OFFICIAL TITTLE—ANTIBACTERIAL EFFECT OF 940NM DIODE LASER, APPLE CIDER VINEGAR AND *GYMNEMA SYLVESTRE* IN DIABETIC PATIENTS WITH APICAL PERIODONTITIS AGAINST PEPTOSTREPTOCOCCUS SPECIES- A RADOMIZED CONTROL CLINICAL STUDY.

Date -1/04/2026

INTRODUCTION

- Diabetic mellitus is a metabolic condition raises the possibility of severe or incapacitating periapical infections as well as drug-resistant bacteria.

- Peptostreptococcus (gram positive strict anaerobe) peptidoglycans and lipoteichoic acids are found in the cell walls ,these compounds regulate inflammatory responses, enhance pain modulation, and also lead to increased insulin resistance and poor glycemic control in diabetes patients.

- In dentistry apple cider vinegar is utilized as an anti-inflammatory, antibacterial and chelating agent.
- Diode lasers is used as an adjunct for effective disinfection of the root canal.
- Anti-microbial efficacy of Gymnema have been shown in a various studies against both gram positive and gram negative bacterial strains.

Literature Review

The meta-analysis data suggest a connection between the endodontic outcome in terms of the presence of periapical radiolucency in root filled teeth in diabetics

Apple cider vinegar is a promising biocompatible natural substance that efficiently affects the penetration of the dentinal tubules and eliminates the smear layer *in vitro*

Diode laser application following conventional irrigation may reduce Post operative pain in single-rooted necrotic teeth with a PAI score of 3 or 4 after RCT performed in two treatment visits

Gymnema extract has showed significant antimicrobial activity against the bacteria P aeruginosa, S aureus and the fungus F oxysporum.

Gymnema sylvestre has strong antioxidant and antimicrobial activity as tested by different methods.

Diode laser disinfection showed the highest reduction of microbial count compared to ultrasonic activated and conventional needle irrigation with 2.5% NaOCl group

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Both 810nm diode laser and 5.25% sodium hypochlorite were effective in reducing bacterial count in root canals of primary teeth. However 810nm diode laser reduced microbial count of root canals more as compared to 5.25% NaOCl.

AIM:

To assess and compare the antibacterial effectiveness of using a 940nm diode laser, apple cider vinegar, and Gudmar (*Gymnema sylvestre)* individually as well as in combination against Peptostreptococcus spp. in primary endodontic infections in diabetic people.

Objectives

1.To determine whether 940nm diode laser effective in reducing the colony forming unit of Peptostreptococcus spp. in diabetic patients with primary endodontic infections.

2.To determine whether apple cider vinegar is effective in reducing the colony forming unit of Peptostreptococcus spp. in diabetic patients with primary endodontic infections.

3.To determine whether Gudmar (*Gymnema sylvestre)* is effective in reducing the colony forming unit of Peptostreptococcus spp. in diabetic patients with primary endodontic infections

4.To determine antibacterial effectiveness of using an 940nm diode laser and apple cider vinegar together in reducing the colony forming unit of Peptostreptococcus spp endodontic infections in diabetic individuals.

5.To determine antibacterial effectiveness of using an 940nm diode laser and Gudmar (*Gymnema sylvestre)* together in reducing the colony forming unit of Peptostreptococcus spp. endodontic infections in diabetic individuals.

6.To compare the antibacterial effectiveness of using an 940nm diode laser, apple cider vinegar and Gudmar (*Gymnema sylvestre)* both individually as well as in combination in reducing the colony forming unit of Peptostreptococcus spp. endodontic infections in diabetic individuals

Data and detailed Methodology

Type of study :analytical/experimental design

Inclusion criteria :

1. Using radiographs, intraoral periapical radiographs (RVG) primary endodontic infection will be selected.

2. Multirooted teeth , root with periapical lesion will be selected

3. Type 2 diabetics between the ages of 25 years and 60 years will be chosen; both male and female patients

4. A fasting blood sugar level of 126 mg/dl and a random blood sugar level of less than 200 mg/dl

5. HbA1c (glycated haemoglobin) $\geq$ 6.5%

Exclusion criteria :

1. Patients with systemic conditions other than type 2 diabetes

2. Women who are pregnant

3. Those who have used antibiotics within the previous three months,

4. Those who have teeth with developmental defects, calcified canals,

5. Teeth that cannot be isolated with rubber dams,

6.Tortuous canals

7.Roots that are fractured

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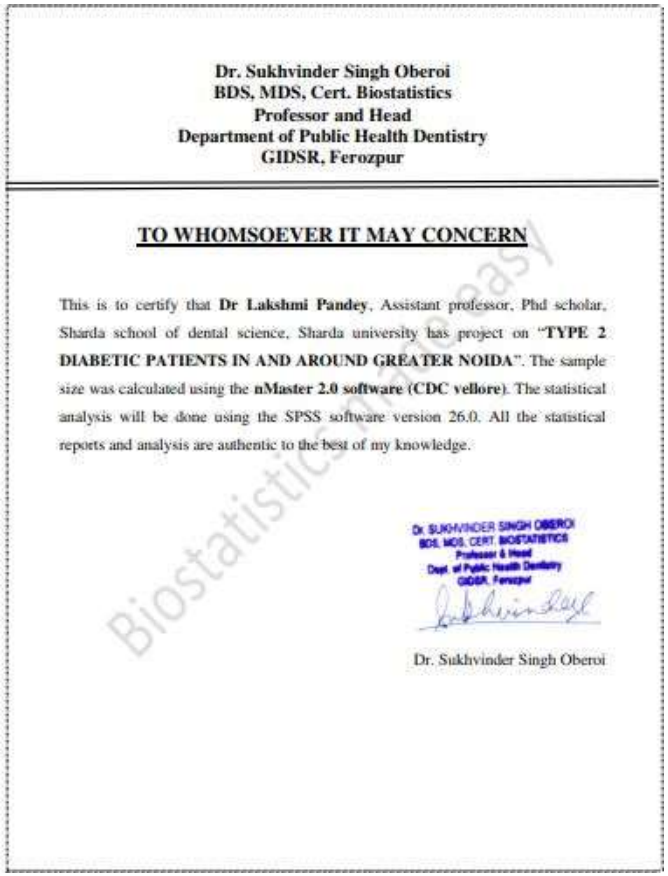
Sample size calculation

The sample size was calculated using the **nMaster 2.0 software**. The power of the study was taken to be 80% and Confidence Interval (C.I.) of 95% was taken. The sample size calculation was done as per the article by *Kaplan et al*. The sample size was estimated to be a minimum of 8 per group.



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TO WHOMSOEVER IT MAY CONCERN

This is to certify that **Dr Lakshmi Pandey**, Assistant professor, Phd scholar, Sharda school of dental science, Sharda university has project on "**TYPE 2 DIABETIC PATIENTS IN AND AROUND GREATER NOIDA**". The sample size was calculated using the **nMaster 2.0 software (CDC vellore)**. The statistical analysis will be done using the SPSS software version 26.0. All the statistical reports and analysis are authentic to the best of my knowledge.

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Date -1/04/2026

Type 2 diabetes patients with informed consent -convenience sampling over a one year period.

The tooth isolated with a rubber dam and the correct disinfection procedures will be carried out.

Access preparation done (access bur #2 and Endo Z carbide bur)

Working length -electronic apex locator (Dentsply Propex II) as well as a radiographic approach.

To enlarge the root canals (ISO size 20 file)

A sterile paper point inside the root canal for one minute until the apex of the root canal is reached

Using T.E Buffer, samples will be delivered to the School Of Basic Life Sciences, Sharda University.

The samples will be subjected to traditional PCR analysis for the detection of Peptostreptococcus spp. in the root canal.

If Peptostreptococcus spp. is not detected in the sample then the patient is not included in the study but the procedure with the ideal irrigation protocol will be followed and the root canal procedure will be completed.

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Grouping

Following instrumentation, 5% NaOCl and 17% EDTA irrigation will be done.

Control group (n=15) The final irrigant will be saline	Group 1 (n=15) irrigation using 5% apple cider vinegar done The final irrigant will be saline	Group 2 (n=15) diode laser is used for disinfection in the presence of saline	Group 3 (n=15) Gymnema Sylvestre Mother Tincture Q 1000 CH solution is used for disinfection in the presence of saline	Group 4 (n=15) diode laser is used for disinfection in the presence of 5% apple cider vinegar. The final irrigant will be saline	Group 5 (n=15) diode laser is used for disinfection in the presence of Gymnema Sylvestre Mother Tincture Q 1000 CH solution. The final irrigant will be saline

A diode laser operating in continuous wave mode at a power of 1.5W will be used. Its wavelength is 940 nm. The fibre optic cone, which has a diameter of 200µm, will point a laser beam into the canal 2 cycles for 40 seconds at intervals of 10 seconds each (total time 80 seconds)

The fibre optic cone's tip will be positioned in the canal 5 mm short of its working length, and while the laser is on, the optic fibre will be lead in slow, circular, spiral-forming movements from the apical to the coronal section.

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After chemomechanical preparation and disinfection, samples will be collected and sent through T.E. Buffer to the School Of Basic Life Sciences, Sharda University.

The samples will be subjected to traditional PCR analysis for the detection of Peptostreptococcus spp. in the root canal.

After the canals will be obturated using ZOE sealer and gutta percha and post endodontic restoration is done using composite restoration

Patient is kept on follow up for 7days, 15 days, 30 days ,45 days and 60 days

Clinical outcome is measure using periapical radiograph (RVG)

Pain is measured using visual analog scale to mark the pain intensity.

Data will be analysed statistically

Statistical analysis

The data will be entered into Microsoft Excel and analysed using SPSS (Statistical Package for Social Sciences) package 26.0 for relevant statistical comparisons.

Inferential statistics will be done using the Chi-square test for the categorical variables and one-way ANOVA test will be used for the comparison of mean values (continuous variables) between more than 2 groups.

Paired t-test will be used for the comparison of values between 2 time intervals and repeated measures ANOVA test for the comparison of values between more than 2 time intervals. Level of statistical significance was set at p-value less than or equal to 0.05.

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CASE HISTORY

Name :.....Age / Sex :.....

Address & Telephone No. :.....

Marital Status :.....

Occupation :.....

DENTAL HISTORY

1. **Chief Complaint**

2. **History of Presenting Illness**

NATURE OF PAIN	NONE	MILD	MODERATE	SEVERE
QUALITY	DULL	SHARP	THROBBING	CONSTANT
ONSET	STIMULUS REQUIRED		INTERMITTENT	SPONTANEOUS
LOCATION	LOCALIZED	DIFFUSE	REFERRED	RADIATING
DURATION	SECONDS	MINUTES	HOURS	CONSTANT
INITIATED BY	COLD	HOT	SWEET	SPONTANEOUS
	BITING	LYING DOWN	PALPATION	NIGHT
RELIEVED BY	COLD	HOT	MEDICINES	

3. **HABITS (GRINDING / CLENCHING / TOBACCO)**

4. **PAST DENTAL HISTORY**

CLINICAL EXAMINATION

(1) Inspection :

a. Extra oral swelling ☐ sinus tract ☐

b. Lymph nodes involved: submaxillary ☐ ; submental ☐ ; other.

c. Tooth discolored ☐

d. Wear facets or signs of occlusal trauma ☐

e. Craze lines ☐

f. Exposed root surface ☐

g. Presence of sinus tract (intra oral) ☐

(2) Palpation / Percussion

a. Tooth tender on percussion ☐

b. Tooth slooth test / Biting test ☐

c. Soft tissue tender on palpation ☐

(3) Radiographs

a. Caries ☐

b. Extensive Restoration ☐

c. Crown root fracture ☐

d. Bone levels ☐

Furcation involvement ☐

Substantial inter-proximal bone loss ☐

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e. Peri-apical radio-lucency present ☐ or absent

f. Thickened periodontal ligament ☐

g. Internal resorption ☐

h. External resorption ☐

i. Calcification ☐

j. Atypical pulpal anatomy.....

k. Horizontal / Vertical bone loss ☐

(4) **Others :**

a. Electric pulp test : control tooth response at No.....Test tooth response at No.....

b. Thermal test : Normal ☐; abnormal response to cold ☐ or heat ☐

No response ☐

(5) **Clinical Diagnosis :**

1. Normal pulp
2. Reversible pulpitis
3. Irreversible pulpitis
4. Pulp Necrosis
5. Previously Treatment Required
6. Previously Initiated Therapy
7. Symptomatic apical Periodontics
8. Asymptomatic apical Periodontitis
9. Acute apical abscess
10. Chronic apical abscess
11. Cellulitis
12. Apical scar
13. Condensing osteitis

CLINICAL FINDINGS

Caries :

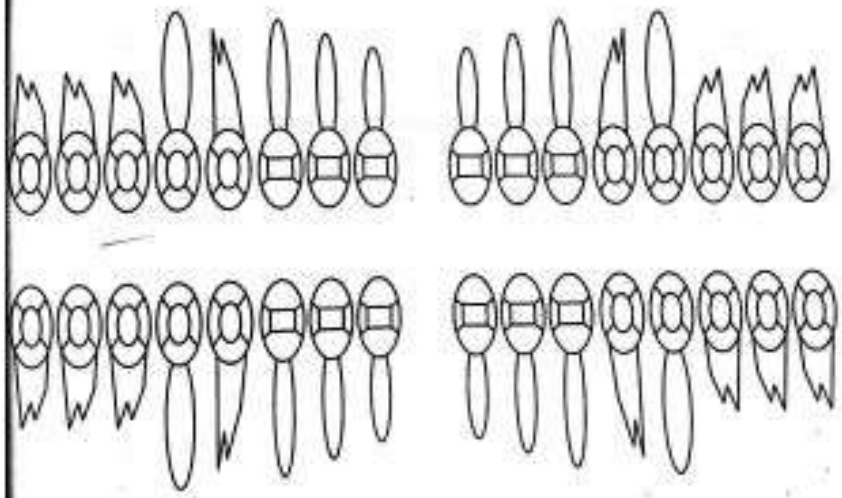
Existing direct Restoration :

Existing Full / Partial Coverage Indirect Restorations :

Carlously exposed teeth :

Fractured teeth :

Others :



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MEDICAL HISTORY

How would describe your health ? Please Circle one.	Excellent	Good	Fair	Poor
When did you have your last physical examination ?				
Are you currently being treated for any illness or medical condition ?			Yes	No
If yes, please describe.....				
.....				
Who is treating you for this condition ?				
Have you ever had any kind of surgery ?.....			Yes	No
When did you have this surgery ?.....				
What type of surgery did you have ?.....				
Have you ever had any trouble with prolonged bleeding after surgery ?			Yes	No
Do you wear a pacemaker or any other kind of prosthetic device ?			Yes	No
Are you talking any medication or drugs at this time ?			Yes	No
Have you ever taken Fen-phen and / or Redux (diet drugs) ?			Yes	No
What medication, drugs or herbs are you taking ?				
Why are you taking these medications ?.....				
.....				
Have you ever had an unusual reaction to an anesthetic or drug (like penicillin) ?			Yes	No
If you please explain ?.....				
Please circle any present or past illness you now have or had in the past ?				

Alcoholism	Blood pressure	Epilepsy	Hepatitis	Kidney or liver	Rheumatic
Allergics	Cancer	Glaucoma	Herpes	Mental	Sinusitis
Anemia	Diabetes	Head/neck injuries	Immunodeficiency	Migraine	Ulcers
Asthma	Drug dependency	Heart/valve disease	Infectious disease	Respiratory	Venereal disease

Are you allergic to latex or any other substances or materials ?	Yes	No
If so, please explain ?.....		
If female, are you pregnant ?	Yes	No
Is there any other information that should be known about your health ?.....		
.....		
Signature of Patient (or Parents).....	Date.....	

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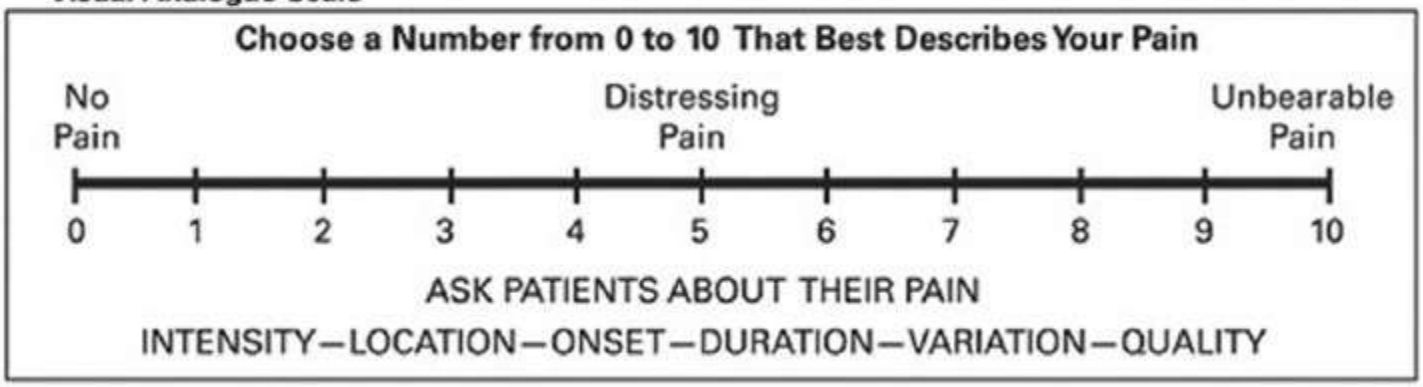
Figures: Tools Commonly Used to Rate Pain

Visual Analogue Scale



DAYS	PAIN RATING
7 DAYS	
15 DAYS	
30 DAYS	
45 DAYS	
60 DAYS	

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PARTICIPANT INFORMATION SHEET

Study Title:

ANTIBACTERIAL EFFECT OF 940NM DIODE LASER, APPLE CIDER VINEGAR AND *GYMNEMA SYLVESTRE* IN DIABETIC PATIENTS WITH APICAL PERIODONTITIS AGAINST PEPTOSTREPTOCOCCUS SPECIES- A RADOMIZ ED CLINICAL TRIAL

I would like to invite you to take part in my research study. Before you decide, it is important that you understand why the research is being done and what it would involve. Please take time to read this information, and discuss it with others if you wish. If there is anything that is not clear, or if you would like more information, please feel free to ask.

What is the purpose of the study?

This study is intended to compare effect of diode laser, apple cider vinegar and *Gymnema sylvestre* on the peptostreptococcus spp. In type 2 diabetic patients.

Why have I been invited?

- You are a type 2 diabetic patient with a primary endodontic infection.
- Your age group lies between 25yrs to 60 yrs.

Do I have to take part?

- Taking part is entirely voluntary.
- You can withdraw if you later change your mind, without giving a reason; however, it is advisable to talk to and inform the research team before withdrawal.
- Withdrawal will not affect your clinical care.

- Your root canal procedure will be initiated and samples will be collected.
- Root canal procedure will be performed and apple cider vinegar, *Gymnema sylvestre* and diode laser will be used.
- Before the finishing of the root canal procedure final samples will be taken.
- Root canal procedure will be complete.

What should I consider?

1. You will not be included in the study.
   - If you have any other systemic disease.
   - If you are pregnant or lactating mother
   - If you have taken antibiotic coverage in last 6 months.
   - If you are not willing to participate and give the informed consent for the same.
2. You can participate, even if you are involved in other research studies.

Are there any possible disadvantages or risks from taking part?

No risk expected.

What are the possible benefits of taking part?

You will have more awareness regarding the effect of diabetes and its complications.
We can provide the dental treatment in more efficient way.

Patients have been involved in the study for examination and data analysis.

Various such studies have already been conducted in similar manner.

Who is organizing and funding the study?

Self organized and Self funded

Who has reviewed the study?

This study has been reviewed and given favorable opinion by INSTITUTIONAL ETHICS COMMITTEE (IEC) Sharda University.

Will my taking part in the study be kept confidential? Yes

Will I be reimbursed for taking part? No

What will happen to my data?

The data collected from the research will be kept confidential. The information about the subject will not be shared outside the research team. When the research will go for publication, identity of the patient will not be revealed.

What will happen if I don't want to carry on with the study?

If you withdraw from the study, unless you state otherwise, the data which has been collected whilst you have been in the study will be used for the research as detailed in this participant information sheet.

What will happen to the results of this study? Alternatively: What happens at the end of the study?

The result of the study can be used for the counselling, publication, conference or any other scientific purposes, but your personal identity will be kept confidential.

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CONSENT FORM

ANTIBACTERIAL EFFECT OF 940NM DIODE LASER AND APPLE CIDER VINEGAR COMBINATION IN DIABETIC PATIENTS WITH APICAL PERIODONTITIS AGAINST PEPTOSTREPTOCOCCUS SPECIES- A RANDOMISED CLINICAL TRIAL

If you agree, please initial box

1. I confirm that I have read the information sheet dated ..... (version.....) for this study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected.	
3. I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from the Sponsor, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.	
4. (If appropriate) *I agree to provide a sample(s) as part of my involvement in this study and I understand I will not gain any direct personal or financial benefit from them.*	
5. (If appropriate) *I agree to audio/video recording and the use of anonymised quotes in research reports and publications.*	
6. (If appropriate) *I agree to my General Practitioner being informed of my participation in the study.*	

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10. I agree to take part in this study.	
11. *I agree to be contacted about ethically approved research studies for which I may be suitable. I understand that agreeing to be contacteddoes not oblige me to participate in any further studies.*	
12. *I agree for my anonymised samples to be used in future research, *here or abroad, which has ethics approval.* (*if some future use may becommercial, state this)	

Name of Participant	*Date*	*Signature*
Name of Person taking Consent	*Date*	*Signature*

Expected Outcomes

940nm diode laser, apple cider vinegar, and Gudmar (*Gymnema sylvestre)* individually as well as in combination will be effective against Peptostreptococcus spp. in primary endodontic infections in diabetic people.

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