10.21608/adjc.2020.22135.1051 Advanced Dental Journal
Volume 2 (2020) | Issue 4| Pages 184-200

Original Article

Diode Laser Application versus Ibuprofen in Treatment of Ligature-Induced Periodontitis in Albino Rats: Histological and Immunohistochemical study

Liza Mohsen Monir[1], Ahmed Mahmoud Halawa[2], Nehad Samir Taha[1], Dina Mohamed Abdel Khalik[2]

[1]Oral Biology Department, Faculty of Dentistry, Misr International University
[2]Oral Biology Department, Faculty of Dentistry, Ain Shams University

Email: lizatakla@gmail.com

Submitted: 9-2-2020
Accepted: 30-4-2020

Abstract

Introduction: Plaque induced periodontitis is the second most common oral disease worldwide after dental caries. Documents from animal experiments proved that NSAIDs can reduce the inflammation and the rate of resorption of alveolar bone thus, stabilize the periodontal condition. In the last 10 years, diode laser also has been highly recommended as a treatment modality for periodontal inflammatory diseases due to its anti-inflammatory effect, ability to accelerate the healing process and its pain reduction ability.

Materials and Methods: 84 albino rats were divided into 4 groups. Group I is the negative control group which did not subjected to any procedure, group II is the positive control group which is subjected to ligature induced periodontitis but did not take any treatment, group III is the diode laser group which is subjected to ligature induced periodontitis and treated by diode laser irradiation after removal of the ligature and group IV is the ibuprofen group which is subjected to ligature induced periodontitis and treated by ibuprofen syrup for 4 days after removal of the ligature.

Results: inflammatory cell count was measured in all specimens stained by H&E and area fraction was measured in all specimens stained by MMP-1 immuno histochemical marker. Total amount of inflammatory cells and MMP-1 decreased by time with a significant difference between treatment groups and positive control group.

Conclusion: Diode laser irradiation showed greater and faster reduction in inflammation than ibuprofen.

Key words: Periodontitis, Diode laser, Ibuprofen, Periodontal ligaments.

Introduction

Periodontal disease is an inflammatory disease which is caused by accumulation of dental plaque in a biofilm, and it's characterized by clinical signs of inflammation such as bleeding on probing and loss of periodontal tissue support **(de Almeida et al, 2008).** Although mechanical control of bacterial biofilm and calculus is the most indicated treatment for

periodontitis, this therapy is not completely effective in removal of bacteria in some conditions .It may fail in eradicating of the pathogenic bacteria in the inaccessible areas such as furcation areas. These concerns are considered limiting factors for long time stability of treatment outcomes of non-surgical techniques **(Saglam et al, 2014, Garcia et al, 2016).** With the increase of progression and severity of periodontal disease in animals and human, there is increase in the level of cyclooxygenase products (COX) especially prostaglandin E2 (PGE2). Cyclooxygenase is the critical enzyme in the formation of prostaglandin E2 from arachidonic acid .Inhibition of PGE2 synthesis by the direct inhibition of the cyclooxygenase enzyme has been achieved by non-steroidal anti-inflammatory drugs (NSAIDs). It has been reported that NSAIDs may slow the rate of alveolar bone loss and attachment loss in many animal models, including ligature-induced periodontitis and naturally occurring periodontitis **(de Vasconcelos Gurgel et al, 2004, Garcia et al, 2010).** The development of high-power lasers that employ fiber optic technology offers a large advantage because such lasers can generate potent and local bactericidal effects. One type of high-power laser, the diode laser (DL), may reduce bacterial loads while causing few unwanted thermal side effects **(Theodoro et al, 2015).**

The Aim of the study was to compare the efficacy of diode laser versus ibuprofen in treatment of ligature induced periodontitis in albino rats.

Materials and Methods

A) Materials:

I. Animals:

The experimental protocol was approved by the Ethics Committee of Ain Shams University.
Eighty four adult male Albino Wistar rats within an average weight 150-200 grams; were obtained from Modern Veterinary Office (MVO), Egypt. The number of rats was determined by sample size calculation for every subgroup (A, B and C) of the main 4 groups and this was based upon the results of **(Theodoro et al., 2015).** Using alpha level of 0.05 (5%) and β level of 0.80 (80%), the estimated minimum required sample size was approximately 5 rats in each subgroup. The animals were housed in controlled environment (temperature 25 + or – 2 and 12 hours dark/light cycles) and fed with standard pellets diet, tap water adlibitum. They were kept in individual cages in a controlled room (temperature 20-25 C; humidity 70-80%). All experiments were conducted in the animal house, Modern Veterinary Office. All efforts were made to reduce the number of animals used and to minimize their suffering.

II. Laser:
Irradiation with diode laser (BIOLASE) was used once with a pulse interval of 20 ms and pulse length of 20 ms delivering 20 s/ cm2, wave length 940 nm, non-initiated tip, at a power of 0.5-1.5 W, continuous wave mode. Irradiation was conducted at Laser Center, Misr International University.

III. Non-steroidal anti-inflammatory drug:

Ibuprofen syrup has been administrated orally by gastric gavage using syringe by the dosage of 20mg/kg once daily for four days after induction of periodontitis and removal of ligature.

IV. Ligature:

Sterile 4/0 non-resorbable silk ligature was used.

Animal Grouping:

84 animals were randomly allocated to four equal groups in three sacrifices periods (sacrifice at 7, 15, and 30 days) 7 animals per sub group **(Oliveira et al., 2017).** Rats were randomly selected using computer generated randomization (www.randomizer.org). Allocation concealment was achieved using a sealed coded opaque envelope containing treatment of the subject.

B) Methods:

Induction of periodontitis:

Group II, III and IV were assigned to ligature. All procedures of periodontal disease induction were performed under general anesthesia by intramuscular injection of a combination of 0.1 ml ketamine hydrochloride (50 mg/ml) and 0.05 ml xylaine hydrochloride (2 g/100ml) for each 100 g body weight. After anesthesia, sterile 4/0 silk ligature was placed in a sub-marginal position on the upper central incisors of each animal to induce experimental periodontitis for 7 days period and the ligature was knotted on the labial side.

The sutures were checked after application every 2 days, and lost or loose sutures were replaced.

After the 7th day, the ligatures were removed and the treatment was begun.

After treatment, Group II, III and IV were equally subdivided into three subgroups A, B and C respectively (7 rats each) for termination **(Theodoro et al., 2016).**

Sub group A: sacrificed at day 7
Sub group B: sacrificed at day 15
Sub group C: sacrificed at day 30
Each sub group was euthanized by administration of a lethal dose of thiopental 150 mg/kg.

Results

Hematoxylin and Eosin Stain:

Group I (Negative Control group):

The negative control group showed normal tissues, PDL fibers are well organized with normal sized interstitial tissues, and the bone is normal with resting lines.

Group II (Positive control group):

Sub group A: The positive control group at day 7 (sub group A) showed complete disorganization and detachment of the PDL fibers with inflammatory cell infiltrate, narrow longitudinal interstitial tissues and cementum PDL detachment Fig.(1). **Sub group B:** At day 15 (sub group B), PDL fibers are still disorganized but with less inflammatory cell infiltrate than sub group A, large interstitial tissues with congested B.V are noticed, PDL bone detachment is still observed at some areas and the bone is showing many resting and reversal lines Fig. (1). **Sub group C:** At day 30 (sub group C), disorganization and tearing of the PDL fibers are still noticed with more less inflammatory cell infiltrate than sub group B, also large interstitial tissues with congested B.V, PDL bone detachment is less and the bone is showing many resting lines and reversal lines Fig. (1).

Group III (Diode laser treated group):

Sub group A: The diode laser group at day 7 showed disorganization of the periodontal ligament fibers and tearing in some areas with some inflammatory cells infiltrate. Irregular bone surface with abundant cuboidal osteoblasts have been noticed also Fig. (2). **Sub group B:** At day 15, PDL fibers are more organized with less inflammatory cells infiltrate than sub group A. Interstitial tissues are wide at some areas. Smoother bone surface lined by osteoblasts and obvious reversal lines were recognized Fig. (2). **Sub group C:** At day 30, PDL fibers have better orientation with no big difference of inflammatory infiltrate than sub group B, interstitial tissues were found in normal size and smooth bone surface with more than one reversal lines have been noticed Fig. (2).

Group IV (Ibuprofen treated group):

Sub group A: The ibuprofen treated group at day 7 showed tearing of the PDL fibers with inflammatory cell infiltrate, wide interstitial tissues with congested blood vessels, some cuboidal osteoblasts resting on bone surface and normal bone appearance with resting lines Fig. (3). **Sub group B:** At day 15, PDL fibers are still disorganized but with less inflammatory cell infiltrate than subgroup A, multiple interstitial tissues with congested blood vessels have been noticed, and there is good bone PDL attachment and normal bone appearance with resting line Fig. (3). **Sub group C:** At day 30, the PDL fibers show better organization, multiple interstitial tissues

have been noticed also, and there is good bone PDL attachment and normal bone appearance with resting line Fig. (3).

MMP1 Immuno-Stain:

Group I (Negative Control):

The PDL fibers and adjacent bone of group I showed normal tissues with immune-negativity to MMP-1 with Hematoxylin counterstain**.**

Group II (Positive control):

Sub group A: Immunohistochemical examination of the PDL fibers of group II at 7th day revealed active MMP-1 expression highlighted as brown cytoplasmic staining of PDL cells (fibroblast/fibrocyte) and extracellular matrix staining between collagen fibers Fig (4). **Sub group B:** PDL fibers of group II at 15th day showed also positive reaction to MMP-1 but lesser than subgroup A, noticed as brown staining of PDL cells (fibroblast/fibrocyte) and extracellular matrix staining between collagen fibers Fig (4). **Sub group C:** At 30th day of group II, PDL fibers showed active MMP-1 expression highlighted as brown cytoplasmic staining of PDL cells (fibroblast/fibrocyte) and extracellular matrix staining as well with almost no difference with subgroup B Fig. (4).

Group III (Diode Laser treated group):

Sub group A: The PDL fibers of group III at 7th day showed less immune-positive reaction to MMP1 than positive control group. It was seen as intracellular cytoplasmic staining of PDL cells (fibroblast/fibrocyte) and extracellularly in the extracellular matrix between collagen fibers as seen in the positive control group Fig. (5). **Sub group B:** Immuno-histochemical examination of PDL fibers of group III at 15th day revealed obvious reduction in MMP1 positive cytoplasmic staining of PDL cells with less staining in the extracellular matrix Fig. (5). **Sub group C:** At 30th day the PDL fibers of group III also showed further reduction of MMP1 positive staining in both intracellular and extracellular matrix Fig. (5).

Group IV (Ibuprofen treated group):

Sub group A: Immunohistochemical examination of PDL fibers of group IV at 7th day revealed that the positive reactivity of MMP1 is less than of group II and shown as cytoplasmic staining of fibroblast/fibrocyte and extracellular matrix staining between PDL fibers Fig. (6). **Sub group B:** At 15th day of group IV, PDL fibers examination continued to show positive staining of MMP1 to fibroblast/fibrocyte cytoplasm and also for extracellular matrix but to a lesser extent than in subgroup A Fig. (6). **Sub group C:** The PDL fibers of group IV at 30th day showed a slight more reduction in the intracellular and extracellular staining of MMP1 Fig. (6).

Statistical Analysis:

A) MMP-1 area fraction

Area fraction was measured by using Image J software program in Misr International University **(Matos et al., 2006).** (Fig. 7), discriptive statistics , comparison and post-hoc analysis are shown in Tables 1 and 2

Tukey's post hoc test: was within the same observation time, means sharing the same superscript letter are not significantly different

Effect of time within the same group

In positive control, the mean value gradually decreased by time with a statistically significant difference (P=0.015). Tukey's post hoc test revealed no significant difference between 15 and 30 days (Table 3, Fig. 8). In Ibuprofen, the mean value gradually decreased by time with a statistically significant difference (P=0.05). Tukey's post hoc test revealed no significant difference between 15 and 30 days (table 3, Fig. 8). In laser group, the mean value gradually decreased by time with a statistically significant difference (P=0.002). Tukey's post hoc test revealed no significant difference between 15 and 30 days (Table 3, Fig. 8).

Tukey's post hoc test: within the same group, means sharing the same superscript letter are not significantly different.

B) Number of inflammatory cells (table 4a)

Tukey's post hoc test: within the same observation time, means sharing the same superscript letter are not significantly different (Table 4b, fig. 9)

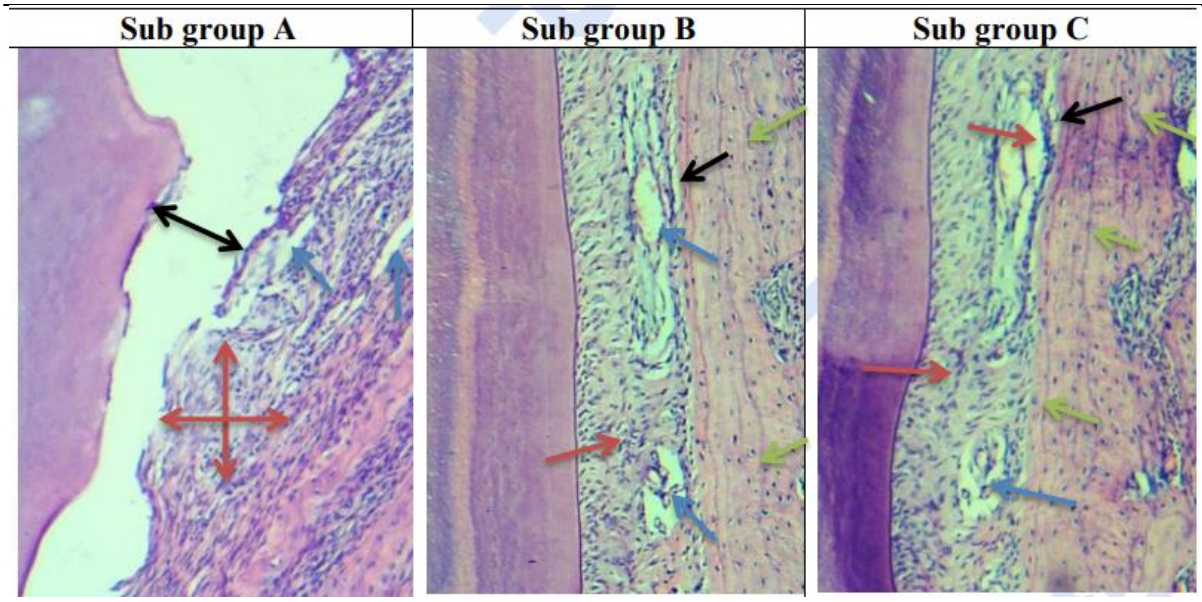

Fig. (1): Sub Gp, A: photomicrograph of the PDL fibers and adjacent bone of group II at day 7 showing complete disorganization of the PDL fibers with inflammatory cell infiltrate (red arrows), narrow longitudinal interstitial tissues (blue arrows) and cementum PDL detachment (black arrows). **Sub Gp B**: photomicrograph of the PDL fibers and adjacent bone of group II at day 15 showing disorganization of the PDL fibers but with less inflammatory cell infiltrate than sub group A (red arrow), large interstitial tissues with congested B.V (blue arrows), PDL bone detachment at some areas (black arrow) and bone with resting lines and reversal lines (green arrows). **Sub Gp C:** photomicrograph of the PDL fibers and adjacent bone of group II at day 30 showing disorganization and tearing of the PDL fibers with more less inflammatory cell infiltrate than sub group B (red arrows), large interstitial tissues with congested B.V (blue arrow), PDL bone detachment at some areas (black arrow) and bone with resting lines and reversal lines (green arrows). (Org. mag. X100)

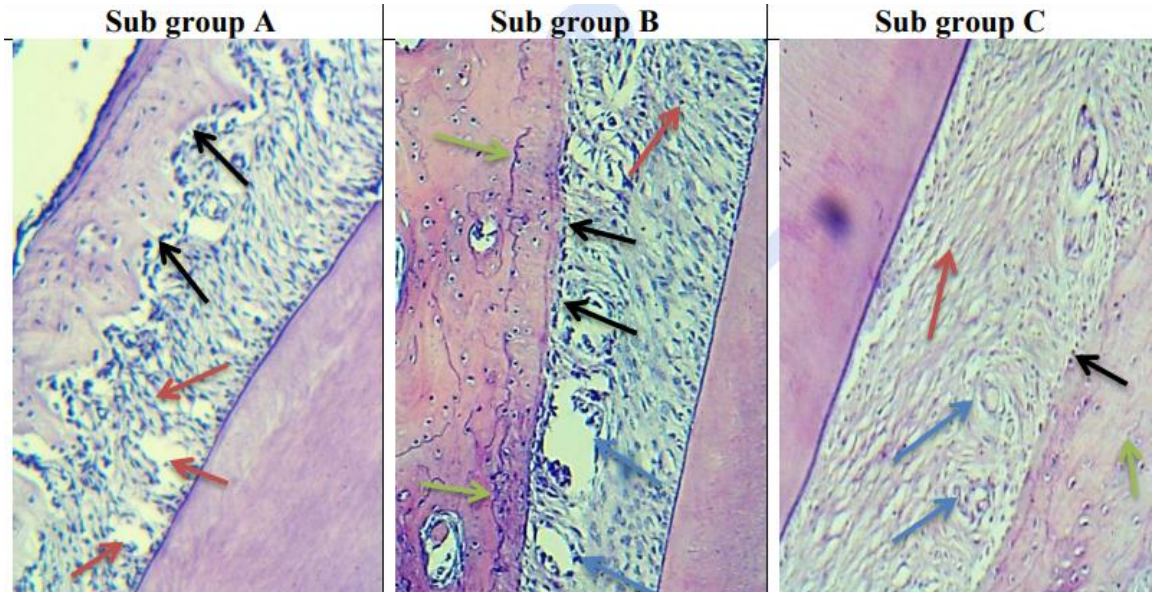

Fig. (2):Sub Gp A: photomicrograph of the PDL fibers and adjacent bone of group III at day 7 showing irregular arrangement of the oblique fibers and tearing in some areas with some inflammatory cells infiltrate (red arrows) and irregular bone surface with abundant osteoblasts on the surface (black arrows). **Sub Gp B:** photomicrograph of the PDL fibers and adjacent bone of group III at day 15 showing normal arrangement of the oblique fibers with less inflammatory cells infiltrate than sub group A (red arrow), wide multiple interstitial tissue (blue arrows), good PDL bone attachment with continuous layer of osteoblasts (black arrows) and clear reversible line denoting new bone formation (green arrows). **SubGp C:** photomicrograph of the PDL fibers and adjacent bone of group III at day 30 showing normal oblique arrangement of fibers with no big difference of inflammatory cell infiltrate than subgroup B (red arrow), normal size interstitial tissue (blue arrows), very good attachment between bone and PDL with few osteoblasts on bone surface (black arrow) and multiple reversible lines (green arrows). (Org. mag. X100)

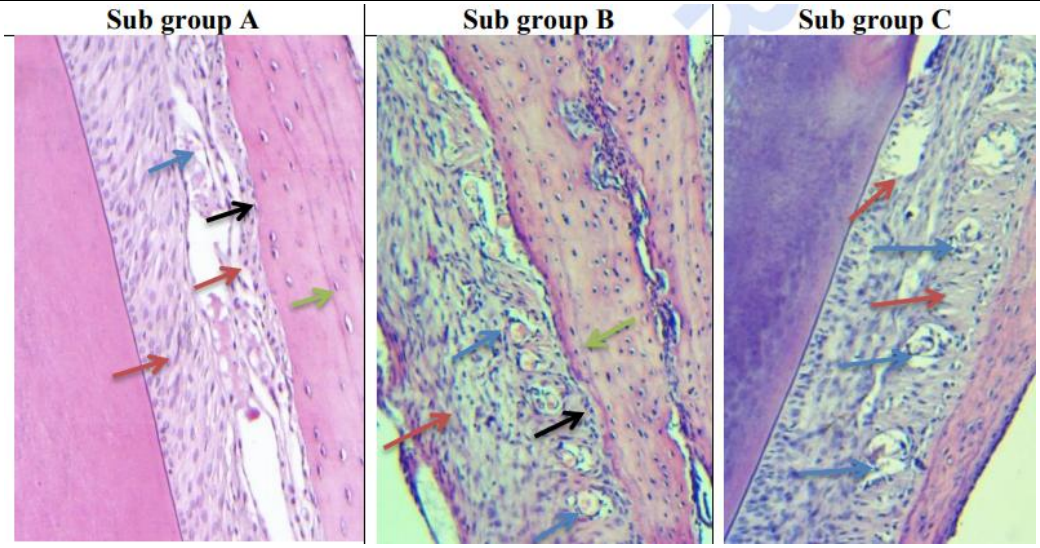

Fig. (3): Sub Gp A: photomicrograph of the PDL fibers and adjacent bone of group IV at day 7 showing tearing of the PDL fibers with inflammatory cell infiltrate (red arrows), wide interstitial tissue with congested blood vessels (blue arrow), good bone PDL attachment with some osteoblasts on the surface (black arrow) and normal bone appearance with resting lines (green arrow). **Sub Gp B:** photomicrograph of the PDL fibers and adjacent bone of group IV at day 15 showing disorganization of the PDL fibers but with less inflammatory cell infiltrate than subgroup A (red arrow), multiple interstitial tissues with congested blood vessels (blue arrows), osteoblasts on bone surface (black arrow) and normal bone appearance with resting line (green arrow). **Sub Gp C:** photomicrograph of the PDL fibers and adjacent bone of group IV at day 30 showing better organization of the PDL fibers at some areas but tearing in others (red arrows), multiple interstitial tissues with congested blood vessels (blue arrows), and normal bone appearance with resting lines (green arrow). (Org. mag. X100)

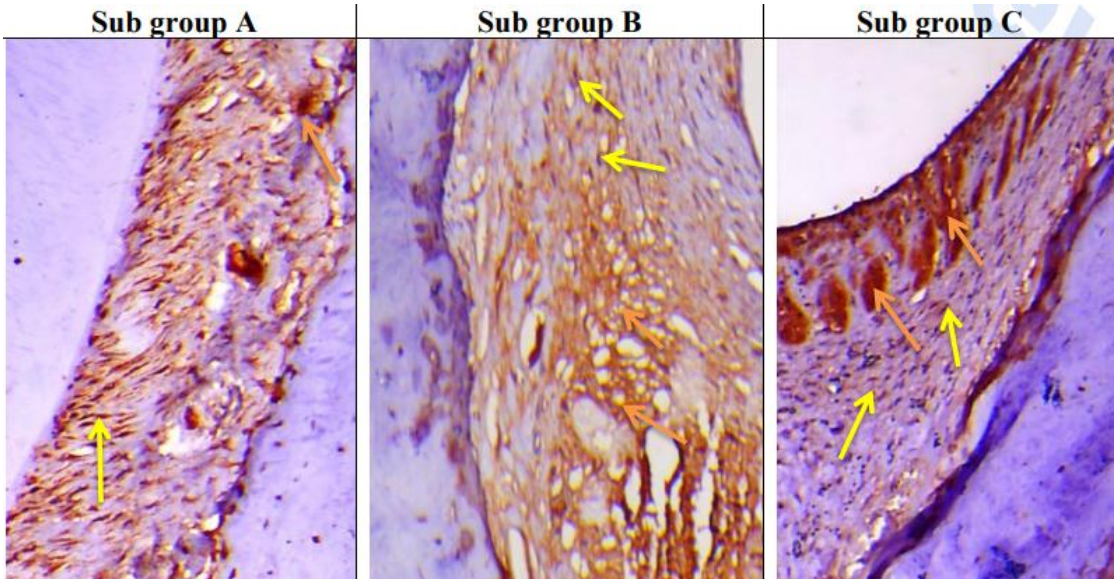

Fig. (4):Sub Gp A: A photomicrograph of the PDL fibers of group II at 7th day showing active MMP-1 expression highlighted as brown cytoplasmic staining of PDL cells, fibroblast/fibrocyte (yellow arrows) and staining of the extracellular matrix between collagen fibers (orange arrows). **Sub Gp B:** A photomicrograph of the PDL fibers of group II at 15th day showing active MMP-1 expression but lesser than subgroup A noticed as brown cytoplasmic staining of PDL cells, fibroblast/fibrocyte (yellow arrows) and extracellular matrix staining between collagen fibers (orange arrows). **Sub Gp C:** A photomicrograph of the PDL fibers of group II at 30th day showing active MMP-1 expression highlighted as brown cytoplasmic staining of PDL cells, fibroblast/fibrocyte (yellow arrows) and extracellularly in the extracellular matrix between collagen fibers (orange arrows). (Org. mag. x200 counterstain: Hematoxylin)

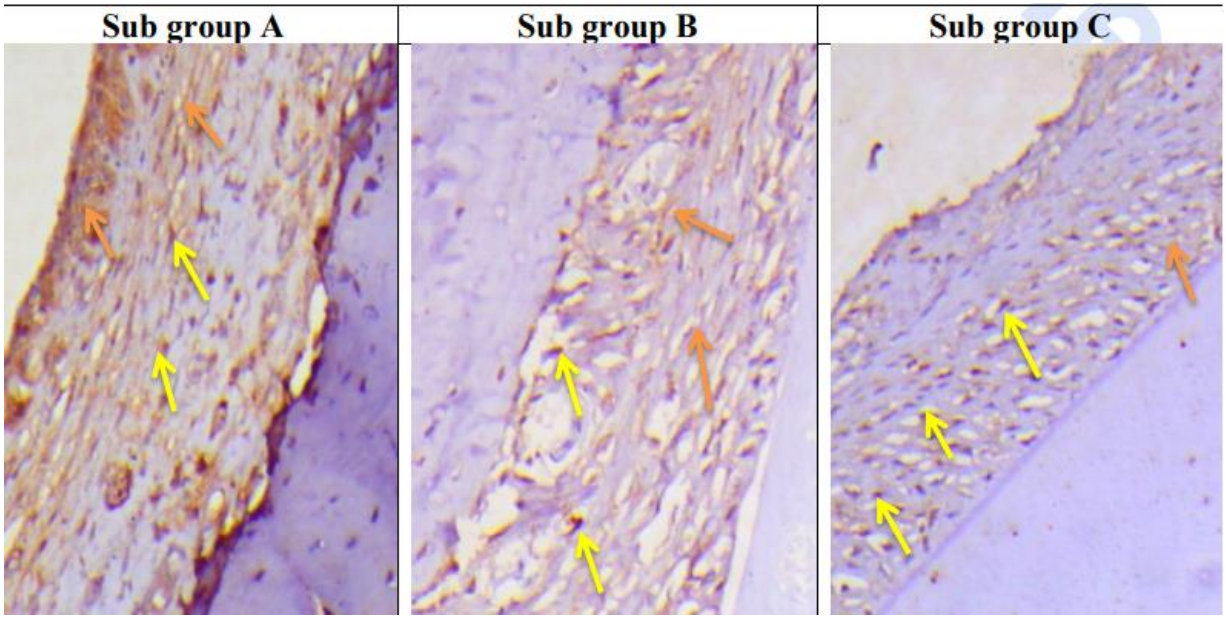

Fig. (5): Sub Gp A: A photomicrograph of the PDL fibers of group III at 7th day showing less immune-positive reaction to MMP1 than positive control group intracellularly in PDL cells, fibroblast/fibrocyte (yellow arrows) and extracellularly in the extracellular matrix between collagen fibers (orange arrows). **Sub Gp B:** A photomicrograph of the PDL fibers of group III at 15th day showing obvious reduction in MMP1 positive cytoplasmic staining of PDL cells (yellow arrows) with less staining in the extracellular matrix (orange arrows). **Sub Gp C:** A photomicrograph of the PDL fibers of group III at 30th day showing further reduction in MMP1 positive cytoplasmic staining of PDL cells (yellow arrows) with less staining in the extracellular matrix (orange arrows**).** (Org. mag. x200 counterstain: Hematoxylin)

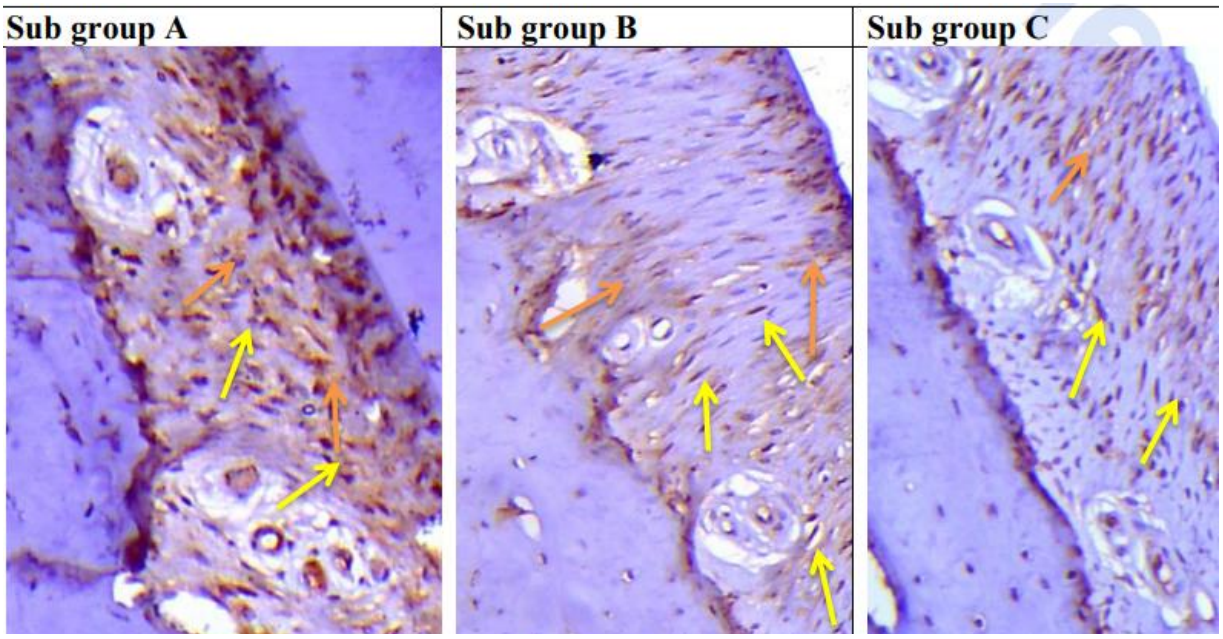

Fig. (6): Sub Gp A: A photomicrograph of the PDL fibers of group IV at 7th day showing positive cytoplasmic cellular staining of fibroblast/fibrocyte (yellow arrows) with extracellular matrix staining between PDL fibers (orange arrows) but less than of group II. **Sub Gp B:** A photomicrograph of the PDL fibers of group IV at 15th day showing a reduction of positive intracellular staining (yellow arrows) and also reduction of extracellular staining (orange arrows). **Sub Gp C:** A photomicrograph of the PDL fibers of group IV at 30th day showing slight more reduction of positive intracellular staining (yellow arrows) and also less staining of extracellular matrix (orange arrows). (Org. mag. x200 counterstain: Hematoxylin)

Table (1) : Descriptive statistics and comparison of mean value of area fraction in different groups (ANOVA test)

		Mean	Std. Dev	Std. Error	95% CI		Min	Max	F	P
					Lower Bound	Upper Bound				
7days	**Negative control**	1.97[c]	.95	.36	1.08	2.85	.81	3.31	117.71	0.00*
	Positive control	38.12[a]	5.44	2.06	33.09	43.15	31.29	48.82		
	Ibuprofen	15.73[b]	4.45	1.68	11.61	19.85	8.45	20.68		
	Laser	7.17[c]	3.21	1.21	4.20	10.14	4.01	12.39		
15days	**Negative control**	1.97[b]	.95	.36	1.08	2.85	.81	3.31	25.58	0.00*
	Positive control	26.68[a]	10.34	3.91	17.12	36.25	10.74	43.94		
	Ibuprofen	9.83[b]	5.51	2.08	4.74	14.92	3.84	19.76		
	Laser	3.37[b]	1.63	.62	1.86	4.88	1.96	6.79		
30days	**Negative control**	1.97[b]	.95	.36	1.08	2.85	.81	3.31	36.18	0.00*
	Positive control	26.14[a]	6.33	2.39	20.28	32.00	19.82	36.87		
	Ibuprofen	9.18[b]	7.50	2.83	2.25	16.12	.65	24.85		
	Laser	2.68[b]	.78	.29	1.96	3.40	1.93	3.96		

Significance level $p \leq 0.05$, * significant

Effect of time

In positive control, the mean value gradually decreased by time with a statistically significant difference (P=0.00). Tukey's post hoc test revealed a significant difference between each 2 observation times (Table 5, Fig. 10). In Ibuprofen, the mean value gradually decreased by time with a statistically significant difference (P=0.003). Tukey's post hoc test revealed no significant difference between 15 and 30 days (Table 5, Fig. 10). In laser group, the mean value gradually decreased at 15 days, then slightly increased at 30 days, with no significant difference between the three observation times (Table 5, Fig. 10).

Table (2): Detailed results of Tukey's post hoc test for pairwise comparison of mean area fraction of MMP1

MMP1-area fraction	(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.	95% CI	
						Lower Bound	Upper Bound
7days	Negative control	Positive control	-36.15714-*	2.08	.00	-41.90	-30.42
		Ibuprofen	-13.76429-*	2.08	.00	-19.50	-8.03
		Laser	-5.20429	2.08	.09	-10.94	.53
	Positive control	Ibuprofen	22.39286*	2.08	.00	16.65	28.13
		Laser	30.95286*	2.08	.00	25.21	36.69
	Ibuprofen	Laser	8.56000*	2.08	.00	2.82	14.30
15days	Negative control	Positive control	-24.71714-*	3.17	.00	-33.47	-15.97
		Ibuprofen	-7.86286	3.17	.09	-16.61	.89
		Laser	-1.40714	3.17	.97	-10.16	7.34
	Positive control	Ibuprofen	16.85429*	3.17	.00	8.11	25.60
		Laser	23.31000*	3.17	.00	14.56	32.06
	Ibuprofen	Laser	6.45571	3.17	.20	-2.29	15.20
30days	Negative control	Positive control	-24.17571-*	2.64409	.000	-31.4697	-16.8817
		Ibuprofen	-7.21714	2.64409	.053	-14.5112	.0769
		Laser	-.71286	2.64409	.993	-8.0069	6.5812
	Positive control	Ibuprofen	16.95857*	2.64409	.000	9.6646	24.2526
		Laser	23.46286*	2.64409	.000	16.1688	30.7569
	Ibuprofen	Laser	6.50429	2.64409	.093	-.7897	13.7983

Significance level $p \leq 0.05$, * significant

Table (3) : Comparison of area fraction in different observation times within the same group (ANOVA test)

	7 days	**15 days**	**30 days**	**F**	**P**
Negative control	1.97±0.95	1.97±0.95	1.97±0.95	------	
Positive control	38.12[a]±5.44	26.68[b]±10.34	26.14[b]±6.33	3.35	0.015*
Ibuprofen	15.73[a]±4.45	9.83[b]±5.51	9.18[b]±7.5	2.59	0.05*
Laser	7.17[a]±3.21	3.37[b]±1.63	2.68[b]±0.78	9.05	0.002*

Significance level p≤0.05, * significant

Table (4a) Descriptive statistics and comparison of mean count of inflammatory cells in different groups (ANOVA test)

		Mean	**Std. Dev**	**Std. Error**	**95% CI**		**Min**	**Max**	**F**	**P**
					Lower Bound	Upper Bound				
7days	**Negative control**	6.71[b]	1.38	.52	5.44	7.99	5.00	8.00	30.98	0.00*
	Positive control	80.14[a]	32.54	12.30	50.05	110.24	45.00	130.00		
	Ibuprofen	22.43[b]	3.78	1.43	18.93	25.92	18.00	27.00		
	Laser	8.14[b]	2.54	.96	5.79	10.50	6.00	13.00		
15days	**Negative control**	6.71[b]	1.38	.52	5.44	7.99	5.00	8.00	23.51	0.00*
	Positive control	26.86[a]	6.28	2.37	21.05	32.67	20.00	38.00		
	Ibuprofen	13.86[b]	7.38	2.79	7.03	20.68	9.00	30.00		
	Laser	7.29[b]	2.93	1.11	4.58	9.99	5.00	12.00		
30days	**Negative control**	6.71[c]	1.38	.52	5.44	7.99	5.00	8.00	13.55	0.00*
	Positive control	15.86[a]	2.54	.96	13.50	18.21	12.00	20.00		
	Ibuprofen	11.71 [a,b]	3.50	1.32	8.48	14.95	8.00	17.00		
	Laser	7.57 [b,c]	3.99	1.51	3.88	11.27	4.00	15.00		

Significance level p≤0.05, * significant

Table (4b) Detailed results of Tukey's post hoc test for pairwise comparison of mean count of inflammatory cells

MMP1-area fraction	(I) Groups	(J) Groups	Mean Difference (I-J)	SE	Sig.	95% CI	
						Lower Bound	Upper Bound
7days	Negative control	Positive control	-73.42857-*	8.79	.00	-97.67	-49.18
		Ibuprofen	-15.71429	8.79	.30	-39.96	8.53
		Laser	-1.42857	8.79	1.00	-25.67	22.82
	Positive control	Ibuprofen	57.71429*	8.79	.00	33.47	81.96
		Laser	72.00000*	8.79	.00	47.75	96.25
	Ibuprofen	Laser	14.28571	8.79	.38	-9.96	38.53
15days	Negative control	Positive control	-20.14286-*	2.73	.00	-27.68	-12.61
		Ibuprofen	-7.14286	2.73	.07	-14.68	.39
		Laser	-.57143	2.73	1.00	-8.11	6.96
	Positive control	Ibuprofen	13.00000*	2.73	.00	5.47	20.53
		Laser	19.57143*	2.73	.00	12.04	27.11
	Ibuprofen	Laser	6.57143	2.73	.10	-.96	14.11
30days	Negative control	Positive control	-9.14286-*	1.62	.00	-13.60	-4.68
		Ibuprofen	-5.00000-*	1.62	.02	-9.46	-.54
		Laser	-.85714	1.62	.95	-5.32	3.60
	Positive control	Ibuprofen	4.14286	1.62	.08	-.32	8.60
		Laser	8.28571*	1.62	.00	3.83	12.74
	Ibuprofen	Laser	4.14286	1.62	.08	-.32	8.60

Significance level $p \leq 0.05$, * significant

10.21608/adjc.2020.22135.1051

Table (5) Comparison of count of inflammatory cells in different observation times within the same group (ANOVA test)

	7 days	15 days	30 days	F	P
Negative control	6.71±1.38	6.71±1.38	6.71±1.38	------	
Positive control	80.14[a]±32.54	26.86[b]±6.28	15.86[c]±2.54	12.14	0.00*
Ibuprofen	22.43[a]±3.78	13.86[b]±7.38	11.71[b]±3.50	8.23	0.003*
Laser	8.14±2.54	7.29±2.93	7.57±3.99	1.24	0.884[ns]

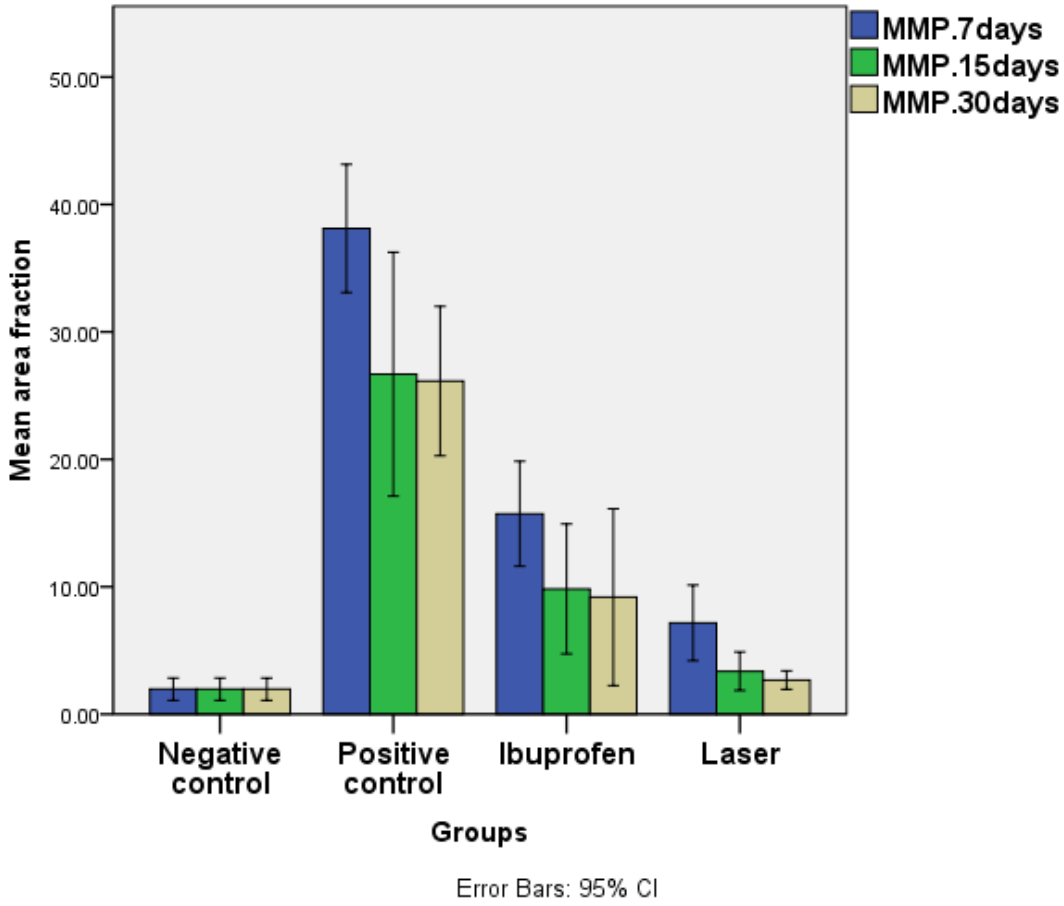

Fig. (7) Bar chart illustrating mean value of area fraction in different groups

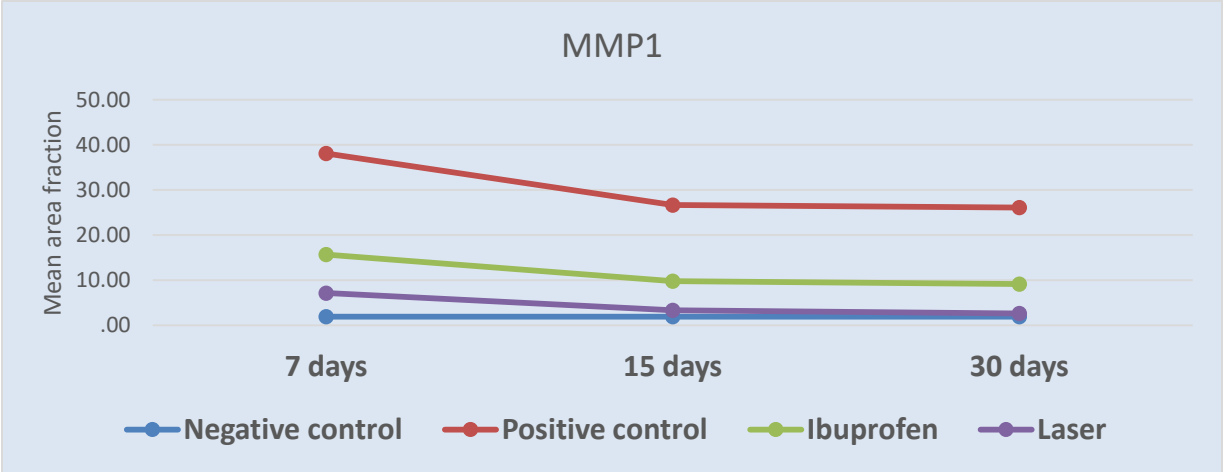

Fig.(8) Line chart illustrating mean area fraction in different observation times within the same group

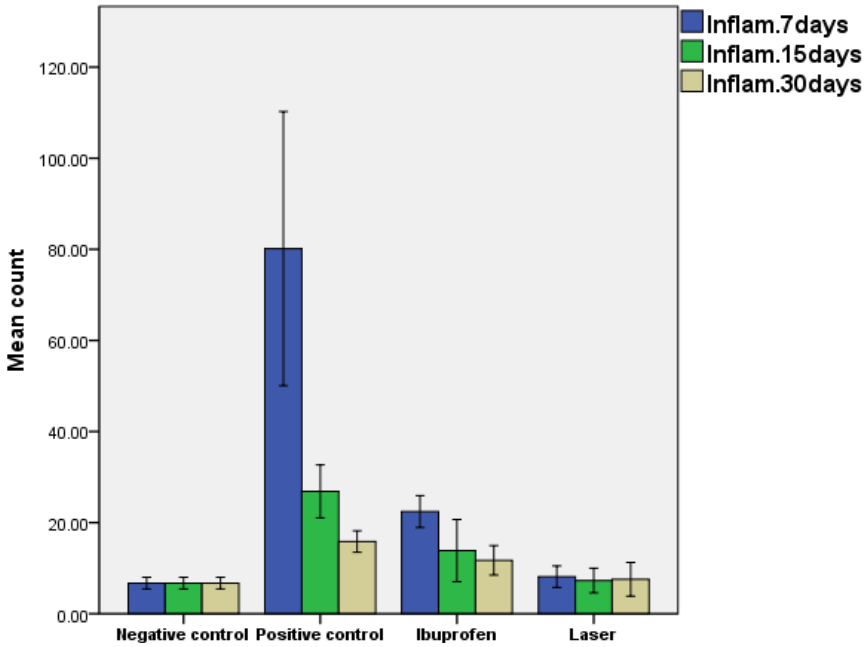

Fig. (9) Bar chart illustrating mean count of inflammatory cells in different groups

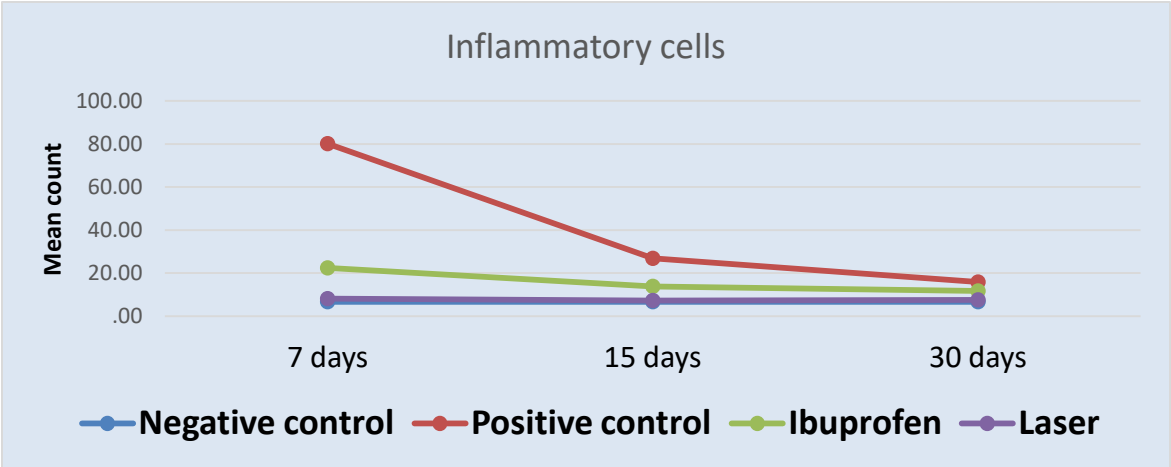

Fig.(10) Line chart illustrating mean number of inflammatory cells in different observation times within the same group

Discussion

Wistar albino rats were chosen for this study as they are considered relevant models for experimental periodontal research due to the similarity between humans and rodents in the dentogingival area **(Struillou et al., 2010).** Due to hard accessibility of posterior teeth of wistar rats and complexity during performing the experiment, modification has been performed in this model which is placement of the ligature around the central incisors instead of molars **(Lonel et al., 2015).** Upper incisors were chosen rather than lower incisors as they have lower rate of growth **(Drevenšek et al., 2008).**

The frequency of periodontal disease occurrence is less in rodents than in humans, so to induce the periodontal disease in this study, placement of ligature around teeth was chosen as a method for bacterial accumulation **(Struillou et al., 2010).** According to **Fontana et al., 2018,** the analysis of microbial samples taken from ligature placed in the sulcus revealed same bacterial composition but in greater number compared to those taken from sulcus directly by using paper points, which proven the efficiency of ligature induced periodontitis method.

Ligatures were removed after 7 days in accordance with **Yang et al., 2013**, who found that most alveolar bone loss occurs in the first 7 days after induction and then stabilizes after that. After removing the ligatures, several histological changes were noted in the periodontium of the rats in our study, mainly periodontal degradation, inflammatory response, and alveolar bone loss. This came in agreement with **Abe and Hajishengallis, 2013** who also found that anaerobic bacteria accumulate by time with a peak in 5th day after ligature placement.

In the present study histopathological analysis was made by examination of sections stained by H&E. H&E stain was useful in review different parameters such as extension of inflammation and cellularity of connective tissue, vascularity status of PDL fibers and presence of resorption in hard tissues. This was made in accordance to **Theodoro et al., 2015.**

In our study, H&E examination of the positive control group revealed the histological characteristics of periodontitis which are; disorganization and detachment of PDL fibers, abundant congested blood vessels and alveolar bone resorption. This came in agreement with **Galvão et al., 2003** who aimed to show the technique and the methodological approach used in describing histological characteristics of induced periodontal disease in rats. In ligature induced groups the following were found, increase of PDL cells and overabundance of blood vessels, looseness and disorganization of PDL fiber bundles and also irregular alveolar bone surface.

The results of group III came in agreement with **Theodoro et al., 2015** who assessed the effect of low-laser therapy as an adjuvant in the treatment of periodontitis induced in rats. He found that the groups subjected to diode laser irradiation showed lesser alveolar bone loss than other groups. He concluded that diode laser reduces the degree of inflammatory process locally thus, accelerating the repair of the destructed periodontal tissues. In our study, the results revealed also good effect of diode laser irradiation such as reorganization of PDL fibers, reduction of inflammatory cell count and new bone formation which indicates good prognosis reached by this treatment modality.

The results of group IV came in agreement with **de Vasconcelos Gurgel et al., 2004** who reported that using NSAIDs in treatment of periodontal disease resulted in reducing inflammation in the periodontal tissues and reducing bone loss as an outcome of blocking the synthesis of prostaglandins, arachidonic acid metabolites and cytokines thus, stopping the progression of periodontal destruction. In the present study, the inflammatory cells are decreased after using ibuprofen but this reduction is less than the diode laser which means that NSAIDs have good effect in reducing inflammation but not as diode laser.

Immune histo-chemical examination of the positive control group of this study revealed positive MMP-1 expression, this active reaction highlighted as brown cytoplasmic staining of

PDL cells (fibroblast/fibrocyte) and extracellular matrix staining between collagen fibers, this came in accordance to **Gölz et al., 2015.** In his study, the expression of interleukin- (IL-) 1β, matrix metalloproteinase-1 (MMP-1), and vascular endothelial growth factor (VEGF) in PDL cells were analyzed and found to be up regulated in accordance with disease progression.

Upon immune-histochemical examination of diode laser treated group, the results of all subgroups A, B and C showed positive reaction to MMP-1 stain which came in agreement with **Saglam et al., 2014** who aimed to examine the clinical and biochemical efficacy of diode laser as an adjunct to scaling and root planing. He reported that MMP-1 levels significantly reduced after laser treatment of periodontal pockets. In our study, the area fraction of MMP1 decreased the most after the treatment by diode laser which indicates the best prognosis of all groups.

Upon immune-histochemical examination of ibuprofen treated group, the specimens of the three sub groups A, B and C showed positive reaction to MMP-1. These results came in agreement with **Sun et al., 2017** who investigated the therapeutic mechanisms of non-steroidal anti-inflammatory drugs and steroids in osteoarthritis. The conclusion of this study was that prednisone, ibuprofen and betamethasone may prevent orthoarthritis by suppressing the expression of IL-6 and IL-8, subsequently decreasing levels of collagen I, MMP-1, and MMP-13.

In the statistical analysis measuring MMP-1 area fraction, the highest mean value was recorded in positive control, while the lowest mean value was recorded in negative control in all sub groups A, B and C. ANOVA test revealed that the difference between groups was statistically significant (p=0.00) but Tukey's post hoc test revealed no significant difference between mean values of laser and negative control in sub groups A and revealed no significant difference between mean values of Ibuprofen, laser and negative control in sub groups B and C.

Measuring the Percent of change in MMP-1 fraction in different groups revealed that there was no statistical significance difference in the first interval (7-15 days) and also in the second interval (15-30 days) in all groups.

Measuring effect of time in each group revealed that, in positive control, the mean value gradually decreased by time with a statistically significant difference (P=0.015), in laser group, the mean value gradually decreased by time with a statistically significant difference (P=0.002), and in Ibuprofen group, the mean value gradually decreased by time with a statistically significant difference (P=0.05). Tukey's post hoc test revealed no significant difference between 15 and 30 days in the three groups.

These findings mean that the diode laser irradiation gives the most effective and the fastest anti-inflammatory effect with keeping that effect for long time period giving good stabilized prognosis when used in the treatment of ligature induced periodontitis. These results came in agreement with **Huang et al., 2014** who investigated the cytological effects of inflammatory periodontal ligament cells in vitro after low-level laser therapy. In periodontal ligament cells, low-level diode laser treatment increased the cell's proliferative ability and decreased the expression of the examined inflammatory mediators.

In statistical analysis measuring the number of inflammatory cells, the highest mean value was recorded in positive control, while the lowest mean value was recorded in negative control in all sub groups A, B and C. ANOVA test revealed that the difference between groups was statistically significant (p=0.00) but Tukey's post hoc test revealed no significant difference between mean values of Ibuprofen, laser and negative control in sub groups A and B but sub group C revealed that Ibuprofen was not significantly different from positive control or laser groups and moreover, there was no significant difference between mean values of laser and negative control.

Upon measuring the percent of change in count of inflammatory cells in different groups of the overall period (7-30 days) the highest mean

percent decrease was recorded in positive control, while the lowest mean percent decrease was recorded in laser. Kruskal Wallis test revealed that the difference between groups was statistically significant.

Measuring effect of time in each group revealed that, in positive control, the mean value gradually decreased by time with a statistically significant difference between each 2 observation times. In laser group, the mean value gradually decreased at 15 days, then slightly increased at 30 days, with no significant difference between the three observation times. In Ibuprofen, the mean value gradually decreased by time with a statistically significant difference. Tukey's post hoc test revealed no significant difference between 15 and 30 days.

These findings revealed that diode laser caused abrupt drop of inflammatory cell count with the early onset of treatment resulting in reducing the inflammatory tissue destruction and speeding up the healing process. These results came in agreement with **de Paula Eduardo et al., 2010** who searched the electronic databases included Medline-PUBMED and ISI Web of Knowledge search engines in view of the importance of low-power lasers in periodontics. It has been reported that laser therapy is able to reduce gingival inflammation and metalloproteinase 8 (MMP-8) expression when applied after scaling and root planing as well as to reduce inflammatory cells on histological examination.

Conclusions

- Diode laser irradiation and ibuprofen when used as a treatment regimen improved the inflammatory process in the rat model of ligature-induced periodontitis.
- Diode laser irradiation showed greater and faster reduction in inflammation than ibuprofen.

Conflict of interest:

The authors declare no conflict of interest.

References

1) Abe, T. and Hajishengallis, G., Optimization of the ligature-induced periodontitis model in mice. *Journal of immunological methods*, *394*(1-2), 49-54, **2013.**

2) Aykol, G., Baser, U., Maden, I., Kazak, Z., Onan, U., Tanrikulu-Kucuk, S., Ademoglu, E., Issever, H. and Yalcin, F., The effect of low-level laser therapy as an adjunct to non-surgical periodontal treatment. *Journal of periodontology*, *82*(3), 481-488, **2011.**

3) de Almeida, J.M., Theodoro, L.H., Bosco, A.F., Nagata, M.J.H., Oshiiwa, M. and Garcia, V.G., In vivo effect of photodynamic therapy on periodontal bone loss in dental furcations. *Journal of periodontology*, *79*(6), pp.1081-1088, **2008.**

4) de Paula Eduardo, C., de Freitas, P.M., Esteves-Oliveira, M., Aranha, A.C.C., Ramalho, K.M., Simões, A., Bello-Silva, M.S. and Tunér, J., Laser phototherapy in the treatment of periodontal disease. A review. *Lasers in Medical Science*, *25*(6), 781-792, **2010.**

5) de Vasconcelos Gurgel, B.C., Duarte, P.M., Nociti, F.H., Sallum, E.A., Casati, M.Z., Sallum, A.W. and de Toledo, S., Impact of an anti-inflammatory therapy and its withdrawal on the progression of experimental periodontitis in rats. *Journal of periodontology*, *75*(12), 1613-1618, **2004.**

6) Drevenšek, M., Volk, J., Sprogar, Š. and Drevenšek, G., Orthodontic force decreases the eruption rate of rat incisors. *The European Journal of Orthodontics*, *31*(1), 46-50, **2008.**

7) Fontana, C.R., Grecco, C., Bagnato, V.S., de Freitas, L.M., Boussios, C.I. and Soukos, N.S., Molecular analyses of two bacterial sampling methods in ligature-induced periodontitis in rats. *Clinical and experimental dental research*, *4*(1), 19-24, **2018.**

8) Galvão, M.P.D.A., Chapper, A., Rösing, C.K., Ferreira, M.B.C. and Souza, M.A.L.D., Methodological considerations on descriptive studies of induced periodontal diseases in rats. *Pesquisa Odontológica Brasileira*, *17*(1), 56-62, **2003.**

9) Garcia, V.G., Knoll, L.R., Longo, M., Novaes, V.C.N., Assem, N.Z., Ervolino, E., de Toledo,

B.E.C. and Theodoro, L.H., Effect of the probiotic Saccharomyces cerevisiae on ligature-induced periodontitis in rats. *Journal of periodontal research*, *51*(1), 26-37, **2016.**

10) Garcia, V.G., Takano, R.Y., Fernandes, L.A., de Almeida, J.M. and Theodoro, L.H., Treatment of experimental periodontal disease by a selective inhibitor of cyclooxygenase-2 with scaling and root planing (SRP). *Inflammopharmacology*, *18*(6), 293-301, **2010.**

11) Gölz, L., Memmert, S., Rath-Deschner, B., Jäger, A., Appel, T., Baumgarten, G., Götz, W. and Frede, S., Hypoxia and P. gingivalis synergistically induce HIF-1 and NF-κB activation in PDL cells and periodontal diseases. *Mediators of inflammation*, **2015.**

12) Huang, T.H., Chen, C.C., Liu, S.L., Lu, Y.C. and Kao, C.T., A low-level diode laser therapy reduces the lipopolysaccharide (LPS)-induced periodontal ligament cell inflammation. *Laser Physics Letters*, *11*(7), 075602, **2014.**

13) Ionel, A., Lucaciu, O., Moga, M., Buhatel, D., Ilea, A., Tabaran, F., Catoi, C., Berce, C., Toader, S. and Campian, R.S., Periodontal disease induced in Wistar rats-experimental study. *Human and Veterinary Medicine*, *7*(2), 90-95, **2015**

14) Matos, L.L.D., Stabenow, E., Tavares, M.R., Ferraz, A.R., Capelozzi, V.L. and Pinhal, M.A.D.S., Immunohistochemistry quantification by a digital computer-assisted method compared to semiquantitative analysis. *Clinics*, *61*(5), pp.417-424, **2006.**

15) Oliveira, G.J.P.L.D., Paula, L.G.F.D., Souza, J.A.C.D., Spin-Neto, R., Stavropoulos, A. and Marcantonio, R.A.C., Effects of avocado/soybean unsaponifiables (ASU) on the treatment of ligature-induced periodontitis in rats. *Brazilian oral research*, *31*, e28, **2017.**

16) Petersen, P.E., The World Oral Health Report 2003: continuous improvement of oral health in the 21st century–the approach of the WHO Global Oral Health Programme. *Community Dentistry and oral epidemiology*, *31*, 3-24, **2003.**

17) Saglam, M., Kantarci, A., Dundar, N. and Hakki, S.S., Clinical and biochemical effects of diode laser as an adjunct to nonsurgical treatment of chronic periodontitis: a randomized, controlled clinical trial. *Lasers in medical science*, *29*(1), 37-46, **2014.**

18) Salvi, G.E. and Lang, N.P., The effects of non-steroidal anti-inflammatory drugs (selective and non-selective) on the treatment of periodontal diseases. *Current pharmaceutical design*, *11*(14), 1757-1769, **2005.**

19) Struillou, X., Boutigny, H., Soueidan, A. and Layrolle, P., Experimental animal models in periodontology: a review. *The open dentistry journal*, *4*, 37, **2010.**

20) Sun, F., Zhang, Y. and Li, Q., Therapeutic mechanisms of ibuprofen, prednisone and betamethasone in osteoarthritis. *Molecular medicine reports*, *15*(2), 981-987, **2017.**

21) Theodoro, L.H., Caiado, R.C., Longo, M., Novaes, V.C.N., Zanini, N.A., Ervolino, E., de Almeida, J.M. and Garcia, V.G., Effectiveness of the diode laser in the treatment of ligature-induced periodontitis in rats: a histopathological, histometric, and immunohistochemical study. *Lasers in medical science*, *30*(4), 1209-1218, **2015.**

22) Theodoro, L.H., Longo, M., Ervolino, E., Duque, C., Ferro-Alves, M.L., Assem, N.Z., Louzada, L.M. and Garcia, V.G., Effect of low-level laser therapy as an adjuvant in the treatment of periodontitis induced in rats subjected to 5-fluorouracil chemotherapy. *Journal of periodontal research*, *51*(5), 669-680, **2016.**

23) Yang, D., Wang, J., Ni, J., Shang, S., Liu, L., Xiang, J. and Li, C., Temporal expression of metalloproteinase-8 and-13 and their relationships with extracellular matrix metalloproteinase inducer in the development of ligature-induced periodontitis in rats. *Journal of periodontal research*, *48*(4), 411-419, **2013.**