EFFICACY OF DIODE LASER 405 NM WITH CHLOROPHYLLS AS PHOTOSENSITIZER ON Enterococcus faecalis

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ABSTRACT
Background: The presence of persistent infections in the root canals by microorganisms causes root canal failure. The most commonly found bacteria that cause persistent infection is Enterococcus faecalis. PDI / photodynamic inactivation is an in vitro approach to inactivation of microorganisms. The combination of light and photosensitivity of chlorophyll in PDI will cause photoinactivation in bacteria. Long radiation of PDT can affect the production of singlet oxygen and ROS (Reactive Oxygen Species) to kill Enterococcus faecalis bacteria. Objective: To prove the effect of 405 nm laser diode with and without chlorophyll photosensitizer and the irradiation effect of 405 nm laser diode on the number of CFU of Enterococcus faecalis bacteria. Method: This study used the Enterococcus faecalis bacteria culture which was divided into 5 groups. Group I as control group, Group II irradiation 30’, III chlorophyll + irradiation 30’, IV irradiation 60’, V chlorophyll + irradiation 60’. After incubation, the bacteria count was calculated with Quebec Colony Counter and analyzed by Shapiro-Wilk test, Levene test and Anova test. Results: There were significant differences (p <0.05) between the number of colonies of Enterococcus faecalis bacteria in each treatment group. Longer duration of PDT exposure (Group II and IV) with chlorophyll showed less number of Enterococcus faecalis bacteria. Conclusion: The longer the PDT irradiation, the less number of Enterococcus faecalis bacteria. The 60-second radiation with chlorophyll showed the least amount of Enterococcus faecalis bacteria.

Keywords: diode laser, chlorophyll, photodynamic therapy, Enterococcus faecalis
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BACKGROUND
The presence of persistent infections in the root canal by microorganisms causes root canal failure. Bacteria that are commonly found to cause persistent infections are: Enterococcus faecalis, Streptococcus sp, Psudoramibacter alactolyticus, Streptococcus windosus, Bacteroides gracilis, Filifactor alocis and Fusobacterium nucleatum. Enterococcus faecalis bacteria responsible for 80-90% of root canal infections. Enterococcus faecalis bacteria have been shown to survive in root canals as single organisms and resistant to commonly used antimicrobial ingredients making it difficult to eliminate from the root canal completely resulting in failure of root canal treatment. Pathological microorganisms can penetrate the root dentin tubules to a depth of 1000 µm, while the irrigation disinfection material only reaches 100 µm depth. The laser beam penetrates into the dentinal tubule to exceed 1110 µm depth for more perfect root canal sterilization, has no toxic content and has a high degree of selectivity to kill bacteria without damaging host cells. Photodynamic inactivation or PDI is an in vitro approach to inactivation of microorganisms. The combination of light and certain photosensitizer in PDI will cause photoinactivation in bacteria. The research currently being developed is using...
chlorophyll as an exogenous photosensitiser. The effect of laser diodes as photoinactivation of bacteria is used with endogenous or exogenous photosensitic porphyrins. Each porphyrin molecule has specific light absorption capabilities with specific wavelengths against specific bacteria\textsuperscript{17}.

**MATERIAL AND METHOD**

The type of this research is laboratory experimental research with Pre-Post Control Only Control Group Design. Sample used in this research is Enterococcus faecalis. Determination of the number of samples used in this study using the Federer formula, obtained the total number of samples as minimum 20. Bacterial culture Enterococcus faecalis done with osse wire that put into a tube containing Brain Heart Infusion (BHI) broth I. Then stirred and incubated (37°C) in an incubator (48 hours) with an anaerobic atmosphere (Forbes et al., 2002). Then from the tube BHI broth I, was taken 0.5 ml with micropipette and inserted into the reaction tube containing BHI broth II and synchronized with Mc Farland scale to obtain bacterial suspension 1.5 x 10^8 CFU / ml. Sampling was obtained from a bacterial suspension reaction tube : 0.5 ml and inserted into each microglass. Group I was a control group (no photosensitiser and no irradiation). Group II irradiation with laser diode light for 30 seconds. Group III is chlorophylls and irradiated for 30 seconds. Group IV irradiation 30 seconds. Group V was given photosensitiser in the form of 0.5 ml of chlorophyll fluid and then irradiated for 60 seconds.

![Figure 1. a) E. faecalis culture sampling (b) 0.5 ml chlorophyll and 0.5 ml Enterococcus faecalis bacteria (c) Irradiation with laser diode according to treatment group](image)

Each microglass is taken 0.1 ml with a micropipette and grown on petridish containing nutrient agar. The petridish contains nutrient agar medium, incubated for 48 hours at 37°C in an anaerobic atmosphere.

![Figure 2. Bacteria Enterococcus faecalis in Nutrient Agar](image)

After incubation, the number of bacterial colonies in petridish was calculated using Quebec Colony Counter by CFU (Colony Forming Unit) method and used for analyzed data\textsuperscript{4}.

**RESULTS**

From the statistical calculation, the average and standard deviation of the colonies of Enterococcus faecalis bacteria after irradiation was done as Table 5.1.

| Description: N = number of samples; Mean = mean; SD = standard deviation |
|-----------------|------|-----------------|
| Kontrol         | 7    | 99.14           | 2.035 |
| Biru 30 detik   | 7    | 58.71           | 2.059 |
| Biru 30 detik + Klorofil | 7    | 27.29           | 1.604 |
| Biru 60 detik   | 7    | 49.14           | 1.952 |
| Biru 60 detik + Klorofil | 7    | 18.71           | 2.215 |

The resulting data are then tested for normality test with Shapiro-Wilk. Result of normality test all data yield p value> 0.05. This means that the resulting data has a normal distribution. The data were tested by Levene Test to see the homogeneity of data, Levene Test test showed p = 0.818 (p> 0.05). This shows that the data obtained by each group is homogeneous. An Oneway Anova test is then
performed to compare the mean of each group and to know the differences between groups. From OneWay Anova test, \( p = 0.000 \) (\( p < 0.05 \)) showed that there was significant difference to absorbance of each data group.

The Post hoc Tukey HSD / LSD test is used to compare each group with significant differences and data with no significant differences. The result is that there are significant differences in each group of data obtained \( p = 0.000 \) (\( p < 0.05 \)) as in table below.

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Kontrol</th>
<th>30</th>
<th>30+K</th>
<th>60</th>
<th>60+K</th>
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<tr>
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<td></td>
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<tr>
<td>30</td>
<td>( p= 0.000* )</td>
<td>( p= 0.000* )</td>
<td>( p= 0.000* )</td>
<td>( p= 0.000* )</td>
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<tr>
<td>30+K</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>60</td>
<td></td>
<td>( p= 0.000* )</td>
<td>( p= 0.000* )</td>
<td>( p= 0.000* )</td>
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</tr>
<tr>
<td>60+K</td>
<td></td>
<td></td>
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<td>( p= 0.000* )</td>
</tr>
</tbody>
</table>

(Note: \( p < 0.05 \) * = significant)

**DISCUSSION**

There are three main factors in photodynamics that cause photochemical reactions. Second, there are light absorbing molecules for chemical reactions and molecular absorption depending on the wavelength of light used. And thirdly, the creation of free radicals is reactive to biological systems, causing cell inactivation events. Each molecule has a light absorption ability that depends on a particular wavelength. The quality and type of molecule used greatly affects the photosensitivity that occurs, so that photodynamic success will be achieved when there is a correspondence between the wavelength of the light source and the photosensitiser.

From the results of the study, the colonies of Enterococcus faecalis bacteria infused with chlorophyll photosensitiser before irradiation with 405 nm laser diode rays for 30 and 60 seconds showed fewer CFUs when compared to the group without giving chlorophyll photosensitiser. This is due to the role of photosensitiser chlorophyll. Photosensitiser chlorophyll will interact with bacterial cell wall.

Photosensitiser is cation (positively charged), while the bacterial cell wall is anion (negatively charged), from the bonding electrostatic interaction between the photosensitiser material and the bacterial cell wall that releases Ca\(^{2+}\) and Mg\(^{2+}\) ions from the cell so that the bacterial cell wall is weaker and its permeability increases. Increased permeability of bacterial cell wall causes the photosensitiser cation to enter the bacterial cytoplasmic membrane, which can lead to increased permeability. This will increase the absorption and binding of photosensitizing cations with bacterial plasma membranes. The irradiation absorbed by the photosensitiser molecule will result in two stages. In the first stage there will be electron transfer between photosensitiser and substrate and produce radical ion called reactive oxygen species (ROS), superoxide anion (O\(^{2-}\)), hydroxyl radical (OH\(^{-}\)) and hydrogen peroxide (H\(_2\)O\(_2\)). This ion is highly oxidative to cells. In the second stage, electrons transfer between photosensitiser with oxygen receptors and produce highly reactive and oxidative singlet oxygen. The results of both stages led to the proliferation of bacterial cell membrane proteins, inactivation of NADH enzymes and lactate dehydrogenase, destroying the K-ion balance, destroying bacterial DNA and ultimately inhibiting bacterial growth that ended in cell death.

From the results of the research, the colony group of Enterococcus faecalis bacteria irradiation with 60 seconds of light laser diode 405 nm showed the least number of CFUs compared to other groups this was caused by the use of photosensitiser chlorophyll which play a good role in the absorption process of 405 nm laser diode, photophysics process described earlier, in addition prolonged exposure can affect the amount of light absorption by photosensitiser to increase energy transfer and generate more ROS and singlet oxygen counts.

**CONCLUSION**

There is a long-standing effect of laser irradiation 405 nm with and without photosensitiser chlorophyll to decrease the number of *E. faecalis* bacteria. There was a
difference in the amount of *E. faecalis* bacterial decrease with and without chlorophyll photosensitiser for 30 and 60 seconds, whereas the number of *E. faecalis* bacteria was at least in the 405 nm laser diode irradiation group with chlorophyll photosensitiser for 60 s.

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