An *in vitro* Evaluation of Radicular Penetration of Hydrogen Peroxide from Bleaching Agents During Intra-Coronal Tooth Bleaching with an Insight of Biologic Response

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***Objectives**: External root resorption is the complication of intra-coronal bleaching done with 30% H_2O_2 alone or mixed with sodium perborate but not with sodium perborate mixed with water. The study was done to comparatively evaluate the H_2O_2 leakage from three H_2O_2 liberating bleaching agents. **Study design**: Fifty one single rooted human teeth were used. After root canal therapy gutta percha was removed below cemento-enamel junction. Three bleaching agents: sodium perborate mixed with water , sodium perborate mixed with 30% H_2O_2 and 30% H_2O_2 alone were used. Teeth without defect , with cervical root defect and with mid root defect constituted group A, group B and group C. According to various bleaching agents groups were subdivided into subgroup 1, 2 and 3. H_2O_2 leakage was measured with the help of spectrophotometer. **Results**: Almost all teeth showed H_2O_2 leakage. It was maximum in B1 followed by C1, B2, A1, A2, C2, B3, A3 and C3. **Conclusion**: Sodium perborate mixed with water was found to be the best bleaching agent.*

***Keywords:** hydrogen peroxide, intra-coronal tooth bleaching.*
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INTRODUCTION

With the increased awareness about esthetics, many patients are visiting clinics concerned with discolored front teeth. As an intact endodontically treated anterior teeth does not always need extra-coronal restoration,[1] the discoloration can be treated effectively with intra-coronal bleaching method. Bleaching is specially beneficial in pediatric patient. Immature discolored nonvital teeth has less dentine thickness with large pulp space. Preparation of these teeth for extra-coronal restoration may render the tooth vulnerable to fracture and thus the longevity.

Endogenous discoloration most commonly is because of a) ptomains and hematoporphyrins from protein degradation, b) iron sulfide derived from cystein, cystine, c) iron derived from hemoglobin, d) silver and other metallic stains.[2,3] For a discolored nonvital tooth intra-coronal walking bleach technique is an effective, economic, less time consuming and a conservative approach. Thirty percent hydrogen peroxide (30% H_2O_2) alone or in combination with sodium perborate (SP), with thermocatalytic[4] or walking bleach[3] method has been used successfully for bleaching discolored root filled teeth. However external cervical root resorption has been observed with the use of these agents.[5] Based on the possible causes, some association with this pathology were correlated, like age of the patient, previous traumatic injuries of the teeth, bleaching agents toxicity and use of thermocatalytic cycle, alteration of the composition of dentin and cementum by the bleaching agents, bacterial penetration on dentinal tubules opened after the bleaching and cut level of gutta-percha.[6] The occurance of root resorption and bleaching relapse are the detering factor among professionals to use these agents for treating discolored teeth. Rotstein *et al*[7] confirmed that H_2O_2 diffuse through radicular dentin into periodontal ligament particularly in presence of cemental defects, which may initiate inflammatory response. This may be the reason related with greater incidence of root resorption in traumatized teeth.

Concentrated H_2O_2 affects the surface morphology of tooth enamel and inorganic components of human dentine and cementum.[8,9,10] These changes combined with residual H_2O_2 in chamber result in increase of microleakage.[11] Floyd[12]

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considered that the tissue's susceptibility to oxidative stress damage depends not only on the applied oxidative stress but also the oxidative status per se thus possibly on the age of person. He suggested the bleaching reaction should cause minimal oxidative stress on already stressed tissues due to aerobic metabolism so that oxygen free radicals can be scavenged by natural body mechanism viz. SOD (superoxide dismutase), catalase and peroxidase. Hence it is important that the bleaching agent should be milder not only to tooth being bleached but also to the vital tissues, yet clinically successful.[10,13]

Spasser[14] was the first to use the SP mixed with water to be used as a bleaching agent in walking bleach technique and found it successful clinically. Many authors had found SP + water to be effective bleaching agent and essentially without external cervical resorption with its use even after ten years of follow up.[5] Oliveira *et.al* [10] concluded that SP + water did not adversely affect the dentin microhardness.[10] SP is easily available, safe, economic, storable at room temperature and has easy handling characteristics. It is mixed with water to liberate H_2O_2 which in turn gives off nascent oxygen and hydroxyl ions. Formation of these ions are slower as compared to from 30% H_2O_2 alone or mixed with SP.[15,16]

In this study some points were raised regarding "SP + water" to be used as intra coronal bleaching agents. These are: a) is there less radicular penetration of H_2O_2 from "SP + water"?, b) if less then how significantly it differs from other oxidative bleaching agents?, c) is there any effect of cementum defect on radicular penetration of H_2O_2 from "SP + water"?, d) if it is there how significant it is as compared to other agents?, e) if it is less then is it within physiologic range?

On the basis of past studies and raised points above a hypothesis was made that there should be less leakage of H_2O_2 from "SP + water" as compared to other bleaching agents which uses concentrated form of H_2O_2. The present study was undertaken to comparatively evaluate the radicular penetration of H_2O_2 through cemental defects at cervical and mid-root level from three walking bleach agents i.e. 30% H_2O_2, SP +30% H_2O_2 and sodium perborate + water. The aim of this study was to find out least H_2O_2 leaching agent which is more tissue friendly in day to day pediatric practice.

MATERIALS AND METHOD

Fifty one, intact single rooted human teeth, freshly extracted for orthodontic or periodontal reasons, were used in this study. Teeth that showed no curvatures, calcifications and internal or external resorption on radiograph were selected. The soft tissue on root surface were removed by placing all the teeth in 5.25% sodium hypochlorite for one hour. Hard deposits were removed manually by scaling. Teeth were then stored in physiologic saline.

All teeth received endodontic treatment using step-back and circumferential technique. All teeth were obturated with gutta-percha points and zinc oxide eugenol using lateral condensation technique. Gutta-percha filling was removed 3mm. short of cemento-enamel junction (CEJ) with the help of hot pluggers. Remnants of gutta-percha was removed with slowly rotating carbide bur and then with cotton soaked in chloroform. The pulp chamber was then thoroughly rinsed with bi-distilled water. The CEJ reference points were either on the lingual or on the buccal side. The teeth were divided into three groups A,B, and C according to cemental defects to be made artificially on root surface. Each group consisted of 17 teeth, in which two teeth served as control (Table 1).

Cementum defects were made to simulate the conditions when root surface could be found devoid of it.[17,18,19] In group A, no cementum defects were made. In group B, with the help of round diamond bur, cementum covering the cervical third of root was removed at four different root surfaces—mesial, distal, buccal and lingual till dentine is exposed in these areas. Same cemental defects were made at a level 4mm below the coronal terminal level of gutta-percha obturation in group C. The smear layer was removed with EDTA. In both B and C groups whole root surface including apical foramen but the cemental defects, was coated with two layers of nail enamel in order to seal the potential defects present on the root surface. Now each group was subdivided into three subgroups 1, 2, and 3 according to various bleaching agents used for intra coronal bleaching (Table 1). Fifteen teeth of each group were repeated to be used as subgroups for various bleaching agents on different days.

Teeth were mounted in Plastic tubes of 3.5 ml capacity. The caps of tubes were perforated and crowns of samples were mounted into caps with the help of sticky wax in a manner to separate the crown from the root at a level about 1mm above the mesial and distal line of CEJ. The roots of samples were immersed completely in bi-distilled water. (Fig. 1)

Prior to experiment the media in which roots were immersed was checked to exclude any possibility of presence of H_2O_2. The mounted samples were then placed in an incubator at 37°C for the period of 20 minutes to simulate the body temperature. The experiment was completed in three days for three bleaching agents. Samples were

Table 1. Distribution Of Samples

GROUPS	SUBGROUPS
GROUP A: Teeth without cementum defect (17 teeth - 15 experimental, 2 control)	A1- 30% hydrogen peroxide A2- sodium perborate + 30% H_2O_2 A3- sodium perborate + H_2O
GROUP B: Teeth with cervical root defect (17 teeth - 15 experimental, 2 control)	B1- 30% hydrogen peroxide B2- sodium perborate + 30% H_2O_2 B3- sodium perborate + H_2O
GROUP C: Teeth with mid-root defect (17 teeth - 15 experimental, 2 control)	C1- 30% hydrogen peroxide C2- sodium perborate + 30% H_2O_2 C3- sodium perborate + H_2O

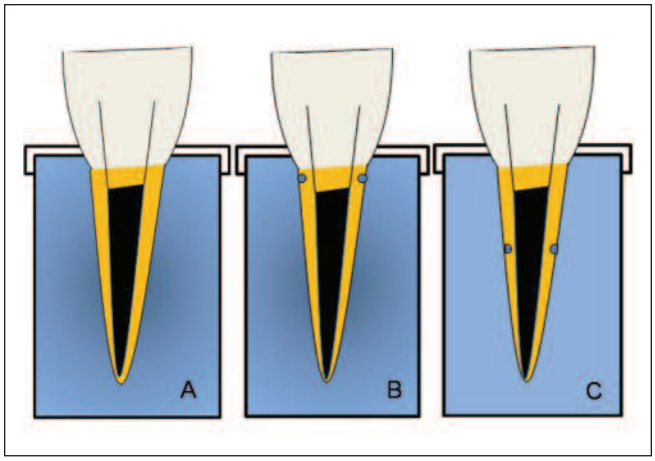

Figure 1. MOUNTING OF SAMPLES: Group A—teeth without cementum defects; Group B—teeth with cervical root defect; Group C—teeth with mid root defect

repeated each day in order to make direct comparison of H_2O_2 leakage from different bleaching agents through same teeth with or without cemental defects.

On the first day 20µl of 30% H_2O_2 (GR, Merck) was pipetted in the pulp chamber and access cavity was sealed with small piece of boxing wax. Samples were then subjected to thermo-catalytic cycle by placing them in a dry incubator at 47°C temperature for the period of one and half hour. Second day, sodium perborate 'tetrahydrate' (Sd fine chem. limited) mixed with 30% H_2O_2 in the ratio of 2gms:1ml,[20] was placed in the pulp chamber and procedure was repeated in the same way. Third day, SP mixed with bi-distilled water in the same ratio was used as the bleaching agent and procedure was performed same way. Every time each day after completion of test the pulp chamber and roots were thoroughly washed with bi-distilled water. All the samples were then stored in 0.9% saline till the next test.

Each day after completion of thermo catalytic cycle 0.5 ml of solution surrounding the root of each samples was taken to test H_2O_2 leakage. The solution was added to 2.5 ml of bi-distilled water to reach a total volume of 3ml. The samples were then added to 0.2 ml of 10 mM of ferrous ammonium sulfate. If H_2O_2 was present, a ferric ion resulted and upon the addition of 0.1 ml of 2.5M potassiumthiocyanate a ferrithiocyanate complex was formed which absorbed light at the wavelength of 480 nm.[21]

A standard curve for the 30% H_2O_2 with known amount of H_2O_2 was established for the study. Samples from each tooth tested were read in the spectrophotometer in terms of optical density, which was then converted to percentage concentration of H_2O_2 with the help of standard curve. The reagents reading served as a negative control while the reading of pure 20 µl of 30% H_2O_2 in 3.5 ml of bi-distilled water served as a positive control.

After the completion of all the test results were tabulated and submitted to statistical analysis using students 't' test and ANOVA test.

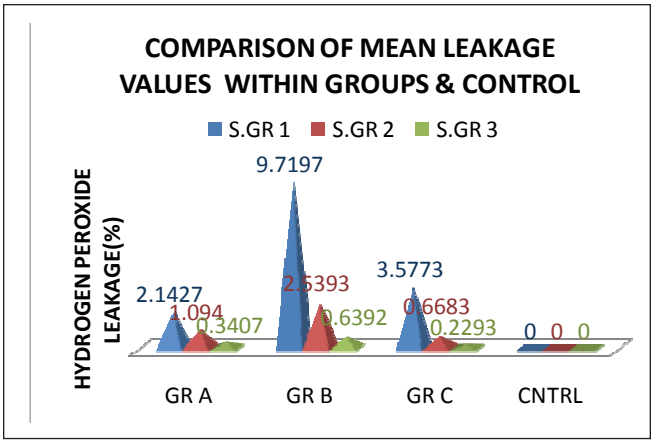

Figure 2. Comparison of mean hydrogen peroxide leakage values within groups and control

RESULTS

During the thermocatalytic cycle H_2O_2 leakage through radicular dentin was observed almost in all teeth except for control teeth in which no H_2O_2 leakage was found. The means and comparison of H_2O_2 leakage of all subgroups are presented in Figure 2.

Among subgroups A1, B1, C1, H_2O_2 leakage was maximum in B1 and minimum in A1. The mean values between these subgroups differed significantly (Table 2). Since "t" value for A1 Vs B1 is significant, leakage differed significantly between these groups and it was more in B1 than in A1. Since "t" value for A1 Vs C1 is significant, leakage differed significantly between these subgroups and it was more in A1. Since "t" value for B1 Vs C1 is significant, leakage differed significantly between these groups and it was more in B1 than in C1.

Among subgroups A2, B2, C2 H_2O_2 was maximum in B2 and minimum in C2 and leakage differed significantly

Table 2. Inter-Comparison of Mean Hydrogen Peroxide Leakage Between Subgroups

Comparison	Degree of freedom	"t"	'p'
A1 Vs B1	28	3.8389	p < 0.001
A1 Vs C1	28	3.8839	p < 0.001
B1 Vs C1	28	3.038	p < 0.001
A2 Vs B2	28	1.6802	NS*
A2 Vs C2	28	0.7152	NS
B2 Vs C2	28	2.2664	p < 0.05
A3 Vs B3	28	1.1822	NS
A3 Vs C3	28	0.8868	NS
B3 Vs C3	28	1.6167	NS
AI Vs A2	28	1.0989	NS
A1 Vs A3	28	2.1382	p< 0.05
A2 Vs A3	28	1.6292	NS
B1 Vs B2	28	3.9378	p<0.001
B1 Vs B3	28	5.2937	p<0.001
B2 Vs B3	28	2.4703	p<0.001
C1 Vs C3	28	2.8256	p<0.001
C2 Vs C3	28	3.5344	p<0.001
C2 Vs C1	28	1.1987	NS

NS*- Non significant

(Table 3). Since "t" value for A2 Vs B2 is not significant, leakage did not differ significantly between these subgroups. Since "t" value for A2 Vs C2 is not significant, leakage did not differ significantly between these subgroups. Since "t" value for B2 Vs C2 is significant, leakage differed significantly between these subgroups and it was more in B2 than in C2.

Among subgroups A3, B3, and C3 leakage was maximum in B3 and minimum in C3. There was no significant difference in mean H_2O_2 leakage between these subgroups (Table 4). Among these subgroups there was no significant difference found in average leakage of H_2O_2.

Within group A, (A1, A2 & A3) mean H_2O_2 leakage was maximum in A1 and minimum in A3 (Table 5). Since "t" value for A1 Vs A2 is not significant, leakage did not differ significantly between these subgroups. Since "t" value for A1 Vs A3 is significant, leakage differed significantly and it was higher in A1. Since "t" value for A2 Vs A3 is not significant, leakage did not differ significantly between these subgroups.

Within group B, (B1, B2 & B3) H_2O_2 leakage was maximum in B1 and minimum in B3 and leakage differed significantly (Table 6). Since "t" value for B1 Vs B2 is significant, leakage differed significantly and it was higher in B1. Since "t" value for B1 Vs B3 is significant, leakage differed significantly between these subgroups and it was more in B1. Since "t" value for B2 Vs B3 is significant, leakage differed significantly and it was more in B2.

Within group C, (C1, C2 & C3) H_2O_2 leakage was maximum in C1 and minimum in C3 (Table 7). The mean leakage differed significantly between C1 &C2 and between C1 & C3 but did not between C2 & C3.

Minimum leakage was found in C group i.e. in teeth with root defects at middle thirds and in which sodium perborate mixed with bi-distilled water was used as a bleaching agent. In this group leakage observed was as minimum as 0.0%. Among all subgroups the maximum mean H_2O_2 leakage was observed in B1 subgroup followed by subgroup C1, B2, A1, A2, C2, B3, A3 and C3.

Figure 3: Reactions of hydrogen peroxide

1. in absence of metal catalyst (Fe^{++})

$H_2O_2(aq) \rightarrow H_2O(aq) + 1/2O_2(g)$

$H_2O_2(aq) \rightarrow OH^*(aq) + OH^*(aq)$

$H_2O_2(aq) \rightarrow OH^-(aq) + OH^-(aq)$

$H_2O_2(aq) \rightarrow H^+ + OOH^-$

$OH^* + H_2O_2 \rightarrow H_2O + HO_2^*$

2. in presence of metal catalyst (Fenton reaction)

A/ Oxidising agent in acidic medium

$Fe^{++} + H_2O_2 \rightarrow Fe^{3+} - OH^* + H_2O$ (1a)

$Fe^{3+} + H_2O_2 \rightarrow Fe^{++} + OH^*$ (1b)

$Fe^{2+} + H_2O_2 + H^+ \rightarrow Fe^{3+} + OH^* + H_2O$ (1)

$^*OH + H_2O_2 \rightarrow OOH^* + H_2O$ (2)

$^*OOH + Fe^{3+} \rightarrow O_2 + Fe^{2+} + H^+$ (3)

$$2H_2O_2 \xrightarrow[\text{catalase}]{Fe} 2H_2O + O_2 \quad (4)$$

B/ Oxidizing agent in alkaline conditions:

$Fe^{2+}(aq) + HO_2^-(aq) + H_2O(l) \rightarrow Fe^{3+}(aq) + 3OH^-(aq)$

[OH^- — Hydroxyl ion; OH*— Hydroxyl radical; OOH*— perhydroxide radical

Fe^{3+} - OH* - Ferryl radical]

DISCUSSION

After H_2O_2 is introduced intracoronally in a non-vital tooth, many reactions start taking place[22,23,24,25] (Figure 3). First within H_2O_2, (figure 3, reaction-1), second fenton reactions and third the decolorisation of chromophores. Hydroxyl radicals (OH*) liberated from H_2O_2 and fenton reaction are responsible for bleaching of chromophores.[22,23] Oxidative potential for OH* 2.8 while for H_2O_2 is 1.8. In acidic medium H_2O_2 disproportionates into water and half oxygen molecule. This reaction is exothermic. Fenton reaction (oxidation in presence of metal ion) too, is exothermic and favored in acidic medium[24,25] (Fig. 3, reaction 2). Imlay[23] *et al* and Wink[26] et.al. postulated that apart from hydroxyl ions some other two types of intermediate ions are also formed. One of which is iron bounded OH* called ferryl ion (Fig 3, reaction-2A,1a). Thus flow of OH* at a time is controlled and gets consumed in the presence of high concentration of H_2O_2 as HO_2* radical are formed. Thus high concentration of H_2O_2 may not be feasible for bleaching.[23] To control temperature rise and rate of reaction, H_2O_2 should be added slowly and medium should remain alkaline.[25] Within access cavity of tooth possibly reaction 1,2,3 occurs while reaction-4 occurs in vital tissues in presence of enzyme catalase[22] after H_2O_2 is penetrated in periodontal tissues (Fig. 3, reaction 2,4). SP tetrahydrate, used in this study, always remains in alkaline range.[18] On the other hand, H_2O_2 in alkaline medium is not a good oxidizing agent which also produces hydroxyl ions (OH^-) with intermediate production of peroxide radical[24] (Fig. 3, reaction 2B,3). OH* produced from these reactions is intensely active.[25] Thus alkaline sodium perborate works slowly but is biologically favorable as amount of H_2O_2 is less and is slowly released which maintains steady bleaching with controlled outflow of reactive oxygen species (ROS) i.e. oxidative radicals/ions and H_2O_2. Because of kinetic hindrance, oxidizing capacity is lost after some time hence paste has to be replaced at regular intervals till required tooth color is achieved. This explanation is consistence with the findings of Ari *et al.*[13] 30% H_2O_2 is supplied as acidic solution to increase its shelf life i.e. to retard its decomposition rate into water and oxygen. Fenton reaction being favored by acidic medium and being exothermic causes rise in rate of reaction in case tooth bleaching is being done with concentrated H_2O_2. This ultimately causes rapid production of OH^- and OH*. Increased temperature as a result of exothermic reaction or externally applied heat causes H_2O_2 solution to disproportionate into its components i.e. water and oxygen (Fig. 3, reaction 1). The rate of destruction is multiplied with 2.2 for every 10° C of rise in temperature.[27] Oxygen being in gaseous form may build up

pressure inside closed cavity. Microcracks may appear because of the rapid decomposition. Also this pressure will tend to push radicals and ions into the periodontal space through dentinal tubules. Physical form of bleaching agent also plays important role. 30% H_2O_2 being in liquid form has wetting property, readily penetrating the space between root canal filling material and canal wall and from there into the dentinal tubules. H_2O_2 penetrated there will cause production of OH^- and OH* there. While penetration of these ions etc. from paste form depends on its rate of production from reaction between SP and water.

After H_2O_2 and ROS penetrated into the periodontal space may cause necrosis (>10mmol), apoptosis (5 – 10mmol), delayed cytotoxicity (0.5 – 0.1mmol) or may be scavenged by catalase, glutathione reductase/peroxidase enzyme systems.[28] It has also been demonstrated that pre exposure of eukaryotic cells to sub-toxic oxidative stress can render these cells resistant to killing by same or related oxidants and other toxic agents.[29] Hence slow rate of nontoxic or sub-toxic amount of ROS penetrating in tissues may not be injurious. Radicals directly injure the cells, while exogenous H_2O_2 again enter the Fenton/ Haber-Weisse cycle.[23] Intensity of these reactions depend on available iron molecules and H_2O_2 produced by oxidative metabolism. Imbalance in Fe/H_2O_2 poses serious threat to aerobic metabolism. According to Imlay[23] et.al actively dividing cells are killed maximally at physiological concentration of exogenous 1.5 - 2.5mmol H_2O_2 and half maximal at 10 – 20mmol H_2O_2. Hence pediatric dental patients should be taken care. Scavenging capacity depends on availability of enzymes in cells, pH of tissues and the concentration of exogenous/endogenously produced ROS with respect to time. If periodontal tissues are already in oxidative stress due to injury/infection, necrosis is inevitable. Studies suggest that glutathione reductase / oxidase is primary defense system against low concentration of H_2O_2 while at higher concentration catalase develop into primary protection.[30] Because both system depend on availability of NADPH which in turn is dependent on G6PD enzyme, patients suffering with G6PD deficiency may find it difficult to scavenge physiologic or excess of ROS. According to studies, catalase can remove metabolic ROS effectively till it is depleted by 98%.[30] It has been mentioned that one molecule of catalase can catalyze the conversion of 6000,000 H_2O_2 molecules into water and oxygen every second.[24]

In order to protect the tissues from oxidative injury from bleaching agent many measures can be done viz. a) selecting milder bleaching agent, b) reducing the heat produced by exothermic reactions[26,27] i.e. to use milder bleaching agent without application of external heat and by adding H_2O_2 slowly, c) access cavity may not be etched, d/ application of effective thickness of barrier[31] preferably 4mm. d) application of some neutralizing/antioxidant agents to remove residual H_2O_2 from access cavity and to reduce intensity of fenton reactions. Some antioxidants like hyperoside,[32] scutellarin and vitamin E[33] had been tested against H_2O_2 induced cytotoxicity and results indicated that these could effectively protect living cells. Catalase[34] and thiourea[35] (reducing agent), in access cavity, in order to remove residual or excess H_2O_2 had been tried and was found successful, e) selection of patient. Patients with history of trauma; depleted catalase level[36] due to anemia, decreased synthesis, genetic acatalasia or genetic peroxisomal disorders[37]; or genetic G6PD deficiency,[30] may be at high risk of root resorption even with non-toxic amount of H_2O_2. Patients suffering with acatalasemia may be asymptomatic and seen as disease in search of some symptom. Its oral clinical features include oral gangrene.[38] Prime of all, we should try to solve a problem at its root level first and then at fruit level i.e. bleaching agent itself should be milder (slowly releasing H_2O_2, work at body temperature and should retain its bleaching action in alkaline medium).

Our study proves that SP + water is the mildest as there was significant difference between A1 Vs A3, B1 Vs B3 and C1 Vs C3. In subgroup 3 (SP + water) itself leakage was non significant. SP tetrahydrate always remains in alkaline range hence should be used for the purposes discussed above. When external heat is applied it not only kinetizes the liquid (30% H_2O_2) but also promotes decomposition of it. Increasing the temperature of radicular dentine by 40º C will result in hydraulic conductance of non-etched dentine increasing by 1.8 folds and 4 folds of etched dentine.[35] 30% H_2O_2 itself is a acidic solution which will etch the dentine, hence there seems no need of etching further by some other agent. Rotstein *et al*[39] found 94.44% and 77.77% reduction in radicular penetration of H_2O_2 when IRM base was placed up to CEJ and 1mm below CEJ respectively. If values of H_2O_2 leakage for group A (teeth without defect and without nail paint to simulate natural tooth) are calculated in terms of mmol/lt, it is 588mmol/lt for A1, 324 mmol/lt for A2 and 100mmol/lt for A3. The values are significantly different. If these values are reduced by 94.44% it becomes 42mmol/lt for A1, 18 mmol/lt for A2 and 5.55 mmol/lt for A3. ED50 for H_2O_2 is 5.0 – 10.0 mmol/lt.[35] Values for SP + water(A3) are within ED50 value. Absence of barrier, thermo catalytic method, cut level of gutta-percha apical to CEJ and etching of dentine were the limitations of this study which led to higher penetration of H_2O_2. Inspite of these conditions SP + water showed leakage within ED50 of H_2O_2. Further study, with actually simulated conditions as would be in vivo may be needed.

Our study again supports the finding that cervical root area is leakage prone area.[7] For subgroup-3 (A3, B3, C3) leakage was not significant even in presence of root defects. If values are calculated and converted as was done previously in terms of ED50, it is 5.55 mmol/lt for A3, 10.44 mmol/lt for B3 and 3.76 mmol for C3. It is proved now that leakage from SP + water always remained in physiologic range though it is slightly higher in B3 (cervical root defect). On the other hand as Crawford[29] stated that pre-exposer to somewhat toxic H_2O_2 render the cells resistant to killing on subsequent exposer by same or other agent. Hence SP+ water paste can be repeated without fear till required color of tooth is achieved. But here it is important to point out that if

cells are rapidly dividing, are at higher risk of killing even in presence of non-toxic H_2O_2 as Imlay[23] stated. Subgroups with mid root defect showed least leakage in there respective groups. It may be because leakage prone area i.e. cervical third of root was nail painted and there was less H_2O_2 available to travel up to mid-root. Farmer *et al*[35] also found leakage of H_2O_2 within ED50, when H_2O_2 combined with thiourea was used.

CONCLUSION

Within the limitations of this study, SP + water can be considered safest bleaching agent. Nonetheless all teeth after bleaching should be monitored for external root resorption. In case it occurs it may be treated with calcium hydroxide.

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