

**COMPARISON ON THE EFFICACY OF THREE DIFFERENT
BLEACHING AGENTS - CARBAMIDE PEROXIDE, SODIUM
PERBORATE AND OZONATED WATER AS
INTRACORONAL BLEACHING AGENTS IN ARTIFICIALLY
DISCOLORED PRIMARY TEETH – AN *IN VITRO* STUDY**

**By
Dr. JANANI.L**

A Dissertation submitted to
Sathyabama Institute of Science and Technology
(Deemed to be University)



In partial fulfillment of the requirement for the Degree of

MASTER OF DENTAL SURGERY

**PEDIATRIC AND PREVENTIVE DENTISTRY
SATHYABAMA DENTAL COLLEGE AND HOSPITAL
CHENNAI 600 119**

2018 – 2021

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Date: 24/03/2022

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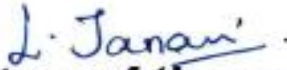
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Facial aesthetics and cosmetology include a thorough assessment of proportion and harmony in relation to individual ethnic and cultural ideals. Dental aesthetics is an essential component of facial aesthetics. The goal of aesthetic dentistry is to provide function and beauty through an attitude, artistic ability, intuition, and technical competence.

There is a plethora of literature on the influence of bad aesthetics on psychology in adults, but there is a dearth of published studies in children. However, it has previously been suggested that youngsters with normal dental appearance are better beautiful, more desirable as friends, more clever, and less prone to behave aggressively.¹

Pediatric dentists need to understand children's aesthetic perception since children are conscious of their own and other children's dental aesthetic look. While Jean Piaget's traditional concept stated that a child's perception of self and care about their appearance only developed by the age of eight years, recent studies in the field of child psychology have challenged this concept, showing that, with increased media exposure, children as young as three to five years of age have a sense of body image consciousness.² During the paediatric and pubertal years, changes in body self-image can have a significant impact on many elements of life, including socializing, emotional and functional components, and familiar interrelationships. As a result, greater emphasis and attention are now being paid to aesthetic rehabilitation in children and adolescents.

Aside from pain, poor aesthetics is typically the major reason for a patient seeking dental care. The desire for better dental aesthetics has fueled advancements in dental materials. Patients, parents, and the news media are increasingly requesting information about teeth whitening for children and adolescents. The diversity and availability of dental bleaching products on the market have been impacted by an increase in demand for bleaching materials and services.

Tooth whitening, often known as bleaching, refers to any treatment that lightens the colour of a tooth. Whitening can be performed through physical stain removal or a chemical reaction to lighten the tooth colour, whereas bleaching is accomplished through the chemical breakdown of the chromogens.³ Bleaching procedures can be executed extracoronal, intracoronal, or a blend of the two. Internal bleaching offers a benefit over external bleaching in that it eliminates gingival irritation and ingestion of the bleaching ingredient, which is highly significant in children.⁴

Many bleaching agents have been employed with variable outcomes over the years, including sodium perborate, hydrogen peroxide, sodium percarbonate, and carbamide peroxide. The hydrogen peroxide (30% and 35%), sodium perborate, carbamide peroxide (10%, 20%, 35%) are the most commonly used bleaching agents for permanent and primary teeth.

Hydrogen peroxide is a colorless liquid with a bitter taste that dissolves readily in water to form an acidic solution. Hydrogen peroxide is a powerful oxidizing agent with numerous industrial applications. This chemical is available in a variety of concentrations, although 30 to 35 percent stabilized aqueous solutions are the most prevalent. There are additional silicone dioxide gel forms containing 35% hydrogen peroxide, some of which are activated by a composite curing light.

External cervical root resorption of the tooth was one of the most serious side effects of intracoronal whitening with hydrogen peroxide. The cervical root resorption in the bleached teeth could have been caused by increased hydrogen peroxide diffusion into the periradicular tissues, presumably through cemental flaws. Although hydrogen peroxide was a powerful and efficient bleaching chemical, there was still some debate about its safety and potential side effects on the teeth.⁵

Carbamide peroxide is a chemical compound that contains hydrogen peroxide and urea. When activated, carbamide peroxide decomposes into HP and urea, the latter of which causes the denaturalization of enamelin and amelogenin, which are proteins found in the matrix component found between the enamel prisms. This could increase the permeability of the enamel and, as a result, create microstructural changes. In contrast, urea produces alkalization, which may result in less demineralization. According to certain studies, bleaching with peroxide products can cause

calcium loss, changes in morphology, and changes in chemical components of the tooth, all of which can diminish the tooth's fracture resistance.⁶

Sodium perborate is a white powder comprising that is water soluble and stable in dry and cool air. In hot and humid air, it decomposes and releases oxygen. It is usually provided in granular form, which must be processed into powder before usage. It contains more oxygen, is more stable during the heating process, and is more soluble in water. Sodium perborate in the form of a crystal is difficult to apply on its own, but it can be transformed into a paste by combining it with 30% hydrogen peroxide or water. The bleaching effect is not weakened if sodium perborate is mixed with water instead of hydrogen peroxide ⁷. Sodium perborate, on the other hand, looks to be a less harmful bleaching agent. and the effect of sodium perborate on microhardness is less significant. (Sharma and colleagues).⁶

Ozone is another chemical that has lately been proposed for use as a bleaching agent.⁸ Ozone is a naturally occurring gaseous molecule composed of three oxygen atoms. It purifies the air and reacts with any pollutant with which it comes into touch. This is the earth's natural method of self-cleansing.⁹ Ozone therapy has numerous uses in practically every department of dentistry. It has immunostimulant, analgesic, anti-hypnotic, detoxicating, antibacterial, bioenergetic, and biosynthetic effects. Its atraumatic, painless, noninvasive nature, and relative lack of discomfort promote patient acceptability and compliance, making it a suitable therapy option for paediatric patients in particular. ¹⁰

A possible explanation for its bleaching efficacy is related to the oxidation-reduction reaction which occurs during the bleaching procedure. According to Dahl and Pallesen, the nascent oxygen attacks the complex highly pigmented carbon ring compounds and converts them into short-chain compounds that are lighter in color. There is limited evidence on the intracoronary bleaching effect of ozonated water and its efficacy particularly in primary teeth.

Hence this in vitro study was aimed to evaluate the bleaching efficacy of ozonated water in comparison with commonly used bleaching agents such as carbamide peroxide and sodium perborate with reported safer concentration.

Aims of the study

The aim of this study was to compare the efficacy of the different bleaching agents carbamide peroxide, sodium perborate and ozonated water as intracoronal bleaching agents in artificially discoloured primary anterior teeth at different time intervals.

Objectives of the study

- To evaluate the bleaching efficacy of 10% carbamide peroxide on artificially discoloured primary anterior teeth on day 7, day 14, and day 21 respectively.
- To evaluate the bleaching efficacy of 10% sodium perborate on artificially discoloured primary anterior teeth on day 7, day 14, and day 21 respectively.
- To evaluate the bleaching efficacy of ozonated water on artificially discoloured primary anterior teeth on day 7, day 14, and day 21 respectively.
- To evaluate the bleaching efficacy of distilled water on artificially discoloured primary anterior teeth on day 7, day 14, and day 21 respectively.
- To compare the bleaching efficacy of 10% carbamide peroxide, 10% sodium perborate, ozonated water and distilled water on artificially discoloured primary anterior teeth on day 7, day 14, and day 21 respectively.

Facial esthetics in children

Smile as one of the most important methods of influencing people.

Shulman et al (2004) found that girls have higher expectations about their tooth color (that is, they are more inclined to disagree with the reference statement) than do boys, and they have higher mean scores on the reference statement scale.¹¹

Aditi kapur (2005) cautioned that with growing general awareness many children even as young as 3 years of age are becoming conscious of their appearance.³ The loss of esthetically essential anterior teeth may affect the child's confidence and normal personality development. Also it may cause abnormal habits and speech difficulties.¹²

L. Tremblay et al (2011) challenged the traditional concept of Jean Piaget and stated that a child's perception of self and care about their appearance only developed by the age of 8 years; there have been recent studies in the field of child psychology that have challenged this concept. He also stated that the knowledge of the children's aesthetic perception is relevant to pediatric dentists because children are conscious about their dental aesthetic appearance.²

Mathew Renu (2013) cautioned that esthetic problems in childhood and adolescence can have a significant effect on psychosocial development and interaction with peers. Abnormalities of shape, size, color and structure of the whole or part of the anterior dentition of children can lead to such problems.¹³

Chadha Tarang et al (2017) found many solutions available for aesthetic problems in Pediatric Dentistry. Dental esthetics is synonymous with facial esthetics. Cosmetic problems at an early age have a huge impact on the psychological development and social interaction with other children at same age. In this respect, the advent of different techniques, devices, and materials help in creating beautiful restorations which help children and adolescents to improve their self-image.¹⁴

Teena Gupta et al (2019) in her study found that the anterior teeth impact negatively on the psychological and social well-being of children. It is postulated that research on awareness about oral health in children may help better define appropriate treatment and subsequent outcomes.¹⁵

Etiology of tooth discoloration

Vogel et al (1975) stated discolorations arise when remnants of root-filling materials or medicaments are left in the pulp chamber and the staining substance infiltrates the dentinal tubules. Although no penetration of the dental enamel takes place, there is an observable difference of color on the tooth.¹⁶

Arens (1989) stated dissemination of blood components into the dentinal tubules caused by pulp extirpation or traumatically induced internal pulp bleeding is a possible reason for discoloration of non- vital teeth. A temporary color change of the crown to pink can often be detected initially. Then, blood degradation products such as haemosiderin, haemin, haematin and haematoidin release iron during haemolysis.¹⁷

Guldener & Langeland (1993) stated that the iron can be converted to black ferric sulphide with hydrogen sulphide produced by bacteria, which causes a grey staining of the tooth. Apart from blood degradation, degrading proteins of necrotic pulp tissue may also cause discoloration.¹⁸

Davis et al. (2002) found the reason for coronal discoloration of root- filled teeth to be caused by some root- filling materials or tetracycline-containing medicaments. Discoloration caused by

root-filling materials can be treated by bleaching depending on the staining substance.¹⁹

According to Ingle et al the cause for tooth discolorations are²⁰ :

Impaired esthetic appearance of maxillary primary anteriors may be the result of a variety of etiological factors. Tooth discolorations can be clinically classified owing to patient - or dentist- related causes. Patient related causes includes, pulpal necrosis, intrapulpal hemorrhage, dentin hyper calcification, age and tooth formation defects. Dentist related causes includes endodontically related causes and restoration related causes.

Pulp necrosis can occur due to bacterial, mechanical, or chemical irritation to the pulp that may result in tissue necrosis and release of disintegration by- products that may penetrate tubules and discolor the surrounding dentin. The degree of discoloration is directly related to how long the pulp has been necrotic. The longer the discoloration compounds are present in the pulp chamber, the greater the discoloration.

Intrapulpal hemorrhage and lysis of erythrocytes occur as a result of traumatic dental injury. Blood disintegration products, presumably iron sulfides, flow into the tubules and discolor the

surrounding dentin. If the pulp becomes necrotic, the discoloration persists and usually becomes more severe with time. If the pulp recovers, the discoloration may be reversed, with the tooth regaining its original shade. The severity of such discoloration is time dependent.

Dentin hypercalcification is excessive formation of irregular dentin in the pulp chamber and along canal walls which may occur following certain traumatic injuries. In such cases, a temporary disruption of blood supply occurs, followed by destruction of odontoblasts. These are replaced by undifferentiated mesenchymal cells that rapidly form irregular dentin on the walls of the pulp lumen. As a result, the translucency of the crowns of such teeth gradually decreases, giving rise to a yellowish or yellow-brown discoloration.

Several other systemic conditions may cause tooth discoloration. Erythroblastosis fetalis, may occur in the fetus or newborn because of Rh incompatibility factors, with resulting massive systemic lysis of erythrocytes. Large amounts of hemosiderin pigment are released, which subsequently penetrate and discolor the forming dentin.

Amelogenesis imperfecta may result in yellow or brown discolorations. Dentinogenesis imperfecta can cause brownish violet, yellowish, or gray discoloration.

Administration or ingestion of certain drugs particularly tetracyclines, during tooth formation may cause severe discoloration both in enamel and dentin. Tetracycline bound to calcium is thought to be incorporated into the hydroxyapatite crystal of both enamel and dentin. However, most of the tetracycline is found in dentin. Repeated exposure of tetracycline- discolored tooth to ultraviolet radiation can lead to formation of a reddish- purple oxidation by- product that permanently discolors the teeth. In children, the anterior teeth often discolor first, whereas the less exposed posterior teeth are discolored more slowly.

Discolorations owing to various dental materials or unsuitable operating techniques do occur; such dentist related discolorations are usually preventable and can be avoided.

Holan G et al stated that necrotic tissue remaining in the pulp chamber left due to improper debridement disintegrates gradually and may cause discoloration. Pulp horns must always be included in the access cavity to ensure removal of pulpal remnants and to prevent retention of sealer at a later stage.

Several intracanal medicaments are liable to cause internal staining of the dentin. Phenolics or iodoform -based medicaments sealed in the root canal and chamber are in direct contact with dentin, sometimes for long periods, allowing penetration and oxidization. These compounds have a tendency to discolor the dentin gradually. Endodontically treated primary teeth may also lead to unesthetic appearance due to the use of iodoform containing paste.²¹

Bleaching in primary teeth

J. Waterhouse (1996) summarized that the method of walking bleaching used was effective for the treatment of discolored non vital teeth in children and adolescents. No side effects were found, Discoloration did occur in some teeth but never matched the original degree of discoloration.²²

Bryan and Welbury (2003) gave the protocol used to reverse dental color in devitalized teeth treated endodontically, known as internal bleaching. They suggest to take an X-ray to confirm the proper root canal seal and the degree in physiological resorption, prior to bleaching. Before beginning bleaching treatment, charting the tooth color must be done as well as a preoperative photograph of the tooth and adjacent ones is recommended.²³

T. Attin (2003) performed intracoronal bleaching successfully by temporarily placing a mixture of sodium perborate - (tetrahydrate) and water into the pulp chamber. For long -term success, it seems to be important to restore the access cavity with an adhesive filling, which prevents leakage of bacteria and stains.²⁴

Carey CM. et al (2014) encouraged that dental bleaching may offer an alternative method that can be completed with conservation of tooth structure, less chair time, low cost, and effective results .³

The **American Academy of Pediatric Dentistry** ²⁵ (2019) encourages the judicious use of bleaching for vital and nonvital teeth. Patients should consult their dentists to determine appropriate methods for and the timing of dental whitening within the context of an individualized, comprehensive, and sequenced treatment plan. Dental professionals and consumers are to consider side effects when contemplating dental bleaching for child and adolescent patients.

Different bleaching agents

Spasser et al (1961) introduced the "walking bleach" technique which involved placement of a mixture of sodium perborate and water into the pulp chamber that was sealed off between the patient's visits to the clinician.²⁶

Truman, (1864) first described bleaching of discolored, pulpless teeth, and a variety of medicaments such as chloride, sodium hypochlorite, sodium perborate, and hydrogen peroxide has been used, alone, in combination, and with and without heat activation.²⁷

Bleaching in primary teeth

Limm MY et al (2004) in his study compared the bleaching efficacy of 35% carbamide peroxide with 35% hydrogen peroxide and sodium perborate stated that samples treated with 35% carbamide peroxide were equally effective for intracoronaral bleaching, and significantly better than sodium perborate after 7 days. After 14 days, there were no significant differences between the groups. Thirty-five per cent carbamide peroxide can be recommended as an equally effective alternative to hydrogen peroxide for intracoronaral bleaching.²⁸

Carrasco et al. (2007) found that 37% carbamide peroxide as an intracoronal bleaching agent increased dentinal permeability, which was not observed for 27% carbamide peroxide. Thus, the use of carbamide peroxide at lower concentrations should be recommended to reduce the frequency of cervical root resorptions.²⁹

Braun et al. (2007) proved that high concentration of bleaching agent promotes faster tooth bleaching; however, considering the biological aspects, it is recommended to use the low-concentration agent to avoid tissue damage.³⁰

Gonjito et al. (2009) compared 37% carbamide peroxide with a diode laser and another group with a halogen lamp was used in an in vivo study on primary teeth. This study proved that both the groups showed similar results.³¹

Volkan Arikan et al (2009) reported a case on bleaching a non-vital primary tooth using sodium perborate with walking bleach technique: A mixture of 2 g of sodium perborate powder and 1 g of distilled water was combined into a paste and applied to a cotton pellet that was placed in the pulp chamber, and the tooth was again temporarily restored with glass ionomer cement. After 7 days, the color of the tooth was recorded as satisfactory. The solution was removed from the pulp chamber, which was

irrigated with distilled water. The final restoration was completed using compomer restorative material . In conclusion, in this study, using sodium perborate with the walking bleach technique was found to be successful i n whitening primary teeth and suggested that this method can be recommended as a safe alternative for treating children with discolored devitalized teeth.³²

Ganesh et al (2013) . compared the intracoronol bleaching efficiency of 10% hydrogen peroxide against 10% carbamide peroxide in an *in- vitro* study in primary incisors and concluded that hydrogen peroxide group showed superior results. ³³

Athimuthu Anantharaj et al (2015) reported a case on walking bleach in primary teeth and concluded that, intracoronol bleaching of discolored primary teeth using sodium perborate is a good alternative, esthetic modality for young children after pulpectomy. Because of the shortened chair time associated with this technique, it may be appropriate for younger children. However, usage of the other esthetic option can also be considered.³⁴

Vagharedin Akhavan Zanjani et al (2015) compared 35 % hydrogen peroxide with ozonated water and a combination of both on permanent teeth. The study showed that the hydrogen

peroxide gel has a more powerful whitening effect than ozone; in addition, ozone has no synergistic effect when used simultaneously with hydrogen peroxide.³⁵

Shaheen et al (2017) conducted an in vitro study on efficacy of 10% carbamide peroxide as an intracoronal bleaching agent in nonvital discolored primary teeth and concluded that intracoronal bleaching using 10 % carbamide peroxide is an effective approach for whitening discolored extracted primary teeth.³⁶

Mahmoud K Al-Omiri (2018) did a comparison of dental bleaching effects of ozone and hydrogen peroxide . This is the first study to compare bleaching effects of ozone and hydrogen peroxide on natural human teeth. It might be suitable to consider ozone for dental bleaching compared to hydrogen peroxide as it could potentially reduce the time, side effects and cost of treatment. Also, application of ozone is better controlled and more convenient and measurable as it is delivered by a machine that can manage the concentration, volume, delivery site and timing of delivered ozone.³⁷

Sharma et al. (2019) performed successful intracoronal bleaching in discolored young permanent and primary teeth using sodium perborate and water. Two months postoperative

radiograph revealed no signs of external resorption and perfect coronal seal clinically. He also concluded that alteration in microhardness using sodium perborate is nonsignificant.⁶

Bhatnagar et al (2020). found that bleaching agents such as carbamide peroxide, sodium perborate and hydrogen peroxide are equally effective in the primary teeth bleaching after 1 week as well as 2 weeks.³⁸

Manisha Behl et al (2020) did a study on the evaluation of three different bleaching agents in permanent and primary teeth, where the test samples were divided into group I (permanent teeth) and group II (primary teeth). The test samples were further divided into three subgroups according to the experimental materials used for bleaching [group IA—10% carbamide peroxide, group IIB—9.5% hydrogen peroxide and group IIIC—10% sodium perborate]. The samples were evaluated at 7 days and 14 days after the completion of intracoronal bleaching procedure. The carbamide peroxide is effective in permanent teeth after 7 days and 14 days of intracoronal bleaching. The three experimental materials are equally effective in the primary teeth bleaching after 7 days and 14 days.³⁹

The present in-vitro study was conducted in the Department of Pediatric and Preventive dentistry, Sathyabama Dental college and Hospital, Chennai.

Other facilities involved in the study

- Life Teck Research Centre, Arumbakkam, Chennai.

Materials and equipment used for the study

Armamentarium for tooth mounting

- 80 primary anterior teeth samples (Fig.1)
- Putty material (Photosil soft putty, DPI, Mumbai)
- Clear acrylic (DPI, Mumbai)
- Black sketch (Cello)

Armamentarium for pulpectomy and sealing of tooth samples (Fig.4)

- Sterile gloves (Medipride, India)
- Airtor handpiece (NSK, Japan)
- Access opening bur #3 (Dentply, USA)
- K file #10,#15,#20,#25, #30 (MANI, INC.Japan)
- 5 ml syringe (Dispovan, India)
- Saline (Aculife Healthcare pvt.ltd. India)
- Paper points 4% taper (Dentply, USA)
- Metapex (Meta biomed, Korea)
- Pluggers (Mani, INC.Japan)

- Type IX GIC (Fuji IX,GC Gold label, Japan)
- Plastic instrument (GDC,India)

**Armamentarium for artificial staining of tooth samples
(Fig.10)**

- Expired human blood (Batch no:ITPOBB3229) (Rotary central Vhs Ttk blood bank, Tharamani, Chennai)
- Eppendorf tubes 1.5ml (Torsans, India)
- 5ml syringe (Dispovan, India)
- Centrifugation machine (Remi Cooling Centrifuge, REMI, India)(Fig 11)
- Incubator (Ausco, India) (Fig.12)
- Vita classical shade guide (VITAPAN classical, Germany) (Fig.13)

**Armamentarium for Bleaching of tooth samples and shade
evaluation (Fig 10)**

- Sterile gloves (Medipride, India)
- Sterile forceps (INLAB Equipments, India)
- Petri dishes (INLAB Equipments, India)
- Watch glass (INLAB Equipments, India)
- Eppendorf tubes 1.5 ml (Torsans, India)
- 1 ml syringe (Dispovan, India)
- Pipette (INLAB Equipments, India)
- Conical flask (INLAB Equipments, India)

- Carbamide peroxide 20% (Opalescence, Ultradent, South Jordan)(Fig.18)
- Sodium perborate tetrahydrate powder (Loba Chemi Pvt.Ltd, India)(Fig.19)
- Ozone water generator (Clean water systems, USA)(Fig.21)
- Distilled water (freshly prepared)
- Magnetic stirrer (REMI, India) (Fig.20)
- Temporary filling material (Prime dental, India)
- Incubator (Ausco, India)(Fig.12)
- Vita classical shade guide (VITAPAN classical, Germany)(Fig.13)

Preparation of tooth samples for artificial discoloration

Eighty non carious primary anterior tooth samples with at least one third of the root structure were collected and disinfected using ethylene glycol. Acrylic moulds were prepared using clear acrylic and the samples were embedded in acrylic mould exposing only the crown part till the cemento enamel junction. To evaluate the shade in natural light with an even contrast, the acrylic moulds were painted with black color sketch pen. Access opening for all the tooth samples was done using access opening bur (No.#3). The canals were cleaned and shaped using K-files from size 10 to size 30 with sufficient saline irrigation. The canals were obturated with calcium hydroxide –

iodoform containing paste (Metapex) and the excess material was removed 1 mm below the orifice by measuring with Williams probe and sealed with type IX GIC and reduced 1 mm below the access cavity.

Artificial discoloration of tooth samples

Expired human blood was used for discoloration of tooth samples. The expired human blood sample was obtained by providing a requisition letter to the authorities of Rotary Central VHS Blood Bank, Tharamani, Chennai. All the collected tooth samples were stained artificially using human blood to attain artificial internal hemorrhage. Allocated tooth samples were immersed in Eppendorf tubes containing 1.5 ml of the expired human blood and centrifuged at 3200 rpm twice daily for three consecutive days. Stained samples were washed thoroughly by rinsing all the tooth samples separately using distilled water and dried completely. Baseline shade (Day 0) immediately after staining procedure was recorded using VITA shade guide.

For convenience purpose, the VITA shade guide values were converted into numerical values and their corresponding ΔE value shown in **Table 1** according to **Jong Gil kim et al** ⁶¹.

Preparation of bleaching agent

Four different bleaching agents were used for this in -vitro study namely:

- **Group I** - Carbamide peroxide 10%
- **Group II** - Sodium perborate 10%
- **Group III** -Ozonated water
- **Group IV (control)** - Distilled water

Carbamide peroxide 10%

Conventionally available carbamide peroxide 20% gel was converted into carbamide peroxide 10% gel by taking 1.2 g of gel and diluting it with 1.2 ml of distilled water and transferred into a 1 ml syringe for easy application into the access canal. This 10% carbamide peroxide gel was prepared freshly during the bleaching procedure.

Sodium perborate 10%

Sodium perborate 10% solution was prepared by dissolving 10 g of sodium perborate tetrahydrate powder in 10ml of distilled water, using a magnetic stirrer for half an hour. This freshly prepared solution was used throughout the study.

Ozonated water

Ozonated water freshly prepared from industrial ozone generator was collected and used immediately.

Application of bleaching agent

After baseline shade evaluation, the discoloured tooth samples were randomly divided based on lottery method and allocated into four groups respectively as

- **Group 1** : Carbamide peroxide 10%
- **Group 2** : Sodium perborate 10%
- **Group 3** : Ozonated water
- **Group 4** : Distilled water

Freshly prepared 0.04 ml of each bleaching agent was taken in a 1 ml syringe and applied directly into the access cavity of corresponding tooth samples. (Fig . 22, 23, 24, 25) Access cavity was then sealed with temporary filling material (IRM) and placed inside Eppendorf tube, which was then placed in the incubator at 37°C to simulate the oral environment.

The change in tooth shade was evaluated and recorded by two independent observers who were trained and calibrated by a senior faculty. Change in tooth shade was evaluated and compared with baseline, day 7, day 14 and day 21 using **Table 1** and recorded in the evaluation sheet by the observers.

Statistical analysis

- Inter- observer reliability analysis was done using **symmetric measure** , Cohen's kappa (k) which measures the agreement between the two observer s accounting for chance.

- The normality of the Data distribution among the groups was evaluated using **Shapiro–Wilk test**.
- The comparison of combined bleaching efficacy of four groups at day 7, day 14 and day 21 were analysed using **Median test**.
- Pair wise comparison was done using **Bonferroni test adjusted for post hoc analysis**.
- Comparison of bleaching efficacy of all four groups between day 7, day 14 and day 21 were analysed using **Median test**.
- Pair wise comparison between the groups on day 7 was done using **Bonferroni test adjusted for post hoc analysis**.

Formulas used for analysis

Shapiro wilk test for normality

$$W = \frac{(\sum_{i=1}^n a_i x_{(i)})^2}{\sum_{i=1}^n (x_i - \bar{x})^2},$$

Median test

$$T = \frac{A/n_1 - B/n_2}{\sqrt{\hat{P}(1-\hat{P}) \left(1/n_1 + 1/n_2 \right)}}; \text{where, } \hat{P} = \frac{A+B}{n_1+n_2}$$

Bonferroni test adjusted for post hoc analysis

$$\text{FWER} = P \left\{ \bigcup_{i=1}^{m_0} \left(p_i \leq \frac{\alpha}{m} \right) \right\} \leq \sum_{i=1}^{m_0} \left\{ P \left(p_i \leq \frac{\alpha}{m} \right) \right\} = m_0 \frac{\alpha}{m} \leq \alpha.$$

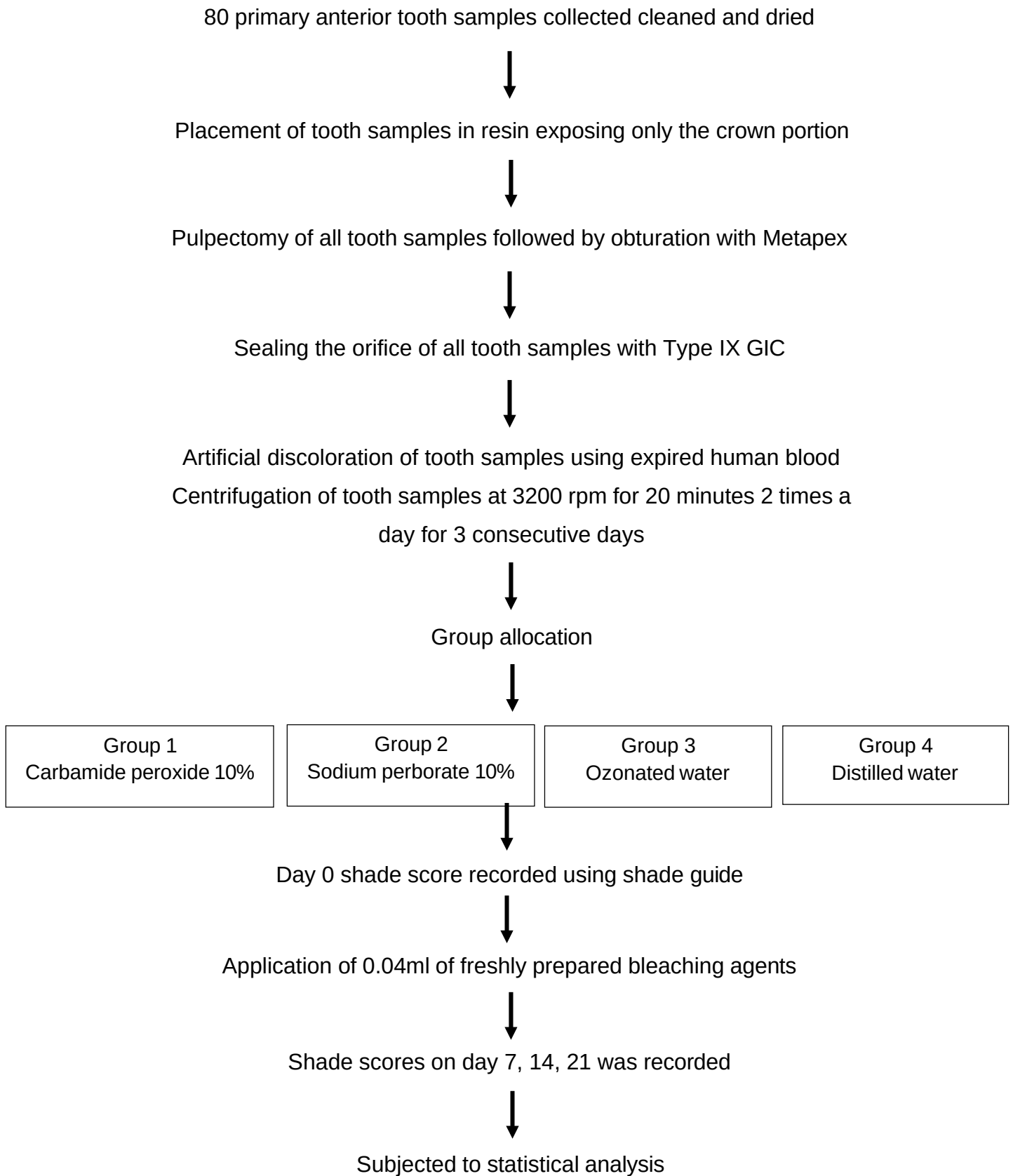




Figure 1 – Tooth samples



Figure 2 – Armamentarium required for tooth mounting





Figure 5 – Access opening done on tooth samples



Figure 6 – Cleaning and shaping of canals



Figure 7 – Irrigation of root canals with saline



Figure 8 – Obturation of root canals with Metapex

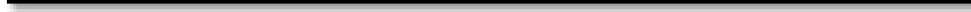




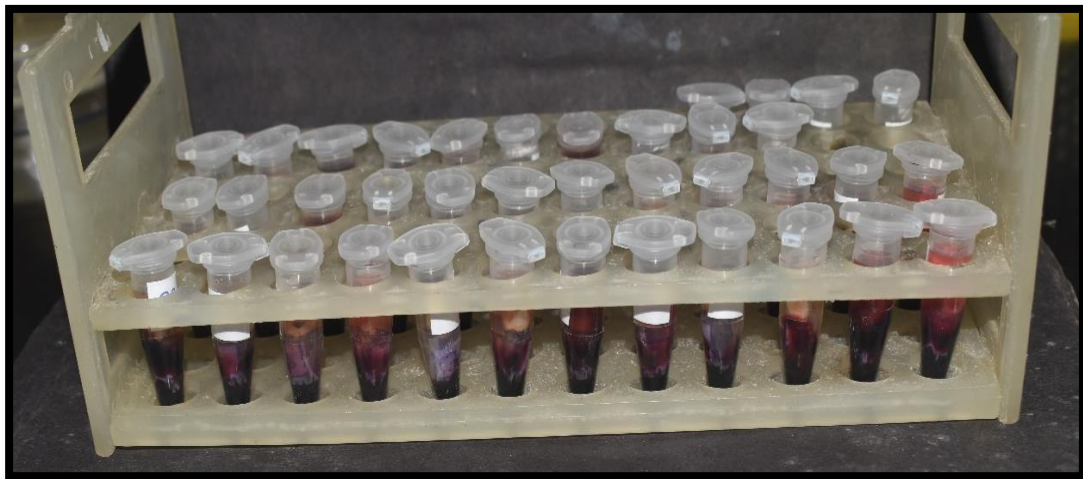
Figure 11 – Centrifugation machine



Figure 12 - Incubator



Figure 13 – VITAPAN classical shade guide



**Figure 14 – Tooth samples immersed in Eppendorf tubes
containing blood**

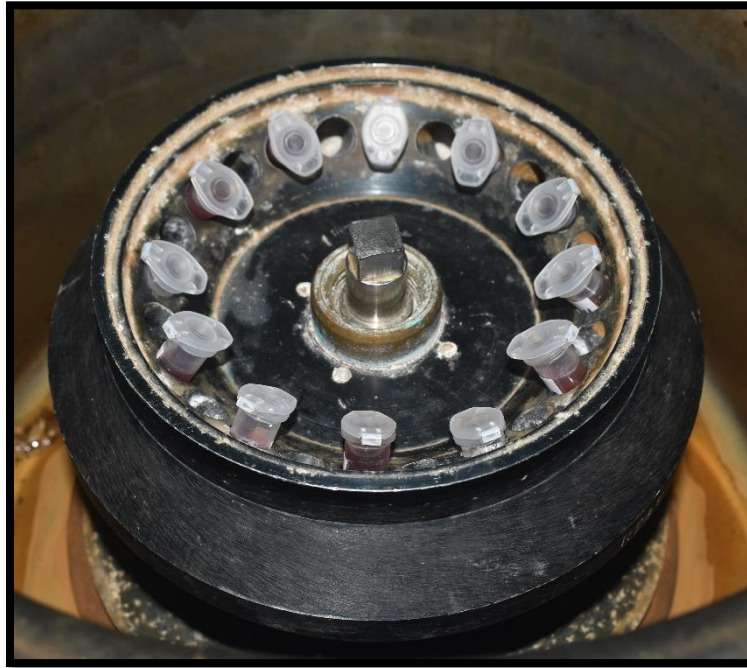


Figure 15 – Centrifugation of tooth samples

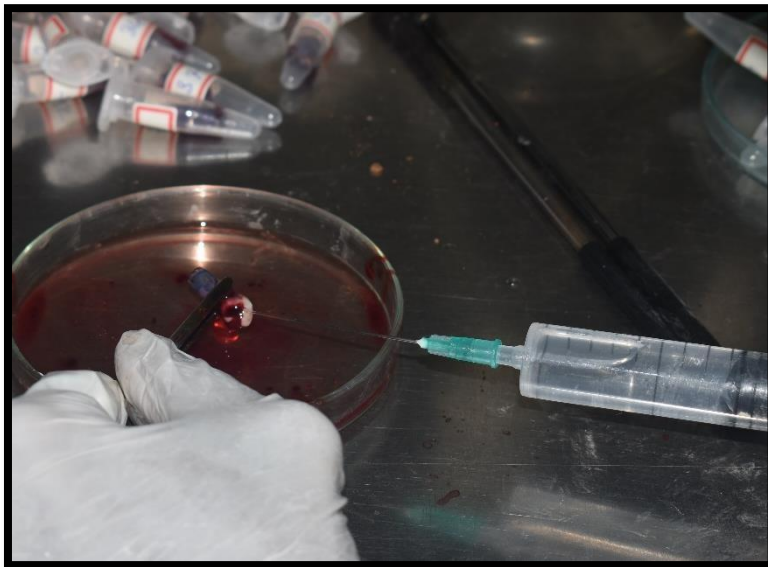


Figure 16 – Removal of excess blood stains from tooth samples using distilled water



Figure 17 – Discolored tooth samples washed and dried



Figure 18 – Carbamide peroxide 20%



Figure 19 – Sodium perborate tetrahydrate powder



Figure 20 – Magnetic stirrer



Figure 21 – 5 g per hour Ozone generator machine



Figure 22 – Application of Carbamide peroxide 10% gel

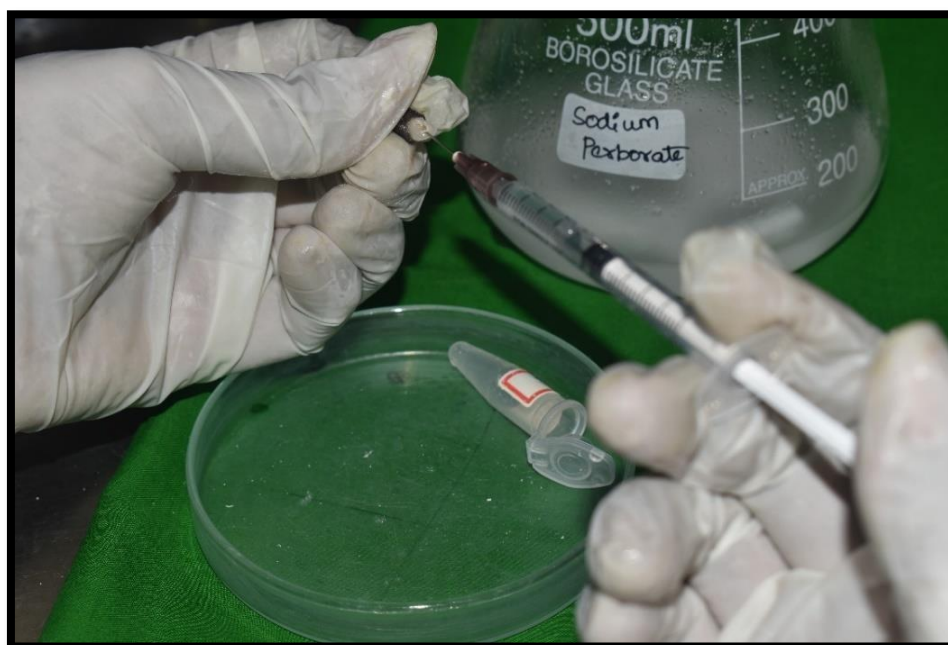


Figure 23 – Application of sodium perborate 10%



Figure 24 – Application of ozonated water

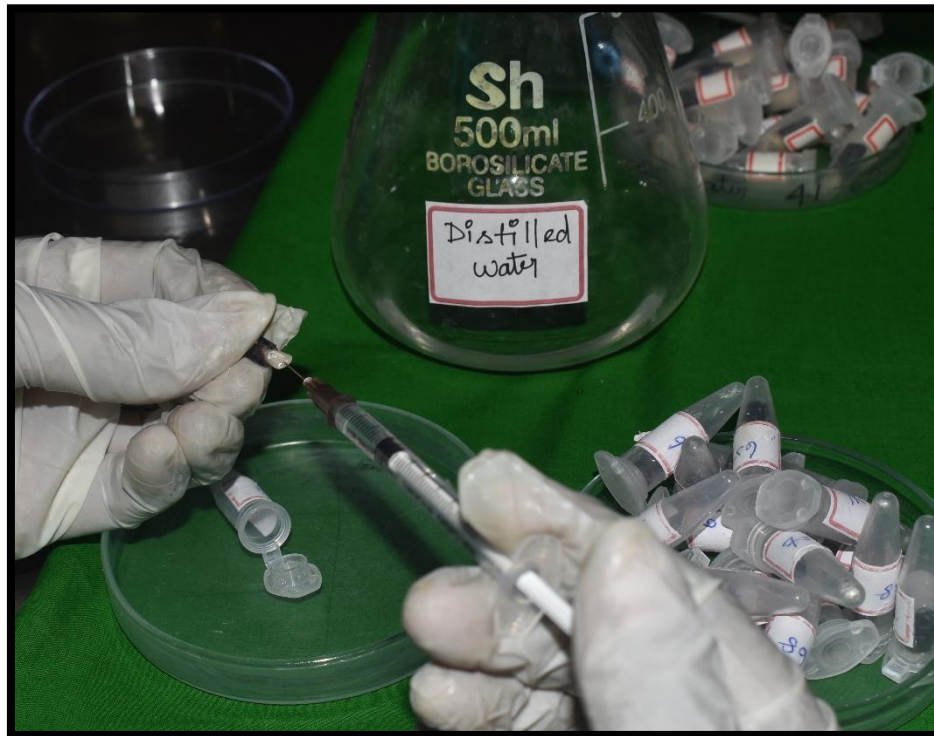


Figure 25 – Application of distilled water



Figure 26 – Sealing of canal with temporary filling material



**Figure 27 – Placement of tooth samples inside incubator
after bleaching procedure at 37°C**

The present in vitro study was conducted at the Department of Pediatric and Preventive Dentistry, Sathyabama Dental College and Hospital, Chennai in association with LifeTeck Research Centre, Arumbakkam, Chennai.

Vita classical shade	B1	A1	B2	D2	A2	C1	C2	D4	A3	D3	B3	A3.5	B4	C3	A4	C4
ΔE value	1.6	1.5	3.5	5.7	3.2	4.8	7.8	9.9	6.6	6.7	10.3	9.5	11.2	10.4	11.9	15.0
Score	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

Table 1 Vita Classic Shade Guide and the readings converted into ΔE value

Groups	Median	75%	25%	Interquartile range	Statistical value*
Carbamide peroxide	6.70	10.40	5.70	4.70	22.071 (P<0.001)**
Sodium perborate	7.80	12.70	6.70	6.00	
Ozonated water	6.60	7.80	4.80	3.00	
Distilled water	5.25	6.70	3.20	3.50	
*-Median test **-Statistically significant					

Table 2. Comparison of medians of combined bleaching efficacy of four experimental groups, carbamide peroxide, sodium perborate, ozonated water and distilled water

Table 2 shows that on comparison, the bleaching efficacy of four experimental groups was statistically significant. ($p < 0.001$) (Table 2). This infers that there is a clinically significant difference in bleaching efficacy of the four experimental groups when compared to control group irrespective of time intervals.

Group	Comparison group	Test statistic value	P-value*
Carbamide peroxide	Sodium perborate	3.609	0.345(p>0.05)
Carbamide peroxide	Ozonated water	0.974	1.000(p>0.05)
Carbamide peroxide	Distilled water	7.619	0.035(p<0.05)**
Sodium perborate	Ozonated water	8.229	0.024(p<0.05)**
Sodium perborate	Distilled water	20.907	<0.001(p<0.05)**
Ozonated water	Distilled water	0.626	1.000(p>0.05)
*-Bonferroni adjusted for post hoc test			

Table 3. Pair wise comparison between carbamide peroxide, sodium perborate, ozonated water and distilled water

On comparison, sodium perborate exhibited better efficacy when compared to ozonated water and distilled water, which was statistically significant ($p < 0.05$). Nevertheless, there is no statistically significant difference between the efficacy of sodium perborate and carbamide peroxide ($p > 0.05$). On pairwise comparison, carbamide peroxide exhibited better efficacy than distilled water ($p < 0.05$). However, bleaching efficacy of ozonated water was comparable with carbamide peroxide ($p > 0.05$) and ozonated water had better bleaching efficacy similar to distilled water ($p > 0.05$). This infers that the bleaching efficacy of sodium perborate and carbamide peroxide are almost similar with the least efficacy shown by ozonated water. (Table. 3).

Day	Group												Median Test*		
	Carbamide peroxide				Sodium perborate		Ozonated water			Distilled water					
	Med.	75%	25%	Med.	75%	25%	Med.	75%	25%	Med.	75%	25%	Overall Median	Test statistic	P-Value
Day 7	10.40	13.45	6.70	10.40	15.00	7.25	7.80	13.45	6.70	5.25	6.70	3.20	7.80	18.032	<.001
Day 14	6.70	9.05	4.80	7.25	10.40	6.70	5.70	7.25	5.25	5.25	6.70	3.20	6.70	5.087	.166
Day 21	4.80	6.20	1.55	6.65	7.80	5.25	3.65	5.70	1.50	5.25	6.70	3.20	5.70	7.086	.069

Table 4. Comparison of bleaching efficacy of all experimental groups carbamide peroxide, sodium perborate, ozonated water and distilled water. between day 7,14 and 21

Results show that the bleaching efficacy of all the experimental groups differed significantly on day 7 ($p < 0.05$) indicating that the bleaching efficacy were same among the groups on day 14 and day 21 ($p > 0.05$). This indicates that the bleaching agents differ in their efficacy on day 7 with no significant effect on day 14 and day 21. (Table 4).

Groups	Comparison group	Test statistic value	P – Value*
Distilled water	Ozonated water	5.227	.133
Distilled water	Carbamide peroxide	6.667	.059
Distilled water	Sodium perborate	12.130	.003
Ozonated water	Carbamide peroxide	.902	1.000
Ozonated water	Sodium perborate	1.600	1.000
Carbamide peroxide	Sodium perborate	.107	1.000
*-Bonferroni adjusted for post hoc test			

Table 5. Pair wise Comparison of bleaching efficacy between the experimental groups carbamide peroxide, sodium perborate, ozonated water and distilled water on day 7

On pairwise comparison between the experimental groups on day 7, sodium perborate exhibited better efficacy than control group ($p < 0.05$) which was statistically significant. However, sodium perborate had better efficacy on day 7 but its efficacy was less when compared to carbamide peroxide ($p > 0.05$). However, ozonated water exhibited the least bleaching efficacy when compared to the other experimental groups on day 7 (Table 5).

Teeth whitening techniques have seen renewed interest from the dental profession as a result of the public's desire for whiter, brighter teeth. Everyone wants whiter teeth to make them feel younger and to provide beautiful smiles, with the accompanying increase in self-esteem. ⁴⁰

Various physiological conditions result in tooth discoloration that may be esthetically improved by lightening tooth color. Among these conditions are tetracycline staining, fluorosis, amelogenesis imperfecta, dentinogenesis imperfecta, chemical or food staining, and hereditary opalescent dentin. Most patients with these conditions desire to have their tooth color changed so that their teeth appear to be within the range of natural tooth color. ⁴⁰

Apart from blood degradation, degrading proteins of necrotic pulp tissue may also cause discolouration. If the access cavity is prepared inappropriately, pulp tissue can be left in the pulp chamber in the pulp horns, which may be another reason for discolouration. ⁴¹

Stewart Ho and Goerig reported that deciduous teeth can also be successfully bleached. As young children are accident - prone and have a significant number of anterior teeth that are

traumatized, the pulps become necrotic, causing discoloration of the teeth.⁴²

In 1961, **Spasser** described the intracoronal bleaching method of sealing by using a mixture of sodium perborate with water into the pulp chamber and leaving it *in situ* for 1 week. This was known as "walking bleach".²⁶ Nutting and Poe modified this by using a combination of 30% hydrogen peroxide and sodium peroxide sealed into the pulp chamber. The most commonly used bleaching agents to produce the desired esthetic color change are hydrogen peroxide and sodium perborate, either used alone or in combination.⁴³

Shaheen et al. have evaluated the efficiency of 10% carbamide peroxide in the primary teeth and observed that intracoronal bleaching using 10% carbamide peroxide is an effective approach for whitening discolored extracted primary teeth.³⁶ Thus, in the present study, 10% carbamide peroxide gel was used.

Sodium Perborate is available in a powdered form or as various commercial preparations. When fresh, it contains about 95% perborate, corresponding to 9.9% of the available oxygen. Commonly used sodium perborate preparations are alkaline, and their pH depends on the amount of hydrogen peroxide

released and the residual sodium metaborate. Sodium perborate is more easily controlled and safer than concentrated hydrogen peroxide solutions. Therefore, it should be the material of choice in most intracoronary bleaching procedures.⁴⁴ So, 10% solution of sodium perborate mixed with distilled water was used in this study.

Ozone is a natural gas formed by three oxygen atoms, and it has been used for medical therapies since World War I.⁴⁵ Currently, health professionals use ozone therapy⁴⁶ for the treatment of several pathologies due to its high oxidation power, immune response, circulatory stimulation, analgesic, anti-inflammatory properties, and parasitological effect.^{47,48,49} In dentistry, Ozone effectively controls infections caused by viruses, protozoa, fungi, and bacteria.⁴⁵ Moreover, it seems to promote tissue repair and healing processes, prevention of dental caries^{47,48,50} remineralization of the tooth surface⁴⁸ treatment of oral ulcers, treatment of gingivitis and periodontitis, pain control in endodontic treatment⁵¹, halitosis, temporomandibular disorders, complementary treatment of non-carious cervical lesions, tooth sensitivity^{52,53} and tooth bleaching.

Using ozone for tooth bleaching is safe in conditions in which diffusion is an important factor, such as in hard dental

tissues, as it works on their organic substances and can be used to reduce tetracycline staining ⁴⁸. However, the effectiveness of ozone therapy in tooth bleaching may depend on the application time, bleaching gel concentration, and gas flow rate. There is still no consensus in the literature on the best usage protocol for ozone for tooth bleaching in primary teeth. Thus, in this study freshly prepared ozonated water was used as the bleaching agent in comparison with 10% carbamide peroxide and 10% sodium perborate. To the author's knowledge this is a first attempt in which ozonated water has been used as bleaching agent in primary teeth.

According to **Lim et al.**, it is well established that visual colour determination is subjective and can be compared to the accuracy of spectrophotometer evaluation. ²⁸ Vachon et al. suggested that although the spectrophotometer readings might be the gold standard, these differences could be clinically identical to the human eye. ⁵⁴ Thus, in the present study, the color changes in each specimen were evaluated using the Vita shade guide under standardized lighting conditions. For comparative evaluation the shades in the Vita classical shade guide were converted to their corresponding ΔE value as cited by **Jong-Gil Kim et al 2009** . The distribution of data was found to be skewed and hence non parametric tests of significance were used in this study. ⁵⁵

In this study, all the experimental groups showed statistically significant differences in their bleaching efficacy on analyzing the combined efficacy when compared to the control group. This correlated with many studies comparing the efficacy of carbamide peroxide and sodium perborate at different concentrations in permanent as well as primary dentition. However, ozonated water could not match the bleaching efficacy of sodium perborate and carbamide peroxide which can be seen on pairwise comparison in our study. This could be due to the use of ozonated water instead of gas which has been used in previous studies. Ozonated water has been tried as a newer bleaching agent in this study in intracoronal bleaching.

Nevertheless, on the whole, ozonated water has shown significant bleaching efficacy in comparison with carbamide peroxide 10% and sodium perborate 10% in accordance with **Azarpazhooh A et al**. The bleaching efficacy of ozone is due to its ability of decomposition and oxygen radical production which could occur more efficiently in the water than in the air.⁵⁶

On day wise comparison of bleaching efficacy between the experimental groups, the bleaching efficacy was similar among the experimental groups on day 7, which is in agreement with the study done by **Ganesh et al.** where there was no significant

difference between the bleaching actions of 10% carbamide peroxide and sodium perborate after two bleaching sessions in primary teeth.³³ These results were in agreement with **Manisha bhel et al.** where 10% carbamide peroxide and sodium perborate does not show statistically significant shade variation on day 0, day 7 and day 14 in primary teeth.³⁹

This study's results are also in concordance with the results of **Perrine et al (2000)** who observed no statistically significant difference between 10% carbamide peroxide and sodium perborate in the tooth whitening.⁵⁷ However, **Carrasco et al** reported that the dentin permeability of carbamide peroxide is higher than sodium perborate. The results could have been different due to the different methodology and evaluation techniques used.²⁹

Ozonated water showed a comparable bleaching efficacy with carbamide peroxide on day 7 in this study. This correlated with the findings of **Thalyta et al.(2022)** in which ozone promoted tooth bleaching with the same effectiveness as 10% carbamide peroxide bleaching treatment in tray. However, ozone gas with the help of customized tray was used with a application protocol of 1 hour every 3 days, for a total of three applications.⁵⁸

Sodium perborate group showed better bleaching efficacy than other groups on day 7. The possible reason for this result is that sodium perborate tetrahydrate presents a small amount of active oxygen and its setting occurs after 7 days. Hence sodium perborate remains active until the bleaching agent is changed.

The conclusion drawn from this invitro study is as follows

- 10% sodium perborate, 10% carbamide peroxide and ozonated water exhibited superior bleaching efficacy when compared to control group distilled water.
- There is no statistically significant difference in the bleaching efficacy of the experimental groups.
- On comparison of the bleaching efficacy at different time intervals of day 7,14 and 21, sodium perborate exhibited comparatively better bleaching efficacy than control groups on day 7.
- However, on Day 7, there was no statistically significant difference between sodium perborate and carbamide peroxide. Ozonated water had least efficacy when compared to other experimental groups.
- On day 14 and day 21, there was no significance in the bleaching efficacy between the experimental groups and control group.

Limitations

To the authors knowledge, this is the first study to use ozonated water as bleaching agent in primary teeth. Within the limitation of this in vitro study, the findings could not be extrapolated. Hence, further studies should be done clinically to compare the efficacy of ozonated water in primary teeth. This could add more value to the scientific and clinical use of ozonated water as bleaching agent in primary dentition.

The present in vitro study was carried out in the Department of Pediatric and Preventive Dentistry, Sathyabama Dental College and Hospital. The study intended to measure the bleaching efficacy of 10% carbamide peroxide, 10% sodium perborate, freshly prepared ozonated water and distilled water as control and also compared the bleaching efficacy within the groups.

Preparation of tooth samples for artificial discoloration

Eighty non carious primary anterior tooth samples with at least one third of the root structure were collected and disinfected using ethylene glycol for this study. Acrylic moulds were prepared using clear acrylic and the samples were embedded in acrylic mould exposing only the crown part till the cemento-enamel junction. To evaluate the shade in natural light with an even contrast, the acrylic moulds were painted with black color sketch pen. Access opening for all the tooth samples were done using access opening bur (No.#3) . The canals were cleaned and shaped using K- files from size 10 to size 30 with sufficient saline irrigation. The canals were obturated with calcium hydroxide – iodoform containing paste and the excess material was removed 1 mm below the orifice by measuring with Williams probe and sealed with type IX GIC and reduced 1 mm below the access cavity.

Artificial discoloration of tooth samples

Expired human blood was used for discoloration of tooth samples in this study. The expired human blood sample was obtained by providing a requisition letter to the authorities of Rotary Central VHS Blood Bank, Tharamani, Chennai . All the collected tooth samples were stained artificially using human blood to attain artificial internal hemorrhage. Allocated tooth samples were immersed in Eppendorf tubes containing 1.5 ml the expired human blood and centrifuged at 3200 rpm twice daily for three consecutive days. Stained samples were washed by thoroughly rinsing all the tooth samples individually using distilled water and dried completely. Baseline shade (Day 0) was noted immediately after staining procedure was recorded using VITA shade guide.

For convenience purpose, the VITA shade guide values were converted into numerical values and their corresponding ΔE value shown in **Table 1** according to **Jong gil kim et al.**

Preparation of bleaching agent

Four different bleaching agents were used for this in -vitro study namely

- **Group I** - Carbamide peroxide 10%
- **Group II** - Sodium perborate 10%
- **Group III** -Ozonated water
- **Group IV (control)** - Distilled water

Carbamide peroxide 10%

Conventionally available carbamide peroxide 20% gel was converted into carbamide peroxide 10% gel by taking 1.2 g of gel and diluting with 1.2 ml of distilled water and transferred into a 1 ml syringe for easy application into the access canal. This 10% carbamide peroxide gel was prepared freshly during the bleaching procedure.

Sodium perborate 10%

Sodium perborate 10% solution is prepared by dissolving 10 g of sodium perborate tetrahydrate powder in 10 ml of distilled water, using a magnetic stirrer for half an hour. Such freshly prepared solution was used throughout the study.

Ozonated water

Ozonated water freshly prepared from industrial ozone generator was collected and used immediately.

Application of bleaching agent

After baseline shade evaluation, the discoloured tooth samples were randomly divided based on lottery method and allocated into four experimental groups respectively as

- **Group 1** : Carbamide peroxide 10%
- **Group 2** : Sodium perborate 10%
- **Group 3** : Ozonated water
- **Group 4** : Distilled water.

Freshly prepared 0.04 ml of each bleaching agent was taken in a 1 ml syringe and applied directly into the access cavity of corresponding tooth samples. Access cavity was then sealed with temporary filling material and placed inside Eppendorf tube, which was then placed inside incubator at 37°C to simulate the oral cavity.

The change in tooth shade was evaluated and recorded by two independent observers who were trained and calibrated by a senior faculty. Change in tooth shade was evaluated and compared with baseline, day 7, day 14 and day 21 by the two observers and recorded in the evaluation sheet.

Statistical analysis

- Inter- observer reliability analysis was done using **Symmetric measure**, Cohen's kappa (k) which measures the agreement between the two observers accounting for chance.
- The normality of the Data distribution among the groups was evaluated using **Shapiro-Wilk test**.
- The comparison of combined bleaching efficacy of four groups at day 7, day 14 and day 21 were analysed using **Median test**.
- Pair wise comparison was done using **Bonferroni test adjusted for post hoc analysis**.

- Comparison of bleaching efficacy of all four groups between day 7, day 14 and day 21 were analysed using **Median test**.
- Pair wise comparison between the groups on day 7 was done using **Bonferroni test adjusted for post hoc analysis**.

Results

Interobserver reliability test was done between the scores given by the two independent observers, which shows 70% agreement between the observers.

Cohen's kappa value from the symmetric measures was found to be 0.856 which is statistically significant (P value <0.05)

On comparison, the bleaching efficacy of four experimental groups was statistically significant. ($p < 0.001$). This infers that there is a clinically significant difference in bleaching efficacy of the four experimental groups when compared to control group irrespective of time intervals.

Pairwise comparison between the experimental groups showed that sodium perborate exhibited better efficacy when compared to ozonated water and distilled water, which was statistically significant ($p < 0.05$). Nevertheless, there is no statistically significant difference between the efficacy of

sodium perborate and carbamide peroxide ($p > 0.05$). On pairwise comparison, carbamide peroxide exhibited better efficacy than distilled water ($p < 0.05$). However, bleaching efficacy of ozonated water was comparable with carbamide peroxide ($p > 0.05$) and ozonated water had better bleaching efficacy similar to distilled water ($p > 0.05$). This infers that the bleaching efficacy of sodium perborate and carbamide peroxide are almost similar and the least efficacy was shown by ozonated water.

Comparison of bleaching efficacy of all groups carbamide peroxide, sodium perborate, ozonated water and distilled water, between day 7, 14 and 21 showed that the bleaching efficacy of all the experimental groups differed significantly on day 7 ($p < 0.05$) indicating that the bleaching efficacy were same among the groups on day 14 and day 21 ($p > 0.05$). This indicates that the bleaching agents differ in their efficacy on day 7 with no significant effect on day 14 and day 21.

Pairwise comparison between the experimental groups on day 7, showed that sodium perborate exhibited better efficacy than control group ($p < 0.05$) which was statistically significant. However, sodium perborate had better efficacy on day 7 but its efficacy was less than carbamide peroxide ($p > 0.05$). However, ozonated water exhibited the least bleaching efficacy when compared to the other experimental groups on day 7.

Conclusion

Based on the results of this study, it can be concluded that,

- 10% sodium perborate, 10% carbamide peroxide and ozonated water exhibited superior bleaching efficacy when compared to control group distilled water.
- There is no statistically significant difference in the bleaching efficacy of the experimental groups.
- On comparison of the bleaching efficacy at different time intervals of day 7,14 and 21, sodium perborate exhibited comparatively better bleaching efficacy than control group ($p < 0.05$) on day 7.
- However, on day 7, there was no statistically significant difference between sodium perborate and carbamide peroxide. Ozonated water had least efficacy when compared to other experimental groups.
- On day 14 and day 21, there was no significant bleaching efficacy between the experimental groups and control group.

Limitations

To the authors knowledge, this is the first study to use ozonated water as bleaching agent in primary teeth. Within the limitation of this in vitro study, findings could not be extrapolated. Hence, further studies should be done clinically to compare the efficacy of ozonated water in primary teeth. This could add more value to the scientific and clinical use of ozonated water as bleaching agent in primary dentition.

1. Modi S, Williams L, Greenwood R, Davey N, Crawford P. Development of an illustrated index of tooth appearance - perception- based quantification of tooth discolouration and surface defects. *Int J Paediatr Dent*. 2010 Jul; 20(4):293 - 304. doi: 10.1111/j. 1365 - 263X. 2010. 01053. x. PMID: 20536591.
2. L. Tremblay, T. Lovsin, C. Zecevic, and M. Larivière, “ Perceptions of self in 3 -5-year- old children: a preliminary investigation into the early emergence of body dissatisfaction,” *Body Image*, vol. 8, no. 3, pp. 287 –292, 2011. View at: [Publisher Site](#) | [Google Scholar](#)
3. Carey CM. Tooth whitening: what we now know. *J Evid Based Dent Pract*. 2014;14 Suppl:70 - 76. doi: 10. 1016/j. jebdp.2014.02.00
4. Arı, H., and M. Üngör. "In vitro comparison of different types of sodium perborate used for intracoronar bleaching of discoloured teeth." *International Endodontic Journal* 35.5 (2002): 433-436.
5. Extraradicular diffusion of hydrogen peroxide and pH changes associated with intracoronar bleaching of discoloured teeth using different bleaching agents. Lee GP, Lee MY, Lum SO, Poh RS, Lim KC *Int Endod J*. 2004 Jul; 37(7):500 -6

6. Sharma DS, Barjatya K, Agrawal A. Intra-coronal bleaching in young permanent and primary tooth with biologic perspectives. J Clin Pediatr Dent 2011;35: 349-52.
7. Jiang, Tao, et al. "Beneficial effects of hydroxyapatite on enamel subjected to 30% hydrogen peroxide." Journal of dentistry 36.11 (2008): 907-914
8. Abu- Naba'a, L. A. "Management of primary occlusal pit and fissure caries using ozone." (2004): 0916-0916
9. Kagan, Val, Al Chambers, and Robert Bray. "Forward to better understanding of optical characterization and development of colored polyamides for the infra-red/ laser welding, part II-family of colored polyamides." Journal of reinforced plastics and composites 22.7 (2003): 593-602.
10. Tiwari, Sansriti, et al. "Dental applications of ozone therapy: A review of literature." The Saudi Journal for Dental Research 8.1-2 (2017): 105-111.
11. Shulman JD, Maupome G, Clark DC, Levy SM. Perceptions of desirable tooth color among parents, dentists and children. J Am Dent Assoc. 2004 May; 135(5): 595 - 604; quiz 654-5. doi: 10.14219/jada.archive.2004.0247. PMID: 15202751.
12. Kapur, Aditi & HS, Chawla & Goyal, Ashima & Gaube, Krishan. (2005). An esthetic point of view in very young children. The Journal of clinical pediatric dentistry. 30. 99 - 103. 10.17796/jcpd.30.2.360k2j4452773418.

13. Text book of Esthetics in Pediatric Dentistry: An overview by Renu Ann Mathew , Amitha M. Hegde , Preeth Shetty
14. Chadha T, Yadav G, Tripathi AM, Dhinsa K, Arora D. Recent trends of Esthetics in Pediatric Dentistry. *Int J Oral Health Med Res* 2017;4(4):70-75
15. Gupta T, Sadana G, Rai HK. Effect of Esthetic Defects in Anterior Teeth on the Emotional and Social Well - being of Children: A Survey. *Int J Clin Pediatr Dent* . 2019;12(3):229-232. doi:10.5005/jp-journals-10005-1628.
16. Vogel, Richard I.. "Intrinsic and extrinsic discoloration of the dentition (a literature review)." *Journal of oral medicine* 30 4 (1975): 99-104 .
17. Arens D. The role of bleaching in esthetics. *Dent Clin North Am* 1989;33:319–36.79
18. Guldener PHA, Langeland K (1993) *Endodontologie*, 3rd edn. Stuttgart . NewYork: Thieme.
19. Davis MC, Walton RE, Rivera EM. Sealer distribution in coronal dentin. *J. Endod.* 2002; 28: 464-6.
20. Text book of Ingle's endodontics 7 th edition.
21. Holan G, Fuks AB. The diagnostic value of coronal dark - gray discoloration in primary teeth following traumatic injuries. *Pediatr Dent.* 1996;18(3):224–227.
22. Waterhouse PJ, Nunn JH. Intracoronal bleaching of nonvital teeth in children and adolescents: Interim results. *Quintessence Int* 1996;27:447–53.

23. Bryan, R. A., & Welbury, R. R. (2003). Treatment of aesthetic problems in paediatric dentistry. *Dental update*, 30 6, 307-13 .
24. Attin T, Paque F, Ajam F, Lennon AM. Review of the current status of tooth whitening with the walking bleach technique. *Int Endod J* 2003;36: 313–29.
25. American Academy of Pediatric Dentistry. Policy on the use of dental bleaching for child and adolescent patients. *The Reference Manual of Pediatric Dentistry*. Chicago, Ill.: American Academy of Pediatric Dentistry; 2021:116-9
26. Spasser HF. A simple bleaching technique using sodium perborate. *NY Dent J* 1961;27:332–334.
27. Truman J (1864). Bleaching of non- vital discoloured anterior teeth. *Dent Times* 1:69-72.
28. Lim MY, Lum SO, Poh RS, Lee GP, Lim KC. An in vitro comparison of the bleaching efficacy of 35% carbamide peroxide with established intracoronal bleaching agents. *Int Endod J*. 2004;37:483–488.
29. Effect of internal bleaching agents on dentinal permeability of non- vital teeth: quantitative assessment. Carrasco LD, Fröner IC, Corona SA, Pécora JD *Dent Traumatol*. 2003 Apr; 19(2):85-9.
30. Braun A, Jepsen S, Krause F. Spectrophotometric and visual evaluation of vital tooth bleaching employing

- different carbamide peroxide concentrations. *Dent Mater.* 2007;23(2):165–169.
31. Gontijo IT, Navarro RS, Ciamponi AL, Zezell DM. Whitening techniques using the diode laser and halogen lamp in human devitalized primary teeth. *J Dent Child (Chic)*. 2008 May-Aug;75(2):164-7. PMID: 18647512.
 32. Arikan, Volkan & SARI, ŞAZİYE & Sönmez, Hayriye. (2009). Bleaching a devital primary tooth using sodium perborate with walking bleach technique: A case report. *Oral surgery, oral medicine, oral pathology, oral radiology, and endodontics*. 107. e80 - 4. 10. 1016/j. tripleo. 2009. 01. 050.
 33. Ganesh R, Aruna S, Joyson M; Manikandan; Deepa. Comparison of the bleaching efficacy of three different agents used for intracoronaral bleaching of discolored primary teeth: an in vitro study. *J Indian Soc Pedod Prev Dent*. 2013 Jan-Mar;31(1):17-21. doi: 10.4103/0970-4388.112394. PMID: 23727737.
 34. Anantharaj A, Patil P, Ramakrishnan S, Jagadeesh R. Walking bleach in primary teeth. *SRM J Res Dent Sci* 2015;6:187-90
 35. Zanjani VA, Ghasemi A, Torabzadeh H, Jamali M, Razmavar S, Baghban AA. Bleaching effect of ozone on pigmented teeth. *Dent Res J (Isfahan)* . 2015;12(1): 20 -24. doi:10.4103/1735-3327.150295

36. Efficacy of 10 percent Carbamide Peroxide as an Intracoronaral Bleaching Agent in Nonvital Discolored Primary Teeth: An In Vitro Study. Shaheen MA, Elkateb MA, Bakry NS, El Meligy OA J Dent Child (Chic). 2017 Jan 15; 84(1):22-29.
37. Al- Omiri MK, Lamfon HA, Al Nazeh AA, Kielbassa AM, Lynch E. Randomized clinical trial on the comparison of bleaching outcomes using either ozone or hydrogen peroxide. Quintessence Int. 2018;49(8): 625 - 634. doi: 10.3290/j.qi.a40783. PMID: 30027174.
38. Dr. Lavina Bhatnagar; Dr Ekta Choudhary; Dr. Priyadarshani Suryawanshi; Dr. Sagrika Grover; Dr. Ankita Bhargava; Dr. Raksha Thakur. "A comparative analysis of different bleaching agents in primary and permanent teeth". *European Journal of Molecular & Clinical Medicine* , 7, 11, 2021, 7890-7895.
39. Behl M, Patnana AK, Khanna V, Chaudhry K. Evaluation of Three Different Bleaching Agents in Permanent and Primary Teeth: An *In Vitro* Study. *Int J Clin Pediatr Dent* . 2020;13(2):130-135. doi:10.5005/jp-journals-10005-1721
40. Christensen GJ. The tooth whitening revolution. J Am Dent Assoc 2002;133:1277-9.
41. Guldener, P. H. A., Langeland, K. (1993). Endodontologie. 3rd ed. Stuttgart, New York. Georg ThiemeVerlag.

42. Stewart HO, Goerig AC. An invitro comparison of different bleaching agents in the discolored tooth. J Endod 1989;15:106-11
43. Nutting EB, POE GS. A new combination for bleaching teeth. J South Calif Dent Assoc 1963;31:289-91
44. Rotstein I, Friedman S. pH variation among materials used for intracoronal bleaching. JOE 1991;17:376.
45. Bocci, V. A. Scientific and medical aspects of ozone therapy. State of the art. Arch. Med. Res. **37**, 425, 435. [https:// doi. org/ 10. 1016/j. arcmed. 2005. 08.006](https://doi.org/10.1016/j.arcmed.2005.08.006) (2006).
46. Bianco, E., Maddalone, M., Porcaro, G., Amosso, E. & Baldoni, M. Treatment of osteoradionecrosis of the jaw with ozone in the form of oil- based gel: 1 -year follow-up. J. Contemp. Dent. Pract. **20**, 270–276 (2019).
47. Nogales, C. G., Ferrari, P. H., Kantorovich, E. O. & Lage - Marques, J. L. Ozone therapy in medicine and dentistry. J. Contemp. Dent. Pract. **9**, 75–84 (2008).
48. Al- Omiri, M. K., Alhijawi, M., AlZarea, B. K., Abul Hassan, R. S. & Lynch, E. Ozone treatment of recurrent aphthous stomatitis: A double blinded study. Sci. Rep. **6**, 27772. <https://doi.org/10.1038/srep27772> (2016).
49. Grocholewicz, K. et al. Effect of nano- hydroxyapatite and ozone on approximal initial caries: A randomized clinical

- trial. *Sci. Rep.* **10**, 11192. <https://doi.org/10.1038/s41598-020-67885-8> (2020).
50. Taşdemir, Z., Alkan, B. A. & Albayrak, H. Effects of ozone therapy on the early healing period of deepithelialized gingival grafts: A randomized placebo- controlled clinical trial. *J. Periodontol.* **87**, 663– 671. <https://doi.org/10.1902/jop.2016.150217> (2016).
51. Lynch, E. Evidence- based caries reversal using ozone. *J. Esthet. Restor. Dent.* **20**, 218– 222. <https://doi.org/10.1111/j.17088240.2008.00183.x> (2008).
52. Kazancioglu, H. O., Kurklu, E. & Ezirganli, S. Effects of ozone therapy on pain, swelling, and trismus following third molar surgery. *Int. J. Oral Maxillofac. Surg.* **43**, 644– 648. <https://doi.org/10.1016/j.ijom.2013.11.006> (2014).
53. Atabek, D., Bodur, H., Yalçın, G. & Kalayci, Ş. Effects of oxidative irrigants on root dentin structure: Attenuated total reflection Fourier transform infrared spectroscopy study. *Oral Health Dent. Manag.* **13**, 753–756 (2014).
54. Internal bleaching with 10% carbamide peroxide in vitro. Vachon C, Vanek P, Friedman S *Pract Periodontics Aesthet Dent.* 1998 Nov-Dec; 10(9):1145-8, 1150, 1152
55. Kim, Jong-Gil & Yu, Bin & Lee, Yong-Keun. (2008). Correlations between Color Differences Based on Three Color- Difference Formulas Using Dental Shade Guide Tabs. *Journal of prosthodontics : official journal of the*

- American College of Prosthodontists. 18. 135 -40.
10.1111/j.1532-849X.2008.00393.x.
56. Azarpazhooh A, Limeback H. The application of ozone in dentistry: a systematic review of literature. J Dent. 2008 Feb; 36(2): 104 - 16. doi: 10.1016/j.jdent.2007.11.008. Epub 2007 Dec 31. PMID: 18166260.
57. Perrine, GA & Reichl, R & Baisden, M & Hondrum, S. (2000). Comparison of 10% carbamide peroxide and sodium perborate for intracoronal bleaching. General dentistry. 48. 264 -70.
58. Ferreira Neves Dias TDRFZ, Ferreira de Campos FU, Turssi CP, Botelho do Amaral FL, Gomes França FM, Basting RT. Color change after tooth bleaching with ozone and 10% ozonized carbamide peroxide for in- office use. Med Gas Res. 2022; 12(3): 100 - 106. doi: 10.4103/2045-9912.330693

Master chart 1

Sample number	ΔE Baseline	ΔE Day 7	ΔE Day 14	ΔE Day 21
1	6.7	5.7	5.7	1.6
2	6.6	6.6	6.6	4.8
3	6.7	6.6	4.8	1.6
4	15.0	10.4	7.8	5.7
5	9.5	6.7	6.7	4.8
6	15.0	15.0	15.0	15.0
7	11.9	6.7	6.7	4.8
8	15.0	15.0	10.4	6.7
9	10.4	6.7	6.7	4.8
10	6.7	7.8	7.8	5.7
11	15.0	6.7	4.8	1.5
12	10.4	10.4	7.8	1.5
13	10.4	10.4	10.4	6.7
14	6.7	6.7	4.8	1.5
15	6.7	4.8	4.8	1.5
16	10.4	11.2	10.3	7.8
17	6.6	7.8	4.2	1.5
18	15.0	15.0	11.2	6.7
19	6.7	7.8	4.8	5.7
20	11.9	6.7	6.6	4.8

Group 1

Sample number	ΔE Baseline	ΔE Day 7	ΔE Day 14	ΔE Day 21
21	10.4	9.5	6.7	4.8
22	15	15	15	15
23	15	6.7	6.7	5.7
24	6.7	6.7	6.7	7.8
25	6.7	6.7	7.8	5.7
26	15	10.4	10.4	6.7
27	10.4	9.5	9.5	6.6
28	10.4	6.7	6.7	7.8
29	15	15	15	15
30	15	10.4	6.7	6.7
31	10.4	6.7	6.7	4.8
32	6.7	6.7	6.7	7.8
33	7.8	7.8	4.8	5.7
34	9.9	9.9	3.2	3.2
35	6.7	7.8	7.8	5.7
36	15	15	15	10.4
37	15	15	10.4	6.7
38	6.7	6.7	7.8	1.5
39	7.8	4.8	4.8	1.5
40	15	15	15	15

Group 2

Sample number	ΔE Baseline	ΔE Day 7	ΔE Day 14	ΔE Day 21
41	7.8	1.5	1.5	1.5
42	6.7	6.7	5.7	5.7
43	6.7	7.8	7.8	5.7
44	10.4	6.7	6.7	7.8
45	6.7	7.8	5.7	5.7
46	7.8	4.8	5.7	1.5
47	15	4.8	5.7	5.7
48	4.8	4.8	5.7	1.5
49	15	4.8	4.8	1.5
50	10.4	6.7	7.8	5.7
51	3.5	1.5	1.5	1.6
52	6.7	7.8	7.8	5.7
53	15	10.4	10.4	10.4
54	15	15	10.4	10.4
55	15	4.8	3.2	1.5
56	9.5	6.7	6.7	6.6
57	6.6	7.8	5.7	1.5
58	6.7	7.8	5.7	1.5
59	11.9	6.7	5.7	1.5
60	6.6	4.8	1.5	1.5

Group 3

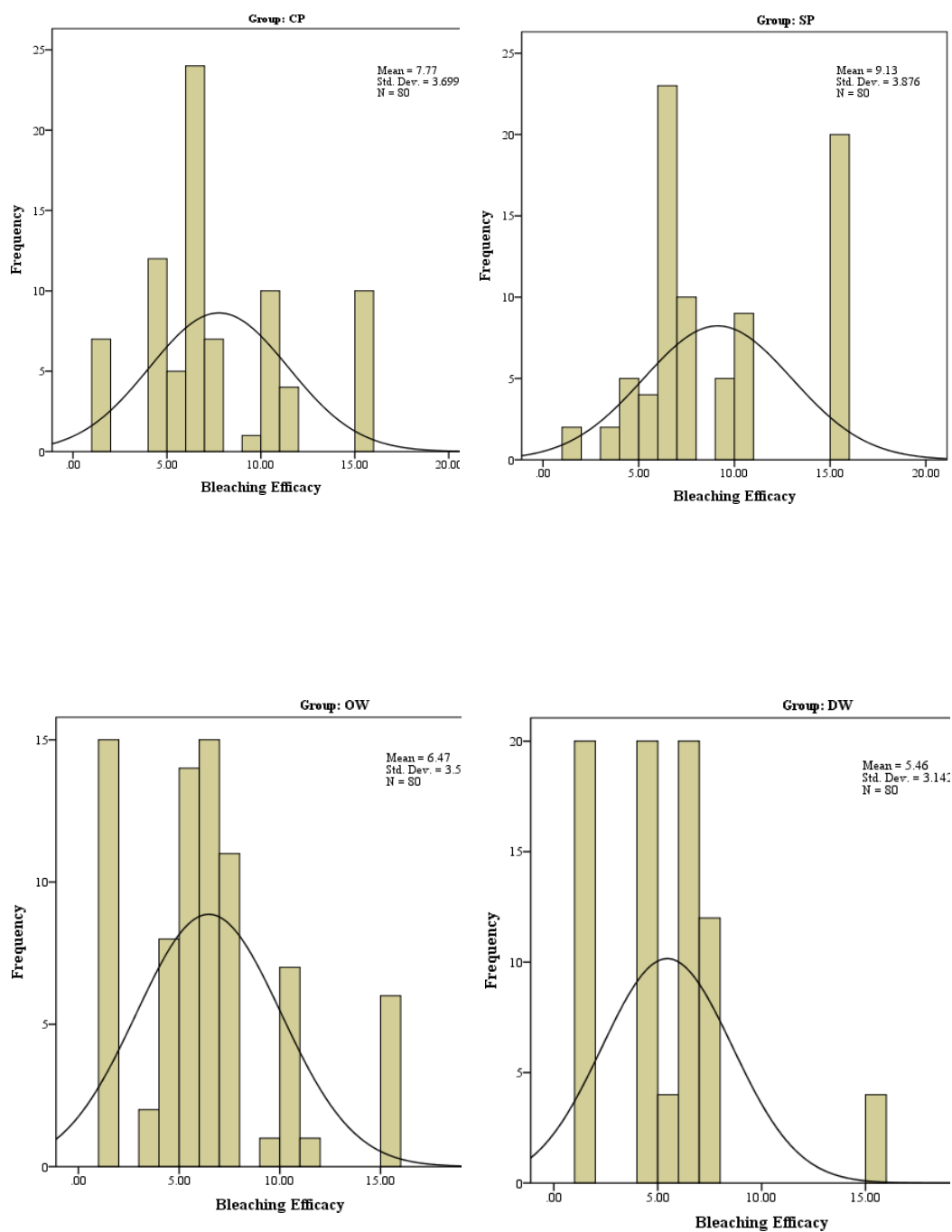
Sample number	ΔE Baseline	ΔE Day 7	ΔE Day 14	ΔE Day 21
61	1.5	1.5	1.5	1.5
62	6.7	6.7	6.7	6.7
63	4.8	4.8	4.8	4.8
64	4.8	4.8	4.8	4.8
65	4.8	4.8	4.8	4.8
66	7.8	7.8	7.8	7.8
67	4.8	4.8	4.8	4.8
68	5.7	5.7	5.7	5.7
69	1.6	1.6	1.6	1.6
70	1.5	1.5	1.5	1.5
71	6.7	6.7	6.7	6.7
72	7.8	7.8	7.8	7.8
73	1.5	1.5	1.5	1.5
74	1.5	1.5	1.5	1.5
75	6.7	6.7	6.7	6.7
76	4.8	4.8	4.8	4.8
77	15	15	15	15
78	7.8	7.8	7.8	7.8
79	6.7	6.7	6.7	6.7
80	6.7	6.7	6.7	6.7

Group 4

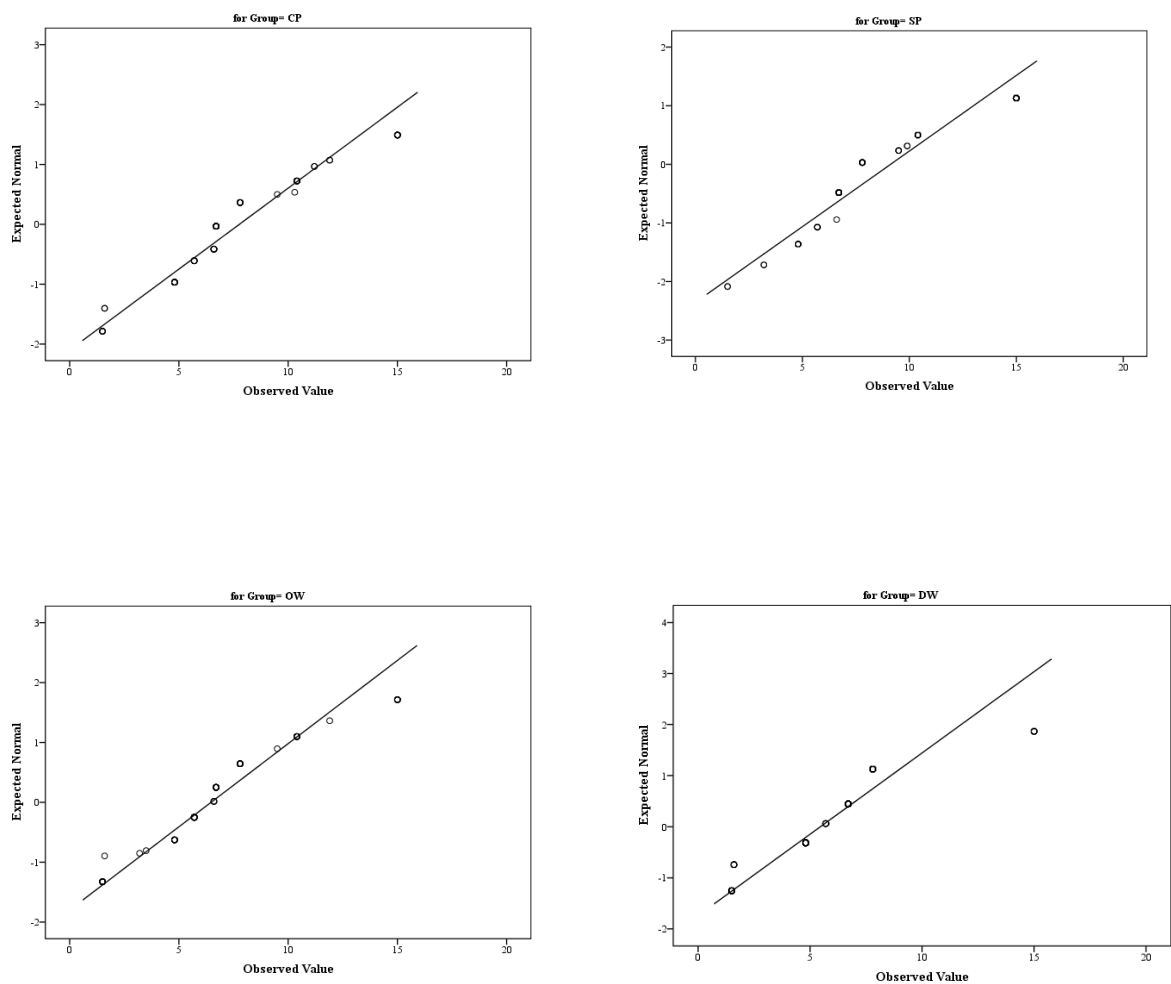
Group	Min.	Max.	Mean	Median	IQR	Skew	SE-Skew	Std.Skew	Kurt	SE Kurt	Std.Kur
Carbamide peroxide	1.5	15	7.765	6.7	4.70	.541	.269	2.011	-.170	.532	-0.320
Sodium perborate	1.5	15	9.126	7.8	7.15	.412	.269	1.532	-.927	.532	-1.742
Ozonated water	1.5	15	6.471	6.6	3.00	.674	.269	2.506	.447	.532	0.840
Distilled water	1.5	15	5.460	5.25	4.30	1.019	.269	3.788	2.258	.532	4.244

**Descriptive characteristics of data and their distribution
assessed by Shapiro – Wilkos test**

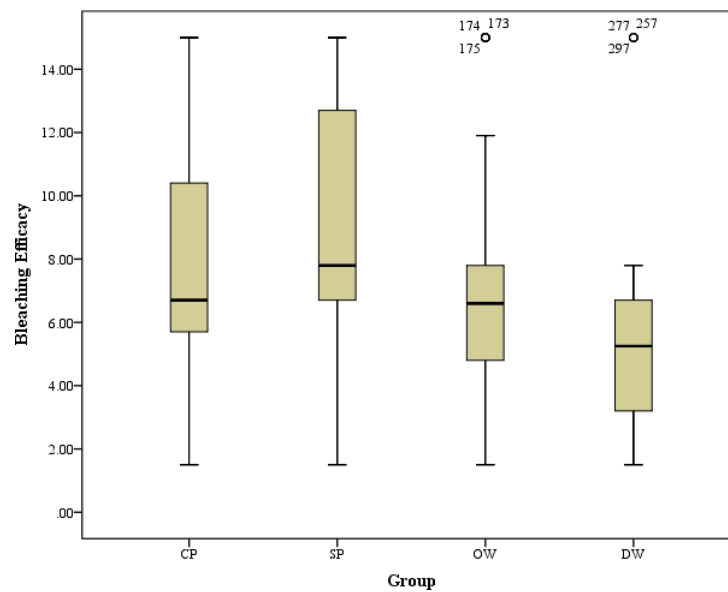
Table 2 shows the data distribution among the four experimental groups carbamide peroxide, sodium perborate, ozonated water and distilled water in histograms and Q-Q plots and box plots indicating that the values are skewed for the four groups, carbamide peroxide with skewness of 0.541 (SE = .269) and kurtosis of -0.170 (SE = .532), sodium perborate with skewness of 0.412 (SE = 0.269), and kurtosis of -0.927 (SE = 0.532), ozonated water with skewness of 0.674 (SE = 0.269) and kurtosis of 0.447 (SE = 0.532), and distilled water with skewness of 1.019 (SE = 0.269) and kurtosis of 2.258 (SE = 0.532) and hence non-parametric tests of significance were used in this study.



Graph 1 – Data distribution in histograms, normal curve



Graph 2 – Q-Q plot



Graph 3 - Box plot

Symmetric Measures					
		Value	Asymptotic Standard Error ^a	Approximate T ^b	Approximate Significance
Measure of Agreement	Kappa	.856	.088	6.662	.000
N of Valid Cases		20			
a. Not assuming the null hypothesis.					
b. Using the asymptotic standard error assuming the null hypothesis.					

Interobserver agreement in assessing the shades of teeth measured by Cohen's kappa



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Ref No: Sathyabama University/ IHEC/study no 067

28/03/2019

To,

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Dean, Head of Oral &
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Sathyabama Dental
College & Hospital

Dr. C. Pravda

Member secretary

Professor & HOD,
Department of Oral
medicine and Radiology
Sathyabama Dental College

I am pleased to inform you that the Institutional Human Ethics Committee (IHEC) has approved your project "**Comparison of the efficacy of three different bleaching agents -carbamide peroxide, sodium perborate, and ozonated water as intracoronal bleaching agents in non vital primary teeth – anin vitro study**" based on the presentation of the proposal made and recommendations of the IHEC committee meeting held on 30th January 2019.

The validity of this ethical clearance is for only one year till 29th March 2020 and in case if the study is not completed within the stipulated time they have to apply to renew for ethical clearance. You are expected to submit six monthly progress and final report of the study to the IHEC.

Thanking you,

Dr. Thayumanavan

Deputy Chairperson



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To whomsoever it may concern

This is to inform that Dr. Janani. L , post graduate student, Department of Pediatric and Preventive Dentistry, Sathyabama Dental College and Hospital has done her research on comparison on the efficacy of three different bleaching agents – carbamide peroxide, sodium perborate and ozonated water as intracoronal bleaching agent in artificially discolored primary teeth – an in vitro study using our facility from 17 – 01 – 2022 to 11 – 02 – 2022 and have made use of equipments present at Life Teck Research Centre.

List of equipments:

- Centrifugation machine (Remi cooling centrifuge, REMI, India)
- Incubator (Ausco, India)
- Magnetic stirrer (REMI, India)
- Ozone water generator (Clean water systems, USA)



Dr. I. Seethalakshmi

Director

11/02/2022