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Research Article

Periodontitis for Dental health

Antibacterial Effect of Longan Extract and Diode Laser Combination on *Peptostreptococcus Species* in Type 2 Diabetic Patients with Apical Periodontitis - A Pilot Study

Dr. Moksha Nayak[1], Dr. Angeline Jose[2*] and Dr. Vidhya Shenoy[3]

[1]*Professor, Dept of Conservative Dentistry and Endodontics, KVG Dental College and hospital, Sullia, Karnataka, India*

[2]*Post graduate student, Dept of Conservative Dentistry and Endodontics, KVG Dental College and hospital, Sullia, Karnataka, India*

[3]*Post graduate student, Dept of Conservative Dentistry and Endodontics, KVG Dental College and hospital, Sullia, Karnataka, India*

Abstract: Diabetes mellitus is a risk factor for developing periapical infections and increases resistance against pharmacotherapy. Microorganisms are the major cause for periradicular diseases. *Peptostreptococcus spp.* is the most common bacterial isolate from root canals of primary endodontic infections in diabetic patients. Herbal irrigants are being researched for its efficacy against root canal microorganisms owing to their resistance against commonly used irrigants and medicaments. Longan extract is known for its antimicrobial and antiglycemic action. Diode laser is a newer modality in the disinfection of root canal system. The aim of this study was to assess the antibacterial effect of longan extract (longan fruit extract) and diode laser on *Peptostreptococcus species* in type 2 diabetic patients with primary endodontic infections using PCR technique. Eleven subjects with type 2 diabetes requiring endodontic treatment were selected for the study based on convenience sampling for a period of 3 months. The preoperative root canal samples were collected after gaining access to the root canal system and initial instrumentation of root canals. Postoperative sample were obtained following a disinfection protocol using 3% NaOCl, 17% EDTA, longan extract as the final irrigant, followed by diode laser irradiation. Both the preoperative and postoperative samples were collected as per Moller's criteria using sterile paper points. The mean percentage reduction following irrigation of the root canal with longan extract was 71.18% whereas diode laser showed 100% disinfection. In conclusion, the combination of longan extract and diode laser provides an effective mode of root canal disinfection in type 2 diabetic patients with apical periodontitis.

Keywords- type 2 diabetes, *Peptostreptococcus species,* apical periodontitis, longan extract, diode laser irradiation

***Corresponding Author**

Dr. Angeline Jose , Post graduate student, Dept of Conservative Dentistry and Endodontics, KVG Dental College and hospital, Sullia, Karnataka, India

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1. INTRODUCTION

Diabetes mellitus is a systemic disorder characterized by high blood glucose as a result of insulin resistance and relative insulin deficiency.[1] This common metabolic disorder is a risk factor for developing large or debilitating periapical infection as well as resistance to pharmacotherapy. Segura *et al*. reported high prevalence of apical periodontitis (81.3%), greater size of the periapical osteolytic lesions, greater likelihood of asymptomatic periapical infections, and a decreased success rate of endodontic treatment in diabetic patients suggesting that diabetes may serve as a disease modifier of periapical lesions.[1,2] The root canal system of diabetic patients are colonized with different microbial profiles which make them more susceptible to severe periradicular diseases.[2] According to Fouad et al, the most prevalent microorganisms in root canals of diabetic patients are *Fusobacterium nucleatum, Peptostreptococcus micros, and Streptococcus spp*.[3] *Peptostreptococcus* is a gram positive, obligate anaerobe frequently isolated from root canals of primary endodontic infections.[4] It is a slow-growing bacterium with high resistance to antimicrobial drugs.[3] Infections caused by *Peptostreptococcus* are synergistic whereby there is mutual induction of sepsis enhancement, increased abscess inducement and enhancement of growth of the bacterial components in diabetic patients.[4] Constant increase in antibiotic resistant strains and side effects of chemical irrigants has led to the search for alternative herbal medicaments.[5] Longan (*Dimocarpus longan)* is a subtropical evergreen tree belonging to the family sapindaceae. The longan fruit contains phenolics and polyphenolic compounds.[6] Longan fruit extract is known to have tyrosinase inhibitory, antioxidant, anti-inflammatory, immunomodulatory, anti-glycated, antimicrobial and anti-cancer activities.[7] Because of its antifungal property it has been incorporated into mouthwashes in dentistry to fight oral infections caused by fungi.[8] Longan extract has not been used as a root canal irrigant for endodontic infections. The high surface tension of root canal irrigants limits its penetration only to a depth of 100–300 µm into dentinal tubules, whereas bacteria can colonize as deep as 1100 µm into the canal lumen.[9] Lasers can penetrate to a depth of >1000 µm into dentinal tubules. It has the advantage of complete canal sterilization, removal of debris and smear layer from the root canal walls following biomechanical instrumentation and has gained acceptance in laser-assisted dentinal disinfection.[10] Hence the aim of the study was to evaluate the antibacterial efficacy of combination of longan extract and diode laser against *Peptostreptoccus spp*. in type 2 diabetic patients with primary endodontic infections.

2. MATERIAL AND METHODOLOGY

2.1. Patient selection

Eleven subjects with type 2 diabetes requiring endodontic treatment referred to the Department of Conservative Dentistry and Endodontics, K V G Dental College and Hospital were selected for the study based on convenience sampling for a period of 3 months. Ethical clearance was obtained from the Institutional Ethics Committee and written consent from the patients were obtained. Both female and male subjects in the age group of 20-60 years with Type 2 diabetics having random blood sugar level ≥ 200mg/dl, fasting blood sugar level ≥126mg/dl and glycated haemoglobin (HbA1c) ≥ 6.5% with primary endodontic infection determined by clinical and radiographic examinations were included in the study(Table 1). Subjects with systemic diseases other than type 2 diabetes, pregnancy and lactation, use of any antibiotics during the past 3 months, teeth that cannot be isolated with rubber dam, calcified canals, tortuous canals, fractured root, teeth with developmental defect, and patients who have participated in any other clinical study during previous 3 months were excluded from this study.

Table1 Glycated haemoglobin level

Level of glycated control	HbA1c
Good	6.5-7%
Fair	7-8%
Poor	>8%

2.2. Sampling procedure

Each tooth was cleaned with pumice and isolated with rubber dam, disinfected and then neutralized according to the protocol by Moller.[6] The efficacy of the disinfection procedure was evaluated using microbial culture. If growth occurred, the sample was disqualified from the study. A standard access preparation was prepared with a sterile high speed endodontic access bur #2 (Dentsply Maillefer) and Endo Z carbide bur and disinfection was carried again using Moller's protocol. The working length is determined using a radiographic method. The root canals were instrumented with hand files up to ISO size 20 file. A sterile paper point was introduced into the full length of the canal (as determined with a preoperative radiograph), and kept in place for 60 sec and transferred to sterile tubes containing sterile TE buffer. In multirooted teeth, the root with the periapical lesion was selected. The samples were transported to Maratha Mandal's NGH Institute of Dental Sciences and Research Centre, Belgaum, for semi-quantitative conventional PCR analysis for detection of *Peptostreptococcus spp*. Longan extract was prepared at K.V.G Ayurveda College using longan fruit.

2.3. Preparation of ethanolic extract of longan

Powdered *Longan pericarp* was extracted with 50% ethanol at room temperature for 6 h. The supernatant was collected after filtering and then was evaporated under reduced pressure using a rotavapor to obtain crude extract. Crude extract was extracted with diethyl ether after which the pHof aqueous fractions were adjusted with 20% $NaHCO_3$. The aqueous parts were extracted with chloroform to obtain chloroform fraction, insoluble fraction, and aqueous fraction, then the pH was adjusted by adding 6 N HCl and then extracted with ethyl acetate to obtain ethyl acetate fraction. The aqueous fraction was finally extracted with n-butyl alcohol.[7]

2.4. The irrigation protocol and microbial identification

After instrumentation, irrigation with 3% NaOCl and 17% EDTA was carried out. Longan extract was used as the final irrigant. The post operative sample collection following irrigation using longan extract was done and transferred to T. E buffer. Following the use of every irrigant saline was used to flush the canal except after the use of final irrigant. This was followed by disinfection of the root canal using a diode laser. The root canals were irradiated with a Denlase diode laser using a wavelength of 810 nm at 1.2 W power in continuous wave mode. Laser beam was directed into the canal by the fiber optic cone with a diameter of 200 µm for 10 sec each for 40 sec. The tip of the fiber optic cone was placed in the canal 1mm short of the working length and optic fiber was led in slow, circular, spiral-forming movements from the apical to the coronal part, while the laser was activated. Post operative sampling was done similar to the initial sampling and placed in a sterile tube containing TE buffer. The samples were transferred to Maratha Mandal's NGH Institute of Dental Sciences and Research Centre, Belgaum, for semi-quantitative conventional PCR analysis for detection of *Peptostreptococcus spp.* The nucleotide sequence for detection and quantification of *Peptostreptococcus spp*. using PCR is Forward Primer: 5'- AGA GTT TGA TCC TGG CTC AG 3'and Reverse Primer : 3'- ATA TCA TGC GAT TCT GTG GTC TC 5'.[4]

3. STATISTICAL ANALYSIS

The obtained data was analyzed using SPSS software (version 21). Chi – square test was used for intragroup comparison. Data were presented as mean and standard deviation. Probability value of less than <0.05 was considered as statistically significant.

Table 2: Mean colony forming unit of Peptostreptococcus spp. following disinfection of root canals using longan extract and diode laser in type 2 diabetics

	Mean Rank	Mean	Std. Deviation	Percentiles			Chi-square	Asymp. Sig.
				25th	50th (Median)	75th		
Preop	3.00	1.66E5	278598.363	6225.00	44600.00	2.26E5		
Longan	2.00	1.0040E5	1.71645E5	3.6510E3	25700.0000	1.3292E5	20.00	0.000
Diode	1.00	0	0	0	0	0		

P value <0.05, (n=11), SD=2.85 E5

Table 3: Intragroup comparison of colony forming units of Peptostreptococcus spp. following disinfection of root canal using longan extract and diode laser in type 2 diabetics

	Longan - preop	Diode - Longan	Diode - preop
Z	-2.803[a]	-2.803[a]	-2.803[a]
Asymp. Sig. (2-tailed)	0.005	0.005	0.005

P < 0.017

4. RESULTS

The present study included a total of 11 subjects of which 8 were male and 3 were female subjects. *Peptostreptococcus spp.* were isolated from a total of 9 teeth of the 11 cases examined. Chi square test showed a significant reduction in the *Peptostreptococcus spp.* colony count following disinfection using longan extract and diode laser. The mean percentage reduction following irrigation of the root canal with longan extract was 71.18% whereas diode laser showed 100% disinfection. Table 2 shows the mean colony count of *Peptostreptococcus spp.* following disinfection of the root canal using longan extract and diode laser. On intragroup comparison, there was a significant difference (p=0.005) (table 3) in colony forming units of *Peptostreptococcus spp.* following disinfection using longan extract and diode laser in type 2 diabetics.

5. DISCUSSION

The challenging part of a root canal treatment is to disinfect the root canal most effectively. Conventional irrigants such as sodium hypochlorite, ethylenediaminetetraacetic acid solution, and chlorhexidine were found to have harmful side effects which led the researchers to investigate ayurvedic alternatives. Herbal alternatives are popular mainly due to their ease of availability, cost effectiveness, increased shelf life and low toxicity, hence are suitable alternatives to an endodontic irrigant.[11,12] Glycosylated haemoglobin assay (HbA1c) is an important diagnostic tool for type 2 diabetes. HbA1c reflects average plasma glucose over the previous eight to 12 weeks. An HbA1c of 6.5% is recommended as the cut point for diagnosing diabetes.[13] It can be performed at any time of the day and does not require any special preparation such as fasting.[14] Due to these properties, this test was preferred for assessing glycaemic control in patients with diabetes in the current study. Baumgartner et al. observed that the cell wall of Gram positive bacteria contain peptidoglycans and lipoteichoic acids, which influence inflammatory reactions and enhance the pain modulation. This can contribute to increased insulin resistance and poor glycemic control in diabetic patients.[15] A reciprocal relationship exists between glycaemic control and chronic periapical lesions. Treating infections of pulp and periodontium will improve glycaemic control and help in healing of lesions similar to non-diabetics.[2] A strong association exist between type 2 diabetes mellitus and presence of *peptostreptococcus* in the root canals.[6] Various irrigants like MTAD, chlorhexidine, doxycycline, NaOCl and irrigation techniques have been evaluated against *Peptostreptococcus spp*.[16,17] Study conducted by Ghoneim et al obtained 82% reduction of *Peptostreptococcus spp* following disinfection using Endovac system in diabetic patients.[17] This is a preliminary study evaluating longan extract as a root canal irrigant against *peptostreptococcus*. Results of the

present study shows that an irrigation protocol of 17% EDTA, 5.25% NaOCl and a final rinse with longan extract resulted in 71% bacterial reduction in the root canals of type 2 diabetic patients with primary endodontic infection. Longan (*Dimocarpus longan* Lour.) belongs to the Sapindaceae family.[7] Longan fruits have a succulent and white ariel with a brown seed. This fruit consists of Gallic acid, corilagin, epicatechin, ellagic acid and its conjugates, quercetin, flavone glycosides, 4-*O*-methylgallic acid, flavone glycosides, glycosides of quercetin and kaempferol, protocatechuic acid, brevifolin.[15] The antimicrobial property of longan extract can be attributed to the ability to form chelates with metal ions, which leads to the disruption of the cell membrane.[4] Various authors have evaluated the antimicrobial property of longan extract against different microorganisms.[5,6,7,8] Biological analysis indicated that polysaccharide fractions from longan fruit pericarp tissues exhibited strong antioxidant ability, anti-glycation activity and antimicrobial acitivity.[18,19] Diabetes mellitus alters many functions of the immune system and is associated with delayed healing and compromised immune responses. The use of longan extract as an endodontic irrigant has the advantage of providing antimicrobial effect, antiglycemic effect and immunomodulatory effect which can provide a good treatment outcome in diabetic patients with periapical lesions. Diode laser of 810 nm was used for the disinfection of root canals following irrigation using longan extract. In the present study, complete eradication of *Peptostreptococcus spp.* was seen following irradiation using a diode laser. The efficacy of diode laser against various root canal organisms have been evaluated by various authors.[20-22] Preethee et al. reported 100% reduction of *E. faecalis* using 908 nm diode laser.[20] Another study conducted by vatkar et al evaluated the antibacterial effect of diode laser against *E. faecalis* using confocal laser scanning microscopy found no viable bacteria in the root canals following irradiation which proves the antibacterial effect of diode laser.[21] The laser irradiation produces denatured protein and induces the bacterial cells to create new proteins to compensate for denaturation.[21-23] The present study is a pilot study for evaluating the efficacy of longan extract and 810 nm diode laser on *Peptostreptococcus spp.* in primary endodontic infections in type 2 diabetic patients. Further research should be conducted with a larger sample size and comparison with the standard irrigation protocol for primary endodontic infections must be done.

6. CONCLUSION

Irrigation protocol of 3% NaOCl, 17% EDTA, final rinse of longan extract, disinfection using 810nm diode laser effectively eliminated *Peptostreptococcus spp* from the root canals of diabetic patients with primary endodontic infections. The added benefit of antimicrobial property along with antiglycemic effect makes longan extract a promising adjunct in the treatment of primary endodontic infections in type 2 diabetic patients.

7. AUTHORS CONTRIBUTION STATEMENT

Dr Moksha Nayak conceptualized and contributed in designing the manuscript of the study. Dr Moksha Nayak, Dr Angeline and Dr Vidhya gathered data for this study. All authors contributed significantly to the final manuscript.

8. CONFLICT OF INTEREST

Conflict of interest declared none.

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