is rather difficult to treat in spite of the availability of various treatment options. [3,25] Applying a desensitizing agent is therefore, consistent with these types of DH treatment. Furthermore, Addy’s suggestion that coating dentinal tubules is effective in over 95% of cases, [1] coincides with the results of our study.

Valid comparison could not be made with other studies since the present study was the pioneering randomized, double-blind, negative controlled clinical trial that compared the efficacy of 30% ethenolic extract of Indian propolis with CPP-ACP containing desensitizing agent, i.e., Recaldent™ in the treatment of DH. Nevertheless, a sincere attempt has been carried out to compare the present study results with similar studies.

The present study had enough statistical power (80%). Which justified the sample size (a total of 74 teeth) and addresses the aims of the study?

Distribution of DH according to severity observed in our study is consistent with Kielbassa’s observation that moderate DH is more prevalent than severe or mild varieties. [26] A mean age of 37 years in the study sample coincides with data reported by Cummins indicating that DH affects primarily adults aged 20-50, with a prevalence of 15-20%. [27]

It is generally recommended that more than one stimulus should be used in clinical studies of DH. This would enhance the measurement of sensitivity. [28] The measurement of hypersensitivity has been primarily evaluated by tactile (probing), air blast from the dental unit air syringe, and thermal stimulus. The stimuli used in our study to evaluate the DH were air blast and probing (where an explorer is passed over the sensitive lesion) stimulus. Ide, Walters, Tarbet and Sowinski et al. and have reported air blast and tactile (probing) stimulus to be the accurate methods for the examination of hypersensitivity levels. [28,29] These stimuli have a good correlation to the hypersensitivity symptoms encountered in daily life. [29]

Attempts to translate subjective feedback to objective data for research purposes have involved both one-dimensional and multidimensional pain measurement systems (Flaherty 1996). One of the most common one-dimensional method is the verbal rating scale and it is widely used in clinical research to assess intensity of acute pain. [22]

In this study, agents, i.e. 30% ethenolic extract of Indian propolis, Recaldent™ effectively reduced the DH. Pain decreased from severe to slight or moderate during various applications of 30% ethenolic extract of Indian propolis and Recaldent™.

Scientific researchers starting in 1960, [30] confirm that folk tradition has known about beneficial aspects of propolis 1000 years ago. It is dispensed in various forms, mouthwash, lozenges, wine, cake, powder,
In 1999, Mahmoud et al. conducted a pioneer study on the effect of propolis on DH in vivo. In this study, propolis was applied twice daily on teeth with hypersensitivity. It was concluded that propolis had a positive effect in the control of DH. In our study, it is seen that there is a significant reduction in the severity of DH among the teeth assigned in 30% propolis group. Its action was slightly slower as compared to Recaldent™ but still the number of teeth with mild pain increased markedly. This could be attributed to high content of flavanoids in propolis which produces the occluding effect. Flavanoids may be able to suppress the information of free radicals by binding heavy metals in ions, which are known to catalyze many processes leading to the appearance of full radicals.

Recaldent™ (GC tooth mousse) was developed by Prof. Reynolds at the University of Melbourne in 1998. It contains CPP and ACP. CPP stabilizes ACP and forms nano complexes with ACP at the tooth surface thereby providing a reservoir of calcium and phosphate ions, which favors mineralization. CPP also buffers the pH of plaque, depresses demineralization and enhances re-mineralization, which also results in anti-cariogenic property. In our study, G.C. tooth mousse was the most effective among the test groups (P < 0.01). The initial observation of the medicine reveals that its action is most effective in the 1st day of application. In the first 2 weeks, 81.2% teeth showed rapid reduction while the overall reduction in DH after 4th application was 91.6%. Perhaps, in order to increase its desensitizing effect, it has been recommended that, the application should be repeated in intervals shorter than 7 days.

The sterile water, which was used as a negative control in our study showed the least (35.4%) reduction in DH. This slight reduction in DH may be attributed to placebo effect and participation bias. The placebo effect is commonly considered as a response to medical intervention that results from the intervention itself and not from any particular mechanism of action.

Several treatment modalities and agents have been used in the management and resolution of dentin hypersensitivity, but their efficacy has varied from one study to another and it is not yet established in the literature. Further, research is needed to clarify the mechanisms and etiology of this uncomfortable clinical condition.

Dentin hypersensitivity studies are subject based. Successful management of DH requires more research into factors such as bad hygiene, diet, lifestyle, salivary flow/content, and other customs. Correcting the factors, which have led to sensitivity in the first place alone can prevent recurrence. It is desirable to develop novel agents that are capable of more effective and lasting tubule occlusion such as methods that mimic or harness the natural defense reactions of the dentin-pulp complex. Furthermore, expanding the use of propolis for DH treatment in dental clinics will help corroborate its effectiveness and safety may result in this product becoming the treatment of choice for moderate and mild DH.

LIMITATIONS OF THE STUDY

- No females participated in the study.
- Although the sample size was calculated to meet the objectives of the present investigation, further clinical studies involving a larger number of patients to evaluate long-term effects of DH treatment with propolis are recommended.

CONCLUSION

Within the parameters of this study on comparison of the clinical efficacy of propolis, CPP-ACP containing Recaldent™ and sterile water in treating DH, the following conclusions were drawn.

- Recaldent™ was the most effective among all three treatment groups followed by 30% ethenolic extract of Indian propolis.
- They have not only shown a rapid reduction in dentinal hypersensitivity, but have also shown a highest patient satisfaction without any side-effect.

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