

# Systematic and Local Effect of Doxycycline and Low Intensity Laser Treatment on Periodontitis in Rats

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## ABSTRACT

**Aim:** To evaluate the systematic and local effect of doxycycline and low intensity laser treatment on periodontitis in rats.

**Study design:** Prospective study

**Place and duration of study:** Department of Periodontology, De'Montmorency College of Dentistry, Lahore from 1<sup>st</sup> January 2021 to 30<sup>th</sup> September 2021.

**Methodology:** One hundred of rats (*Rattus norvegicus albinus*) were selected and divided into two groups. In Group I, 50 rats were given induction of periodontal disease while other 50 as Group II were not given the induction of the periodontal disease and were designated as non-treatment group. Lower left 1<sup>st</sup> molar was covered with ten-number cotton threads using altered forceps. Group I rats were further divided into slots of ten rats depending upon the local-treatment they would be receiving including doxycycline and or low intensity laser. After seven days of receiving periodontal disease the ligature was removed from each rat.

**Results:** The animal was euthanized after completion of 30 days follow-up. Post-euthanasia the jaws were removed and preserved in 10% solution of formalin for a period of 48 hours. The left side was then subjected to the x ray imaging after its separation from the right side. The imaging illustrated that there was significant bone loss in the Group II. Considering the bone loss in all the groups it was observed that reduced bone loss was presented in Sub group I c, d and e than Sub group I a and Group II on day seven.

**Conclusion:** Periodontitis was greatly reduced in doxycycline as well as low intensity laser groups systematically as well as locally in comparison with no treatment group.

**Key words:** Periodontitis, Doxycycline, Low intensity laser

## INTRODUCTION

Periodontal disease is caused by host related inflammatory response which is triggered through bio-filming of microbes. These microbes are accumulated around the teeth and causes tissue destruction. Periodontal infections are complex and involved multiple reasons. The progression of this disease is totally dependent on imbalances in host-response. In cases where host-response is poor then periodontium deterioration is exacerbated.<sup>1,2</sup> At the initial stages the tissue of periodontal region presents gingivitis which further progresses into cleavage of collagen fibers and pocket formation and further hyperemia, gingival bleeding and edema.<sup>2</sup> As a result of immune responses cytokines are released and the endotoxins in the periodontal-pockets causes systematic inflammatory-response.<sup>3</sup>

The resorption of bone is seen in the periodontal disease which is protein-controlled mechanism. The receptor-activator of the nuclear factor  $\kappa$ B as well as RANKL also known as RANK ligand and also the osteoprotegerin are the proteins which control this mechanism. Osteoclast-membrane have the presence of RANK in them and allows the dimerization of this membrane by forming tartrate resistant acid-phosphate which are +ve multinuclear cells and are accountable for bone resorption.<sup>4</sup> Forming a complex structure with RANK the RANKL also performs bone resorption.<sup>5</sup>

Root planning as well as scaling are some treatment methods for periodontal bacterial infections which protects the periodontal from the tissue destruction.<sup>6</sup> The disadvantage of scaling and the root planning method is that during the procedure many regions which cannot be accessed by the instrument are left not cleaned from the microbial agents.<sup>7,8</sup> Therefore, a combinational therapy of conventional and adjunctive procedure is considered as best possible method of treatment.<sup>9-11</sup> Doxycycline (DOX) is extensively used antibiotic in therapeutic treatment of periodontitis as it has an anti-inflammatory response inhibiting enzyme release which destroys the collagen. This drug has shown

to protect bacterial protein-synthesis as well as bone resorption.<sup>12</sup> Low intensity laser treatment in combination with scaling and root planning is an adjuvant method of treatment which has shown positive effects on inhibition of inflammation and bone resorption.

The present study was designed to analyze the systematic as well as local doxycycline effect in addition to low-intensity laser treatment on the rat's periodontitis.

## MATERIALS AND METHODS

This prospective study was conducted at Department of Periodontology, De'Montmorency College of Dentistry, Lahore from 1<sup>st</sup> January 2021 to 30<sup>th</sup> September 2021. This study was in consistent with the guidelines provided for conductance of animal studies. A total of 100 of rats (*Rattus norvegicus albinus*) were selected for this study and divided into two groups. Group I have 50 rats given induction of periodontal disease while other 50 as Group II were not given the induction of the periodontal disease and were designated as non-treatment group. The age of the rats was between 2-3 months. The rats were given standard food and protocol with maintained temperature and a dark and light cycle kept maintained twelve hourly. The weight of the rat was between 200-250 grams. The animals were given an anesthetic injection (IM: 75mg/kg of ketamine-hydrochloride and 6mg/kg xylazine-hydrochloride) before induction of the periodontal disease. Lower left 1<sup>st</sup> molar was covered with ten-number cotton thread using altered forceps. Group I rats were further divided into slots of ten rats depending upon the local-treatment they would be receiving. After seven days of receiving periodontal disease the ligature was removed from each rat. 1<sup>st</sup> sub group (Sub group I a) received scaling and root planning treatment while other 2<sup>nd</sup> subgroup received (Sub group I b) Doxycycline and 3<sup>rd</sup> group received (Sub group I c) Doxycycline and scaling and root planning treatment while 4<sup>th</sup> group (Sub group I d) received low intensity laser treatment and 5<sup>th</sup> (Sub group I e) received Doxycycline along intensity laser treatment. The scaling and root planning were performed at buccal a lingual level. An irrigation was performed for 10% Doxycycline gel was made by 10% DOX-hydrochloride, 15% ethyl-cellulose gel, 3% tri-ethanolamine as well as distilled water. A

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1 ml was injected in subgingival region which was then aspirated after a minute. Gel was then placed tropically. In the low intensity laser group with Doxycycline the low intensity laser was administered post a minute of drug deliverance. Low intensity laser used 660nm wavelength and a beam area of 0.04cm<sup>2</sup>. The applied dosage was formulated by already reported equation in literature.<sup>13</sup> Each rat was followed up till 30 days. The periodontitis results were studied through radiological imaging and jaw removal; post rat euthanized. Histopathological analyses were also performed. Data was analyzed by using SPSS version 26.

**RESULTS**

The animal was euthanized after completion of 30 days follow-up. Post euthanasia the jaws were removed and preserved in 10% solution of formalin for a period of 48 hours. The left side

was then subjected to the x ray imaging after its separation from the right side. The imaging illustrated that there was significant bone loss in the Group II while no significant bone loss was observed in Group I (Fig. 1).

Considering the bone loss in all the groups it was observed that reduced bone loss was presented in Sub group I c,d and e than Sub group I a and Group II on day seven , day fifteen and day thirty (Table 1). The histopathological slides showed that an induction of periodontal disease was illustrated in the furcation-region of the mandibular first left molar in non-treatment group at day 15. While similar results were observed at day 30. The immunostaining for tartrate-resistant acid phosphatase was performed in various histopathological slides (Fig. 2).

Table 1: Comparison of bone loss in various groups and sub groups (n=100)

Group I: 5 subgroups (n=50) Group II (n=50)	n.	Time points		
		7 days	15 days	30 days
Negative Control	20	0.32±1.01 <sup>Ba</sup>	0.36±0.02 <sup>Ba</sup>	0.30±1.10 <sup>Ba</sup>
Group II non-treatment	30	1.90±0.17 <sup>Aa</sup>	1.80±0.11 <sup>Aa</sup>	1.53±0.09 <sup>Ab</sup>
Sub group I a	10	1.44±0.27 <sup>Aa</sup>	1.41±0.14 <sup>Aa</sup>	1.36±0.05 <sup>Aa</sup>
Sub group I b	10	0.60±0.21 <sup>Ba</sup>	0.57±0.12 <sup>Ba</sup>	0.53±0.11 <sup>Ba</sup>
Sub group I c	10	0.57±0.12	0.51±0.18	0.48±0.10
Sub group I d	10	0.54±0.22 <sup>Ba</sup>	0.51±0.05 <sup>Ba</sup>	0.50±0.134 <sup>Ba</sup>
Sub group I e	10	0.45±0.07 <sup>Ba</sup>	0.43±0.13 <sup>Ba</sup>	0.40±0.02 <sup>Ba</sup>



Fig. 1: Day 7 radiological image of Group II no treatment given with a scale of 1mm used

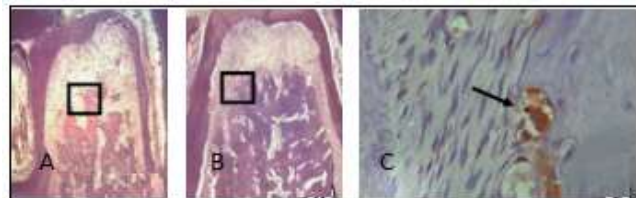


Fig. 2: Histopathological illustration of periodontal disease at day 15(A) and Day 30 (B) and TRAP staining(C)

**DISCUSSION**

This study was in continuation of the proposed model in earlier studies for inducing periodontal disease which also used cotton threads around the rat molars.<sup>14</sup> The result of the ligature is formation of the periodontal disease. This method was preliminary presented by Theodoro et al which explained that antimicrobial activity effects are presented by antimicrobial-photodynamic therapeutics in context to the treatment plan of periodontal disease<sup>15</sup>. Various bacterial accumulation was observed in the ligatures applied. The current study also reported the similar results and found this model as an efficient method for inducing bacterial infection and plaque in the rats as the ligature applied produced inflammation. The main features of the periodontal

disease were gingival-inflammation in addition to redness, edema and loss of gingival reliability<sup>16,17</sup>.

A lower level of infection was presented by the usage of doxycycline as also seen in the current study. This reduction was higher than that observed in the day 15 or day 30 of scaling a root planning group.<sup>18</sup> This treatment plan can be applied in the humans as well with reduction in the periodontal disease by the assistance of doxycycline.<sup>19</sup> The doxycycline seems to be an efficient method in comparison to scaling and root planning. However, in cases where both procedures are combined has given much more reliable results and increases radiographical bone density with significantly improved periodontal factors<sup>20</sup>.

The low intensity laser has also shown well developed connective tissue as well as bone tissue formation with a very less number of tartrate-resistant acid-phosphatase cells in comparison with the non-treatment group or even when compared with the scaling and root planning, doxycycline groups at day seven.<sup>21,22</sup> Similar results have been demonstrated in the present study as well.

**CONCLUSION**

Clinical parameters as gingival-index, bleed by probing and periodontitis were greatly reduced in doxycycline as well as low intensity laser groups systematically as well as locally in comparison with no treatment group.

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