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RESEARCH ARTICLE

DENTINAL TUBULE OCCLUDING EFFECT OF A HERBAL TOOTH PASTE AND MOUTHWASH- AN INVITRO SEM ANALYSIS

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ABSTRACT

Aim - To evaluate the effects of a herbal desensitizing dentifrice in different forms on dentinal tubule occlusion by scanning electron microscopy (SEM).

Methods and Materials - 120 dentine blocks were prepared from the cervical region measuring 1.5 ×1.5 ×1.5 mm, was etched with 6% citric acid for 2 minutes, rinsed in distilled water and were divided into 4 groups:

Group 1 specimens were brushed with distilled water, Group 2 specimens were rinsed in Hiora K mouth wash, Group 3 specimens were brushed with Hiora K tooth paste, Group 4 specimens were brushed with Hiora k tooth paste and the rinsed with Hiora K mouth wash

From each group 5 samples each were taken on 3rd, 7th, 21st, 28th, 45th and 60th day. Specimens were washed with distilled water, dried in dessicator for 2 days, sputter coated with thin gold layer and subjected for SEM analysis and was scored in accordance with the tubule occlusion classification scoring system.

Results –All the groups excluding control showed complete occlusion of dentinal tubule on the 45th and 60 th day. On the remaining days better results were shown by the tooth paste group followed by combination group and least occlusion was seen in mouth wash alone group.

Conclusions - As Hiora K toothpaste and mouthwash occluded the dentinal tubules, they may be useful for the treatment of dentine hypersensitivity.

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INTRODUCTION

Dentine hypersensitivity is an important issue that needs addressing both from a diagnostic and a management perspective. It is described clinically as an exaggerated response to a non noxious sensory stimulus. It is viewed as originating from the underlying exposed dentin after the enamel or cementum has been eroded away (Joshi, 2013). It is characterized by short, sharp pain arising from exposed dentin in response to external stimuli, typically thermal, evaporative, tactile, osmotic or chemical, and which cannot be ascribed to any other form of dental defect or disease (Pillai, 2013). Treatment of dentin hypersensitivity is challenging for both the patient and the healthcare provider. Hypersensitivity can resolve without treatment or may require several weeks of desensitizing agents before improvement is seen. There are two principal treatment options:- *plug the dentinal tubules preventing fluid flow*, or *desensitize the nerve,* making it less responsive to stimulation (Jacobsen, 2001).

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Currently only potassium nitrate claims to desensitize the nerve. Agents which occlude the dentinal tubules includes ions or salts which are hypothesized to precipitate within the tubule like stannous ion in stannous fluoride, Oxalate ions which react with the calcium in the tooth to form insoluble calcium oxalate crystals that occlude the tubules, Strontium ions also have the property of precipitating together forming a mass that occludes the tubules, precipitates like glutaraldehyde, resins like dentin sealers, methyl methacrylate etc (Jacobsen, 2001). Recently herbal products like hiora k tooth paste and mouth wash (Himalaya) are introduced in market which claim to reduce sensitivity with both desensitizing and occluding action. Hiora K tooth paste contains *Suryakshara, Palakya, Lavanga, triphala, clove* and *cinnamon. Suryakshara* contains Potassium nitrate which inhibits pain in hypersensitive teeth through its desensitizing effect on dentinal nerves. *Palakya* (spinach) contains natural oxalate compounds, which help in occluding the dentinal tubules (Kumari, 2013). Hiora K mouth wash contains *Suryakshara, Lavanga* and extracts of *holy basil, cloves, mace* and *peppermint*. Suryakshara contains potassium nitrate which inhibits pain in hypersensitive teeth through its desensitizing effect on dentinal nerves (Kumari,

2013). However, there are no published data regarding the effectiveness of Hiora K mouth wash and tooth paste as a desensitizing agent, when used alone and in combination for different duration. Hence the present study was undertaken to evaluate the tubule occluding ability of two desensitizing herbal products namely Hiora K toothpaste and Hiora K mouthwash using SEM analysis at various time interval.

MATERIALS AND METHODS

The present study was conducted in the Department of Conservative Dentistry and Endodontics, Dayananda Sagar College of Dental Sciences.

Selection of teeth

Inclusion criteria

- 72 intact human maxillary premolars indicated for extraction for orthodontic or periodontal reason were selected for the study and stored in saline.

Exclusion criteria

- Carious teeth
- Restored teeth
- Hypoplastic teeth
- Fractured teeth

Armamentarium

- High speed contra angle hand piece
- No 245 carbide bur
- Straight hand piece
- Mandrel
- Diamond discs
- Power brush
- Scanning electron microscope

Materials used

- 6%citric acid
- Hiora K mouth wash(Himalaya)
- Hiora K tooth paste(Himalaya)
- Cold cure acrylic resin(DPI)
- Distilled water
- Plaster of Paris

MATERIALS AND METHODS

Seventy two extracted premolars were collected, cleaned of gross debris and sectioned labio-lingually. The specimens were placed in deionized water till dentine blocks were prepared. Each surface were wet ground to remove enamel and to expose a flat underlying dentine surface in the cervical region. Dentine blocks were prepared from the cervical region measuring 1.5 ×1.5 ×1.5 mm. One block is obtained from each flat cervical dentine surface. The blocks were ultrasonicated in distilled water for 30 s to remove the particulate matter resulting from cutting. Blocks were mounted in acrylic resin to stabilize while brushing. Smear layer was removed by etching the blocks in 6% citric acid for 2 minutes and rinsed in distilled water. The blocks were stored in distilled water during the experimental period.

The specimens were divided into 4 groups:

Group 1: Specimens brushed for 2 minutes twice daily for 60 days with distilled water
Group 2: Specimens were rinsed for 1minute twice daily for 60 days with Hiora K mouth wash.
Group 3: Specimens brushed for 2 minutes twice daily for 60 days with Hiora K tooth paste.
Group 4: Specimens brushed with Hiora K tooth paste for 2 minutes and then rinsed with Hiora K mouth wash for 1 minute, twice daily for 60 days.

For the tooth paste group slurries were prepared in a dilution of 1: 3 tooth paste: water mixture. The blocks were brushed using a power brush for 2 minutes twice daily. For the mouth wash group, the specimens were dipped in mouthwash for 1 min twice daily. For the combined group ,blocks were first brushed for 2 minutes and then dipped in mouth wash for 1 minute. To remove the excess slurry or aqueous solution test samples were rinsed indistilled water for 10 seconds, after each procedure. From each group 5 samples each were taken on 3rd, 7th, 21st, 28th, 45th and 60th day. Specimens were washed with distilled water, dried in dessicator for 2 days, sputter coated with thin gold layer and subjected for SEM analysis and was scored in accordance with the tubule occlusion classification scoring system (Pathan, 2016).

Scores

- Occluded (100% of tubules occluded);
- Mostly occluded (50% to <100% of tubules occluded);
- Partially occluded (25% to <50% of tubules occluded);
- Mostly unoccluded (<25% of tubules occluded);
- Unoccluded (0%, no tubule occlusion).

RESULTS

In the mouth wash group, on day 3 ,25 % of tubules were occluded in 40% samples; on day 7,60 % samples showed occlusion of upto 50% tubules;on day 21 more than 50% tubules were occluded in 80% samples;on day 28,80% samples showed complete occlusion and on day 45 and 60 all the samples showed 100% occlusion (Fig-1, Graph-1). In the tooth paste group, on day 3,all the samples showed occlusion of 25% tubules; on day 7 more than 50 % tubules were occluded in 40% samples, on day 21,60% of the samples showed complete occlusion of the tubules; on day 45^{th} and 60^{th} all the tubules were occluded in all the samples. (Fig 2, Graph 2). In the combination group, on day 3, 80% samples showed occlusion of 25% of tubules, on day 7^{th}, 80% of samples showed occlusion of upto 50% tubules;on day 21and 28^{th}, 40 % and 80 % of the samples showed occlusion of all the tubules respectively. On day 45 and 60 all the samples were showing 100% tubule occlusion (Fig 3, Graph 3). All the tubules were open in all the sample in the control group from day 3 to 60.

DISCUSSION

Dentine hypersensitivity is a painful experience which for the majority of sufferers, generates a very unpleasant perception. It is a widespread condition with a reported prevalence varying from as low as 4.5% to as high as 57% (Cummins 2009), depending upon the population and the criteria used to assess sensitivity.

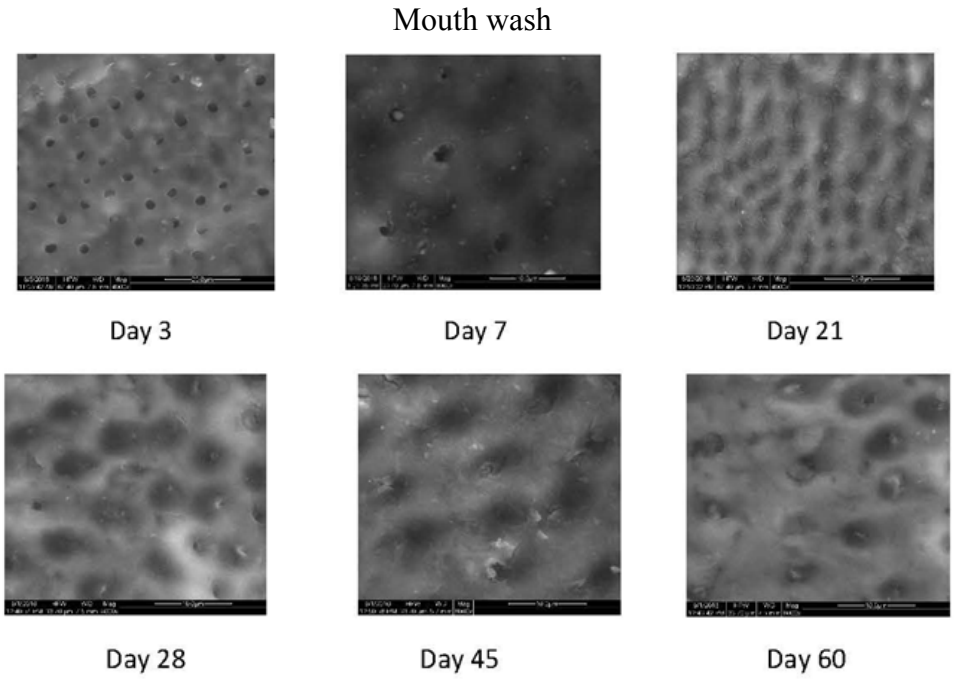
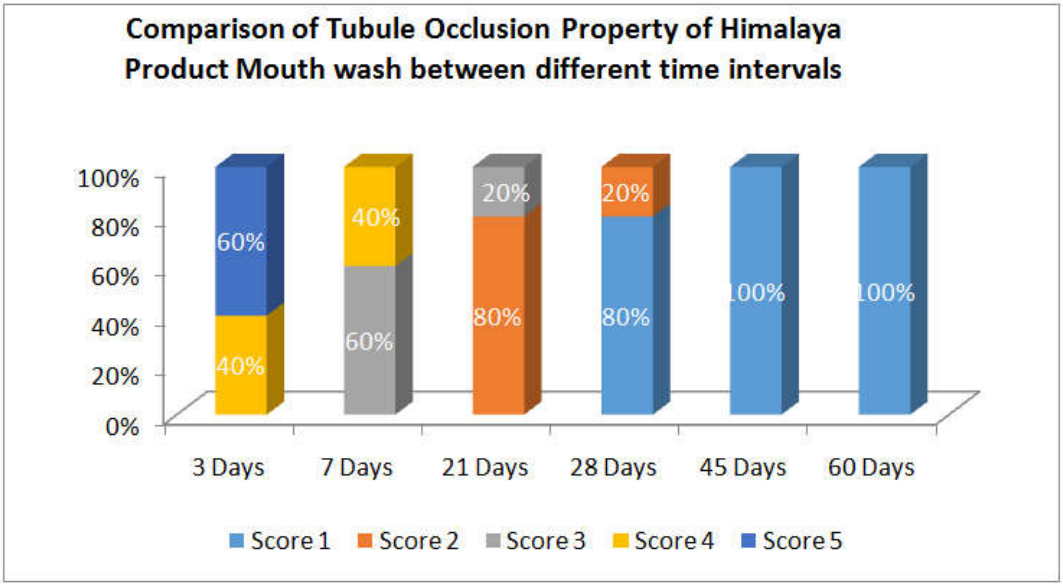


Figure 1. SEM pictures of Hiora k mouth wash between different time intervals



Graph 1.

This study focuses on the use of herbal products in the treatment of dentine hypersensitivity. The products included are HIORA K tooth paste and mouth wash used alone and in combination. The main ingredientsin HIORA Kcomposition, that cause reduction in dentine hypersensitivity are potassium nitrate and oxalate compounds. As stated earlier Potassium nitrate acts mainly by desensitizing the nerves. By increasing the potassium ion concentration adjacent to the dentinal nerve terminals, there is depolarization and activation of nerve fibres (Pillai, 2013). A prolonged period of depolarization results in inactivation of the action potential. Divalent cation solutions stabilize the nerve membrane without changing the membrane potential (Pillai, 2013; Markowitz, 1992). One of the most important points that was stressed in all these clinical trials is that, potassium based toothpastes must be used for a minimum of 2 weeks, twice daily to bring about a reduction of sensitivity; and for a period of 4-8 weeks to demonstrate significant pain relief.

The cited reasons for this are that the potassium ions must diffuse from the oral cavity into the dentinal tubules, and further against dentinal fluid flow to the site of action, which is the nerve endings. The concentration of potassium ions must also build up to significant quantities to achieve depolarization and pain relief, which takes 4-8 weeks. Furthermore, when the use of these pastes is stopped, the potassium ions at the site of action are diffused, and the relief of sensitivity is lost (Pillai and Neelakantan, 2013). Oxalate compounds acts by occluding the open tubules. Oxalates were introduced as agents to treat DH in the late 1970s to mid-1980s, based on work done primarily in vitro. Several studies reported significant decreases in hydraulic conductance across dentin disks treated with oxalates, suggesting that oxalates limit fluid flow in exposed dentin in vivo, thereby reducing pain (Pashley *et al*., 1978, 1984; Greenhill and Pashley, 1981; Pashley and Galloway, 1985).

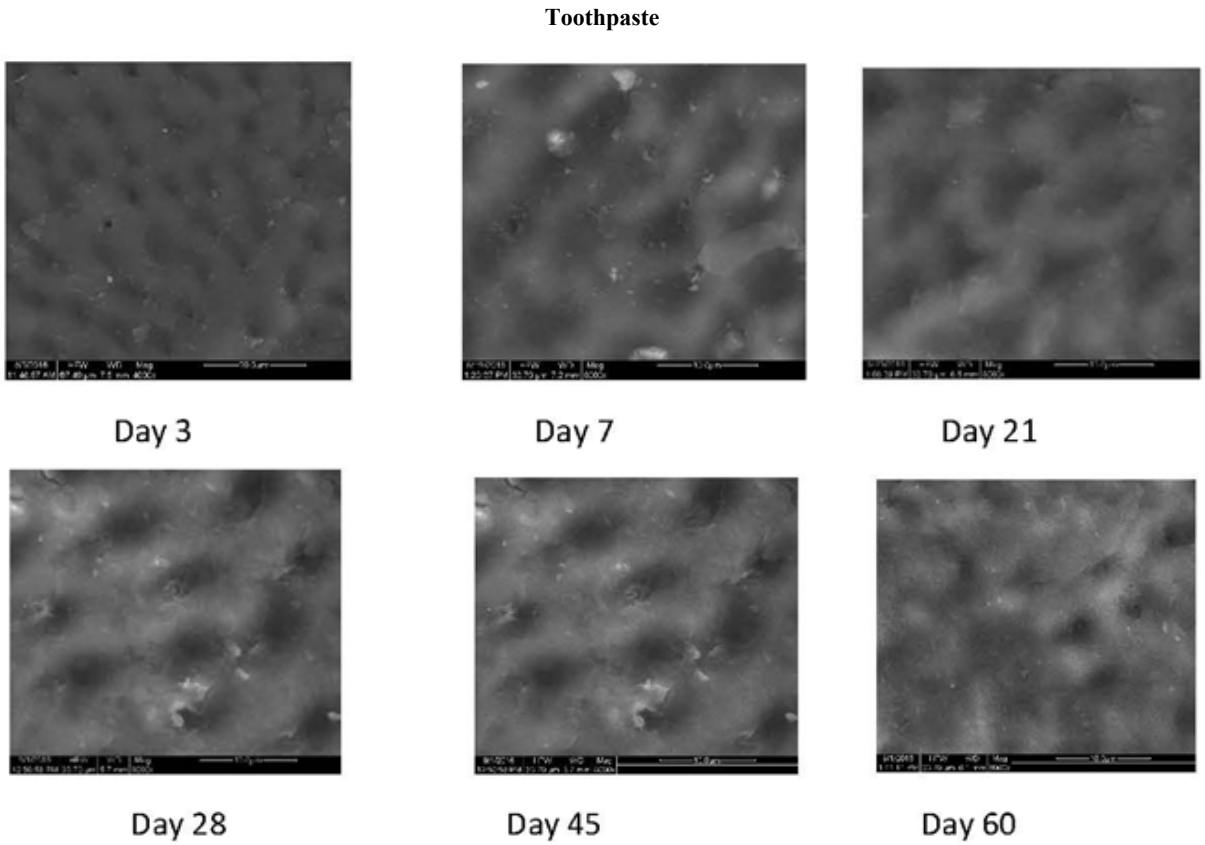
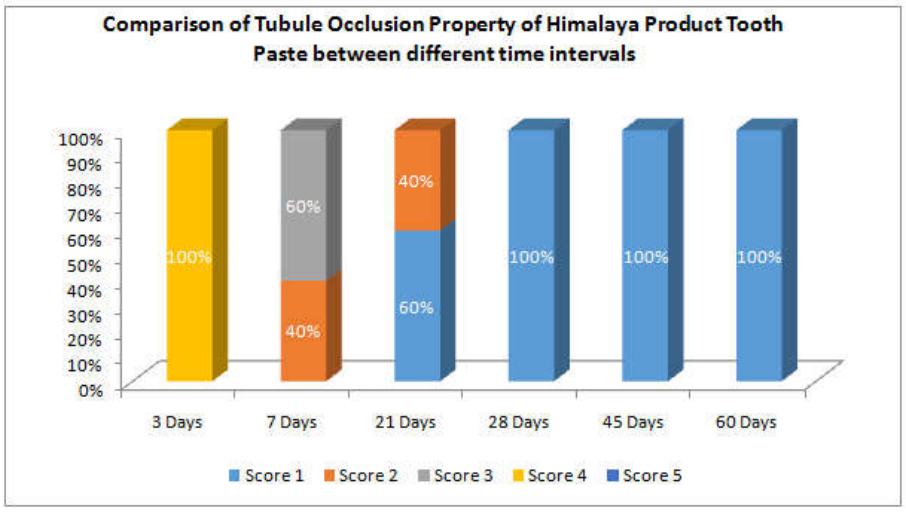


Figure 2. Sem pictures of Hiora k tooth paste between different time intervals



Graph 2.

Subsequent work showed that oxalates formed precipitates within dentin tubules that blocked dentinal fluid flow (Cuenin *et al*., 1991). Oxalates reportedly have the added advantage of relative insolubility in acid, making them resistant to dissolution after treatment (Pereira *et al*., 2005)10. Hiora K tooth paste contains Suryakshara, Palakya, Lavanga, triphala, clove and cinnamon. Suryakshara contains Potassium nitrate which inhibits pain in hypersensitive teeth through its desensitizing effect on dentinal nerves. Palakya (spinach) contains natural oxalate compounds, which help in forming phytocomplexes on the teeth. This occludes dentinal tubules and blocks the transmission of pain from the surface to the tooth's nerves. These oxalate compounds produce protective films and thus, helps to prevent tooth destruction.

Lavanga (Clove) contains an anesthetic chemical compound called eugenol, which numbs nerves and controls pain. The *essential oil of clove* is also an antiseptic which helps to eliminate oral bacteria (Markowitz, 2009). Hiora K mouth wash contains *suryakshara, Lavanga* and extracts of *holy basil, cloves, mace* and *peppermint*. Suryakshara contains potassium nitrate which inhibits pain in hypersensitive teeth through its desensitizing effect on dentinal nerves. *Lavanga* (clove) contains an anesthetic chemical compound called eugenol, which numbs nerves and stops pain. The *essential oil of clove* is also an antiseptic, which helps eliminate oral bacteria (Markowitz, 2009). In this study, the specimens were brushed for 60 days with tooth paste alone in Group 1, mouthwash alone in Group 2 and combination of both in Group 3.

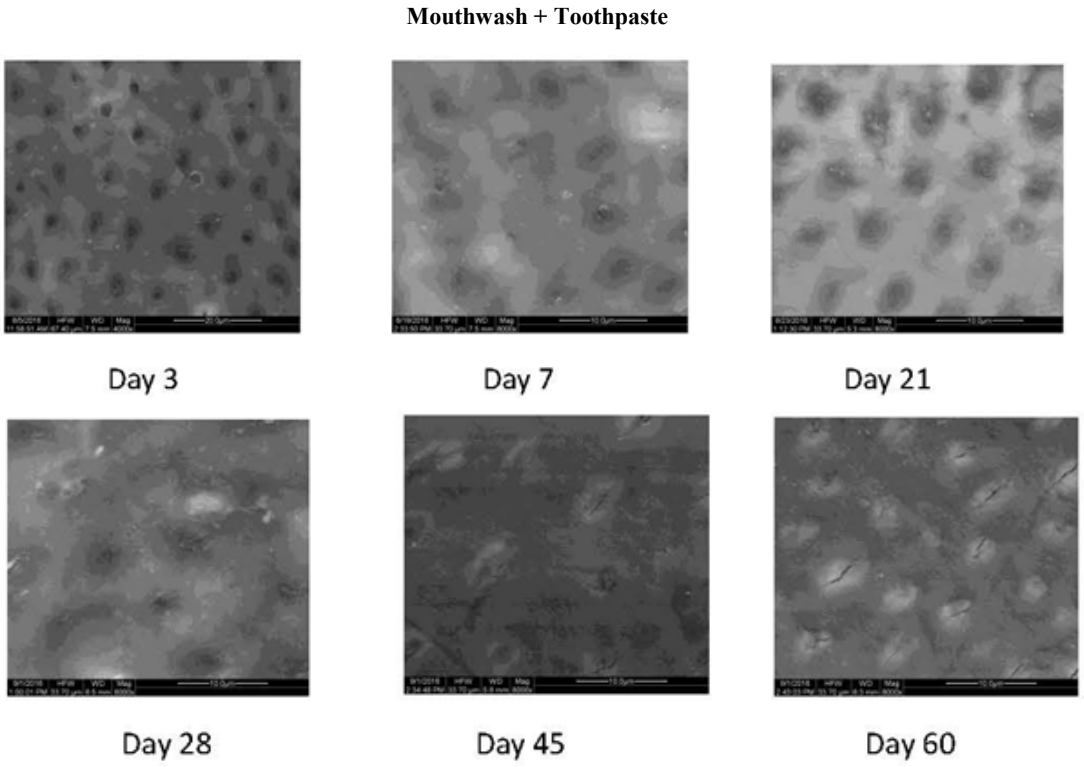
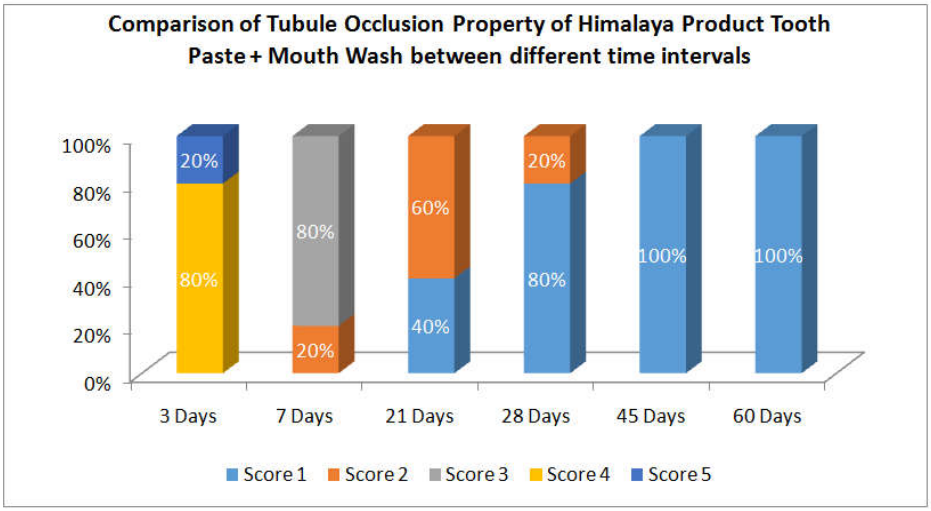


Figure 3. SEM pictures of hiora k mouth wash plus tooth paste between different time intervals



Graph 3.

Few specimens from each group were subjected to scanning electron microscopic analysis on day 3,7,21,28,45 and 60 respectively and scored according to the tubule occlusion classification scoring system where score 1 indicate 100% of tubules occlusion, score 2 - 50% to <100% of tubules occluded; score 3 -25% to <50% of tubules occlusion; score 4 - <25% of tubules occlusion and finally score 5 indicating no tubule occlusion. The results obtained are as follows:

On day 3, all the tooth paste samples showed score 4,60% of mouth wash samples showed score 5 and 40% showed score 4.80 % of sample in combination group showed score 4 and remaining 20% score 5. This goes to say that on day 3,tubule occlusion was highest in tooth paste samples followed by combination group and least by mouth wash group. On day 7,60% of tooth paste samples showed score 3 and 40% score 2,80 % of sample in combination group showed score 3 and remaining 20% score 2. In the mouth wash group 60% samples showed score 3,40% score 4. On 21st day, 60 % of tooth paste samples showed score 1 and 40% score 2, combination group 60%samples showed score 2 and 40% score ,80% of mouth wash samples showed score 2 and 20% score 3. On 28th day all the tooth paste samples showed score 1 occlusion,80% of both combination and mouth wash group showed score 1 occlusion while the remaining 20% of both groups showed score 2.So,on the 28th day we can see improved performance of mouth wash group similar to combination group. On 45th and 60th day all the groups except control showed score 1 tubule occlusion. All the samples of the control showed score 5 on all the test days. Hiora k toothpaste started showing tubule occlusion from day 3 itself. The occluding effect of this tooth paste is attributed to the presence of Palakya (Spinicia Oleracea). It consisted of 10.0 mg Spinacia oleracea per gram of dentifrice. It has been found that soluble oxalates and oxalic acid in phytocomplexes present in Spinacia oleracea (spinach leaves) form calcium oxalate crystals by reacting with dentinal calcium (Sauro *et al.,* 2006). Calcium oxalate crystals present in lyophilized phytocomplexes may penetrate inside dentinal tubules if their dimensions are less than 1– 2 mm. The ratios of

calcium and oxalate/calcium determine the effect of phytocomplexes on dentinal tubule occlusion. Low amounts of calcium and excess oxalate (oxalate/calcium ratio >1) induce binding of oxalate to calcium, producing calcium oxalate directly inside dentinal tubules (Pashley, 1988). Oxalate crystals are small enough to penetrate the tubules and occlude tubular orifices. A study has shown that treatment with oxalate-containing phytocomplexes induce microcrystal deposition on dentine and inside dentinal tubules and thus reduce the tubular diameters by forming crystals or crystallike structures (Sauro, 2006). Though the main ingredient of hiora K tooth paste is suryakshara (30 mg per gram of toothpaste) which contains potassium nitrate. Studies have found that pastes containing 5% potassium nitrate significantly decreased DH. It has been found that potassium nitrate does not diminish dentine hydraulic conductivity, or promote obstruction of dentinal tubules by the deposition of crystals (Peacock, 1995). According to Wilchgers and Ermert and Kim, potassium nitrate has an effective desensitizing action (Wichgers, 1997). The increase in the concentration of extracellular potassium around the nerve fibres causes their depolarization, avoids repolarization and blocks the axonic action and passage of nerve stimulus, thus inactivating the action potential (Peacock, 1995 and Wichgers, 1997). In this formulation Spinacia oleracea may have a possible mechanism of having a synergistic effect along with potassium nitrate in reducing DH by its dentinal tubule obliterating property (Kumari, 2013). Recently there has been a growing interest in natural products. Herbal based toothpastes have been found as effective as the conventionally formulated dentifrice in the control of plaque and gingivitis. Herbal formulations have also been found to be effective in the prevention of dental caries. From the results of this study, it can be concluded that herbal formulations are also effective in the treatment of dentine hypersensitivity and this novel herbal dentifrice can be recommended for treatment of DH. However, further studies are require to evaluate the retention of occluding material after discontinuation of the dentrifice, changes in surface morphology of dentine after its prolonged use, composition of the occluding material and also long-term clinical trials are required in which other commercially available dentifrices containing potassium salts, fluorides or calcium sodium phosphosilicate, are compared with this herbal formulation to confirm the findings of this study.

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