Al-Khassaki & Ahmed *J Pure Appl Microbiol,* **13**(**1**), 619-628 | March 2019
Article 5418 | https://dx.doi.org/10.22207/JPAM.13.1.71

Print ISSN: 0973-7510; E-ISSN: 2581-690X

RESEARCH ARTICLE

Clinical Assessment of Anti-inflammatory Activity of 940 Nanometer Low Level laser Therapy on Carrageenan Induced Arthritis in Temporomandibular Joint in Wistar Albino Rats

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Abstract

The aim of this study was to evaluate the possible anti-inflammatory effect of 940nm diode laser in treating carrageenan induced arthritis in temporomandibular joint in Wistar albino rats, using different energy density of LLLT, by clinical assessment of the inflammation. it was concluded that 74J/cm2 LLLT had very efficient in edema reduction and restore function with good anti-inflammatory effect, on the other hand 171J/cm2 had appear to be in or near the borderline between non-thermal and thermal lasers eradiation in considering to Westar rat skin.

Keywords: Anti-inflammatory activity, Temporomandibular joint, Wistar albino.

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(Received: 28 December 2018; accepted: 10 January 2019)

Citation: Ali Imad Al-Khassaki and Jamal Noori Ahmed, Clinical Assessment of Anti-inflammatory Activity of 940 Nanometer Low Level laser Therapy on Carrageenan Induced Arthritis in Temporomandibular Joint in Wistar Albino Rats, *J Pure Appl Microbiol*., 2019; **13**(**1**):619-628 doi: 10.22207/JPAM.13.1.71

INTRODUCTION

Temporomandibular joint disorders (TMDs) are considered as the most common non-dental-related orofacial pain conditions which are usually presents with an inflammatory component [1,2] many types of non-surgical treatments are usually used to treat TMDs to improve and enhance function and to reduce or cure symptoms [3]such as physical therapy[4] , drug therapy[2,5], various types of occlusal splints[4,6] , acupuncture7 and low level laser therapy[3] which is very promising method to treat TMDs[2,3,8]. Carrageenan is an extract of seaweeds; Chondrus spp. and Gigartinaspp which is commonly known as Irish moss, chemically it is a sulphatedmucopolysaccharide[9], for many years it had been used to induce inflammation in rat joints as in footpad , paw[10] and temporo-mandibular joint[2,5], carrageenan induced arthritis is the most common and reliable method to test new anti-inflammatory drugs effectiveness and assessment of new strategies to treat inflammatory arthritis[2,11]. Low level laser therapy (LLLT) is a light-source therapeutic method that utilize light of a single wavelength, and acts through photochemical non-thermal reactions in the cells[5,12], LLLT is a good option to treat arthritis in the temporomandibular joint due to its known analgesic, anti-inflammatory and regenerative effects[13], another important advantage of LLLT is patient acceptance and reduce or stop using drugs thus get rid of its side effects[2,14] such as gastric ulcer and nephrotoxicity[15].Previous studies have shown very good effects of LLLT of different wavelength such as 780, 850, 660 on carrageenan induced arthritis in rat TMJ[2,5,15-18]. The aim of this study was to clinical evaluation of the effectiveness of 940nm LLLT on carrageenan induced arthritis in Wistar albino rats TMJ, using two different energy density (171J/cm^2 and 74J/cm^2).

MATERIALS AND DISCUSION

Subjects

Following approval by the Committee of the college of Dentistry of the University of Baghdad, sixty male Wistar rats (150-200g) obtained from the animal house of the higher institute of infertility diagnosis and research assisting production of the University of Al-Nahrain were used in this study. Animals kept in acrylic cages lined with wood chips and maintained at 24°C in a day/night light cycle with limitless access to food and water. Procedure was carried out in the animal house during light period of the day from 8:30 a.m. to 12:30 p.m.

Preparation and calibration of laser device

Epic X diode laser device and deep tissue handpiece were used in this experiment, the handpiece was covered with tin foil diaphragm with central 10mm radius circular opening, the tin foil diaphragm was used to cover and protect eyes and ears from laser beam. Laser power was calibrated using Laser power and energy meter (Mastero, Gentec, Quebec, Canada), in put power was set to 4 watts as manufacturer recommendation to pain therapy and the output power measured by power meter was around 1.48 watts, then the input power set at 1.6 watts and the output power were measured by the power meter which was around 0.650 watts.

Formation of the experimental groups

Animals were randomly assigned to four experimental groups as following:

Negative control group: injected by normal saline into TMJ area and applying deep tissue handpiece with 0 J/cm^2 .

Positive control group: injected with carrageenan solution into TMJ area with and applying deep tissue handpiece with 0 J/cm^2 .

LLLT with 171 J/cm^2 group: injected with carrageenan solution into TMJ area with and applying deep tissue handpiece with 171 J/cm^2 .

LLLT with 74 J/cm^2 group: injected with carrageenan solution into TMJ area with and applying deep tissue handpiece with 171 J/cm^2 .[2]

Injection of saline and applying 0 J/cm^2 were made to ensure that all animals undergo the same circumstances and stressful procedures.

Carrageenan injection

Freshly prepared 1% carrageenan solution was made by dissolving carrageenan powder (Modernist, USA) in normal saline (Pioneer Co. IRAQ). The animals were anesthetized by inhalation of Diethyl ether (stab/ BHT, Netherlands) in close plastic container then hair on TMJ area were removed using hair removal cream and plastic spatula. thepostero-inferior border of the left zygomatic arch was palpated and a needle of an insulin syringe was inserted immediately below this point and then advanced in the anterior direction until reaching the postero-lateral aspect

of the condyle. Then, 50 µL of the carrageenan solution was injected into the left TMJ[2,5,19,20].

LLLT procedure

Low level laser therapy procedure was made transcutaneously in one contact point facing the central position of left TMJ, immediately after carrageenan injection while the animal is under anesthesia, using diode laser device (Biolase epic X, USA) and deep tissue handpiece (Biolase epic, USA), using wavelength 940 nm, spot radius 10mm, irradiation time 90 second and energy density for first experimental group was 171J/cm^2 and 74 J/cm^2 for the second experimental group, the procedure were repeated every 48 hours for the next seven days (four session of irradiation).

Clinical assessment

Each animal was weighted before the induction of arthritis and every next day till the end of experiment (eight times) to evaluate weight changes[21,22], and percentage of weight changes were recorded for each animal.Circumferences of animal head were measured using measuring tape in a circle around the head just anteriorly to the ears in line over TMJs in both sides before the induction of arthritis and every next day till the end of experiment (eight times) to evaluate circumferences changes which represent edema in TMJ [23] and percentage of circumferences changes were recorded for each animal.

Statistical analysis

Data were analyzed using SigmaStat for Windows version 4.0 build 4.0.0.37, Systat, USA. and the figures were made using Microsoft office 2016. Comparison between study groups were made using one-way ANOVA and multiple post hoc comparisons were performed using the Tukey test. A probability level of less than 0.05 was considered to indicate statistical significance

RESULTS AND DISCUSION

Clinical assessment was done by evaluation of weight changes and edema in TMJ area caused by injection of carrageenan solution into TMJ between study groups, 128 Westar albino rats were used in this experiment divided into four study groups; negative control, positive control, 171J/cm^2 LLLT and 74J/cm^2 LLLT, 30 animals were randomly assigned in each experimental group and eight were died during or briefly after induction of arthritis.

Weight changes

Each animal was weighted by digital weight meter after light anesthesia early in the morning from 8-9 am from day of arthritis induction by carrageenan (day zero) and for the next seven days, statistically there was highly significant increase of weight in the negative control group (p=<0.001) and non-significant decrease in weight in positive control and 171J/cm^2 laser therapy group (P = 0.903 and 0.426 respectively) and there was non-significant increase in weight in 74J/cm^2 laser therapy group (table 1), a non-significant increase in weight was reported from day one to day four in LLLT group treated by 171J/cm^2 laser radiation then a non-significant decrease of weight was reported till the end of the experiment (Fig. 1). Comparison of weight changes between study groups on each day of the experiment (table 2) showed a non-significant differences between them in the first four days of the experiment, but a highly significant differences were reported from day 4-7 so Pairwise Multiple Comparison Procedures using Holm-Sidak method were done to clarify the particular differences in every study group for the day 4-7. Analysis of day four (Table 3) results reveals highly significant differences in weight between negative and positive study groups (p=0.002) and non-significant differences between all other groups. In day five of the experiment; mean of weight of the positive control group was highly-significantly lower than that of negative control group (P= <0.001) and there was statistically significant decrease in weight of 171J/cm^2 group in comparing

Table 1. Comparison of weight changes

Group	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	P value
negative	182.103	184.345	189.750	193.792	203.316	206.526	206.895	209.895	<0.001
Positive	182.241	182.379	179.200	180.080	172.750	173.133	173.933	174.643	(P = 0.903)
171J/cm^2	181.241	183.138	186.417	188.542	189.632	186.474	182.632	178.579	(P = 0.426)
74 J/cm^2	183.172	183.034	183.308	189.000	190.143	191.316	194.368	196.632	(P = 0.166)

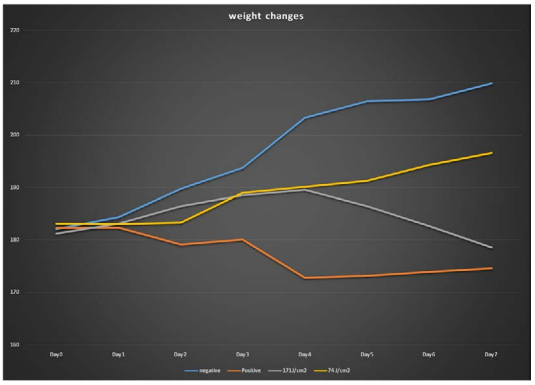

Fig. 1. Weight changes through the experiment

to negative control (P=0.029), regarding 74 J/cm^2 groupa non-significant differences were recorded(table 4). Day six results; weight mean of positive control group continued to be highly significantly lower than that of negative control one with P= <0.001, 171 J/cm^2 group also continued to be highly significantly lower than that of negative control group with P=0.005, regarding 74 J/cm^2 there were non-significant differences in comparing to negative control, but there was significant increase in weight when comparing 74 J/cm^2 to the positive control group with P= 0.035 as shown in table (5).in the last day of the experiment, day seven; weight differences were continued to increase between groups, regarding positive control group and 171 J/cm^2 group the mean of weight in both were statistically highly significantly lower than that of negative control(P=<0.001), regarding 74 J/cm^2 group there was non-significantly different from mean of negative control group, but significant increase in weight were obvious in comparing to positive and 171 J/cm^2 croup and the P value were 0.021 and 0.038 respectively as shown below in table (6).

Edema assessment

Injection of carrageenan solution into TMJ area caused marked edema (figure 2) was obvious with naked eye, to assess the validity of LLLT treatment the circumference of rat head was measured before and every successive day to the end of the experiment (figure 3), one-way ANOVA to compare between means of head circumferences with negative control group which was injected with normal saline solution instead of carrageenan solution. The analysis of one-way ANOVA of head circumferences in day zero showed non-significant differences between study groups (P=0.648) which indicate no edema present at this moment, 24

Table 2. Comparison of weight changes

day	Negative control		Positive control		171 J/cm		274J/cm2		P value
	mean	SD	mean	SD	mean	SD	mean	SD	
0	182.103	22.626	182.241	29.693	181.241	18.820	183.172	30.482	(P = 0.968)
1	184.345	22.263	182.379	29.499	183.138	19.860	183.034	32.546	(P = 0.994)
2	189.750	23.266	179.200	28.490	186.417	18.354	183.308	31.638	(P = 0.538)
3	193.792	22.950	180.080	28.614	188.542	18.101	189.000	31.797	(P = 0.322)
4	203.316	21.380	172.750	23.067	189.632	16.550	190.143	30.552	(P = 0.004)
5	206.526	23.595	173.133	23.964	186.474	14.664	191.316	23.620	(P = <0.001)
6	206.895	21.807	173.933	23.819	182.632	15.334	194.368	25.771	(P = <0.001)
7	209.895	22.285	174.643	23.581	178.579	15.806	196.632	26.764	(P = <0.001)

Table 3. Comparison of weight changes in day four

Comparison between		Diff of Means	t	P	P<0.050
Negative	Positive	30.566	3.804	0.002	Yes
74J/cm2	Positive	17.393	2.214	0.142	No
171 J/cm2	Positive	16.882	2.101	0.148	No
Negative	171 J/cm2	13.684	1.781	0.219	No
Negative	74 J/cm2	13.173	1.757	0.160	No
74J/cm2	171J/cm2	0.511	0.0682	0.946	No

hours later highly significant differences with P value <0.001, for the next two days till day three the same results were reported with the exact P value, then in day four and day five the differences begun to decrease but the differences still statistically significant with P value 0.017, 0.027 respectively, in the last two days of the experiment (day 6 and 7) the decrease in differences continued to reach a non-significant level as shown in table (7). Post hoc analysis were done to compare all

Table 4. Comparison of weight changes in day five

Comparison between		Diff of Means	t	P	P<0.050
Negative	Positive	33.393	4.459	<0.001	Yes
Negative	171 J/cm2	20.053	2.850	0.029	Yes
74 J/cm2	Positive	18.182	2.428	0.070	No
Negative	74 J/cm2	15.211	2.162	0.099	No
171 J/cm2	Positive	13.340	1.781	0.152	No
74 J/cm2	171 J/cm2	4.842	0.688	0.494	No

Table 5. Comparison of weight changes in day six

Comparison between		Diff of Means	t	P	P<0.050
Negative	Positive	32.961	4.352	<0.001	Yes
Negative	171 J/cm2	24.263	3.411	0.005	Yes
74 J/cm2	positive	20.435	2.698	0.035	Yes
negative	74 J/cm2	12.526	1.761	0.228	No
74 J/cm2	171 J/cm2	11.737	1.650	0.196	No
171 J/cm2	positive	8.698	1.149	0.255	No

Table 6. Comparison of weight changes in day seven

Comparison between		Diff of Means	t	P	P<0.050
Negative	Positive	35.252	4.610	<0.001	Yes
Negative	171 J/cm2	31.316	4.424	<0.001	Yes
74 J/cm2	Positive	21.989	2.876	0.021	Yes
74 J/cm2	171 J/cm2	18.053	2.550	0.038	Yes
Negative	74 J/cm2	13.263	1.874	0.126	No
171 J/cm2	Positive	3.936	0.515	0.608	No

Table 7. Head circumferences comparison among study groups

Day	-ve Control Mean±SD	+ve control Mean±SD	171J/cm2 Mean±SD	74J/cm2 Mean±SD	P value
0	82.75±7.62	84.12±5.49	85.06±4.18	85.12±3.48	0.648
1	82.8±7.61	96.76±3.63	93.47±6.38	92.12±3.87	<0.001
2	83.67±8.06	96.75±3.86	92.67±6.15	88.5±4.08	<0.001
3	83.8±8.18	96.5±3.4	89.17±5.1	87.33±3.89	<0.001
4	84.2±9.9	95.43±4.12	89.86±5.21	85.71±5.15	0.017
5	84.2±9.95	95.29±4.54	88.57±4.54	85.2±4.21	0.027
6	84.2±9.95	93.86±6.39	88±4.43	85.2±4.27	0.093
7	84.3±9.83	93.67±7.42	87±4.4	84.8±3.96	0.131

pair of study groups in each day of the experiment, in day zero the analysis revealed as it was expected a non-significant differences between study groups (table 8). Twenty-four hours later the numbers dramatically changed with highly significant increase of head circumferences (P=<0.001) in positive control, 171J/cm2 and 74J/cm2 groups in comparing to negative control group (table 9) which were due to the massive edema caused by carrageenan solution injected in TMJ in these groups, non-significant differences were recorded between all three group which were injected with carrageenan solution which revealed that the swelling was almost identical at this stage between them. In day two the head circumferences were still highly significantly higher in positive control and 171J/cm2 groups when compared with negative control group thus the edema was still obvious in these groups 48 hours after the initial induction of inflammation by carrageenan solution, but things were different in 74J/cm2 LLLT group, a non- significant difference with P value = 0.197 was reported between 74J/cm2 LLLT group and negative control group and highly significant differences between 74J/cm2 LLLT group and

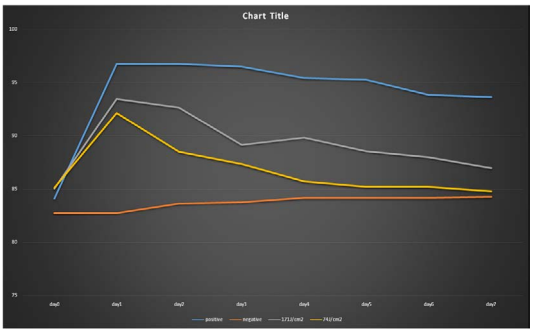

Fig. 3. Head circumference changes during the days of the experiment

Fig. 2. Edema caused by carrageenan injection in TMJ area

Fig. 4. Samples of burnings caused by 171J/cm2 laser therapy

Table 8. Head circumferences comparison in day zero between every pairs

1st group	2nd group	P value
-ve Control	+ve control	0.935
-ve Control	171J/cm2	0.669
-ve Control	74J/cm2	0.660
+ve control	171J/cm2	0.985
+ve control	74J/cm2	0.982
171J/cm2	74J/cm2	1.000

Table 9. Head circumferences comparison in day one between every pairs

1st group	2nd group	P value
-ve Control	+ve control	<0.001
-ve Control	171J/cm2	<0.001
-ve Control	74J/cm2	<0.001
+ve control	171J/cm2	0.435
+ve control	74J/cm2	0.123
171J/cm2	74J/cm2	0.956

positive control group with P value =0.007 which indicate that the edema begun to subside only 48 hours after the initial induction of inflammation, also there were a non- significant difference with P value = 0.407 between 171J/cm2 and positive control which mean that this parameter are still not effective in this stage (table 10). Day three results analysis with post hoc showed that the edema was still present in positive control group and the highly significant increase in head circumference compared to negative control group with P value =<0.001, both LLLT groups (171J/cm2 and 74J/cm2) showed non- significant differences compared to negative control and significant / highly significant lower head circumferences in comparing to positive control group indicating the edema begun to subside in both groups as shown in table (11).Post hoc analysis of day four showed significant increase in head circumference in positive control group compared to negative control group with P value =0.014, other pairs comparison results were non-significantly different compared to each other as shown below in table (12).Day five results almost the same as day before with significant increase in head diameter in positive group comparing to negative group (p= 0.015), all other pairs comparing were non-significantly different (table 13).In day six there was non-significant increase in head diameter of the positive control group in comparing to negative control group (p= 0.059) which indicated that inflammation in positive control group started to subside. All other pairs were non significantly different when compared to each other (table 14). Comparison of head circumferences of all pairs in the last day of the experiment revealed non statistically significant differences between all pairs, indicating that the inflammation end in all groups (table 15). Induction of arthritis was made using the most common method used to evaluate the effects of different types of treatment protocols on inflammatory arthritis which is induction of arthritis using carrageenan solution injected directly into TMJ, carrageenan is polysaccharide obtained from sea weed, it have potent pro-inflammatory effect in animal joint when injected into the joint space[11], previous studies had proof the carrageenan effectiveness in arthritis induction in TMJ of rats[19,20,24,25]. Pilot study revealed massive edema into TMJ area and decrease amount of food intake by rats suffering from arthritis comparing to normal rats and apparently that decrease in food consumption caused these rats look thinner, thus we made an

Table 10. Head circumferences comparison in day two between every pairs

1st group	2nd group	P value
-ve Control	+ve control	<0.001
-ve Control	171J/cm2	0.001
-ve Control	74J/cm2	0.197
+ve control	171J/cm2	0.407
+ve control	74J/cm2	0.007
171J/cm2	74J/cm2	0.387

Table 11. Head circumferences comparison in day three between every pairs

1st group	2nd group	P value
-ve Control	+ve control	<0.001
-ve Control	171J/cm2	0.112
-ve Control	74J/cm2	0.487
+ve control	171J/cm2	0.018
+ve control	74J/cm2	0.002
171J/cm2	74J/cm2	0.929

Table 12. Head circumferences comparison in day four between every pairs

1st group	2nd group	P value
-ve Control	+ve control	0.014
-ve Control	171J/cm2	0.431
-ve Control	74J/cm2	0.990
+ve control	171J/cm2	0.526
+ve control	74J/cm2	0.071
171J/cm2	74J/cm2	0.769

Table 13. Head circumferences comparison in day five between every pairs

1st group	2nd group	P value
-ve Control	+ve control	0.015
-ve Control	171J/cm2	0.665
-ve Control	74J/cm2	0.999
+ve control	171J/cm2	0.336
+ve control	74J/cm2	0.093
171J/cm2	74J/cm2	0.904

attempt to evaluate these clinical findings caused by inducted TMJ arthritis and the effect of treatment protocol on head circumference changes caused by edema and weight changes caused by decrease food consumption due to loss proper function of inflamed TMJ.According to our knowledge there was no similar articles available that used head circumference and/or weight changes as clinical parameters for evaluating treatment protocol in induced arthritis, although several authors were report relations between similar parameters and pan-arthritis or joint arthritis (other than TMJ) in human studies as well as animal experimental studies including studies on rats, Vierboom et al., reported significant relation between weight changes and several diseases including arthritis in observational study in human[26], Leech et al., found relation between lower extremities arthritis with weight and growth in human study[27], Borbʹly et al., mentioned significant weight loss in mouse after induction of experimental autoimmunity arthritis[28],in the same way Hasan et al., reported similar weight loss in induced paw arthritis inSprague–Dawley rats using complete Freund's adjuvant[29], identical weight loss secondary to induced arthritis were reported by several authors in Westar rats induced arthritis[21,22,30,32], also there were several articles used body weight as parameter to assess TMJ function as secondary outcome depending on the fact that limited mouth opening would interfere with normal feeding such as the study of Angelo et al., and Richmond who did randomized clinical trial on Black Merino sheep temporomandibular joint andMiyamoto et al., who used body weight of mice as valuable index for evaluation TMJ diseases [33,35]. As mentioned above no specific head circumference measurement as parameter to evaluate TMJ edema was previously reported, but many authors used similar way to assess edema in arthritic joints by measuring diameters or volume changes, it's good to mention some examples of these techniques; Uttra et al., used Vernier caliper to measure paw diameter to evaluate paw induced arthritis in rats[31], others relied on volume changes in paws to assess induced arthritisusing displacement plethysmometry[29,36,37], apparently this method can precisely measure edema progression and regression but unfortunately cannot applied in our study due to possible rat suffocation in water during the procedure hence it was anesthetized, thus our study relied on circumference measurement as parameter to assess edema in area TMJ. Before discussion inflammatory results it's important to mention An unexpected observation noticed during this experiment in the study group treated with 171J/cm^2 which is developing of red lesion of burns in the area of TMJ in 21 of the 30 rats exposed to two or more session of laser radiation(figure 4) noticed in the day after the session, none of these lesions were reported in other groups (P = <0.001), thus it was concluded that 171J/cm^2 was in or near the borderline between non-thermal and thermal lasers eradiation in considering to Westar rat skin. Another observation was the slight hair growth noticed in 74J/cm2 LLLT and in rats which didn't developed burns in 171J/cm^2 LLLT group at last day of the experiment, no such growth was observed in other rats, which can be explained by biostimulation of hair follicles by LLLT[38,40]. The results of this study showed that irradiation of the TMJ area of rats with 940 nm diode laser with 74 J/cm^2 lead to faster resolution of the inflammation regarding swelling size and normal weight gain of the rat. This laser dose didn't cause burning or negative impact to skin overlying the TMJ area.

Table 14. Head circumferences comparison in day six between every pairs

1st group	2nd group	P value
-ve Control	+ve control	0.059
-ve Control	171J/cm2	0.797
-ve Control	74J/cm2	0.999
+ve control	171J/cm2	0.518
+ve control	74J/cm2	0.231
171J/cm2	74J/cm2	0.957

Table 15. Head circumferences comparison in day seven between every pairs

1st group	2nd group	P value
-ve Control	+ve control	0.100
-ve Control	171J/cm2	0.934
-ve Control	74J/cm2	1.000
+ve control	171J/cm2	0.445
+ve control	74J/cm2	0.253
171J/cm2	74J/cm2	0.983

Typically, edema is caused by intensification in vascular permeability, fluid retention, extravasation of protein and other cellular component at the site of inflammation[41], LLLT with suitable dosimetry can revert these effects by increasing blood supply, clearing inflammatory agent and downgrading inflammatory response[3,42,44].Slowing the process of weight gain is caused by difficulty in eating due to induced arthritis followed by limited mouth opening[33]. Using the lower laser setting of 74 J/cm^2 had led to nearly normal weight gain which was close to the negative control group. This finding further support the results of rat head circumference (edema suppression). This means that the reduction in the head circumference passing the TMJ area has led to normal mouth opening and normal feeding process[45]. on the other hand, LLLT using 171J/cm^2 caused initial good result when edema decreased and body weight was somewhat identical to 74J/cm^2 till day four when result begun to change and weight started to decrease reaching similar level of positive control group, these finding can be due to burnings in TMJ area occurred in some of the rats which was an indication that this dose represents the threshold settings. This finding accomplices with Arndt-Schulz law which states that biostimulation falls in a window area and doses over this window will have no or even adverse effect to the biological tissues[46].

CONCLUSION

The results of this study demonstrate that LLLT using 74J/cm^2 have a very powerful anti-inflammatory effect on inflamed TMJ.

ACKNOWLEDGMENTS

We gratefully acknowledge the all kind of technical supports from the Department of oral diagnosis, College of Dentistry, University of Baghdad, Baghdad, Iraq.

CONFLICT OF INTERESTS

The author declares that there are no conflict of interest.

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