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### RESEARCH ARTICLE

#### SINGLE AND COMBINED EFFECT OF GRAPE SEED EXTRACT AND NANOHYDROXYAPATITE ON REMINERALIZATION OF BLEACHED ENAMEL.

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#### Abstract

**Aim:** to evaluate the single and combined effect of Grape Seed Extract and nanoHydroxyApatite on surface topography and calcium content of bleached enamel using Scanning electron microscope and Energy-Dispersive X-ray Analysis.

**Materials and methods:** 35 maxillary first premolars were assigned to five groups, seven premolars each. According to the procedure done: Group I (control group), group II (bleached), group III (bleached treated with grape seed extract), group IV (bleached treated with nanoHydroxyapatite), and group V (bleached treated with both materials). After that teeth of each group were left in artificial saliva for 24hrs.

**Results:** Both groups III & IV revealed less pronounced perikymata ridges and grooves and precipitations scattered on rodless enamel or caused partial occlusion of enamel rod ends. Group V enamel rod ends were small in diameter so appear as pitting on the surface of the enamel. Another variant presented almost homogenous deposition of the precipitates covered most of the enamel surface leading to almost occluded enamel rod ends that could hardly be identified.

Energy-Dispersive X-ray Analysis showed that Ca mean weight percentage of group V were similar to group I with no statistical significant difference while it was significantly higher than group II while groups III & IV Ca mean weight percentages were significantly lower than the control group (group I) while there was no significant difference when compared to the bleached group (group II).

**Conclusion:** it was found that combining Grape Seed Extract and nanoHydroxyApatite encouraged remineralization after tooth demineralization by a bleaching agent.

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#### Introduction:-

Color and aesthetics of teeth is an important topic for patients, as they influence self-esteem and professional relationships. Patients have been progressively requesting a perfect smile. They spend large amounts of money and time to improve the appearance and color of their teeth (Karadas & Duymus, 2015).

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Tooth bleaching gained interest among people (Access, 2014). Since it comprises a safe, conservative and simple option for the aesthetic treatment of stained and discolored teeth, it also happens to be the least destructive procedure for treatment of tooth discoloration (Elfallah et al., 2015).

Bleaching products include different composition of gels with different peroxide concentrations. (Access, 2014). These peroxides penetrate through enamel and dentin eventually reaching the pulp in the course of which it undertakes chemical breakdown and the peroxide decomposes forming oxygen free radicals. These free radicals are highly unstable and capable of oxidizing and disintegrating a wide range of organic and inorganic materials including chromophores (Elfallah et al., 2015).

The changes in the organic and inorganic content of enamel cause erosion-like changes to be observed in enamel after bleaching treatments, especially when low pH and high-concentrated peroxides are being used (Elfallah et al., 2015; Santos et al., 2015). Longer application time and/or multiple treatments sessions contribute and increase the risk of tooth sensitivity which has been shown by a large number of patients during the first days of treatment.

GSE, is a naturally occurring plant metabolite widely available in fruits like cranberries, vegetables, nuts, flowers, bark and the leaves of bilberry, birch, ginkgo, Hawthorne (Fine, 2000; Prabhakar et al., 2012).

GSE is considered a rich source of proanthocyanidin (PA), which consists mainly of free monomeric flavanols and various polyphenolic compounds i.e., the PAs, as well as their dimeric, trimeric, tetrameric, and higher oligomeric forms, named the OPAs (Oligomeric Proanthocyanidin) which forms about 80%-90% of PAs, and is known as grape seed proanthocyanidin extract (GSPE) (Gunjima et al., 2004).

It is hypothesized that GSE inhibits the growth and biofilm formation of *S. mutans*, thus preventing the progression of artificial enamel caries. Therefore, PAs rich foods or beverages are consumed in order to benefit oral health, with its antigingivitis and anticaries properties. It was also found that GSE exhibited antioxidant potency 50 times greater than sodium ascorbate (Abraham et al., 2013; Zhao et al., 2014).

Gallic acid is one of the major constituents of grape seed extract, it increases their free radical scavenging ability (Fine, 2000). Moreover, gallic acid was revealed to facilitate mineral deposition, predominately on the surface layer. So it can promote remineralization (Mirkarimi et al., 2013).

Hydroxyapatite is one of the most important bioceramics for medical applications (Lu et al., 2007). It and its derivatives can be exploited as a perfect compound to study biomineralization in the human body and can be used in various biomedical applications (Sadat-Shojai et al., 2013).

nHA is considered one of the most biocompatible and bioactive materials and has gained extensive acceptance in medicine and dentistry in recent years. Whilst former attempts to use HAs clinically did not succeed, synthesis of nano-scaled hydroxyapatite yielded a significant progress where it was shown that nHA could possibly repair dental enamel damaged by bleaching (Tschoppe et al., 2011).

Another study also revealed that nHA could deposit strongly onto the etched enamel surface and significantly prevent subsequent mineral loss from the enamel surface under acidic solutions (Huang et al., 2011). And thus nHA-based remineralizing agent was presented in the market (da Costa Soares et al., 2012), and it was revealed to restore luster to enamel (Kutsch et al., 2013), to be used instead of the previously used polishing materials (Heshmat et al., 2014).

Consequently a product like nHP, in theory, encourages repair of the microscopic defects caused by bleaching and can minimize tooth sensitivity by diminishing the diffusion of hydrogen peroxide into the pulp (Loguercio et al., 2015).

Therefore this study aimed to evaluate the single and combined effect of GSE and nHA on surface topography and calcium content of bleached enamel using Scanning electron microscope and Energy-Dispersive X-ray Analysis (EDXA).

**Materials and methods:-****Samples:**

Thirty five recently extracted permanent first premolars were selected and stored after cleaning and removing debris under refrigeration in saline solution until testing commenced. The decayed and damaged teeth were excluded.

**Grouping:**

The teeth were divided into five groups, seven premolars each.

Group I (control group), group II (bleached), group III (bleached treated with grape seed extract), group IV (bleached treated with nanohydroxyapatite), and group V (bleached treated with GSE followed by nHA).

**Sample Preparation:-**

In the middle third of the buccal surfaces of all teeth a window 2x3 mm was created by covering the selected area with pink wax then the remaining of the buccal surfaces was covered by nail varnish (Luna 280). After the varnish was applied the pink wax was removed.

The bleaching agent (Opalescence Xtra Boost®) was supplied as two syringes attached together one contained the bleaching agent and other contained the activator. It was prepared by mixing the activator with the bleaching agent by pressing the two syringes back and forth for 20 times rapidly (10 each side).

A thin layer of the bleaching agent was applied directly on the enamel surface of the area to be examined in teeth of groups II,III,IV&V for ten minutes. The surfaces were rinsed off. The procedure was repeated 3 times. Then after the last application the teeth were rinsed with water for 30 seconds.

Then immediately after bleaching and rinsing, teeth of groups III & V were treated with 5% proanthocyanidin solution prepared by dissolving 5gm of grape seed extract powder in 100ml of distilled water (Abraham et al., 2013) for 1 hour and then rinsed off.

Teeth of groups IV & V were immersed in distilled water plus 10% nHA solution prepared by dissolving 10gm nHA powder in 100 ml of distilled water for 12 hrs. (Haghoo et al., 2014)

All samples were stored in artificial saliva for 24 hrs (Kutsch et al., 2013) before testing begins. The artificial saliva used in this present study were prepared according to (McKnight-Hanes & Whitford., 1992).

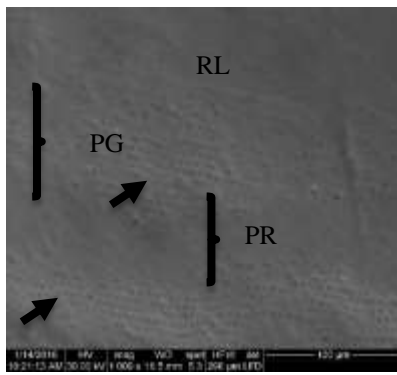
Examination was done to the middle third of labial surface, which was cleaned from any remaining solution then refrigerated until examined by scanning electron microscope at magnification 1000 and Energy Dispersive X-ray Analysis (EDXA) model FEI Inspect S was used to evaluate the surface mineral content of Calcium (Ca).

The statistical analysis was performed using the statistical software “IBM Statistical Package for Scientific Studies, SPSS version 22”.

**Results:-****Scanning electron microscope: (SEM) examination:**

SEM examination of group I (control group) revealed few perikymata grooves and ridges with observed enamel rod ends (EREs). Areas of rodless enamel were observed on the perikymata ridges and they appear as areas with no structure and with uniform surface. EREs appeared as areas of shallow saucerizations of enamel surface. These saucerizations almost had regular margins and apparently uniform diameter on the perikymata grooves. Their depth didn't variably differ. Fig. (1)

While group II showed irregular pitted enamel surface in some areas but other areas showed EREs that were less observed with ill-defined boundaries. Perikymata were less defined where the ridges were apparently less elevated and the grooves were apparently shallower. Areas of perikaymata ridges presented relatively shallow EREs while the EREs on perikymata grooves were more accentuated and apparently deeper than those of group I. Areas with rodless enamel were apparently fewer. Fig. (2)



fig(1)

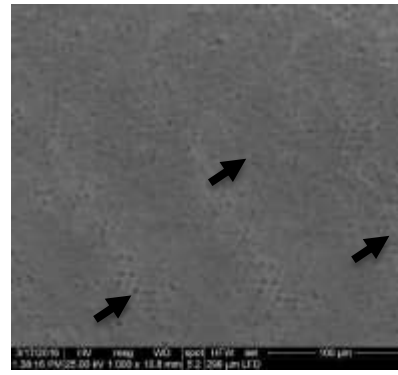


fig (2)

**Fig. 1:-** SEM of group I showing perikymata grooves (PG) and ridges (PR) with obvious EREs (black arrows) and areas of rodless enamel (RL), Fig. 2: SEM of group II showing ill-defined perikymata with areas of EREs with ill-defined prism boundaries (black arrow). (x1000)

SEM of groups III, IV and V (bleaching + GSE, bleaching + nHA and bleaching + GSE + nHA respectively) showed less pronounced perikymata where the ridges were apparently less elevated and the grooves were apparently shallower which was similar to group II.

Partially occluded EREs with precipitates of variable sizes were scattered on the enamel surface. Some of these precipitates were deposited on top of the rodless enamel, others were encountered in the concavities of the EREs, which presumably lead to their partial occlusion. In group V EREs were small in diameter so appeared as pitting on the surface of the enamel. Another variant presented almost homogenous deposition of the precipitates covered most of the enamel surface leading to almost occluded EREs that could hardly be identified.

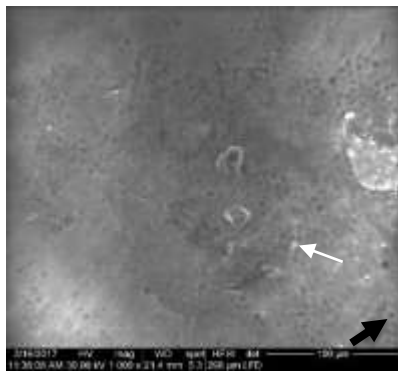


fig (3)

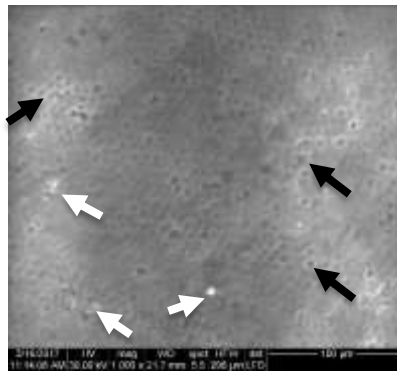


fig (4)

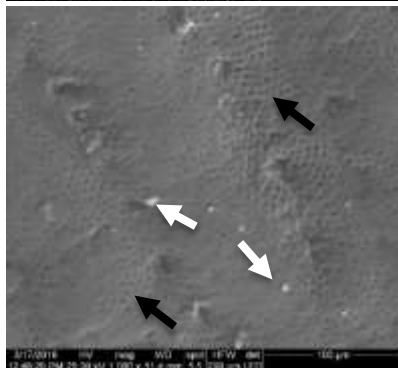


fig (5)

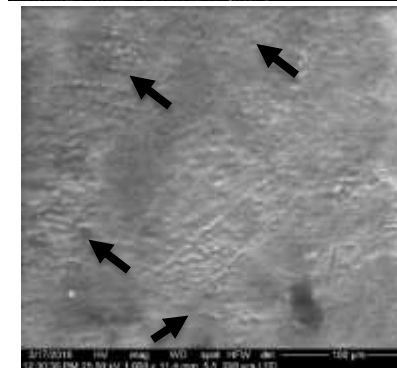


fig (6)

**Fig. 3 & 4:-** SEM of subgroup III&IV showing partially occluded EREs (black arrows) and precipitates of different sizes (white arrows) were observed on the enamel surface. Fig. 5: SEM of subgroup V showing occluded and narrowed EREs (black arrows) with precipitates of different sizes (white arrows) observed on the surface. Fig. 6: showing completely occluded EREs (black arrows) with evidence of a precipitate covering the entire surface.

Energy Dispersive X-ray Analysis (EDXA) and statistical results:

Calcium (Ca):

One Way Analysis of Variance (ANOVA) was used to compare the Ca mean weight percentage among group II and groups III, IV & V. It showed the presence of statistically significant difference among the treatment groups ( $P \leq 0.001$ ).

A pairwise comparison was held between Ca mean weight percentage in group I and the other groups using unpaired Student's t-test. The results showed a statistically significant difference between group I and all the other groups where the p value was less than 0.05 except group V where the results showed no statistically significant difference with the control group where the p value was 0.470 i.e.  $p > 0.05$

A pairwise comparison was held between Ca mean weight percentage in group II and groups III, IV & V using unpaired Student's t-test. The results showed no statistical significant difference between group II and groups III and IV ( $p > 0.05$ ). But, group V showed statistically significant increase in Ca percentage mean in comparison to group II ( $p < 0.05$ ).

A pairwise comparison was held between Ca mean weight percentage in group III and Subgroup IV using unpaired Student's T-test and it showed statistically non-significant difference in Ca percentage mean ( $P > 0.05$ ). Comparing group III and group V, unpaired Student's T-test showed statistically non-significant difference between both groups ( $P > 0.05$ ). As for the comparison between group IV and group V, unpaired Student's T-test showed a statistically significant difference between both groups ( $P < 0.05$ ).

### Discussion:-

GSE is a natural product that is rich in proanthocyanidins. And although it has not been widely investigated, what research was done has proven GSE useful in the field of medicine and specifically in dental research. nHA is a biocompatible calcium-phosphate compound with a similar structure to that of the mineralized part of the tooth which has been used in many forms and examined by different authors.

In this study, results of group II revealed illdefined perikymata grooves and ridges with irregular pitted enamel surface. Moreover Ca mean weight percentage was significantly lower than that of the control group. Our results were in accordance with da Costa Soares et al., in 2012, where in their study they observed that after the application of bleaching agent and after the samples were left in artificial saliva for 24 hrs that there were no calcium depositions and some irregularities such as depressions were observed on the enamel. They also stated that the amount of Ca is reduced after bleaching compared to the sound enamel.

Moreover, Coceska et al., in 2016 used a similar bleaching agent (Opalescence Xtra Boost®) on permanent molars. They stated in their results that there were areas with partial damage and erosion seen on the enamel surface and areas that were not so severally damaged were also seen. These results were in accordance with our study, in spite of the difference in the contact time and in the fact that they didn't use the artificial saliva. This could indicate that artificial saliva is not enough to restore the bleached enamel to normal surface topography.

In the present study, group III showed some surface depositions with partial occlusion of EREs. These results were in agreement with Mirkarimi et al., in 2013, despite the difference in experimental methods. They reported spherical deposits on the enamel surface in groups treated with GSE indicating that GSE can induce surface calcific deposition. But in their study they tested the remineralization process on primary teeth after a process of artificial caries induction in enamel. They claimed that by interacting with the mineral content of the enamel surface GSE facilitated mineral deposition and thus helps initiate surface remineralization.

Even though GSE showed signs of calcific deposition it was not effective alone to fully remineralize the bleached enamel to reach levels close to control level since there was significant decrease in group III Ca mean weight percentage than group I, but when compared with group II (bleaching group) there was no significant difference in Ca mean weight percentage. Xie et al., in 2009, who was among the first people to work on GSE, explained GSE action by deposition of minerals on superficial layer of the lesion by combining with  $\text{Ca}^{2+}$  from the remineralizing solution and also by forming insoluble compounds with Ca.

In the present work, group IV revealed depositions on the enamel surface with partial EREs occlusion. these results were in accordance with da Costa Soares et al., in 2012 in which their results showed a large number of nHA deposits twenty four hours after the application of Nano-P (a nanohydroxyapatite-based agent), regardless of the bleaching agent used.

Moreover, Kutsch et al., in 2013, was also in agreement with our results. They reported the presence of nHA crystals on enamel surface of the group of teeth that were being treated with the remineralizing gel that contained nHA. This indicates that nHA can induce surface remineralization of enamel.

However, EDXA statistical results of this group concluded despite being the same element as the enamel itself and showed signs of calcification; nHA was not enough on its own to remineralize the damaged enamel into normal surface topography nor was GSE.

A study done by Da Costa Soares et al., in 2012 reported increased microhardness of groups treated with nHA based gel after bleaching as opposed to groups treated with other remineralizing materials. Moreover Kutsch et al., in 2013, revealed that there is no difference in weight percentage of Ca among control group and groups treated with the nHA remineralizing gel after being stored in artificial saliva 24hr. Both attributed these results to incorporation of nHA crystals into the superficial layer of enamel after bleaching and subsequent mineral uptake. Similar results of these studies were obtained in spite of the differences in type of bleaching agent and in concentrations of hydrogen peroxide, nHA and sodium fluoride.

Both results were not in accordance with our results. This could be explained by their usage of sodium fluoride in combination with nHA. This also supports that nHA was not enough on its own – as in group IV in our study- to remineralize the damaged enamel into normal.

Meanwhile, the results of Haghgoo et al., study in 2014, who was previously mentioned to have used several concentrations of nHA on demineralized premolar teeth and compared them to each other and to sodium fluoride, concluded from their experiment that 10% nHA solution yielded the best results compared to other concentrations of nHA but similar results to NaF using microhardness test. That was consistent with our results despite using demineralizing agents prior to immersion in the remineralizing solutions while we used hydrogen peroxide based bleaching agent and we tested them using EDXA while they used microhardness test.

On the other hand, SEM findings of group V could be attributed to a synergistic action of both GSE and nHA that could enhance remineralization after bleaching and consequently reduce the post bleaching sensitivity. And this is supported by the Ca mean weight percentage results which revealed that combining GSE and nHA could enhance remineralization and increase Ca content to reach almost normal levels of untreated enamel. And this may be due to the fact that GSE contains Gallic acid that has the potential to induce surface mineral deposition and to obtain Ca from surrounding media. These results were in accordance with Mirkarimi et al., in 2013. Therefore the presence of GSE could enhance the deposition of nHA on the surface of the enamel and also enhance the deposition of Ca from the surrounding media.

### **Conclusion:-**

This study concluded that combining GSE and nHA encouraged remineralization after tooth demineralization by a bleaching agent and increase surface Ca deposition.

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