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Evaluation of the Antibacterial Effect of Aloe Vera versus Diode Laser in Management of Deep Carious Lesions (In Vivo Study)

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ORIGINAL STUDY

Evaluation of the Antibacterial Effect of Aloe Vera Versus Diode Laser in Management of Deep Carious Lesions (In Vivo Study)

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Abstract

Purpose: This clinical study was designed to evaluate the antibacterial effect of Aloe Vera versus Diode laser in the management of deep carious lesions. **Material and methods:** patients with deep occlusal caries in molars were enrolled in the study. Patients had a total of forty-five permanent molars with deep occlusal carious lesions were used in this study. The teeth were randomly assigned after stepwise caries removal into three groups ($n = 15$), according to the applied treatment agent (A): Group (A1): no treatment was applied (control), Group (A2): the teeth were treated with Aloe Vera, Group (A3): the teeth were treated with Diode laser. After cavity preparation and selective caries removal, carious dentin samples were collected for bacterial count then the treatment agents were applied. Glass ionomer was placed in the cavities till the second visit. The second visit of Stepwise caries removal was employed after three months. The samples were collected at baseline and after three months follow-up. These samples were subjected to microbiological evaluation, for Streptococcus mutans counts on mitis salivarius (MS) agar. **Results:** The results showed that all groups showed a significant reduction in bacterial count when compared to baseline However, inter group comparison revealed that Aloe Vera and Diode laser groups showed a significantly greater reduction in *S. mutans* than the control group. **Conclusion:** both Aloe Vera and Diode laser are effective antimicrobial therapy tools in deep caries management. They demonstrated successful clinical outcome when used with stepwise caries removal.

Keywords: Aloe vera, Diode laser, Microbiological assessment, Stepwise caries removal

1. Introduction

The management of deep carious lesions presents a significant challenge, as during caries removal there are chances for pulp exposure inducing severe inflammation that may cause pulp necrosis [1]. Deep caries lesion treatment options include complete caries removal, partial caries removal, and stepwise caries excavation. Complete caries removal is the removal of soft dentin until the hard dentin, which is considered overtreatment and is no longer recommended. Partial caries removal is the removal of peripheral soft caries while leaving residual soft carious dentin in the pulpal floor under a permanent restoration. The stepwise excavation technique refers to the removal of caries in two separate appointments separated by months to reduce pulpal exposure and allow reparative dentin formation. The dentist removes the soft dentin and places a temporary restoration for 6–12 months during the first appointment of the stepwise excavation technique. Complete removal will perform in the second visit [2]. Clinical trials with long-term follow up demonstrated that cariogenic microorganisms persist under the restorations and have an important role in the development of secondary caries [3,4]. It was also reported that numerous bacteria might remain in the dentinal tubules after cariostatic-sealed restorations [5,6]. The use of an antibacterial agent was found to be very important to reduce the residual bacteria before restoring the cavity [7].

'Phytotherapeutics' and 'ethnopharmacology' are terms used to describe the growing trend of using natural medicines as part of medical and dental

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therapies [8]. *Aloe barbadensis* Mill (A. Vera), which is found in nature, is proving to be an effective antibacterial agent in a variety of dental applications. A. Vera is a cactus-like succulent herb with green daggers, curved meaty, spiky, and marginated leaves loaded with a transparent viscous gel that possesses significant anti-inflammatory, antibacterial, antifungal, and antiviral properties [9,10].

In the mid-1990s, dental laser systems made a breakthrough. Diode Laser systems quickly established themselves as small, affordable, and adaptable additions to the dentist's toolkit [11,12]. The Diode Laser can be used for a variety of dental operations, mostly soft tissue procedures like pulpotomy, frenectomy, and gingivectomy [11], as well as some hard tissue procedures like root canal disinfection and tooth whitening [12]. When compared to chemical disinfectants, the use of a Diode laser in root canal disinfection demonstrated encouraging results in efficiently reducing the microbial burden in infected root canals. Yet there is no enough availability of reports regarding the cavity disinfection, so it is worth considering testing the effect of using Diode laser in cavity disinfection.

Therefore, the purpose of this study was to investigate the antibacterial effect of Aloe Vera and Diode laser on deep carious dentin in vivo after minimally invasive excavation of dental caries.

2. Material and methods

2.1. Trial design and sample size calculation

The research was planned as an interventional, randomized, prospective, single-center controlled clinical trial. This trial was conducted at restorative dental clinic (RDC) at the Faculty of Dental Medicine for Girls, Al-Azhar University, Cairo; Egypt; from February 2021 to October 2021. Recruitment of participants was done between February 2021 and April 2021. According to Prabhakar et al., 2015 [13], by adopting an alpha level of (0.05) a beta of (0.2) i.e. power = 80% and an effect size (f) of (0.541); the predicted sample size (n) was a total of (36) cases (i.e. 12 cases per group). The total sample size was increased by (25%) to account for possible dropouts to be (45) samples i.e. (15) for each group. Sample size calculation was performed using G*Power version 3.1.9.7 [14].

2.2. Eligibility criteria

2.2.1. Inclusion criteria

The age range was from 20 to 40 years of both genders. Patients were presented with at least one permanent molar with deep carious occlusal lesion (ICDAS code 6) with no signs or symptoms of irreversible pulpitis. The carious lesion extended into the inner 1/3 of dentin as shown by bitewing radiograph [15].

2.2.2. Exclusion criteria

Patients with poor oral hygiene or evidence of rampant caries or any chronic debilitating disease were excluded. Also previously restored teeth, cracked enamel or pregnancy/breastfeeding were not included. Irreversible pulpitis and Internal or external resorption cases were also excluded.

2.3. Trial registration and ethical approval

The trial was registered online in clinicaltrials.gov database. Protocol Registration and Results System (PRS) with identification number **(ID: NCT05327049)** was given. Data regarding the study description, conditions, study design, arms and interventions, outcome measures, eligibility and contacts/locations were provided. The research protocol, patient information sheet as well as consent form were analyzed and approved by the Ethical Research Committee, Faculty of Dental Medicine For Girls, AI-Azhar University, Cairo; Egypt **(approval code: REC-OP-22–01).**

2.4. Patient risk assessment

ADA caries risk assessment (Ages >6) [16] was used to formulate an individualized caries risk for developing future caries lesion based on patient past caries experience and clinical examination for current caries lesions. The assigned patients were categorized as medium or high-risk.

2.5. Grouping of patients and informed consent

`Each patient presented at least one permanent molar with deep occlusal lesion. A total of 45 human teeth (upper and lower, first and second molars) were selected for this study. Patient's teeth were classified into three groups (n = 15) according to the applied treatment agent (A): Group (A1):, Control group with no treatment applied, Group (A2): treated with Aloe Vera, and Group (A3): treated with Diode laser. Microbiological assessment was done at different time intervals (B). The baseline sample was obtained after stepwise excavation and before application of treatment agent (B0). Then after 3 months follow up period, the second sample was taken (B1). All procedures, purpose, risks, benefits, number of visits and expected duration of the clinical trial were explained to participants in colloquial language. A chart was made for

each patient along with a written consent for willingness to participate in the study.

2.6. Randomization, blinding, allocation sequences

An excel sheet with random list of numbers was created, in which each randomly assigned participant in this list occupied a sequence no (ID) from '1 to 15' to be assigned to one of the three groups either no treatment control group, Aloe Vera or Diode laser. The study was double blinded trial where the outcome assessor and patients were blinded to treatment.

2.7. Clinical procedures

2.7.1. First visit

2.7.1.1. Patient preparation. Patient's preoperative data was recorded in predesigned patient's chart. Each patient first received full mouth scaling and polishing to clean the teeth surfaces. Patients were given proper oral hygiene instructions.

2.7.1.2. Cavities preparation. Forty-five cavities were prepared in both upper and lower first and second molars. Preoperative radiographs were taken using digital X-ray machine (Kodac 2200, France) with plate (digital sensor size 2 Image plate, Durr Dental, Germany). Local anesthesia was administrated by infiltration technique and nerve block technique for upper and lower teeth, respectively. Class I cavities were prepared after heavy sheet rubber dam isolation. Caries was completely removed from the walls and selective caries excavation technique was used on the pulpal floor using a double ended spoon excavator until leathery dentin was reached. All the procedures were done by the main researcher.

2.7.1.3. Sampling procedure. After dryness carious dentin samples were obtained from the center of the floor of the carious cavity with a single, full stroke by using sterile spoon excavator for microbiological analysis (sample 1). Photographs were taken for the sampling area in order to identify the same area in the next sample. Carious dentin samples were immediately placed in a sterile vial containing saline and transported to the microbiology lab for processing within maximum 2 h [17].

2.7.1.4. Application of treatment agents. After cavity preparation, each group received treatment according to the following instructions (Fig. 1):

Group A1 control group was not treated by any material. Group A2 was treated by Aloe Vera paste which was prepared by mixing Aloe Vera powder by distilled water until a thick paste is formed. The paste was applied to the prepared floor by condenser to cover the remaining carious lesion. Group A3 was treated by Diode laser using the following parameters: Irradiation time was 15 s and repeated three times for 15 s interval with contact, pulsed mode and the output power was adjusted at 1.30 W. The laser light was transferred through a 400 μm flexible fiber optic tip by a special hand piece. According to manufactures instructions the fiber optic tip was inserted inside the cavity with a spiral continuous movement clockwise from the top to the floor and anti-clockwise in the reverse direction [18].

2.8. Restorative procedures

2.8.1. Temporary restoration

2.8.1.1. First treatment visit. The cavities were sealed by using highly viscous GIC (Riva self-cure) restoration material. A carver was used to remove excess material and allowed to set for 6 min High-speed finishing stones were used to finish the restoration; Riva coat was applied to the surface and cured with LED intensity of 1200 mW/cm2 for 20 s. Patients were recalled for a second dentin sample after 3 months (B1).

2.8.1.2. Second visit. After three months, patients were recalled for their second visit. Teeth were re-examined clinically and radiographically for any symptoms of failure (irreversible pulpitis, pulp necrosis or periapical pathosis). No patients presented signs of failure. The temporary glass ionomer restoration was removed and a second dentin sample was taken from the same place of the base line sample [19]. removal of the residual carious tissue was performed using a sharp excavator. The excavation end point was the detection of firm dentine at the pulpal floor using a sharp dental explorer [20]. A light cured glass ionomer base was put on the pulpal floor and the final composite restoration was done.

2.8.2. Microbiological assessment

Streptococcus mutans count (colony forming units/ml: CFU/ml) was assessed at baseline and after 3 months. Bacterial count was assessed according to Scaffaro R et al., 2013 [21] using Selective Mitis Salivaris Agar Base (MSB). Dentin samples were transported in a cool ice box to maintain the viability of the bacteria immediately after collection. 0.5 ml of the sample was diluted in a 10-fold saline solution. A sterile inoculum spreader was used to evenly distribute the inoculum over the surface of

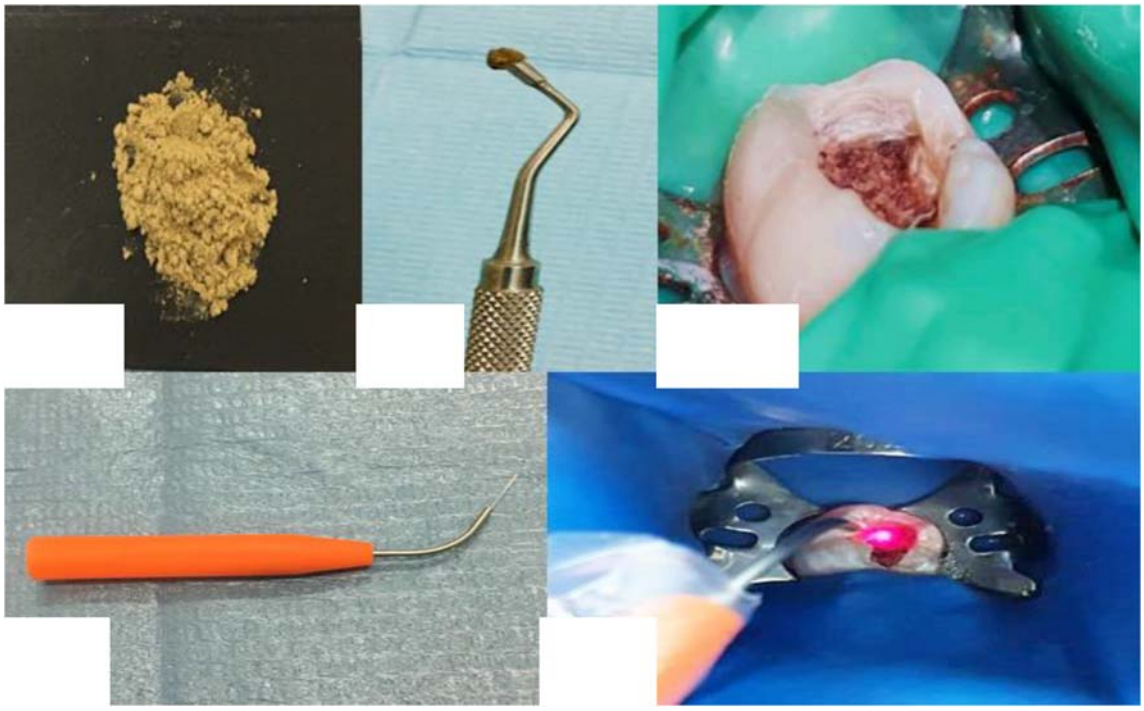

Fig. 1. Application of treatment agents in each group A: Aloe Vera powder from B: Aloe Vera paste. C: after application of Aloe Vera paste on the pulpal floor. D: laser tip and E: application of Diode laser n the pulpal floor.

the agar media. All plates were incubated for 24 h at 35° ± 2 °C. After incubation, the colonies were identified by morphological bacterial characteristics. Colony forming units (CFU) were counted using manual colony counter [22].

2.9. Statistical analysis

Numerical data were explored by using Shapiro–Wilk test, Bacterial count data showed non-parametric distribution and extreme skewness. Log transformation of the data was carried out to correct for the skewness. Intergroup comparisons were analyzed using one-way ANOVA followed by Tukey's post hoc test, while intragroup comparisons were analyzed using repeated measures ANOVA followed by Bonferroni post Hoc test. The significance level was set at P£0.05 for all tests. Statistical analysis was performed with R statistical analysis software version 4.1.2 for Windows.

3. Results

The results revealed that pretreatment values of bacterial count (B0) for the three groups (control, Diode laser, Aloe Vera) were significantly higher than those after three months (B1). Percentage change (%) for different groups revealed that the highest percent change was found for Laser (A3), followed by Aloe Vera (A2) with nonsignificant difference, while the Control group (A1) showed a significantly lower percent change value compared to the two groups ($P < 0.001$).

3.1. Antimicrobial assessment results (Tables 1 and 2)

Table 1. Mean and Standard deviation (SD) values for log bacterial count at base line and after 3 months within each group.

Group	Log bacterial count (Mean ± SD)		t value	P value
	Pre-count (B0)	After 3 months (B1)		
Control (A1)	9.51 ± 1.39	7.29 ± 0.74	7.50	<0.001[a]
Aloe Vera (A2)	8.69 ± 1.95	3.13 ± 0.47	10.27	<0.001[a]
Laser (A3)	10.34 ± 1.54	3.84 ± 0.81	20.87	<0.001[a]

[a] significant ($P \leq 0.05$) ns; non-significant ($P > 0.05$).

Table 2. Mean and standard deviation (SD) values of percent change of bacterial count for different groups (ΔT).

Bacterial count percentage change (%) (Mean ± SD)				P value
Control (A1)	Aloe Vera (A2)	Laser (A3)	f value	
90.31 ± 6.70[B]	99.57 ± 0.43[A]	99.79 ± 0.16[A]	10.16	0.002[a]

Different superscript letters indicate a statistically significant difference within the same row.

[a] significant (P£0.05).

4. Discussion

This study was designed to evaluate the antibacterial effect of Aloe Vera and Diode laser on deep carious dentin. An alternative technique to complete caries dentine removal is the stepwise excavation technique. It was used in this study as it allows the defense reaction of the pulp to avoid the costly endodontic treatment. If the procedure is done in sterile settings and followed up with an adhesive restoration, this intervention might be very promising [23]. Because bacteria remain in the cavity even when excavation is carried to firm tissue, many restorations fail as a result of secondary caries development [24]. Traditional restorative dentistry was founded on the belief that bacterial infection of demineralized dentine required operational intervention. The more modern notions of least invasive dentistry, on the other hand, emphasize the importance of creating a favorable environment for halting caries progression with bare minimum operative intervention [25].

'Phytotherapeutics' and 'ethnopharmacology' are terms used to describe the growing trend of using natural medicines as part of medical and dental therapies. *A. barbadensis* Mill (A. Vera), which is found in nature, is proving to be an effective antibacterial agent in a variety of dental applications (as a tooth gel, Mouthwash, denture adhesive, root canal irrigation). Aloe Vera is a succulent, tender plant containing high water content (99–99.5%). Solid contents range from 0.5 to 1% and consist of a variety of active components i.e. fat and water soluble minerals, vitamins, simple/complex polysaccharides, organic acids, enzymes and phenolic compounds [9,10].

Different mechanisms, including thermal and photo disruptive effects, allow the Diode laser to generate an antibacterial effect on carious tissue with little thermal disruption to the tooth, which are regarded the main reasons for the laser to eliminate bacteria [18]. The use of a Diode laser in root canal disinfection has shown to be efficient in lowering microbial load in diseased root canals.

Class I carious lesions were selected to decrease the probability of leakage and communication with the external environment, which is important especially in the in vivo study where teeth are embedded in mineral-containing natural saliva. Carious dentin samples were taken after removing the superficial dentin layer to ensure that the sample contained the largest amount of organisms originally situated in the body of the lesion, not in the dental plaque [26].

All the tested materials were restored with self-cure highly viscous glass ionomer which offer a good seal which is critical factor for success treatment of deep carious lesion [27]. Glass ionomer also offer potential advantages over other restorative materials for cavity sealing following partial caries removal. For instance, GIC chemically bonds well to tooth structure and releases of fluoride, which may aid in the remineralization of carious lesions [28].

S. mutans is a gram positive cocci facultative anaerobic bacterium commonly found in the human oral cavity and it is a significant contributor to tooth decay. For isolation and identification of S. mutans, the culture medium Mitis salivaris bacitracin agar was used as it allowed the selective recovery of S. mutans while ensuring maximum suppression of other oral streptococci [29].

The results of this clinical trial revealed that treatment of active carious dentin with Diode laser and Aloe Vera showed a significant decrease in the bacterial count when compared to base line total bacterial count. When comparing the effect of different materials on bacterial count after three months period, the results showed that there was a significant difference in the percent change of bacterial count. The highest value of percent change was found in the Laser treated group, followed by Aloe Vera treated group with non-significant difference between them, while the lowest value was found in the control group with a significant difference from other groups.

The improvement in antibacterial activity of Aloe Vera group can be attributed to a number of pharmacologically active compounds, including anthraquinones, aloin, Aloe-emodin, Aloetic acid, anthracene, Aloe mannan, Aloeride, antranol, chrysophanic acid, resistanol, and saponin [30,31]. Aloin and Aloe emodin have potent antibacterial and antiviral properties [32]. They can stop bacterial cells from making protein, which explains their antibacterial properties [33]. Moreover, some chemicals in A. Vera gel as anthraquinones and saponin, exhibit direct antibacterial activity, whereas other compounds, such as acemannan, are thought to have indirect bactericidal effect by stimulating phagocytosis [34]. This was in agreement with a study investigated the role of Aloe Vera and Propolis as cavity disinfecting agents after minimally invasive dental caries hand excavation. When compared to the untreated control group, and the results demonstrated a substantial drop in bacterial counts [13]. It was also suggested that at optimum concentration, Aloe Vera gel might be used as an antibacterial to prevent tooth cavities and periodontal disease [35].

Similarly, the Diode laser group showed a significant decrease in total bacterial count after three months. This could be due to a variety of mechanisms relating to the antibacterial impact of Diode

lasers, such as Thermal and photo disruptive effects that thought to be the main causes for the laser's ability to kill bacteria [36]. The disintegration of cell wall integrity, as well as possible protein denaturation, are all examples of lethal damage. Cell growth and subsequent cell lysis will be halted if the cell wall is damaged. The cellular protein, on the other hand, is extremely sensitive to heat fluctuations [37]. Another cause could be due to the likelihood of occluding the dentinal tubules, which occurs when dentin melts, trapping invading bacteria and reducing the amount of dentinal fluid available for nourishment [38]. Another theory for laser's antibacterial effect is that it generates intra tubular water expansion and water vapor collapse as deep as possible, which is capable of producing an acoustic wave loud enough to discredit intratubular bacteria [39]. This was in accordance with the findings of other studies [40,41] that evaluated the antibacterial effect of Diode laser which revealed a significant antibacterial activity against strept mutans bacteria.

Regarding the control group (dentin restored only with conventional glass ionomer without treatment), it exhibited significant decrease in bacterial count but the least among the other groups. It may be directly attributed to the antibacterial effect of fluorine from the glass ionomer cement. The fluoride ion fits well in the structure of a hydroxyapatite crystal, actually better than the hydroxyl group and this could result in lower solubility of fluoridated apatite when compared with fluoride-free apatite. As a result, it slows down demineralization by precipitating calcium and phosphate ions that dissolve and by enhancing the precipitation of fluoridated apatite above the critical pH [42]. Also, when cavities were sealed efficiently by glass ionomer caries was arrested and also mineral deposition occurred. This could be attributed to the absence of bacteria that can produce enzymes cleaving the terminal sugars from the glycoproteins. These factors allow host–defense reactions of the dentin-pulp complex with the resulting deposition of calcium [19]. This is also supported by (Neves et al., 2019) [43] who measured the mineral changes on a longer term and showed increased radiographic density during the 10–15 month follow-up period, indicating mineral gain and dentin remineralization after glass ionomer restoration.

4.1. Conclusion

(1) Sealing only with glass ionomer has a crucial antibacterial effect in deep carious lesions.
(2) Both Aloe Vera and Diode laser have a more remarkable antibacterial effect on carious dental tissues than sealing with glass ionomer alone.

4.2. Recommendation

(1) Further clinical trials are required to evaluate the pulpal outcome, the long term survival and clinical performance of the applied materials.
(2) Further clinical trials also are required to investigate other natural materials.

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Conflicts of interest

There is no conflict of interest.

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