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EVALUATION OF REMINERALIZATION EFFECT OF ALOE VERA OF MILD AND SEVERE CARIES LESIONS IN PERMANENT TEETH :AN IN VITRO STUDY

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ABSTRACT

Background: The Aloe vera plant is a cactus-like plant in the Liliacea family that has been known and utilized for its medical benefits for millennia. It has been attempted to be used as a remineralizing agent.

Aim of the study: Assessment of the remineralizing effect of aloe vera gel compared to sodium fluoride gel by scanning electron microscope and Energy Dispersive X-Ray Analysis (SEM-EDX).

Materials and methods: 40 extracted premolars were used in this in vitro study. Crowns were separated mesiodistally to give 80 enamel specimens then divided and randomly allocated into demineralizing solution for 4 days to obtain mild caries like lesion (group I) and for 10 days to obtain severe caries like lesions (group II) each group subdivided in to sub group 1 (aloe vera group) and sub group 2 (sodium fluoride group) each sub group had 20 specimens. All specimens were scanned with (SEM-EDX), analysis was carried out at baseline, post demineralization and later post 21 days of remineralization.

Results: SEM-EDX evaluation showed improvement in the enamel appearance and the surface became smooth after remineralization. The surface minerals content mean value in the group treated by aloe vera gel was higher than the recorded mean value for group treated with sodium fluoride.

Conclusion: aloe vera gel could be acceptable alternative to fluoride for caries prevention in terms of safety and efficiency.

KEY WORDS: Aloe vera, Fluoride, Remineralization, SEM.

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INTRODUCTION

Caries is a chronic condition that arises when bacteria's products attack enamel and dentin and degrade the mineral content of teeth, but the incidence of dental caries is determined by a dynamic balance between demineralization and remineralization [1, 2].

Oral fluids contain supersaturated amounts of phosphate and calcium, under physiological settings which are frequently deposited on enamel or redeposit on previously lost enamel sites [3].

During the early stages of this process, the diagnosis and treatment is possible. As a result, modern dentistry has recently turned its attention to the development of early identification approaches for carious lesions as well as non-invasive alternative treatments such as remineralization [4].

The presence of white spot lesions on the enamel surface is the primary clinical indication of enamel surface caries. This can be prevented or delayed by applying fluoride, phosphate or calcium ions to the tooth surface [5,6].

Fluoride has long been used for remineralization, but excessive amounts can produce fluorosis and toxicity. As a result, Attempts to reach effective anti-caries and remineralizing products with little side effects have been stepped up [7].

Several herbals and other natural products have been investigated as remineralizing remedies. According to their content, they could boost mineral saturation and precipitation, serve as antimicrobials, or stabilize collagen to increase mineral deposition [8]. The present study used a scanning electron microscope and Energy Dispersive X-Ray Analysis (SEM-EDX) to assess the remineralizing capacity of aloe vera gel over sodium fluoride gel.

MATERIALS AND METHOD

In the current in vitro study, 80 specimens were allocated in 4 parallel groups (20 specimens each). The study was approved from research ethic committee, Faculty of Dentistry, Minia University number (388) in 2020 .

Test agents used

UltraEZ gel is a sustained-release 3% potassium nitrate desensitizing gel with fluoride (0.25% neutral NaF) made in USA was brought from dental market in minia.

Aloe vera gel from aloe vera. the aloe vera leaves were brought from Faculty of Agriculture, Minia University. Gel was obtained after cutting the aloe Vera leaves, the aloin content was removed by allowing them to sit for 20 minutes. The external green component was then scraped away with a sterile knife to extract the Aloe vera gel without scraping too much to avoid aloin content in the collected gel [9]. Ten grams of sodium carboxy methyl cellulose was added gradually to one liter of extracted aloe vera gel to prevent clump formation. KOH was used to adjust the pH to 7.

Demineralizing solution was prepared at Faculty of Pharmacy, Minia University. It was composed of 2.2 mM calcium chloride (CaCl2), 0.05 mM lactic acid, 0.2 parts per million (ppm) fluoride and 2.2 mM monosodium phosphate (NaH2PO4). The pH of the solution was maintained at 4.5 by adding 50% sodium hydroxide (NaOH) [10].

Artificial saliva was prepared at Faculty of Pharmacy, Minia University by mixing 2 g/L methyl p-hydroxybenzoate, 10 g/L sodium carboxy methyl cellulose, 8.38 mmol/L of KCl, 29 mmol/L of MgCl2 .6H2O, 1.13 mmol/L of CaCl2.2H2O, 4.62 mmol/ L of KH_2PO_4 and 2,40 mmol/L of K_2HPO_4 [11].

Specimens collection

Forty freshly extracted, for orthodontic purpose, human premolars were collected from orthodontic clinic of Minia University Dental Hospital. At the outset, the patients signed informed consent for approval of the use of their teeth in research work. Selected teeth were free of caries, enamel

malformations, cracks erosions or abrasions on buccal or lingual/palatal surfaces.

Specimens preparation

Teeth were cleaned and stored in saline for maximum 1 month before use. A carborundum disk under coolant was used to section the crowns 2mm below the cemento-enamel junction. Then crowns of teeth were mounted in acrylic blocks and each crown was sectioned mesiodistally into buccal and palatal sections. To standardize the area of treatment and evaluation, the surface of every enamel sample was polished and covered with 4x4 mm adhesive tape, the rest of the surface was covered with nail polish.

Caries like lesions were induced by immersion the specimens in the demineralizing solution at 37˚C. Visual detection was performed daily under the clinic light, if white spot lesions were visible under moist conditions, the sample was categorized into the "severe carious lesions" group and moved to distil water. If the lesion couldn't be detected, the sample would be dried for 5 seconds using compressed air before being re-evaluated in a dry environment. If white spot lesions could be detected, the sample would be classified into the "mild carious lesions" group and then placed in distil water; minor lesions could be detected in 3:4 days, whereas advanced lesions needed 9:10 days [12].

Randomization, allocation and grouping the specimens

After preparation of enamel specimen and demineralizing regimen to give mild lesion specimens (group I) and severe lesion specimens (group II), specimens of each group were randomly allocated into two subgroups by computer generated block randomization, as following:

Sub group 1 : enamel specimens treated with aloe vera gel.

Sub group 2 : enamel specimens treated with sodium fluoride gel.

Each sample was exhibited to a pH-cycling process. Daily, each group's samples were solely immersed in 7 mL of fresh lactic acid for 3 hours, simulating the acid challenges of the mouth cavity, and then stored in 20 mL of fresh artificial saliva, simulating the remineralizing oral fluid. Therapeutic substances were applied three times a day for one minute each time with an applicator [13].

Specimens evaluation

All the enamel specimens from all groups were prepared to be examined by SEM-EDX for surface topography and minerals contents (Ca and phosphorus P), at the baseline, post demineralization and post reminerlaization. The scanning with SEM-EDX was performed at Central Laboratory for Microanalysis, Minia University.

Statistical analysis

To compare between more than two groups in non-related samples One-way ANOVA followed by Tukey post hoc test was used. Repeated measure ANOVA was used to compare between more than two groups in related samples.

Paired sample t-test was used.To compare between two groups in related samples

Statistical analysis was performed with IBM® SPSS® Statistics Version 20 for Windows. The significance level was set at $P \leq 0.05$.

RESULTS

Scanning of enamel surface before demineralization showed normal pattern of enamel topography with honey comb appearance.

After demineralization enamel surface became rough with increased width of pores. The pores were more and wider in severe group (fig.1) than in mild group (fig.2).

But after remineralization the surface in mild group regain its smoothness (fig 3,4) and became less porous than in severe group (fig 5,6) where

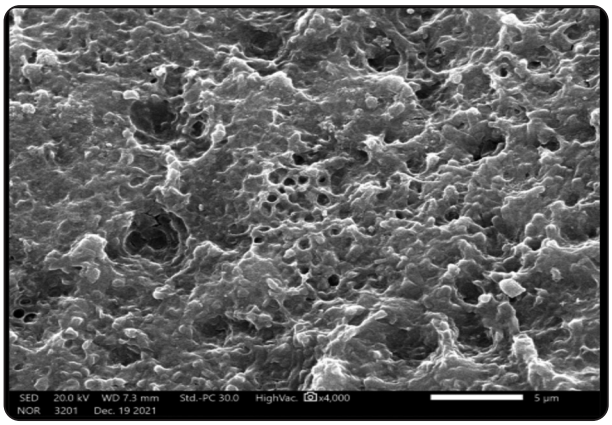

Fig (1) Severe caries like lesion

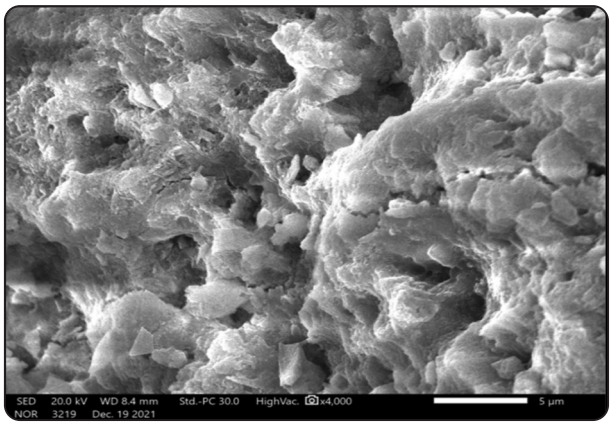

Fig (2) Mild caries like lesion

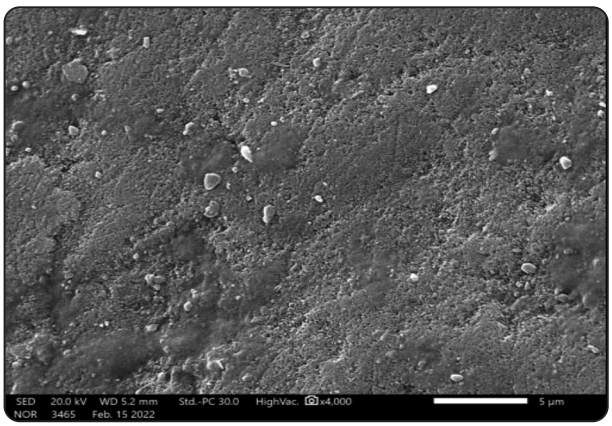

Fig (3) Post remineralization of mild caries like lesion with sodium fluoride

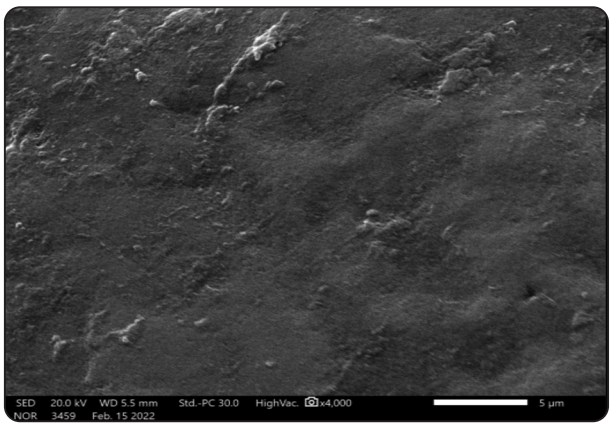

Fig (4) Post remineralization of mild caries like lesion with aloe vera

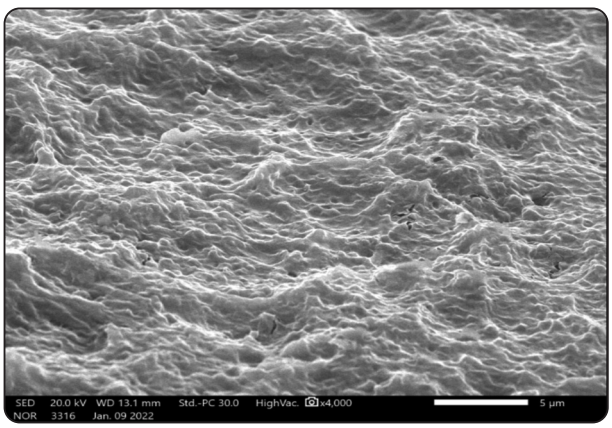

Fig (5) Post remineralization of severe caries like lesions with sodium fluoride

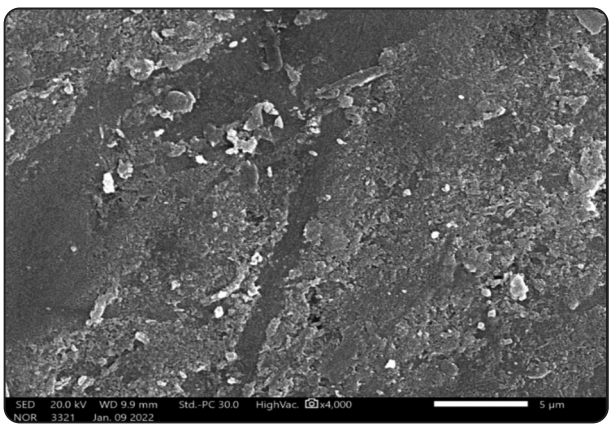

Fig (6) Post remineralization of severe caries like lesions with aloe vera

surface roughness of enamel still could be seen. The surface was smoother in aloe vera group than in sodium fluoride group.

Energy dispersive X-ray analysis: Calcium and phosphorus levels:

After the demineralization process, there was a statistically significant drop in mean calcium and phosphorus values in both groups, with no differences between them (p<0.001).

Post-remineralisation, a statistically significant difference in calcium and phosphorus levels was discovered in both group I and II, however, for calcium and phosphorus, subgroup 1(aloe vera) had a greater mean value in Post-remineralisation than subgroup 2 (sodium fluoride).

GROUP I

1-* ALOE VERA*: (*CALCIUM Content*)

The mean values of enamel specimens at baseline before demineralization was (52.76 ±0.21) and became (28.63 ±0.18) post demineralization and raised to (43.37 ±0.18) after 21 days of remineralization.

There was a statistically significant difference between (Baseline), (Post demineralization) and (After 21 days) groups where (p<0.001).

2- SODIUM FLUORIDE

The mean values of enamel specimens was (52.76 ±0.21) and became (28.63 ±0.18) post demineralization and raised to (41.46 ±0.12) after 21 days of remineralization.

There was a statistically significant difference between (Baseline), (Post demineralization) and (After 21 days) groups where (p<0.001).

2- Phosphorus content

1- Aloe vera:

The mean values of enamel specimens was (16.67±0.22) and became (12.54±0.11) post demineralization and raised to (15.36±0.21) after 21 days of remineralization.

There was a statistically significant difference between (Baseline), (Post demineralization) and (After 21 days) groups where (p=0.003).

2- Sodium fluoride:

The mean values of enamel specimens was (16.67±0.22) and became (12.54±0.11) post demineralization and raised to (14.96±0.27) after 21 days of remineralization.

There was a statistically significant difference between (Baseline), (Post demineralization) and (After 21 days) groups where (p=0.003).

GROUP II: (severe lesion)

1- Aloe vera: (calcium content)

The mean values of enamel specimens was (52.76 ±0.21) and became (20.15 ±0.28) post demineralization and raised to (40.38±0.12) after 21 days of remineralization.

There was a statistically significant difference between (Baseline), (Post demineralization) and (After 21 days) groups where (p<0.001).

2- Sodium fluoride

The mean values of enamel specimens was (52.76 ±0.21) and became (20.15 ±0.28) post demineralization and raised to (38.24±0.25) after 21 days of remineralization.

There was a statistically significant difference between (Baseline), (Post demineralization) and (After 21 days) groups where (p<0.001).

2- Phosphorus content

1- Aloe vera

The mean values of enamel specimens was (16.67±0.22) and became (9.38±0.12) post demineralization and raised to (13.88±0.11) after 21 days of remineralization.

There was a statistically significant difference between (Baseline), (Post demineralization) and (After 21 days) groups where (p<0.001).

2- Sodium fluoride

The mean values of enamel specimens was (16.67±0.22) and became (9.38±0.12) post demineralization and raised to (13.56 ±0.13) after 21 days of remineralization.

There was a statistically significant difference between (Baseline), (Post demineralization) and (After 21 days) groups where (p<0.001).

DISCUSSION

Dental caries is a common diet-related disease that has escalated into a major public health concern. One of the goals of modern dentistry is to use remineralization to treat non-cavitated carious lesions to reduce disease progression and improve strength, function, and aesthetics [14].

Fluoride is the gold standard in the struggle against tooth decay. Fluoride's effect, in particular, improves saliva-driven remineralization of demineralized enamel and has a good impact on solubility [15]. Despite fluoride-containing dentifrices are commonly used, their use as a daily dentifrice is controversial due to observed local and systemic adverse effects [16]. There has been a rising emphasis in advanced countries on the significance of developing novel remineralizing agents for the management of early caries [17].

The Aloe vera plant has anti-inflammatory, antiviral, antibacterial and antioxidative effects, its use as a herbal remedy in dental conditions and in remineralization due to its unique contents is promoting [18].

The pH cycling model applied in this research is based on an approach that disrupts an acidic environment on a regular basis. It aims to simulate the in vivo periodic pH alternation that occurs when carbohydrates are digested in the mouth to create a caries lesion. The pH-cycling model's advantage is that these combined studies are meant to imitate the kinetics of mineral loss and gain involved in caries formation [19].

The results of EDX analysis of this study showed that Aloe Vera gel was able to increase the minerals level more than fluoride in two types of white spot lesions (mild and severe) bypassing that of demineralized enamel indicating an effective remineralization potential but with no statistically significant difference.

The remineralising effect of aloe vera and sodium fluoride was also confirmed by SEM evaluation where, smooth enamel surface in mild group figures (3,4) and severe groups figures(5,6) compared to demineralized carious enamel as shown in figures (1,2)

Remineralization potential of aloe vera gel may be attributed to the unique composition as water makes approximately 98-99 percent of it. Active ingredients like methylchromones, flavonoids, saponin, sterols, amino acids, and vitamins; enzymes like acid phosphatase, alkaline phosphatase, amylase, lactic dehydrogenase, and lipase, and various inorganic substances like aluminium, boron, barium, calcium, iron, magnesium, sodium, phosphorous, silicon, and strontium make up the remaining 1-2 percent. Because of the porous nature of the demineralized enamel it facilitate the passage of those active ingredients into the enamel improving the remineralization [20,21].

The results of this study was parallel to results reported by **Silva et al**. (2016) that in terms of preventing white spot lesions, an aloe vera-based dentifrice is just as beneficial as a fluoride-based dentifrice [22].

Al Haddad et al. (2021) [23] reported that aloe vera gel promotes remineralization in the same way that a 1,450-ppm fluoride toothpaste does. Those

findings are in consistent with this study where a significant rise in calcium and phosphorus ratio was found after application. More concentrated fluoride treatments (5000 vs. 1500 ppm) were found to improve remineralization of advanced enamel lesions [24]. Those findings are in parallel with results of this study where a significant increase in remineralization of severe enamel lesion by aloe vera gel was found.

Results of the current study are in disagreement with those reported by **Ranjana,et al** (2021) [25] as they reported that herbal pediatric dentifrice (aloe vera dentifrice) shows an insignificant result compared with fluoride based dentifrice making it not a very effective remineralizing agent for primary teeth.

This disagreement may be as a result of short duration of the study (7 days) and delivery of aloe vera was in the form of toothpaste not in gel form.

This in vitro study has the following limitations

1. Simulating oral conditions in terms of biological factors of caries and the complexity of intraoral variables that contribute to dental caries is extremely difficult to mimic effectively in laboratory studies, and the involvement of enzymes is also ignored.
2. Because the current study uses inorganic ion solutions only, so, the effects of salivary pellicle, proteins and plaque on mineralization inhibition are not considered.
3. The extraction method, packaging, storage, harvest times and chemo types are different leads to varying in the chemical composition of the extracts.
4. The presence of experimental faults and various micro-structure of the enamel between specimens.
5. There are just a few studies (with limited quantitative data) obtainable for confirmation or comparison.

Recommendations:

Further studies are needed to test other parameters specially: micro hardness, colour and microroughness.

CONCLUSIONS

Based on the findings of this study, the following can be concluded:

1. Both aloe vera gel and sodium fluoride gel showed effective remineralisation of both mild and severe enamel caries.
2. Aloe vera gel showed slightly higher degree of remineralisation than sodium fluoride gel.
3. Aloe vera gel applied to enamel produces smooth surface through remineralization.

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