ORIGINAL RESEARCH

Comparative Evaluation of Ozonoid Olive Oil and Calcium Hydroxide as an Indirect Pulp Capping Agent in Primary Mandibular Second Molar: A Randomized Controlled Trial

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Abstract

Aim: The aim of this study was to evaluate clinical and radiographical success of ozonoid olive oil as an indirect pulp capping (IPC) agent in primary mandibular second molar when compared to calcium hydroxide and to evaluate the antimicrobial efficacy of ozonoid olive oil on *Streptococcus mutans* and *Lactobacilli*.

Materials and methods: A split-mouth randomized controlled trial was conducted on 30 primary mandibular second molars in 15 children of age 5–9 years with deep dentinal carious lesion. Teeth were randomly allocated to two groups of 15 each. After achieving local anesthesia and rubber dam isolation, an IPC procedure was performed using ozonoid olive oil in group I and calcium hydroxide in group II. Teeth were evaluated clinically and radiographically at 6 and 12 months of follow-up for success or failure of IPC. The bacterial counts of *S. mutans* and *Lactobacilli* were measured before and after application of ozonoid olive oil for 60 seconds on dentinal tissue in group I and recorded as colony-forming units per mL (CFU/mL).

Results: There were no statistically significant differences found between the materials used for IPC ($p > 0.05$). About 93.33% and 100% clinical and radiographical success rates were seen in group I and group II, respectively. Statistically significant differences were observed for bacterial reduction after the application of ozonoid olive oil ($p < 0.05$) for both the microorganisms.

Conclusion: The results of this study showed that the success of IPC is independent of capping material. Ozonoid olive oil, an antimicrobial agent, can also be used for IPC. The success of the IPC procedure depends on a reduction in the bacterial count and sealing of the tooth with hermetic restoration. More clinical studies with a larger sample size and longer follow-up duration are required for understanding the efficacy of this material.

Clinical significance: Ozonoid olive oil can be used as an IPC agent in primary molars and also for a bacterial reduction in dentinal caries.

Keywords: Calcium hydroxide, Indirect pulp capping, Ozonoid olive oil, Primary molars, Split-mouth randomized controlled trial.

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Introduction

Dental caries remain the most common oral health problem affecting mankind. The prevalence of childhood caries is 49.5% in India. It is a multifactorial disease that is characterized by local destruction of the hard tissue of the tooth that involves an interplay of four factors: tooth, saliva, microflora, and diet.[1] Bacteria, predominantly *Streptococcus mutans* and *Lactobacilli,* play an important role in dental caries.[1]

Due to the uncooperative behavior of children, management of deep carious primary molar is sometimes a challenging procedure for the clinician. The current approaches for the management of deep carious lesions in primary teeth without any signs or symptoms indicative of irreversible pulpitis are indirect pulp therapy (IPT) and pulpotomy.[2] Indirect pulp therapy is a less invasive procedure than pulpotomy. The rationale of IPT is to arrest the carious process by decreasing bacterial count and provide conditions that form reactionary dentine beneath the affected dentine and cause remineralization of remaining carious dentine to promote pulpal healing that preserve or maintain the vitality of pulp tissue.[2]

Various materials have been used for IPT in primary teeth, such as calcium hydroxide,[3–5] dentin bonding agents,[4,6] mineral trioxide aggregate (MTA),[5] glass ionomer cement,[7] zinc oxide and eugenol,[8] calcium silicate,[9] Portland cement,[10] or biodentine.[11] Currently, calcium hydroxide, mineral trioxide aggregate, and biodentine are used for IPT in primary and permanent teeth. However, these materials have some disadvantages. It has been reported that 89% of dentin bridges formed by calcium hydroxide contained tunnel defects. The tunnel defects fail to provide a hermetic seal to the underlying pulp against recurring infection

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due to microleakage. Also, calcium hydroxide has a poor adhesive capacity to the dentinal wall. Mineral trioxide aggregate has a long setting time, high cost, and potential for discoloration. The main disadvantages of biodentine are its setting time, material cost, and it requires special equipment for mixing. Though biocompatible materials are indicated over the remaining demineralized tissue, inactivation of the active carious lesion has also been shown by clinical, radiographical, and microbial studies[12–14] even when inert materials such as wax or gutta-percha were used as an IPC materials. The main requisite of the material is to completely seal the involved dentin from the oral environment avoiding nutrient supply to the microorganisms, thereby compromising their metabolism.[15,16]

In recent years, in dentistry, new methods like ozone therapy have become popular for the management of dental caries.[17] Ozone molecule is a highly unstable form of oxygen and rich in energy. It is a strong oxidizing agent for cell walls and cytoplasmic membranes of microorganisms and is thus considered as one of the best bactericidal, antiviral, and antifungal agents. In literature, it is documented that ozone can be used in various forms. Ozone has strong antimicrobial property, and its dentine regenerative property is also been observed. The mechanism of action of ozone is that it releases nascent oxygen when applied to the tissue, thus destroying microorganisms. Ozone can be applied to oral tissue in three forms: Ozonated water, ozonated or ozonoid oil, and ozone gas.[18] Ozonated water and ozonoid olive oil have the capacity to entrap and then release ozone. Ozonoid olive oil is reservoir of ozone that slowly releases it. It is prepared by mixing ozone gas to olive oil by a process called ozonation. Thus, the ozonoid olive oil contains free ozone in higher concentrations. Owing to its strong antimicrobial properties, which disrupts bacterial cell membranes and causes bacteria to die, resulting in a decrease in bacterial count.[19] Laboratory studies have shown that ozone significantly reduces the number of microorganisms in carious dentin in a very short period of time and improves dentine remineralization.[20] In the past decade, various clinical studies[21–25] have proved that ozone therapy can be used to treat deep dentinal carious lesions. In these clinical studies, ozone was used in gaseous form which required special equipment to deliver it on the dentinal tissue. In this study, the ozone was used in oil form due to its ease of application to the dentinal tissue which did not require special equipment.

Therefore, the present clinical, radiographical, and microbiological study was planned to evaluate the efficacy of ozonoid olive oil as an IPC agent in primary molars.

Materials and Methods

This split-mouth randomized controlled trial was conducted on 30 primary mandibular second molars in 15 children aged 5–9 years with deep dentinal carious lesions after obtaining written consent from the parents. The study was approved by the Institutional Ethical Committee, in accordance with 1975, Helsinki declaration (IEC/01/013 dated: 09/10/2019) and was registered in the clinical trial registry – India (CTRI/2020/03/023763).

Sample size of 12 per group was calculated using the formula $n = 2(Z\alpha + Z\beta)^2\ S^2/d^2$; where $Z\alpha = 1.96$ at α-error of 5%; $Z\beta = 1.26$ at β-error of 10%. Finally, 15 samples per group were taken to compensate for the 25% loss to follow-up. Thus, 30 teeth from 15 children were selected for the study which was divided into two groups: Group I–ozonoid olive oil (15 teeth) and group II–calcium hydroxide (15 teeth).

Randomization and Blinding

Initially, the participants for this study were selected by convenience sampling technique according to inclusion and exclusion criteria. The inclusion and exclusion criteria are mentioned in Table 1. Randomization of samples was done using a computerized generated sequence method. The tooth was then allocated to either group I or group II. The principal investigator performed the clinical procedure. The principal investigator was not blinded to the treatment groups because of the study design. Evaluators for clinical and radiographical assessment were blinded to the treatment group. Also, patients were blinded to the treatment groups. The allocation flow diagram according to the CONSORT guidelines is shown in Flowchart 1.

Clinical Procedure

Local anesthesia (Lignocaine hydrochloride 2% with adrenaline 1:80,000, ICPA Health Products Ltd. Ankleshwar, India) was achieved by giving inferior alveolar nerve block and long buccal nerve block, and complete isolation was performed using a rubber dam and saliva ejector with suction tip. For each patient, as per a computer-generated sequence, the primary mandibular second molar on one side was treated with ozonoid olive oil: Group I (ADC INC DENTOZONEINDIA, Navi Mumbai, India) while the primary mandibular second molar on the other side was treated with calcium hydroxide: Group II (Prime Dental Products, Pvt. Ltd. India).

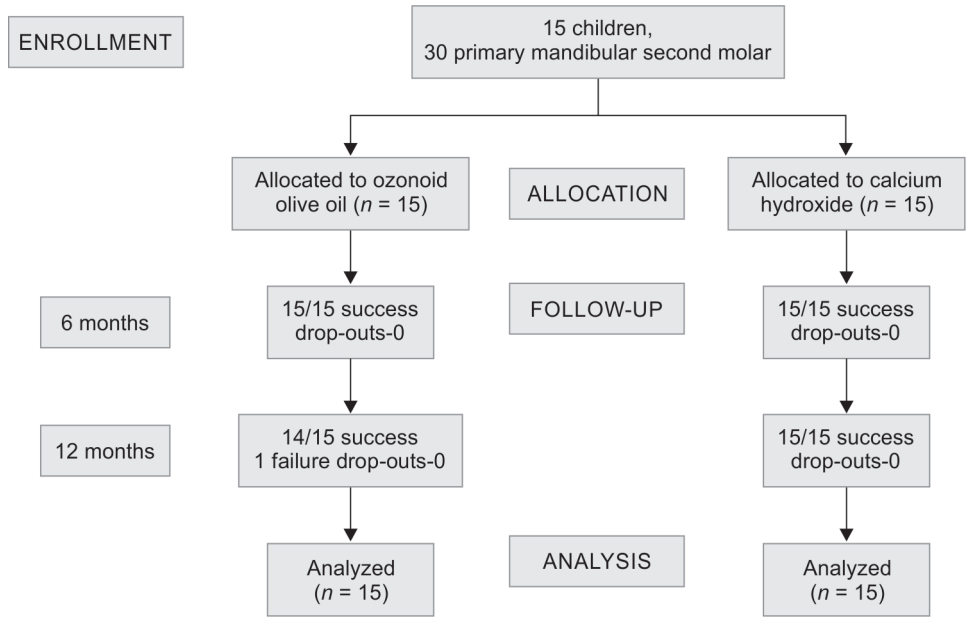
In Group I

Superficial caries were removed with sterile diamond round and straight fissure bur. Then dentinal tissue sample was taken using

Table 1: Inclusion and exclusion criteria

Inclusion criteria	*Exclusion criteria*
• Children in the age-group of 5–9 years • Cooperative patients with no associated systemic illness • Bilateral, restorable, primary mandibular second molars with deep dentinal carious lesion without pulpal involvement as evident by clinical and radiographical examination • History of pain suggestive of reversible pulpitis • No clinical signs of pulpal degeneration such as throbbing pain, tenderness to percussion, mobility, abscess, or gingival swelling • No radiographical signs of pulpal degeneration such as internal root resorption, periapical or interradicular radiolucency	A) Clinical criteria • Non-restorable tooth • Spontaneous or nocturnal pain • Tenderness on percussion • Gingival redness or swelling • Tooth mobility • Sinus tract formation B) Radiographical criteria • Radiographical signs suggestive of pulpal degeneration such as internal root resorption • Periapical or interradicular radiolucency • External root resorption

Flowchart 1: Allocation flow diagram



a sterile slow rotating round carbide bur (SS White®, NJ 08701, United States) for microbiological evaluation of *S. mutans* and *Lactobacilli*. The carbide bur was removed from the handpiece by sterile tweezers and placed in a sterile tube containing 1 mL normal saline (Beryl Drugs Ltd. India) as transport fluid. The tube was shaken to dislodge the adherent dentin from the bur, and then the bur was removed from the tube with a sterile tweezer. Then ozonoid olive oil was applied on the remaining dentinal tissue with the help of an applicator tip for 60 seconds. Then another dentinal tissue sample was taken with the help of a sharp spoon excavator. Both dentinal tissue samples were immediately transported to the microbiological laboratory. After that, ozonoid olive oil was again applied to dentinal tissue, and a thin layer of OpalDam (Ultradent Product, Inc. South Jordan) was applied and light-cured. The tooth was restored with glass ionomer cement (GC Corporation, Tokyo, Japan). A stainless steel crown (3M ESPE™, Dental Products, USA) was given after 1 week.

In Group II

Superficial caries were removed with sterile diamond round and straight fissure bur. Infected carious dentin was removed with a sharp spoon excavator avoiding pulpal exposure. Then cavity was lined with calcium hydroxide base material and restored with glass ionomer cement. A stainless steel crown was given after 1 week.

Each patient was recalled after 6 months and 12 months for clinical and radiographical evaluation.

Microbiological Procedure

The sample transported to the laboratory was diluted in normal saline to obtain 1/10,000 (Serial dilution up to 10-4 dilution). A 100 µL of the sample of 10-4 dilution was plated into freshly prepared media Mitis salivarius agar (HiMedia Laboratories Pvt. Ltd. India) for *S. mutans* and Rogosa agar (HiMedia Laboratories Pvt. Ltd. India) for *Lactobacilli*. Then the plates were incubated anaerobically at 37°C for 72 hours. After a predetermined incubation period, the colony-forming units per mL (CFU/mL) were calculated on each media utilized (CFU/mL = no. of colonies × volume of dilution plated/dilution factor). All bacterial counts were recorded in case record form for statistical analysis.

Table 2: Average age and gender distribution of participants included in the study

Average age (in year)	*Gender*		
	Male n (%)	*Female n (%)*	*Total*
6.66 ± 1.10	6 (40%)	9 (60%)	15 (100%)

Method of Measurement

The parameters used for clinical evaluation were history of spontaneous pain or night pain, tenderness on percussion, tooth mobility, sinus tract formation, and periapical abscess. The parameters for radiological evaluation were furcation involvement, periapical pathology, internal root resorption, external root resorption, and discontinuation of lamina dura. In microbiological evaluation, the bacterial count was measured before and after the application of ozonoid olive oil and recorded as CFU/mL.

Evaluators for clinical and radiographical assessment and patients were blinded to the treatment groups. A single operator, who was not blinded to the treatment groups, performed the clinical procedure.

Statistical Analysis

The Chi-square test was used for comparison of the clinical and radiographical success rate of IPC between the groups. Paired *t*-test was used for comparison of CFU/mL (at 10-4 dilution) before and after application of ozonoid olive oil for *S. mutans* and *Lactobacilli*.

Results

A total of 30 primary mandibular second molars from 15 children of age 5–9 years were included for IPC. Out of 15 children, 6(40%) were males and 9(60%) were females with an average age of 6.66 ± 1.10 years (Table 2). Clinical and radiographical follow-up of the teeth treated with IPC was done at 6 and 12 months (Tables 3 and 4). There were no drop-outs in this study. All the teeth were available for analysis. At 6 months of follow-up, both the IPC agents showed 100% (15/15) clinical and radiographical success, whereas at 12 months of follow-up, group I (ozonoid olive oil) showed 93.33% (14/15) as compared to group II (calcium hydroxide), which showed

Table 3: Comparison of clinical success rate of indirect pulp capping between group I (ozonoid olive oil) and group II (calcium hydroxide)

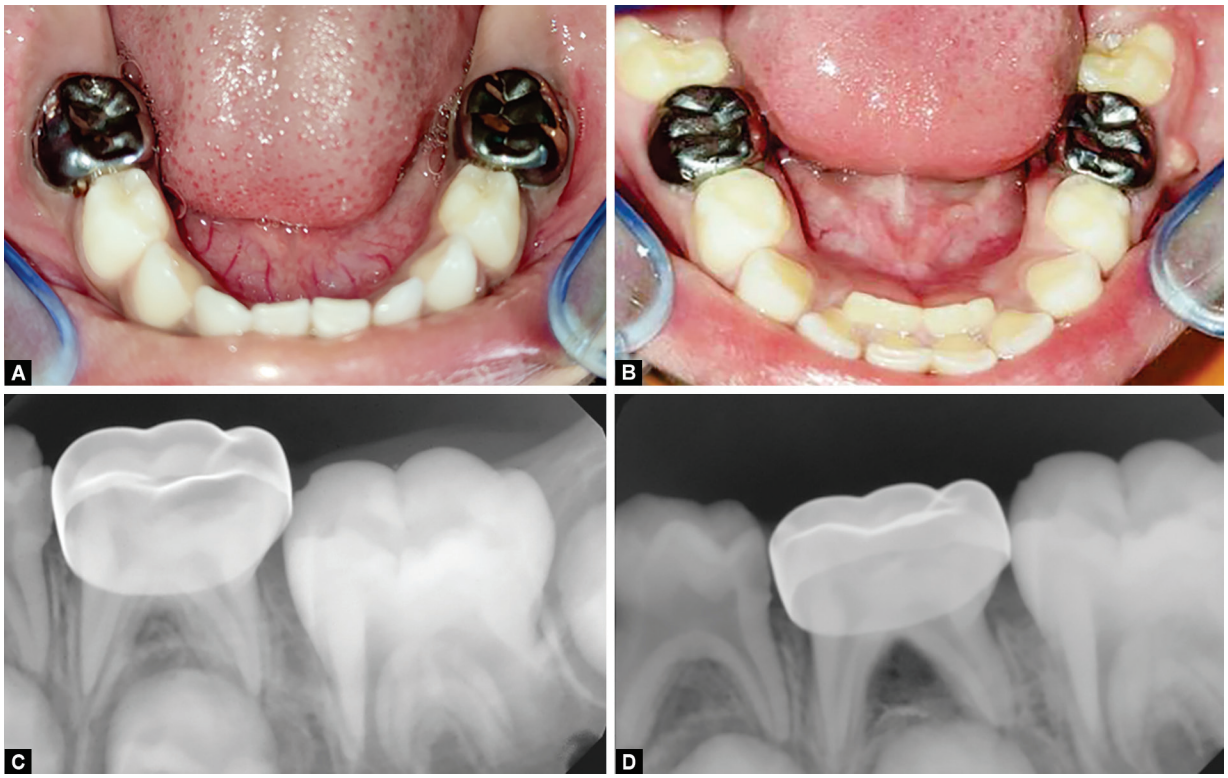
	At 6 months			*At 12 months*		
Groups	*Sample n*	*Success n (%)*	*Failure n (%)*	*Sample n*	*Success n (%)*	*Failure n (%)*
Group I (Ozonoid olive oil)	15	15 (100%)	0 (0%)	15	14 (93.33%)	1 (6.67%)
Group II (Calcium hydroxide)	15	15 (100%)	0 (0%)	15	15 (100%)	0 (0%)
p value		–			1.000 (NS)	

Chi-square test; NS, non-significant difference

Table 4: Comparison of radiographical success rate of indirect pulp capping between group I (ozonoid olive oil) and group II (calcium hydroxide)

	At 6 months			*At 12 months*		
Groups	*Sample n*	*Success n (%)*	*Failure n (%)*	*Sample n*	*Success n (%)*	*Failure n (%)*
Group I (Ozonoid olive oil)	15	15 (100%)	0 (0%)	15	14 (93.33%)	1 (6.67%)
Group II (Calcium hydroxide)	15	15 (100%)	0 (0%)	15	15 (100%)	0 (0%)
p value		–			1.000 (NS)	

Chi-square test; NS, non-significant difference



Figs 1A to D: (A) Clinical photograph immediately after the procedure; (B) Clinical photograph of failure at 12 months of follow-up; (C) Intraoral periapical radiograph immediately after the procedure; and (D) Intraoral periapical radiograph of failure at 12 months of follow-up in group I

100% (15/15) clinical and radiographical success. In group I, clinical and radiographical failure which occurred in the same patient meant the tooth which showed the clinical symptom of failure (sinus tract formation) had the radiographical failure (furcation involvement) as well (Fig. 1). When both groups were compared at 12-months follow-up, no statistically significant differences were found. But, on microbiological evaluation, statistically significant differences were found for *S. mutans* and *Lactobacilli* after the application of ozonoid olive oil. The microbiological evaluation in terms of bacterial count (CFU/mL) was performed before and after the application of ozonoid olive oil in group I. The mean value of CFU/mL for *S. mutans* before application of ozonoid olive oil was 78.93. This value reduced significantly after the application of ozonoid olive oil with a mean value of 15.67. Similarly, the mean value of CFU/mL for *Lactobacilli* before application of ozonoid olive oil was 35.53. This value reduced significantly after the application

Table 5: Comparison of colony-forming units/mL (at 10-4 dilution) of microorganisms before and after application of ozonoid olive oil

Microorganism	*Interval*	*Mean*	*SD*	*Difference*	*p value*
S. mutans	Before	78.93	±76.19	63.27	0.002*
	After	15.67	±14.97		
Lactobacilli	Before	35.53	±37.03	23.73	0.007*
	After	11.80	±9.36		

Paired *t* test; *Indicates significant difference at $p \leq 0.05$

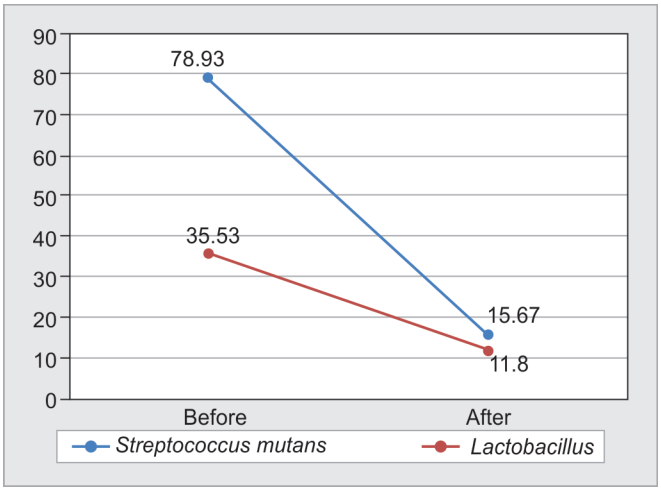


Fig. 2: Comparison of colony-forming units/mL (10-4 dilution) before and after application of ozonoid olive oil

of ozonoid olive oil with a mean value of 11.80. When the statistical analysis was performed for microbiological evaluation, a significant difference was found for both the bacteria (Table 5 and Fig. 2).

Discussion

Indirect pulp capping is an effective, simple, and conservative vital pulp therapeutic modality in deep dentinal carious lesions of primary teeth. As a minimally invasive therapy, it decreases the need for pulpotomy in primary molars and improves the quality of tooth since more tooth structure is preserved. Calcium hydroxide has been used in IPC for a long time.[26] In the present study, the need to find an alternative to the gold standard material for IPC–calcium hydroxide has been addressed to overcome its drawbacks like the formation of tunnel defects and poor adhesive capacity.[11] The use of ozone therapy has increased recently in the management of deep carious lesion in children.[21–28] Ozone with its strong oxidizing property is considered as an effective bactericidal, antiviral, and antifungal agent.[20] Ozone, when applied to the tissue, releases nascent oxygen, thereby destroying microorganisms. The antimicrobial property of ozone disrupts the cell membrane leading to bacterial death, thereby reducing their count.[29] This prompted us to conduct a study wherein ozone was used in oil form. In this study, ozonoid olive oil was evaluated as an IPC agent.

In the present study, the clinical and radiographical success at 6 months follow-up was 100% in both the groups; however, one clinical and radiographical failure was evident at 12 months follow-up in the experimental group. The clinical and radiographical failures occurred in the same tooth. At 12 months follow-up, the calcium hydroxide group showed 100% clinical and radiographical success as compared to the experimental group which showed 93.33% success. No significant differences were observed for both the materials used for IPC treatment. Also, a significant reduction in colony counts of the microorganisms was observed after the application of ozonoid olive oil. When comparing the antimicrobial effect of ozonoid olive oil on both organisms, it was found that it was more effective on *S. mutans* than *Lactobacilli*. Statistically significant differences were found for both the bacteria. Thus, ozonoid olive oil has proved its efficacy as a potent antimicrobial agent on microorganisms of deep dentinal caries. Thus, the present study results showed that ozonoid olive oil was as successful as calcium hydroxide as an IPC agent and has potent antimicrobial properties. The high success rate of ozonoid olive oil may be attributed to its antimicrobial capacity and hermetic seal of the tooth which avoided the chances of microbial invasion after the treatment.

Safwat et al.[30] conducted an *in vivo* study to evaluate the effect of ozone on deep dentinal carious lesions in immature first permanent molars that were indicated for IPC. The teeth were treated with ozone therapy and calcium hydroxide. No significant differences were observed between ozone treatment and calcium hydroxide during follow-up periods. Safwat et al.[23] conducted a microbial study on immature first permanent molars evaluating the effect of ozone and calcium hydroxide on *S. mutans*, *Lactobacilli*, and *Candida albicans*. *S. mutans*, *Lactobacilli*, and *C. albicans* counts were significantly reduced immediately after ozone application. Yeşilöz Gökçen et al.[28] compared the effects of clearfil protect bond, dycal, physiologic saline, and ozone on *S. mutans* in primary mandibular molars indicated for IPC. This study concluded that there was a reduction in bacterial count in all groups except physiological saline. Ozone was most effective in bacterial reduction followed by clearfil protect bond and dycal. The antimicrobial results of the present study are similar to these[23,27] studies.

Marchi et al.[7] compared calcium hydroxide (Dycal) with glass ionomer cement clinically and radiographically for 48 months and found no statistically significant differences between these two groups. Renata et al.[31] studied Hydro-C (a lining material containing calcium hydroxide) and gutta-percha sheets as IPC agents in primary teeth and evaluated them clinically and radiographically for 36 months. No significant differences were found between the two groups. It was also observed that a sealed continuous restoration determines the success in IPC treatment irrespective of the capping material used. Büyükgüral and Çehreli[32] conducted a study on primary teeth with 24 months of clinical and radiographical observation. In this study, three adhesive systems were compared with calcium hydroxide. At 24 months follow-up, the success rate was 100%. All the groups showed similar results. It was noted that resin-based composite material could stop the caries progression at the base of the cavity, and, thus, it was concluded that calcium hydroxide was not a determining factor for the success of IPC in primary teeth. Casagrande et al.[4] observed primary teeth clinically and radiographically for 60 months after indirect pulp treatment with a self-etching adhesive system and calcium hydroxide material. It was found that there was no statistically significant difference between the two groups with overall success rate. George et al.[5] evaluated calcium hydroxide (Dycal) and MTA, clinically and radiographically, as an IPC agent in primary molars for 6 months of period. No failure rates were seen in the MTA group, however, in dycal group, one patient had reported with sinus opening at a 6 month follow-up. In George's study, permanent restorations were given in the second visit, but in the present study, permanent restorations were given in a single visit, and stainless steel crown was given after a week. Mathur et al.[33] conducted study on primary teeth using glass ionomer cement (GIC-type VII), MTA, and calcium hydroxide (Dycal). No statistically

significant differences in clinical and radiographic success were observed among the materials used for IPC treatment. Garrocho-Rangel et al.[34] evaluated primary teeth using bioactive tricalcium silicate (Biodentine) and light-activated calcium hydroxide for IPC treatment. The teeth were observed clinically and radiographically for 12 months. The overall clinical and radiographic success for tricalcium silicate and calcium hydroxide was similar. No significant differences were found in success rates between the groups. Gurcan and Seymen[35] treated second primary molars and first permanent molars with IPC materials–Dycal, ProRoot MTA, and TheraCal LC. The teeth were evaluated for 24 months clinically and radiographically and found no statistically significant differences among the groups. This stated that IPC treatment is independent of the capping materials used. The clinical and radiographical results of the present are in accordance with these studies.[4,5,7,31–35]

Silva et al.[36] conducted a systematic review that compared microbial reduction in root canals after treatments with ozone and sodium hypochlorite in extracted mature human teeth or randomized clinical trials. Some of the studies in this systematic review incorporated ozone in an aqueous form for irrigation of root canals. The conclusion of this review was that aqueous ozone was as effective as sodium hypochlorite in the reduction of the microbial count. In the present study, ozonoid olive oil also had similar results on cariogenic bacteria. Badhe H et al.[37] recently conducted a systematic review on antimicrobial effects of ozone therapy and concluded that ozone therapy can be used for reduction of microbial count in deep dentinal carious lesion in primary and permanent teeth.

The present study has similar clinical, radiographical, and microbiological results with the aforementioned studies when compared. These findings may be due to the hermetic seal of the carious lesion and strong antimicrobial property of ozonoid olive oil.

Limitations of the Study and Future Perspective

The aforementioned literature and the findings of this study show that ozonoid olive oil is an effective IPC agent in primary teeth and is a potent antimicrobial agent against caries causing bacteria. However, there are few limitations in this research which include a smaller sample size and a short duration of follow-up period. Furthermore, in radiological evaluation, it was not possible to evaluate digitally the increase in dentine thickness because of the restoration with stainless steel crown. Although we studied 30 primary mandibular second molars for IPC treatment using a split-mouth study design, there were no drop-outs through the study duration. Therefore, all the samples were available for evaluation at the decided follow-up period.

Future studies should consider a larger sample size and longer duration of follow-up with split-mouth design in primary and permanent teeth, evaluating the increase in dentine thickness digitally on a radiograph. To study pulpal response after ozonoid oil application, researchers should also consider the histologic parameter. However, ethically histologic studies can only be performed in teeth with deep carious lesions requiring IPC treatment which are subsequently indicated for extraction in future orthodontic treatment.

Conclusion

The results of this study showed that no statistically significant differences were found for clinical success rate of IPC between both the groups (ozonoid olive oil and calcium hydroxide).

The results of this study showed that no statistically significant differences were found for radiographical success rate IPC between both the groups.

However, statistically, significant differences were found in microbiological evaluation for both the bacteria. Additionally, the results of this study showed that ozonoid olive oil application was more effective on *S. mutans* in reducing bacterial count when compared to *Lactobacilli*. In the present study, ozonoid olive oil has proved its efficacy as a potent antimicrobial agent on microorganisms of deep dentinal caries.

More studies with a larger sample size and longer duration of follow-up with split-mouth design are required to confirm the use of ozonoid olive oil as an alternative to calcium hydroxide for IPC in primary teeth.

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