Grape seed extract as a potential remineralizing agent - A structured review

Maheswari Elumalai, Meignana Arumugham Indiran, Pradeep Kumar Rathinavelu, Sri Sakthi Doraikannan*, Jayashri prabhakar

INTRODUCTION
Among the oral diseases, dental caries is the most common chronic disease of mankind. Dental caries development is considered to involve a triad of indispensable factors that can be concluded as bacteria in dental plaque, carbohydrates in the diet, and susceptible teeth. Grape seed extract (GSE) is a rich source of proanthocyanidin (PA), mainly composed of monomeric catechin and epicatechin, gallic acid and polymeric, and oligomeric procyanidins. It verified that GSE, composed mainly of PA, can positively affect the tooth structure, thus offering a new therapy for carious lesions. Aim: The aim of this systematic review was to analyze the existing literature on the remineralizing effect of GSE on caries-like lesion compared to other remineralizing agent. Materials and Methods: Search strategy- The Data Bases of PubMed, Cochrane, Science direct, Lilacs, and Google Scholar were searched for the period from January 2000 to October 2017. References of selected articles and relevant reviews were searched for any missed publications. Selection criteria - a in vitro study evaluating the remineralizing potential of GSE on enamel, dentin, and root caries on human permanent, primary teeth, and bovine teeth. Results: The systematic search revealed a total of 340 publications from PubMed, Cochrane, Science direct, Lilacs, and Google Scholar. The articles were scrutinized based on present inclusion and exclusion criteria. 11 publications fulfilled all the inclusion criteria, and 329 publications were excluded from the review. Conclusion: With the available evidence, GSE was found to have remineralizing effect on dental caries. In the future, GSE with its antimicrobial, antigingivitis, and anticaries effect can be utilized in preventive and restorative materials to preserve the dental health.

KEY WORDS: Artificial caries, Enamel caries, Knoop hardness, Proanthocyanidins, Remineralization

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Department of Public Health Dentistry, Saveetha Dental College, Saveetha University, Saveetha Institute of Medical and Technical Sciences, Chennai, Tamil Nadu, India

*Corresponding author: Sri Sakthi Doraikannan, Department of Public Health Dentistry, Saveetha Dental College, Saveetha University, Saveetha Institute of Medical and Technical Sciences, Chennai, 162, Poonamallee High Road, Velappanchavadi, Thiruverkadu, Chennai – 600 077, Tamil Nadu, India. E-mail: drsrsakthiphd@gmail.com

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of tooth structure is hampered majorly by the loss of dentin. Restorative strategies have been continuously developing to repair and replace lost tooth structures.[7]

Restorative materials have been relied on to restore the teeth affected with dental caries. The most commonly used was glass ionomer cement. The origin of this glass ionomer cement is the inclusion of nanofillers, which constitute up to two-thirds of the filler content. The presence of nano-sized glass particles and clusters of nanosilica particles in this material has been claimed to improve its physical properties.[9] Dental caries restorative procedure includes removal of caries-affected tooth part and restored with a restorative cement. Pradeep kumar conducted a study on Papacarie, he stated that the chemomechanical caries removal is a non-invasive technique for caries removal which aims at the elimination of infected dentin.[9]

Dental caries is one of the most prevalent diseases in the world that causes demineralization and destruction of the hard tissues of teeth. The dental profession has, of course, been aware of this for many years and, consequently, has developed preventive and therapeutic regimens for these diseases based on mechanical removal of the biofilms.[10]

Due to modern life, people have changed their lifestyle and dietary habits. Dietary habits such as consumption of confectionary food items was seem to be increased in both developing and developed countries, which are potential to cause dental caries on tooth due to high sugar content in those food items. The sugar in the form of sucrose is the major sugar substitute being added in confectionary products. Sucrose is the main culprit for the occurrence of dental caries. Sucrose is metabolized by Streptococcus mutans and lactobacillus both are the microbial organism involved in the dental caries process.

The traditional use of plants as medicine provides the basis for indicating specific medical conditions. It is important to scientifically analyze these plants which have been used in traditional medicines.[11,12] Natural sources possess inherent medicinal properties, it includes all the parts of the trees, plant, vegetables, and fruits. Natural products are important source of new drugs which are having importance in modern medicine.[13] In recent years, due to the increased consumer desire in healthy living, there has been a dramatic increase in the consumption of natural foods and the use of dietary supplements.[14]

Natural products have been used in medicines for thousands of years and are promising sources for novel therapeutic agents, especially in oral diseases such as dental caries.[15] Grape seed extract (GSE) is a rich source of proanthocyanidin (PA), mainly composed of monomeric catechin and epicatechin, gallic acid and polymeric, and oligomeric procyanidins. It verified that GSE, composed mainly of PA, can positively affect the tooth structure, thus offering a new therapy for carious lesions.[16] Polyphenols are plant-derived substances which have antioxidant and anti-inflammatory properties.

Enamel demineralization leads to the dissolution of hydroxyapatite and diffusion of calcium and phosphate ions in the direction of its surface.[17] One of the main reasons for enamel demineralization is undoubtedly a decrease in pH below the critical point that leads to the dissolution of hydroxyapatite. Demineralization of tooth structure involves various factors such as calcium and phosphate ion in the saliva, pH of plaque fluid, and the dietary factors.

One such polyphenol is PA which is a bioflavonoid-containing benzene-pyran-phenolic acid molecular nucleus. PA, a naturally occurring plant metabolite is a natural antioxidant and free radical scavenger. The PA accelerates the conversion of soluble collagen to insoluble collagen during development and increases collagen synthesis.[18] PA s are present in grapefruit, apples, blueberries, cranberries, hazelnut, pistachios, etc. PAs present in some fruits prevent the damage caused by reactive oxygen species, inhibiting the glycosyltransferases and possible acid production by Streptococcus mutans. Studies have shown that agents such as glutaraldehyde and extracts rich in PA can be effective in improving the function of the dentin tissue, improving the mechanical stability and reducing collagen degradation. In a study done by Han B et al, proanthocyanidin (PA) obtained from grape seed extract was selected to fix biological tissues showed that a 6.5% GSE solution could interact with demineralized dentin and increase its modulus of elasticity and strength, due to the stability and increase in the amount of cross-linked collagen.[19]

GSE s inhibit the growth of anaerobic bacteria, such as Porphyromonas gingivalis and Fusobacterium nucleatum, associated with periodontal disease.[20] Berden-Russo et al. demonstrated that GSE, as a collagen cross-linker, increased the stiffness of demineralized dentin in a study on changes in stiffness of demineralized dentin following the application of collagen cross-linkers. Furthermore, GSE has been shown to improve the ultimate tensile strength of demineralized dentin.[21] It is reported that GSE effect on remineralization of artificial root caries improved the bond strength of dentin matrix.[22] GSE is rich in polyphenols to enhance the collagen matrix of dentin. Anti-bacterial effect of grape seed extract on periodontal pathogens such as F. nucleatum and P. gingivalis was investigated by Aurelie Furiga et al, which showed that GSE can increase the remineralization of carious enamel lesions, for it could be an effective natural agent for noninvasive dentistry.[21]
Aim
The aim of this systematic review was to analyze the existing literature on the remineralizing effect of GSE on artificial caries lesion against other remineralizing agent.

MATERIALS AND METHODS

Structured Question
Does GSE possess a remineralizing potential on caries-like lesion in human permanent and primary teeth.

PICO Analysis
- **Population/Problem**: Artificial caries lesion, Dental caries, Primary teeth, and permanent teeth.
- **Intervention**: GSE.
- **Comparison**: Fluoride, saline, glutaraldehyde, brain heart infusion solution, distilled water, naringin, and quercetin.
- **Outcome**: Lesion depth and mineral content of caries-like lesion after use of remineralizing (GSE) agent.

Inclusion Criteria
Criteria for considering studies for the review
The search was narrowed down manually by the reviewer according to the inclusion criteria of the present systematic review.
- An *in vitro* study
- Observational study
- Longitudinal study
- Prospective studies and retrospective cohorts
- Literature in other languages which can be translated by the reviewer was included in the study.

Exclusion Criteria
- Studies evaluating the effectiveness of other remineralizing agent combined with GSE were excluded from the study.

Search strategy
The Data Bases of PubMed, Cochrane, Science direct, Lilacs, and Google Scholar were searched for the period from January 2000 to October 2017. References of selected articles and relevant reviews were searched for any missed publications. Search strategy was inclusive of controlled vocabulary, free text terms, and mesh terms.

Data collection and analysis
The Data Bases of PubMed, Cochrane, Science direct, Lilacs, and Google Scholar were searched. Electronic search was carried out using the keywords. In the search flowchart, the number of articles obtained in Google Scholar (6), PubMed (35), Cochrane (0), Science direct (299), and Lilacs (0). Total published trials were 340. Among this after reading titles, the number of articles excluded from PubMed (25), Google Scholar (6), Science direct (297), and Lilacs (0). The number of articles identified after reading titles was 12. The number of articles excluded after reading abstract was 1. The number of articles excluded after reading full text was none. Hence, the number of full text articles assessed for eligibility for review was 11.

Data extraction
About 11 articles was reviewed. Based on the variable of interest enamel caries, dentin caries and root caries lesion was noted. The teeth utilized are primary teeth, permanent teeth (Third molar), permanent teeth (premolar), and bovine teeth. Total number of studies which have analysed remineralizing effect of grape seed extract on enamel caries was three, enamel and dentin caries was one, root caries was four and dentin caries was three. Sample size calculation was not done in none of the studies. The outcome variables are Vickers microhardness, Knoop microhardness (KHN), transverse microradiography, confocal laser scanning, scanning electron microscope, and polarized light microscope.

Meta-analysis
Due to the heterogeneity of full text eligibility articles, meta-analysis could not be done Tables 1-5 and Figures 1-5.

DISCUSSION
In present times, natural products have been the focus of much research, to be used as new therapeutic agents.[19] Potent caries prevention activity has been attributed to hydrophilic fractions of natural extracts whose major components are high molecular weight substances like PA, consisting of approximately 22 catechin units in their structure. GSE has recently been advocated for its beneficial antioxidant, antibacterial, and free radical scavenging properties. GSE has high PA content. PA from GSE has been demonstrated to increase collagen synthesis and accelerate the conversion of soluble collagen to insoluble collagen during development.[22] PA-treated collagen matrices are nontoxic and inhibit the enzymatic activity of glucosyltransferase F-ATPase and amylase.

In the obtained available literature KHN was utilized as an outcome measure in four studies. In a study done by Xie *et al.* and Silva *et al.*, the KHN value for treatment group containing GSE was 61.30 ± 12.17 (root caries), 347.21 ± 19.19 (enamel), and 46.16 ±

<table>
<thead>
<tr>
<th>Table 1: Variables of interest</th>
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<td>S. No</td>
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</tbody>
</table>

\[^{24}\] Xie Q, Bedran-Russo AK, Wu CD, Maheswari Elumalai, et al |


(Contd..)
<table>
<thead>
<tr>
<th>S. No</th>
<th>Article</th>
<th>Author and journal</th>
<th>Study design</th>
<th>Sample size</th>
<th>Group</th>
<th>Methodology</th>
<th>Parameters</th>
<th>Statistical analysis</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Effectiveness of plant-derived proanthocyanidins on demineralization on enamel and dentin under artificial cariogenic challenge</td>
<td>Silva AP, Gonçalves RS, Borges AF, Bedran-Russo AK, Shinohara MS.</td>
<td>In vitro study</td>
<td>Sample size -90</td>
<td>Three groups: Group 1: GC (control), Group 2: GSE (grape seed extract), Group 3: fluoride-1000 ppm.</td>
<td>Teeth utilized: Bovine incisors. Ninety bovine incisors were obtained. The enamel and root specimens. The 6.5% grape seed extract was prepared. The enamel and root specimens were placed in 50 ml of treatment solution (CG, GF, GSE) for 10 min, then in 50 ml of demineralizing solution for 30 min and finally in 50 ml of buffered solution for 10 min.</td>
<td>Microhardness polarized light microscope.</td>
<td>ANOVA to compare three groups.</td>
<td>Enamel: Enamel surface microhardness (KHN) of treatment groups: Group 1: 348.34±18.24 Group 2: 247.21±19.19 Group 3: 351.76±19.81 Dentin: (1) Dentin surface hardness (KHN) groups before and after treatment with fluorides and grape seed extract: Group 1: 44.78±5.12 Group 2: 46.16±4.15 Group 3: 47.16±2.81</td>
</tr>
<tr>
<td>4</td>
<td>Grape Seed Extract as a Potential Remineralizing Agent: A comparative in vitro study</td>
<td>Benjamin S, Sharma R, Thomas SS, Nainan MT. Grape seed extract as a potential remineralizing agent: A comparative in vitro study.</td>
<td>In vitro study</td>
<td>Sample size-20. Sample size calculation was not mentioned.</td>
<td>Group A: 6.5% (w/v) GSE solution prepared in phosphate buffer 0.025M KH2PO4, 0.025M K2HPO4 Group B: 0.05% CaGP with 0.17% sodium monofluorophosphate Group C: 0.5% CaGP Group D: Deionized water (dH2O)</td>
<td>Twenty sound human teeth extracted for periodontal reasons were obtained. Root fragments were placed in a demineralizing solution (pH 4.6) for 96 h at 37°C to create artificial carious lesions. All the samples were pH cycled in the following order. Treatment solutions (10 min), acidic buffer at pH 5.0 (30 min) and neutral buffer at pH 7.0 (10 min) for 8 days consisting of 6 cycles per day.</td>
<td>Confocal laser scanning microscope</td>
<td>ANOVA to compare three groups.</td>
<td>(1) ROD of artificial caries lesion treated by GSE, 0.05% CaGP, sodium monofluorophosphate, 0.5% CaGP and deionized water: Group A: 78.37±12.43 Group B: 45.32±22.44 Group C: 41.52±20.74 Group D: 44.08±23.42</td>
</tr>
<tr>
<td>5</td>
<td>Remineralization of artificial caries in primary teeth by grape seed extract: An in vitro study</td>
<td>Mirkarimi M, Eskandarion S, Bargtizan M, Delazar A, Kharazifard MJ. not mentioned.</td>
<td>In vitro study</td>
<td>Sample size-17. Sample size calculation was not mentioned.</td>
<td>Groups: Group 1: Grape seed extract solution in phosphate buffer, Group 2: Distilled water.</td>
<td>The morphology of enamel surfaces was evaluated under a scanning electron microscope.</td>
<td>Independent t-test</td>
<td>(1) Enamel Knoop microhardness values of the case and control group: Group 1: 358.66±83.42 Standard error mean -</td>
<td></td>
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</table>

(Contd..)
6 The preventive effect of grape seed extract on artificial enamel caries progression in a microbial biofilm-induced caries model.[29]

In vitro study
Sample size-30
Sample size calculation not mentioned.

Six treatment groups (n=12): Group 1: inoculated brain heart infusion with 1% sucrose (BHIS) GSE Group 2: 1 mg/mL GSE Group 3: 2 mg/mL GSE Group 4: 3 mg/mL GSE Group 5: 10 ppm fluoride as NaF Group 6: uninoculated BHIS.

Teeth utilized: Bovine teeth (enamel blocks).

LD and ROD were determined by polarized light microscopy and confocal laser scanning microscopy

ANOVA to compare more than three groups

7 The role of grape seed extract in the remineralization of demineralized dentine: micromorphological and physical analyses.[30]
Tang CF, Fang M, Liu RR, Dou Q, Chai ZG, Xiao YH, et al. The role of grape seed extract in the remineralization of demineralized dentine: Micromorphological and physical analyses. Arch Oral

In vitro study
Sample size-25. Sample size calculation not mentioned.

One group: GSE preconditioner 15% concentration

Teeth utilized: Human third molars. (Dentin block).

Vickers Microhardness was assessed.

One sample t-test.

(Contd...)
### Table 2: Data extraction table (Continued)

<table>
<thead>
<tr>
<th>S. No</th>
<th>Article Title</th>
<th>Author and journal</th>
<th>Study design</th>
<th>Sample size</th>
<th>Group</th>
<th>Methodology</th>
<th>Parameters</th>
<th>Statistical analysis</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>Site-specific properties of carious dentin matrices biomodified with collagen cross-linkers.</td>
<td>Bedran-Russo AK, Karol S, Pasley DH, Viana G. Site-specific properties of carious dentin matrices biomodified with collagen cross-linkers. Am J Dent 2013;26:244-8</td>
<td><em>In vitro</em> study</td>
<td>Sample size-15. Sample size calculation not mentioned</td>
<td>Groups Group 1: Demineralized dentin specimen</td>
<td>Teeth utilized: Human molars (dentin blocks). (1) superficial demineralization (using 32% phosphoric acid gel (Uni- Etched) for 60 s ($n=15$), and (2) teeth specimen immersed in GSE solution for 1 h treatment with cross-linking agents.</td>
<td>Elastic modulus values are measured</td>
<td>ANOVA to compare three groups</td>
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<td><em>Continued</em></td>
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### Table 2: Data extraction table (Continued)

<table>
<thead>
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<th>Article</th>
<th>Author and journal</th>
<th>Study design</th>
<th>Sample size</th>
<th>Group</th>
<th>Methodology</th>
<th>Parameters</th>
<th>Statistical analysis</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>Effect of flavonoids on remineralization of artificial root caries.</td>
<td>Epasinghe DJ, Yiu C, Burrow MF.</td>
<td>In vitro study</td>
<td>Sample size-40. Sample size calculation not mentioned</td>
<td>Groups: (1) 6.5% proanthocyanidin; (2) 6.5% naringin; (3) 6.5% quercetin; (4) 1000 ppm Fluoride; (5) deionized water (control).</td>
<td>Teeth utilized: Human third molars (root blocks). The root fragments were placed in a remineralizing solution for 96 h at 37°C. All specimens were pH-cycled through the treatment solutions (10 min), acidic buffer 30 min for and neutral buffer. Six remineralization cycles were performed each day and continued for 8 days.</td>
<td>The remineralization effects were evaluated using Knoop microhardness, transverse microradiography (lesion depth and mineral loss) and confocal laser scanning microscopy.</td>
<td>Microhardness at different lesion depths was analyzed with two-way ANOVA and Tukey’s test, while lesion depths and mineral loss were analyzed with one-way ANOVA and Tukey’s test.</td>
<td>(1) Cross-sectional microhardness (KHN) value; Group 1: 44.8±7.38 Group 2: 73.6±12.02 Group 3: 66.2±16.24 Group 4: 62.9±19.94 (2) Effect of remineralization treatments Group 1: Mineral loss - 42.1±70.42 Lesion depth - 164.3±40.63 Group 2: Lesion depth - 62.5±15.84 Group 3: Lesion depth - 101.5±16.58 Group 4: Lesion depth - 90.9±27.09 The percentage SMHR was obtained for GSE, NaF, GSE+NaF, and DDW group (40.87±9.24, 44.60±12.48, 48.54±9.27 and 15.98±8.33)</td>
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<tr>
<td>11</td>
<td>In vitro remineralization effect of grape seed extract on artificial dentin caries.</td>
<td>Shi JH, Li H, Wang YN.</td>
<td>In vitro study</td>
<td>Sample size - 60. Sample size calculation not mentioned.</td>
<td>Four groups: Group 1: 10% GSE; Group 2: 1 mg/L NaF; Group 3: 10% GSE+1 mg/L NaF; Group 4: distilled water (DDW, as control).</td>
<td>Sixty human dentine fragments were stored in a remineralizing solution for 24 h at 37°C to induce artificial dentin caries lesions. The remineralized samples were pH-cycled for 8 days and surface dentine microhardness was assessed before and after pH-cycling regimen.</td>
<td>The percentage SMHR was calculated. The samples were subsequently analyzed with SEM and EDXS.</td>
<td>The date was analyzed using ANOVA and Fisher’s tests.</td>
<td>The percentage SMHR was obtained for GSE, NaF, GSE+NaF, and DDW group (40.87±9.24, 44.60±12.48, 48.54±9.27 and 15.98±8.33)</td>
</tr>
</tbody>
</table>
In a study done by Mirkarimi et al. and Epasinghe et al., the KHN value for treatment group containing GSE was 358.66 ± 83.42 (enamel) and 44.85 ± 7.38 (root caries). The KHN value in enamel sections is found to be similar.

The Vickers microhardness was assessed in three studies, in a study done by Rubel et al., the VHN value was 357.56 ± 5.53 (enamel). Whereas in a study done by Tang et al. and by Shi et al., the VHN value was 42.23 ± 3.31 (dentin) and 40.87 ± 9.92 (dentin). The VHN values of the above two studies were found to be similar.
Table 4: Risk of bias - minor criteria

<table>
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<tr>
<th>S. No</th>
<th>Study</th>
<th>Sample justified</th>
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<th>I/ E criteria</th>
<th>Method of error</th>
<th>Risk of bias</th>
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<tr>
<td>1</td>
<td>Xie et al., 2008</td>
<td>No</td>
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<td>2</td>
<td>Rubel et al., 2016</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
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<td>3</td>
<td>Silva et al., 2015</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
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<td>4</td>
<td>Benjamin et al., 2012</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
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<td>5</td>
<td>Mirkarimi et al., 2013</td>
<td>NA</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
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<td>6</td>
<td>Zhao et al., 2014</td>
<td>NA</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Low</td>
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<tr>
<td>7</td>
<td>Tang et al., 2013</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Low</td>
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<tr>
<td>8</td>
<td>Pavan et al., 2011</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
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<td>9</td>
<td>Ana et al., 2013</td>
<td>Yes</td>
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<td>No</td>
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<td>10</td>
<td>Epasinghe et al., 2016</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
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<td>11</td>
<td>Shi et al., 2015</td>
<td>No</td>
<td>Yes</td>
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Table 5: Data on teeth utilized and the region of dental caries focused on selected articles

<table>
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<tr>
<th>Teeth specimens/blocks</th>
<th>Total no of studies</th>
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<th>Total no of studies</th>
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<tr>
<td>Enamel caries</td>
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<td>Primary teeth</td>
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<tr>
<td>Dentin caries</td>
<td>3 studies</td>
<td>Permanent teeth (Third molar)</td>
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<tr>
<td>Root caries</td>
<td>4 studies</td>
<td>Permanent teeth (premolar)</td>
<td>3</td>
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<tr>
<td>Enamel and dentin caries</td>
<td>1 study</td>
<td>Bovine teeth</td>
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Figure 1: Screenshot of PubMed search
In two studies, transverse microradiography was utilized as an outcome measure, in a study done by Epasinghe et al. and Pavan et al., the lesion depth value obtained was 164.36 ± 40.63 (root block) and 109 ± 20 (root block). The study population and outcome measure are similar; however, the results are dissimilar.

Confocal laser scanning microscope was assessed for relative optical density in two studies. In a study done by Xie et al and Benjamin et al, the relative optical density values (ROD) obtained for root caries lesion were 81.86±28.84 and 78.37±12.43. The results obtained were similar. Lesion depth was assessed using confocal laser microscope in two studies. In a study done by Zhao et al. and Epasinghe et al., the values were 11.92 ± 11.39 (enamel) and 164.36 ± 40.63 (root block). The results obtained are dissimilar due to the difference in the region of teeth specimen involved in the studies.

In three studies, polarized light microscope was utilized as outcome measure. In a study done by Zhao et al., the lesion depth obtained was 122.86 ± 13.41 μm. In a study done by Anapaula et al., in the analysis of polarized light microscopy, in all specimens, as well as the enamel, also had negative birefringence showing areas of demineralization more pronounced in GSE than fluoride group. In one study done by Xie et al., mineral precipitation band depth in the GSE treated group was 40 μm.

Scanning electron microscope was a qualitative analysis, utilized as outcome measure in three studies. In all three studies, scanning electron microscopy of the treatment groups revealed greater mineral deposition on dentinal tubules. Elastic modulus values are measured in a study done by Ana et al., it revealed that the highest increase of endoplasmic reticulum (ER) was observed for GSE treatment about 0.02 ± 0.01.

GSE positively affects the remineralization process. Proposed mechanism of its action is that it contributes to mineral deposition on the superficial layer of the lesion. In vitro demineralization and remineralization, studies are important research tools which provide vital information regarding clinical outcome. The dynamic and fluctuating variation in natural caries process is well-simulated by the in vitro pH cycling model.[17]

In three studies, permanent premolars were used. Permanent third molars are utilized in five studies, primary incisor was used in one study, and bovine incisor was used in two studies. Remineralization of root caries was assessed in three studies. Both enamel and dentin specimens are included in one study. In the polarized light microscopic analysis, fluoride had a greater preventive potential on enamel than the GSE in one study.
In three studies on scanning electron microscope, the reaction products of GSE are seen as amorphous clumps. Spherical, globular agglomerates were observed on the surface of the enamel. Samples treated with GSE had significantly higher microhardness values compared with the control group. The outcome analysis in the included articles was microhardness test assessed in seven studies, whereas lesion depth analyzed using polarized light microscope was done in three studies. Mineral content was assessed using confocal laser scanning microscope in two studies.

In evaluating the compiled literature, it was revealed that GSE is a promising source for its remineralizing effect on dental caries. It is stated that GSE might contribute to mineral deposition on the superficial layer of the lesion by the formation of insoluble complexes when mixed with bufferic phosphate solution; in addition, GSE might interact with proteins to induce cross-links by four different mechanisms: Covalent interaction, ionic interaction, hydrogen bonding interaction, and hydrophobic interaction.[19]

According to Zhao et al. (2014) and Mikarimi et al. (2013), showed that Grape seed extract has a remineralizing effect on carious enamel lesions and so, it could be used as an effective natural agent for noninvasive dentistry.

**Interpretation of Results**

In this review, 11 articles are included, which assessed the remineralizing effect of GSE on artificial caries lesion in enamel and dentin. These are included articles (Xie et al., Rubel et al., Silva et al., Benjamin et al., and Mikarimi et al.). The remineralization effect can be evaluated only by in vitro study models. In the included studies eight studies utilized permanent tooth, bovine teeth were used in two studies, and primary teeth were utilized in one study.

The outcome analysis in the included articles is microhardness test assessed in seven studies, whereas lesion depth analyzed using polarized light microscope was done in four studies. Mineral content was assessed using confocal laser scanning microscope in four studies. The micromorphological features of enamel and dentin were assessed by scanning electron microscope in three studies. One included study used energy dispersive X-ray spectrometry to analyze the Ca: P ratio in tooth specimens after demineralization and remineralization. One study assesses mineral content by transverse microradiography.

In all the included studies, GSE was found to show a higher remineralizing potential than compared with control groups. Fluoride was the comparative group in six studies where the concentration used was 1000 ppm and 10 ppm. In one study saline was used as control group, deionized (distilled water) was used as a control group in four studies. One study they compared with naringin and quercetin. One study glutaraldehyde was used as a comparative group. Brain heart infusion solution was utilized as comparative group in one study.

Out of the six studies, only three studies had a low risk of bias and three studies had high risk of bias. High risk of bias in three studies due to unclear information on the randomization sequence generation or allocation or blinding which are important to overrule the selection bias, performance bias, and detection bias, respectively.

**Limitations**

The present systematic review limits the studies included are to be in English language only. This limited the number of studies assessing the aim of this review. This systematic review also considers only the published data for result interpretation. The unpublished and the raw data of the studies have not been included for interpretation. Due to the heterogeneous nature of the various assessment techniques and interventions included in this review, the pooling of data was not possible.

**Inference**

**Implications for practice**

GSE with its remineralizing potential on caries lesion and antimicrobial property on oral microflora, it can be used as a cost-effective caries preventive measure.

**Implications for research**

Further studies must be performed with standard research methodology protocols to assess the effectiveness of GSE containing formulations on dental caries. Trials must be conducted to assess the effectiveness of GSE as a restorative material.

**Summary**

The aim of the systematic review was to systematically evaluate the effectiveness of GSE on dental caries.

An electronic search was carried out on PUBMED, COCHRANE, SCIENCE DIRECT, and LILACS, database for the articles which could be used for evaluating the effectiveness of GSE containing formulations on dental caries. Article search was narrowed down based on the pre-stated inclusion and exclusion criteria. A total of 11 articles were included in this systematic review for detailed evaluation.

Remineralizing effect of GSE was assessed on enamel and root caries. GSE showed a significant difference compared with other groups with an increase in microhardness value and increased in Ca: P ratio. The lesion depth was decreased after immersion of the teeth specimens in GSE solution.

Based on the results of this systematic review, we can conclude that GSE is effective against prevention...
and control of dental caries. A properly designed randomized controlled study must be performed to give concrete evidence on the clinical performance of GSE as anticariogenic and trials must be conducted to assess the effectiveness of GSE as a restorative agent.

CONCLUSION

With the available evidence, in vitro studies are included. In five studies, samples treated with GSE and fluoride were significantly higher in microhardness values when compared to the control group. A significantly wider mineral precipitation band was observed in the GSE treated group when compared to those of fluoride and control groups in three studies. Relative optical density values of the GSE group were higher in two studies. In the polarized light microscopic analysis, fluoride had a greater preventive potential on enamel than the GSE in one study. GSE could be a better alternative to fluoride, where is known to adversely affect dental and systemic health by causing dental fluorosis and skeletal fluorosis. In the future, GSE with its antimicrobial, anti-inflammatory, and anti-caries effect can be utilized in preventive and restorative materials to preserve the dental health.

REFERENCES


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