

The Effect of Low Level Laser Therapy on the Eruption of Teeth

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ABSTRACT

Aims of study: Evaluation of the effects of low level laser on the eruption of teeth, on BAP,TRACP, MMP1, MMP3 concentration, on the number of osteoblasts, osteoclasts, bone trabecular thickness, blood vessels and on new bone formation.

Materials and Methods: The eruption rates were measured using Digital Caliper in parts of millimeters. Experimental groups included: A-Laser group: Consisted of 12 rabbits ,the right lower incisor of each rabbit was subjected to laser irradiation with a wave length of 810 nm and output power of 100mW.The animals were divided into3 subgroups according to the doses of laser they received 1-Laser Week One (Lw1) The rabbits received laser in days 0,1and 2. 2-Laser Week Two (Lw2),the rabbits received laser in days 0, 1,2,7,8 and 9. .3-Laser Week Three (Lw3),the rabbits received laser on days 0,1,2,7,8,9,14,15 and 16. B-Control group: Consisted of 12 rabbits which received no treatment .They were randomly divided into three subgroups: 1- Control Week One (Cw1) -Control Week Two (Cw2). 3-Control Week Three (Cw3). Histological evaluation hematoxylin and eosin (HandE) stain was used .Biochemical assay: (ELISA) biochemical tests were employed .These testswere: Rabbit Bone Specific Alkaline Phosphatase (BAP), Rabbit Tartrate Resistant Acid Phosphatase (TRACP), Matrix metalloproteinase 1 (MMP1) and Matrix metalloproteinase 3 (MMP3).Statistical analysis SPSS program: Descriptive statistics and Independent Sample T-Test was performed between the two groups. Values of $p \leq 0.05$ were considered statistically significant.

Results: Laser caused an increase in BAP about two folds in Lw2 to six folds inLw3. Area of new bone formation recorded a significant increase when compared to control and the number of osteocytes was significantly reduced.

Conclusions: Laser enhanced tooth eruption, the rates of eruption was significantly higher than control in week one, week two and week three periods.

Key words: laser, acceleration of eruption, matrix metalloproteinase.

INTRODUCTION

Tooth eruption is a dynamic process that encompasses completion of root development, establishment of the periodontium, and maintenance of a functional occlusion^[1].In order for a tooth to erupt, two obvious requirements are needed. First, there has to be alveolar bone resorption of the bone overlying the crown of the tooth such that an eruption pathway is formed. Second, there has to be a biological process that will result in the tooth moving through this eruption pathway^[2].

Laser irradiation has a variety of effects on tissues^[3]. The effect in tissue depends on the wavelength of laser. The effects of laser radiation which are not accompanied by local temperature increase in tissues by more than 1 °C are called ‘biostimulating effects’. Treatments that take effect via biostimulation potency of laser radiation are called ‘low level laser therapy’^[4].

Laser irradiation has a variety effects on tissues, ranging from biostimulation to photodisruption. Arising effect in the tissue depends on the irradiation time and the energy density. The effects of laser radiation which are not accompanied by local temperature increase in tissues by more than 1°C are called ‘biostimulating effects’. Treatments take effect via biostimulation potency of laser radiation are called ‘low-level laser therapy’ (LLLT) ^[5]. The aims of Studyis to evaluation of the effects of low level laser on the eruption of teeth, studying the effects of low level laser on BAP,TRACP, MMP1, MMP3 concentration, and investigating the effects of low level laser on the number of osteoblasts, Osteoblasts, fibroblasts, blood vessels, collagen fibers and on new bone formation.

MATERIALS AND METHODS

Animals:

The sample consisted of 24 female's new Zealand white rabbits which were obtained from the local farms, of an average weight 600gm (500gm-750gm) housed in an iron cages and fed with vegetables, corn, and grains and supplied with tap water *ad libitum*. They were randomly divided into two groups, one experimental group and one control group of twelve rabbits each. The scientific committee of college of dentistry /University of Mosul approved our protocols. Prior to each treatment, the rabbits were anaesthetized with xylazine 0.2 cc/100 g body weight (interchemie, Holland 20mg/ml) and ketamine 0.2 cc/100 g body weight (Hamlet pharmaceuticals GmbH, Germany 50mg/ml injection) combination.

The eruption rates were measured by marking the right lower incisor of each rabbit by drilling a small hole on the labiodistal side of the tooth near the gingival margin with small round carbide bur in low speed hand piece. A new mark was drilled at every measurement and the distance between the gingival margin and the mark was measured using Digital Caliper in parts of millimeters. The eruption rates were measured twice at three days interval before starting to give the different agents used in this study to provide a record for normal eruption ^[6].

A-Laser group:

Consisted of 12 rabbits, the right lower incisor of each rabbit was subjected to laser irradiation (Laser Diode elexxion /Germany) (Fig.1) with a wave length of 810 nm and output power of 100mW.

The irradiation was performed by a continuous waves by a fiber probe of 8mm in diameter and held perpendicular and in contact with the mucosa. The root of the right lower central incisor was irradiated from five points (two points at distobuccal, one at the distal approximate and two at the distopalatal side) for 108 second each (10.8J/point or 225J/cm²). The energy dose corresponding to at 9 minutes exposure was 45J.(540s,100mw)^[7](Fig.2). The animals were divided into 3 subgroups according to the doses of laser they received

1-Laser Week One (Lw1): The rabbits received laser in day's 0,1 and 2. The eruption rates were measured in day 3 and day 7. The collection of blood and animals scarification was made on day 7.

2-Laser Week Two (Lw2): The rabbits received laser in days 0, 1,2,7,8 and 9. The eruption rates were measured in days 3,7,10 and day 14. The collection of blood and animal's scarification was made on day 14.

3-Laser Week Three (Lw3): The rabbits received laser on days 0,1,2,7,8,9,14,15 and 16. The eruption rates were measured on days 3, 7, 10, 14, 17 and day 21. The collection of blood and animals scarification was made on day 21.

B-Control group: Consisted of 12 rabbits which received no treatment. They were randomly divided into three subgroups:

1- Control Week One (Cw1): The eruption rates were measured on days 3, 7. the collection of blood and animals' scarification was made on day 7.

2-Control Week Two (Cw2): The eruption rates were measured on days 3, 7, 10, 14. The collection of blood and animal's scarification was made on day 14.

3-Control Week Three (Cw3): The eruption rates were measured on days 3,7,10,14, 17 and day 21. The collection of blood and animals scarification was made on day 21.

Histological Evaluation:

The lower jaw of each rabbit was dissected from the head. The anterior teeth with their supporting bone were cut (the cutting was made anterior to the premolars bilaterally). They were then fixed in 10% buffered formalin solution after that they were decalcified in 10% nitric acid for 72 hours. After decalcification they were embedded in paraffin blocks in such a way that the sectioning is made parallel to the long axis of the tooth starting from the inferior border of the jaw. The sectioning was made in 5 micron thickness, stained with hematoxylin and eosin (HandE) stain.

The teeth together with the periodontium and supporting bone were examined under light microscope 450x by a specialist in histology. Bone osteoblasts, osteoclasts, osteocytes, bone trabeculae thickness, blood vessels and areas of new bone formation were examined and recorded in six bone areas around the right lower incisor (mesial and distal) in the coronal, middle and apical levels of the tooth. Recording the number of osteoblasts, number of osteoclasts, number of osteocytes, number of blood vessels and bone trabeculae thickness was performed directly on the

microscope while measuring the percentage of area of new bone formation was done on by Image J computer program by measuring the percentage of number of pickcells of new bone to the number of pickcells of the examined area.

Biochemical assay:

The blood samples were collected into tubes and after settling of about half an hour they were centrifuged for 15 minutes (3000 rpm). The extracted serum sample was stored in -20 °C until they were assayed. They were allowed to melt at room temperature and four Enzyme-linked immunosorbent assay (ELISA) biochemical tests (Biosource USA) were performed in accordance with the manufacturer instructions. These tests were: Rabbit Bone Specific Alkaline Phosphatase (BAP), Rabbit Tartrate Resistant Acid Phosphatase (TRACP), Matrix Metalloproteinase 1 (MMP1), and Matrix Metalloproteinase 3 (MMP3).

Statistical Analysis:

The eruption rate clinical measurements, BAP, TRACP, MMP1 and MMP3 concentration in blood and the histological finding including osteoblasts and osteoclasts, bone trabeculae thickness, blood vessels and areas of new bone formation were statistically analyzed using SPSS program: Descriptive statistics and Independent Sample T-Test between the two groups. Values of $p \leq 0.05$ were considered statistically significant.

RESULTS

The eruption rates are shown in (Table 1) Laser subgroups revealed higher rates of eruption when compared to control and the elevation was significant from week one. The highest mean value was recorded in (Lw3) while (Cw1) showed the lowest eruption rate. (Table 2) demonstrates descriptive statistics of BAP, TRACP, MMP1 and MMP3. The highest BAP concentration was noticed in (Lw3) which also showed the highest maximum value, range and standard error. The lowest mean was observed in (C). The concentration of TRACP was higher in (Lw2) which also recorded the highest maximum value. On the other hand the lowest mean value and standard error was seen (Lw1). The highest elevation seen in MMP1 concentration was in (Lw3) followed by (Lw1) and (Lw2) respectively while (C) showed the lowest mean. A decline in the level of MMP3 concentration in relation to (C) was seen in the three laser subgroups where (Lw3) showed the lowest value followed by (Lw2) and (Lw1) respectively. Independent sample T-Test between control and laser subgroups of MMP1, MMP3, BAP and TRACP Concentrations are shown in (table 3) (Lw1) showed a significantly higher mean value than (C) on the other hand (C) showed a significantly higher MMP3 concentration than (Lw2) and (Lw3).

Blood vessels number was higher in (Lw3) and lower in (Lw1). Bone trabeculae thickness was the highest in (Lw3) which also recorded the highest standard error while the lowest thickness was seen in (C). Bone osteoblasts showed an increase in (Lw3) followed by (Lw2), (Lw1) and (C) respectively. The highest standard error is seen in (Lw1). Bone osteocytes were higher in (Lw3) while (Lw2) recorded the lowest value. A rise in the percentage of new bone formation was noticed in laser subgroups when compared to (C) and the increase was more obvious in (Lw3) followed by (Lw2) and then (Lw1). The comparison between Control and Laser groups is seen in (Table 5). Bone trabeculae thickness was significantly higher in (Lw3) in comparison to Control. On the other hand osteocytes number were significantly lower in (Lw1) and (Lw2) when compared to control. The percentage of area of new bone formation was significantly higher in (Lw2) and (Lw3) in comparison to control.

DISCUSSION

Low level laser therapy (LLLT) have been reported to stimulate bone formation but no information had been documented that laser have an effect on bone resorption.

Acceleration of eruption was achieved in all experimental subgroups when compared to control starting from week one and the acceleration continued to ascend in week two and week three. This rise in the eruption rate was significant in all laser subgroups when compared to control. Ozawa *et al*, 1995^[8] Reported that LLLT resulted in a significant increases in cellular proliferation, bone nodule formation and alkaline phosphatase (BAP) activity. We reported an increase in the level of Alkaline phosphatase concentration and this comes in agreement with the findings of Stein *et al* (2005)^[9]. But it disagrees with the findings of Coombe *et al* (2001)^[10] who found no effect of LLLT on BAP level also it disagrees with the findings of Yoo *et al* (2015)^[11] who found that LLLT decreased bone volume by decreasing mRNA expressions of alkaline phosphatase. We reported no effect of laser on TRACP concentration.

Laser had no effect on MMP1 concentration and this comes in agreement with the findings of Sousa *et al* (2009)^[12], who reported no effect of laser on MMP1 concentration. Low level laser therapy (LLLT) reduced the level MMP3 concentration and this comes in agreement with the findings of Hsieh *et al* (2014)^[13], who reported a significant reduction of MMP3 concentration in rats treated with low level laser therapy.

Laser irradiation has a variety of effects on tissues, ranging from biostimulation to photo disruption. The effects of laser include the stimulation of DNA and RNA synthesis^[14], fibroblast and chondrocyte proliferation^[15,16] and increasing the formation of new capillaries^[17], nerve faster regeneration^[18], wound healing^[19], bone regeneration and enhance new bone formation^[20,21].

In this study no significant difference was found in number of osteoblasts between laser and control groups and this comes in agreement with the findings of Coommo *et al*(2000)^[10] who found that osteoblasts division and proliferation were not affected by low level laser irradiation and also in agreement with the findings of Ninomiya *et al*(2007)^[21] who stated that laser irradiation increases osteoblasts activity not number. Our findings disagreed with those of Ko *et al*(2013)^[22] who found a significant increase in number of osteoblasts in laser irradiated samples compared to control. Renno, *et al* (2007)^[23] found that osteoblasts proliferation increased significantly after irradiation with 830 nm laser (at 10 J/cm²) and decreased osteoblasts proliferation after irradiation with 780nm laser (at 1.5 and 10 J/cm²).

Although the osteocytes number was significantly reduced when compared to control in Lw1 and Lw2 subgroups, no significant difference was recorded in Lw3 which disagrees with the findings of (Ko *et al* 2013)^[22] who recorded a significant increase in the number of osteocytes.

On the other hand bone trabeculae thickness showed a significantly higher value when compared to control in Lw3 and this comes in agreement with the findings of Ko *et al*(2013)^[22] and Ninomiya *et al*(2007)^[21].

Blood vessels showed no significant increase when compared to control and this disagrees with the findings of Corazza *et al* (2007)^[24] and Colombo *et al*(2013)^[25] who found a significant increase of blood vessels number after laser treatment. The varying results obtained might be attributed to the different laser wave length, doses and the energy employed and could be also related to the fact that these studies were applied to soft tissues and not to bone.

CONCLUSIONS

Low level laser therapy enhanced tooth eruption and the acceleration in eruption rates were noticed in week one and raised in week two and week three of laser application when compared to control.

Table 1: Descriptive statistics and Independent Sample T-Test Of Eruption Rate Between Control and Laser Subgroups Groups mm/d

	Range	Minimum	Maximum	Mean	Std. Error	T-value	Sig.
LW1	.663	.732	1.395	1.100	.076	-5.237	0.000**
Cw1	.145	.611	.756	.676	.021		
LW2	.411	.899	1.310	1.055	.047	-3.038	0.000**
Cw2	.174	.573	.747	.721	.023		
LW3	.075	1.140	1.215	1.172	.010	-8.352	0.000**
Cw3	.223	.567	.765	.733	.027		

*significant at $P \geq 0.05$

Table 2: Descriptive statistics (Range, Minimum, Maximum, Mean, Std. Error of BAP, TRACP, MMP1 and MMP3

BAP	Range	Minimum	Maximum	Mean	Std. Error
Control	39.000	3.000	42.000	15.425	9.070
Lw1	70.600	69.700	140.300	98.650	14.892
Lw2	39.700	17.300	57.000	36.925	9.470
Lw3	219.100	4.900	224.000	108.800	49.077
TRACP					
Control	48.300	3.600	51.900	31.050	10.480
Lw1	19.700	10.700	30.400	20.900	4.191
Lw2	38.400	18.400	56.800	38.500	10.216
Lw3	37.200	12.800	50.000	26.100	8.390

MMP1					
Control	4.100	3.800	7.900	5.650	.856
Lw1	3.900	4.400	8.300	6.050	.835
Lw2	2.800	4.000	6.800	5.750	.606
Lw3	3.400	4.900	8.300	7.075	.792
MMP3					
Control	4.300	12.000	16.300	14.100	1.106
Lw1	4.200	11.100	15.300	12.650	.928
Lw2	1.200	10.400	11.600	10.800	.273
Lw3	1.000	9.800	10.800	10.500	.234

Table 3: Independent Sample T-Test between Control and Laser SubgroupsofMMP1, MMP3, BAP and TRACP Concentrations

	MMP1		MMP3		BAP		TRACP	
	T value	Sig	T value	Sig	T value	Sig	T value	Sig
Lw1	-0.334	0.750	1.004	0.355	-4.773	0.005**	0.889	0.420
Lw2	-0.095	0.928	2.894	0.028*	-1.640	0.152	-0.905	0.629
Lw3	-1.221	0.268	3.182	0.019*	-1.871	0.111	0.369	0.770

*significant at $P \geq 0.05$

Table 4: Descriptive statistics (Range, Minimum, Maximum, Mean, Std. Error Of Blood Vessels , BTT, Osteoblasts, Osteocytes and Percentage of Area of New Bone Formation

Group	Range	Minimum	Maximum	Mean	Std. Error
Control	3.00	1.00	4.00	2.500	.428
Lw1	3.00	1.00	4.00	2.166	.600
Lw2	5.00	1.00	6.00	2.500	.846
Lw3	4.00	1.00	5.00	2.666	.557
BTT					
Control	232.00	88.00	320.00	140.666	36.477
Lw1	120.00	80.00	200.00	124.666	19.055
Lw2	220.00	180.00	400.00	240.000	33.862
Lw3	260.00	140.00	400.00	316.666	42.713
Osteoblasts					
Control	9.00	3.00	12.00	7.666	1.333
Lw1	12.00	6.00	18.00	9.445	2.170
Lw2	12.00	6.00	18.00	10.666	1.837
Lw3	12.00	6.00	18.00	11.333	1.977
Osteocytes					
Control	10.00	10.00	20.00	14.833	1.720
Lw1	4.00	4.00	8.00	6.166	.749

Lw2	12.00	2.00	14.00	6.000	1.693
Lw3	18.00	8.00	26.00	15.333	3.602
%A.N.B.F.					
Control	.847	.770	1.617	1.131	0.194
Lw1	1.448	.987	2.435	1.730	0.309
Lw2	1.887	2.100	3.987	2.966	0.387
Lw3	1.654	5.657	7.311	6.312	0.356

Table 5: Independent Sample T- Test between Control and Laser Subgroups of Blood Vessels, BTT, Osteoblasts, Osteocytes and Percentage of Area of New Bone Formation

	Blood Vessels		BTT		Osteoblast		Osteocyte		% A.N.B.F.	
	T. value	Sig.	T.value	Sig.	T. value	Sig.	T. value	Sig.	T. value	Sig.
Lw1	1.414	0.190	0.389	0.706	0.435	0.673	4.618	0.001*	-1.986	0.162
Lw2	0.452	0.662	1.996	0.074	0.168	0.872	3.659	0.004*	5.586	0.011*
Lw3	0.000	1.000	3.133	0.011*	0.472	0.647	0.125	0.903	15.417	0.001*

*significant at $P \geq 0.05$



Figure 1: Laser Diode elexxion /Germany



Figure 2: A rabbit during Laser application

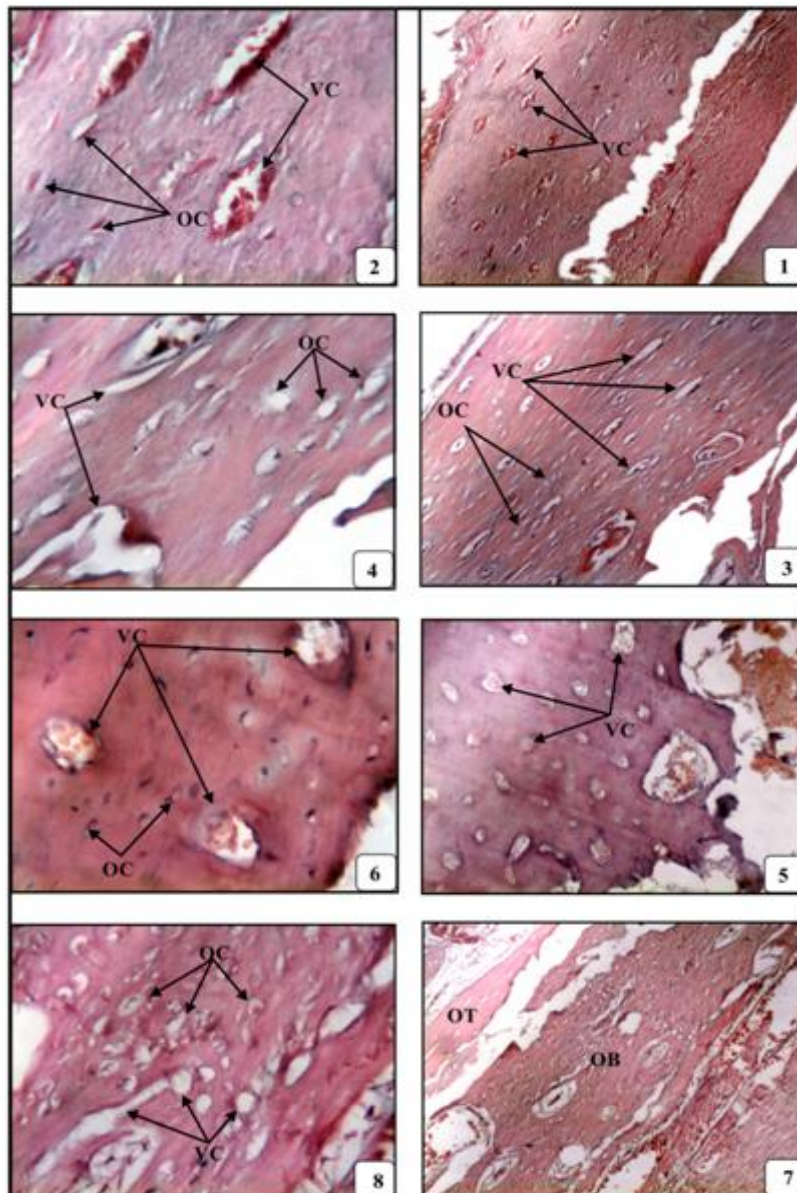


Figure 3: Histological Evaluation Figures

- 1- Laser Week One photomicrograph of decalcified bone from a rabbit one week after treatment with laser .vascular channel (VC) and osteocytes (OC) are visible. H&E, 100X.
- 2- Laser Week One Higher power magnification of an area of the previous section. Note the vascular channels (VC) and osteocytes (OC). H&E, 400X.
- 3- Laser week Two photomicrograph of decalcified bone from a rabbit two week after treatment with laser . Numerous vascular channels (VC) are discernible. H&E, 100X.
- 4- Laser Week Two Higher power magnification of an area of the previous section. Vascular channels (VC) and osteocytes (OC) are visible. H&E, 400X .
- 5- Laser Week Three photomicrograph of decalcified bone from a rabbit three weeks after treatment with laser. New mature osseous tissue (OT) adjunct to old bone (OB) could be visualized. H&E, 100X.
- 6- Laser Week Three Higher power magnification of an area of the previous section. Vascular channels (VC) and osteocytes (OC) could be seen. H&E, 400X .

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