

## Research Article

### **Effect of an indigenously available herbal tooth whitening system on human enamel microhardness and micromorphology-an in-vitro study.**

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**Abstract:** The aim was to study the effects of indigenously available herbal tooth whitening product on the human enamel surface micro hardness and micro morphology. The methodology was forty enamel fragments were made from 20 extracted human teeth. The fragments were equally divided into two groups for application of a professionally prescribed bleaching agent and an indigenously available herbal tooth whitening agent. The products were applied onto the samples as per the application protocols. Micro hardness of each sample was analyzed pre and post bleaching treatment. Intra- group and inter-group comparisons were made. Two samples from each group were selected and were prepared for Scanning Electron microscopy (SEM) analysis. The images were obtained and compared to study the erosive effects of each agent. The Intra – group comparison of micro hardness values before and after treatment were analyzed using Wilcoxon signed rank test and the inter-group comparison of the micro hardness values were analyzed using Mann- Whitney U test. The level of significance was set as 0.05 and p values less than 0.05 was considered statistically significant. The results showed that there existed no statistically significant difference in the micro hardness of the enamel specimen at baseline among both the groups, there was a statistically significant reduction in the micro hardness level in both post 14 days treatment. The fall in the micro hardness value in group A was 19.80 and that for group B was 35.31. The SEM images of the samples from both the group showed similar type of erosions. The present study showed that an indigenous herbal based tooth whitening system, marketed as an over the counter product produced similar changes in the enamel surface micro hardness and micro morphology comparable to that of a professionally prescribed bleaching agent.

**Keywords:** Indigenous, herbal, bleaching, Micro hardness, Scanning electron microscopy analysis.

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#### **INTRODUCTION**

Appearance is central to social experience and interaction throughout one's life. Esthetics in Oral health plays an important role in deciding the psychological health of an individual. Discolored or stained teeth can affect one's social well being and also can worsen the oral health related quality of life [1-3]. Noticeable discoloration of the teeth should not only be regarded as a condition of cosmetic importance, it also sometimes becomes a physical handicap to one's lifestyle in various aspects. Hence, an esthetic smile is what everyone desires to maintain their social well being.

Cosmetic dentistry has emerged as a field of dentistry, which specializes in satisfying the esthetic desires of patients. According to the American Academy of Cosmetic dentistry (AACD), the average amount spent on cosmetic dental treatment ranges from 300 USD to 4000 USD every year by each patient.

About 71% of the patients opting for cosmetic dental treatment are females and the majority of the population is under the age group of 40-49 years [4]. Tooth-whitening (bleaching) has become one of the most popular and successful esthetic dental services offered to patients. There is also a dramatic increase in the public demand for bleaching which has led to the development of various products and methods of application. Tooth whitening treatments are categorized into Professionally applied bleaching system and home based bleaching techniques. Professionally, hydrogen peroxide or carbamide peroxide is used at various concentrations for bleaching. Hydrogen peroxide whitens the teeth by oxidizing their organic matrix and releasing free radicals. The surface gets roughened and whitens the enamel surface. There are studies which indicate the safety of using hydrogen peroxide [5,6]. However, hydrogen peroxide has the following demerits, which includes, cervical root resorption [7] associated with non-vital bleaching, increased tooth

sensitivity [8] associated with vital bleaching, alteration in the topography of the enamel and also has the ability of being carcinogenic [9]. Hence, to overcome this, alternative medicines free of hydrogen peroxide or other chemicals have been developed and are available over-the-counter (OTC) for tooth whitening. Traditionally in India, various herbs have been used in dentistry for treating oral diseases like dental caries, oral malodor, gingivitis, poor oral hygiene, tooth sensitivity etc, which includes, *Acacia arabica*, *Datura fastuosa* Linn., *Cassia alata* L, *Coriandrum sativum* Linn, *Mangifera indica* L etc [10]. Though various studies have been done on the effects of these indigenous products on oral health promotion and interventions, very few literature is available on the effects of these herbal products as a bleaching or tooth whitening agent. A variety of tooth whitening products are also available in the Indian market claiming to have herbal formulations, though their effectiveness have not been studied in the dental literature. One of the popular brands marketed in the Indian market is “Top Dent Drops”, which has a herbal composition and is also marketed OTC, thus making increase accessibility to the patients. Its low cost and better accessibility is presently making it a popular choice of tooth whitening among the general population. Since, there exists a lacunae on the in vivo and in vitro research on this product, it becomes imperative to find out the effectiveness of this material on enamel surface on short term and long term use. Hence, this in-vitro study was contemplated to find out the effects of this indigenously available herbal tooth whitening product on the human enamel micro hardness and micro morphology.

## MATERIALS AND METHODS

Prior to the start of the study, the protocol was submitted and approved by the Institutional review board.

### Sample Preparation

Twenty human teeth (incisors, canines and premolars) were collected, which were extracted either due to periodontal reasons or for orthodontic reasons from the department of Oral and Maxillofacial surgery of the same college. During, the extraction procedure, care was taken to ensure that the forceps was engaged in such a way that there was no or minimal trauma to the crown of tooth, without hampering the normal morphology of the crown structure. All the collected teeth were thoroughly cleaned of debris and stains. Teeth which had dental caries, cracks, extensive attrition or abrasion or erosion up to middle third of the crown were not included for the study. The teeth were then stored for approximately 24 hours in a 0.1% thymol solution (pH = 7) [5]. The crowns of the teeth were longitudinally sectioned with double-faced diamond disks, using a low-speed hand piece and deionized water was used as a coolant during sectioning. 40 enamel fragments were produced with a minimum of 4 x 4 mm from the facial (for anteriors) or

middle of the buccal or lingual aspect (for premolars) and the roots were discarded. The fragments presenting cracks were not used. After completing the sectioning, the specimens were soaked in deionized water for 24 hours at  $37^{\circ} \pm 1^{\circ}\text{C}$  [5]. Each tooth was made into two identical enamel sections which was allotted to two groups ‘A’ and ‘B’. Group A fragments were allotted for the application of professionally prescribed bleaching agent, and Group B fragments, for application of indigenously available herbal tooth whitening agent. The enamel fragments were embedded individually in a self-curing resin (DPI-RR Cold cure, Acrylic resin material) in a polyvinyl chloride ring mold with approximately 2.0 cm in diameter, allowing the external surface of the enamel to be exposed (Figure 1 and 2). The molds were removed in order to shape a plane enamel surface for micro hardness testing.



Fig-1: Sample after mounting.



Fig-2: Labeling of the samples.

The specimens were allotted to both the groups and were labeled individually with the number and the group. A total of forty specimens were prepared for twenty under each group. Prior to the start of the study, the micro hardness for each sample was estimated using Vicker's micro hardness tester (Matsuzawa, Japan,

Model-MMT X7) and was noted as the baseline micro hardness value. Three indentations were made on the enamel surface and the mean value of the three readings was considered for each sample. Artificial saliva was prepared [11] and all the samples were stored in a tray containing artificial saliva during the study period.

**Bleaching treatment**

A group sample was applied with professionally prescribed bleaching agent comprising of 35% hydrogen peroxide bleaching agent (*Mix one supreme, Villievie*). The material was applied onto the tooth surface and rinsed once weekly for 2 weeks (14 days), as per the manufacturers’s instructions [12]. Group B samples were applied with the indigenously available herbal tooth whitening agent (“Top Dent Drops” - A proprietary siddha medicine, with the Drug license number – 1157) available as a paint-on solution was applied onto the tooth surface for ten minutes daily with a 12 hour interval for 14 days as per the manufacturer’s instructions. Composition of the indigenously available tooth whitening agent as presented by the manufacturer, consisted of, *Ficus bengalensis* – 9%, *Acacia arabica* – 9%, *Syziglum aromaticum* – 0.7%, *Cinnamomum camphora* – 0.4%, *Mentha arvensis* – 0.3%, *Alum* – 0.3%, *Sodium chloride* – 0.1%, *Azadiracta indica* – 6%, *Carum roxburghianum* 2%, *Punica granatum* – 3%, *Triphala*- 6%, *Vitex negando* – 3%, *Embelica ribes* – 1%, *Citrus limonum* – 0.5%, *Rock salt* – 0.3%, *Allium sativum* – 2%, *Brassica juncea* – 7%, *Cocos nucifera* – QS. All the fragments were stored in artificial saliva, which was changed every 24 hours throughout the study period.

**Microhardness and SEM Analysis**

After the 14 day treatment, the teeth samples were subjected to microhardness testing using Vicker’s

microhardness tester similar to the baseline. Two samples from each group were thoroughly cleaned and prepared for scanning electron microscopy (SEM) examination according the preparation protocols [13]. The samples were examined with a high resolution Scanning Electron Microscope (COXEM, South Korea, Model- CX 200) at 20 KV acceleration voltage and magnifications of 300x, 500x and 1.0kx. The images were viewed on a monitor and were evaluated by the expert professional qualified in SEM analysis and interpretation employed in the lab where the tests were done (MICROLABS, Chennai).

**Statistical Analysis**

Statistical analyses were carried out using Statistical Package for Social Sciences version 19 (IBM, 2010). The micro hardness values before and after treatment within the same group was analyzed using Wilcoxon signed rank test and the micro hardness values before and after treatment between the two groups were analyzed using Mann- Whitney U test. The level of significance was set as 0.05 and p values less than 0.05 was considered statistically significant.

**RESULTS**

**Microhardness**

Table 1 shows the difference in the mean micro hardness values in group A and group B at baseline and post treatment after 14 days. Though there existed no statistically significant difference in the micro hardness of the enamel specimen at baseline among both the groups, there was a statistically significant reduction in the micro hardness level in both posts 14 days treatment. The fall in the micro hardness value in group A was 19.80 and that for group B was 35.31.

**Table-1: Distribution of mean micro hardness values of group A and group B at baseline and post-treatment.**

Variable	Microhardness value at baseline (mean ± S.D)	Microhardness value after 14 days (mean ± S.D)	Mean Rank	P- value
Professionally prescribed bleaching agent (Group A)	281.96±40.40*	262.08±40.84†	10.50	0.01
Indigenously available herbal bleaching agent (Group B)	281.38±39.81*	246.01±38.40†	10.50	0.01

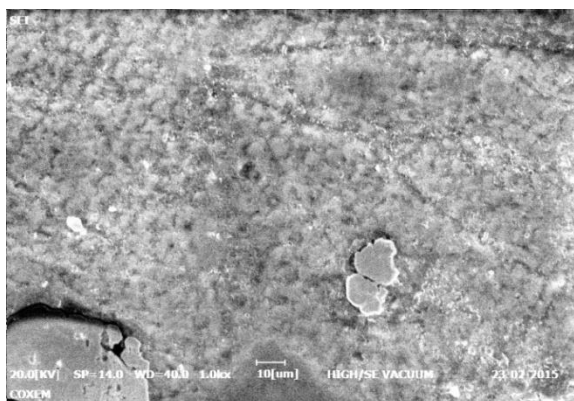
\* Mann Whitney U test: -0.081, p= 0.935

† Mann Whitney U test: - 1.298, p= 0.194

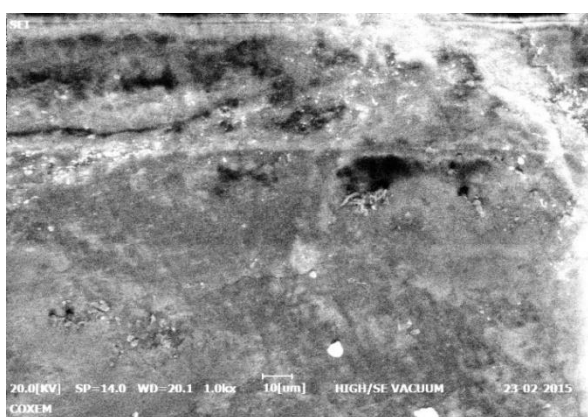
**SEM Analysis**

Figures 3 shows the SEM image of the professionally prescribed bleaching agent after the study period (post – treatment after 14 days). The images shows the erosion levels of the enamel at magnification of 1.0kx. Figure 4 shows the SEM images of the indigenously available herbal tooth whitening agent at a similar magnification. Samples from both the group showed similar type of erosions.

The enamel surface showed depressions and irregularities with alterations in the inter-prismatic structure.



**Fig-3: SEM image of the enamel showing erosions after application of the professionally prescribed bleaching agent at 1.0 kx magnification.**



**Fig-4: SEM image of the enamel showing erosions after application of the herbal agent at 1.0kx magnification.**

## DISCUSSION

The present study focused on the effect of an indigenously available herbal tooth whitening agent sold OTC when compared to a professionally prescribed home bleaching agent on enamel surface micro hardness and micro morphology. Presently, professionally prescribed bleaching techniques are the most commonly opted tooth whitening treatments and accounts for about 23% of the total cosmetic dental treatments done [4]. Most of these agents contain hydrogen peroxide and carbamide peroxide at various concentrations, which as a result of releasing free radicals, produces a change in the tooth shade, thus providing whitening effects. Studies indicate that though there is an effective tooth whitening using various concentrations of hydrogen peroxide and carbamide peroxide, however, there is an alteration in the micro hardness and micro morphology of the tooth structure post bleaching [14-16].

Micro hardness measurement is a simple method for determining the mechanical properties of enamel and dentine surfaces and it is related to a loss or gain of mineral of the dental structure. It has been previously shown to be a suitable method for determining small changes in surface micro hardness of

enamel and dentine following erosive challenges due to bleaching procedures [15].

In the present study, Vicker's micro indentation tester was chosen for estimating micro hardness values, because in the Vickers test, the load is applied smoothly, without impact, forcing the indenter into the test piece. The indenter does not alter the shape or morphology of the sample, which enables us to reuse the same sample for further evaluation if required. Because the shape of the Vickers indentation is geometrically similar at all test loads, the micro hardness value is constant, within statistical precision, over a very wide test load range [17].

Our present study results showed that the mean difference in the micro hardness values was 19.80 in the professionally prescribed group, and 35.31 in the herbal based whitening agent group. These results were comparable to the study conducted by, Yeh et al [18], which showed that a 35% hydrogen peroxide treatment for 30 min on human enamel showed significant reduction of micro hardness from baseline to after 21 days post application. Similarly, an in vitro study conducted by Lewinstein et al [19], observed a slight reduction in enamel micro hardness, which was completely reversed when treated with a 0.05% fluoride solution.

Scanning electron microscopy (SEM) is a rapid and convenient method for qualitatively analyzing the surface morphology of enamel and dentine specimens following bleaching. In this present study, 1.0 kx magnifications images from samples of both the group showed similar erosive changes, indicating that both these agents have similar bleaching effects on the enamel surface. Our study results were similar to SEM findings of studies conducted by Yeh et al [18], and Spalding et al, who observed minor surface erosions at localized areas of enamel surface, but considered these changes to be within the normal variations existing in natural teeth.

Of late, there is an emerging market for the use of herbal based products for a variety of health conditions, including prevention of oral diseases. In our study, we used a product, which claim to have a herbal composition. The formulation of this agent contains herbs which have found to have preventive effect on various diseases. Studies indicate that herbs like *Acacia arabica* and *Azadiracta indica* have found to be effective in reducing dental caries. *Cinnamomum camphora*, *Embelica ribes* and *Citrus limonium* are effective in maintaining oral hygiene. These ingredients are used for tooth cleaning are commonly used in tooth paste formulations [10]. However, the key ingredient in this preparation, which contributes to tooth whitening remains not known. There are numerous studies done to prove the various dental uses of herbal formulations, but there are no studies done to find out the effect of herbal

preparations on tooth whitening. Since, this is one of the first studies done to find out the effectiveness of a herbal based tooth whitening agent, comparison with previous studies were not possible. It can be suggested that, since this herbal tooth whitening system has results which can be comparable to professionally prescribed bleaching agent, in terms of enamel surface micro hardness and micro morphology, the potential of these agents warrants further research, especially of the tooth shade improvements capacity. An important advantage of these herbal agents are that these agents are more cost-effective and that the traditionally available professional bleaching agents. The herbal based agent used in the present study costing around 0.80 USD (as mentioned in the product label) when compared to the cost of professionally prescribed bleaching product which ranges from 15 USD to 100 USD.

There existed some limitations in this study which should be considered while interpreting the results. Though the study was done in-vitro with artificial saliva as storage medium for human enamel slabs, the intra-oral exchange of minerals in in-vivo conditions would contribute to the micro hardness and micro morphology of enamel [14]. Hence, the results on the in vitro effects of these agents will throw more light on the interaction of enamel surface and the changes produced by the application of these agents over a period of time.

## CONCLUSION

The present study showed that an indigenous herbal based tooth whitening system, marketed as an OTC product produced similar changes in the enamel surface micro hardness and micro morphology comparable to that of a professionally prescribed bleaching agent. If future researches prove them as an alternative to the professionally prescribed agents, they can act as a cost effective viable mode of delivery of aesthetic dentistry, especially for a developing country where herbal products are available at abundance.

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