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RESEARCH ARTICLE

Design and Enhancement Low-Level Laser Therapy System for Rheumatoid Arthritis

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ABSTRACT

Rheumatoid arthritis (RA) is inflammatory and autoimmune disease has a high prevalence in the population, while therapy is available, it often required injection of drugs causing discomfort to patients and side effect in most cases. The purpose of this study is to evaluate the clinical effect of low-level-laser-therapy (LLLT) with different wave length as an alternative treatment.

This study is case control study. Forty-eight male rats were separated to six experimental groups: group I (negative control), group II (RA positive control), group III [RA treated with LLLT 650nm], group IV [RA treated with LLLT 910nm], group V [RA treated with LLLT 1064nm], and group VI [RA treated with methotrexate (RA+MTX)]. Paw measurement and Biochemical parameters for all groups [Rheumatoid-factor (RF), interleukin-6 (IL -6), interleukin-1β (IL-1β), tumor-necrosis-factor-alpha (TNF-α), malondialdehyde (MDA), and superoxide-dismutase (SOD)] were measured and analyzed statistically.

There was a reduction in paw measurement due to LLLT effect after four weeks of therapy also there was a noticeable significant decrease in the levels of all biochemical parameters (IL-6, IL-1 β, TNF-α and MDA except the level of SOD, there was a significant increase as compared to the positive control group.

LLLT effectively reduced inflammation signs in rat model of CFA but in different quantities, the effects of LLLT appear mostly in Nd:YAG laser with 1064 nm wavelength, intermediate effect in diode laser with 910 nm wavelength, and less effective in diode laser with 650nm wavelength.

INTRODUCTION

Rheumatoid Arthritis (RA) is a systemic autoimmune illness known as rheumatoid arthritis (RA) is characterized by persistent inflammation, mostly affecting the joints and other organs, particularly the articular tissues and synovial membranes. Women are more likely than males to experience it, and it can happen at any age [1]. The immune systems of people with RA attack healthy synovial joints, creating persistent polyarticular synovitis. This condition eventually results in bone damage, irregularities in the joints, and deterioration of the articular cartilage, which causes excruciating pain, swelling, and fever. As the illness worsens, the patient's quality of life may decline and their financial burden may increase [2]. As of right now, there is no effective RA medicine that doesn't have side

effects. To treat soreness and stiffness in the joints caused by inflammation, doctors often recommend nonsteroidal anti-inflammatory drugs (NSAIDs) and steroids. These medications do not provide a permanent cure for RA, but they do successfully reduce its symptoms. Drugs that affect rheumatoid arthritis Although DMARDs take time to take effect, they can significantly halt the progression of the illness, stop RA from starting, and relieve joint abnormalities. All of the current RA pharmaceutical treatments, however, have adverse effects, and patients may experience financial difficulties if they use these prescriptions for an extended period of time. The number of people with arthritis is increasing along with life expectancy, which highlights the urgent need for efficient, side-effect-free RA treatments [3].Since 1981, low-level laser therapy (LLLT) has been utilized in clinical settings to treat a variety of inflammatory diseases [4]. Several authors have suggested therapeutic benefits of LLLT for inflammatory pathologies, including R. C. Pallotta et al [5] They examined the impact of low-level laser treatment (LLLT; infrared, 810 nm) on inflammation in rats that was created experimentally, and A. C. A. Alves et al [6] who evaluated histological aspects of low-level laser therapy (LLLT: 780nm) effects in different RA progression stages in the knee. It was done using a collagen-induced RA model. LLLT is used to promote tissue healing, reduce inflammation and edema, and provide pain relief. It can be utilized to stimulate cellular function[7]. Laser photons are expected to be absorbed by chromophores within cells, such as cytochrome c oxidase found in the mitochondria [8].]. Adenosine triphosphate, or ATP, is the main source of cellular energy that normalizes cell function, reduces pain, and promotes healing when cytochrome c oxidase activity is altered. LLLT has been used in the treatment of RA because the primary goals of therapy are to decrease pain and inflammatory activity, avoid tissue degeneration, increase function, and improve quality of life. Its mode of action is characterized by the chromophores' absorption of red and infrared radiation, which can enhance analgesic effects by increasing enzymatic activity, adenosine triphosphate (ATP) production, protein synthesis, and cell proliferation [9]. Furthermore, it might lessen stiffness and soreness, which helps protect joints. Some authors have noted no effect on inflammation, and others have questioned the effectiveness of LLLT as an anti-inflammatory. On the other hand, more recent studies have found some dose-dependent effects on in vivo tests [10] . Reducing prostaglandin synthesis and chemotactic factor in the early phases of inflammation is one of LLLT's other main functions [11]. The laser's parameters—wavelength, application technique, dosage, duration, and location—determine the anti-inflammatory, analgesic, and healing properties of LLLT radiation. With its biophysical and biological characteristics, LLLT is a non-invasive therapy that blocks the inflammatory process. Additionally, LLLT can shield joints by controlling arthritis-related cells by lowering inflammation and promoting tissue [12]. The purpose of this study is to examine the impact of LLLT on RA disease at three different wavelengths: 650 nm, 910 nm, and 1064 nm, along with various laser settings.

MATERIALS AND METHODS

According to the aim of this study, types of lasers with different wavelengths were proposed which are continuous mode diode laser with 650nm wavelength, 5 mW power, 0.6 cm2 spot size, continuous mode diode laser with 910nm wavelength, 150 mW power, 0.6 cm2 spot size, and pulsed mode Nd:YAG laser with 1046nm wavelength, 25 J energy, 1 cm2 spot size. Using the same circumstances and number of sessions to reach the purpose which is anti-inflammatory effect of the laser on tissues. To achieve this study a rat model has been used by inducing complete Freund's adjuvant (CFA) which, as a result of increased inflammatory cell infiltration in the synovium, caused prolonged synovial inflammation and hyperplasia, simulating the actual symptoms of rheumatoid arthritis in humans. Figure 1 shows the schematic diagram of this study.

Animal model and treatment

Forty-eight male Wester rat weighted between 125g to 200g were kept in polypropylene cages of eight rats each in the biotechnology research center, Al-Nahrain University, Baghdad. Iraq, with free

access to food and water, with constant temperature (20 -25 C) and with light/dark cycle 12 h. the study was approved by the ethics of Biomedical engineering department, Al-Nahrain university, Baghdad, Iraq. Animals were acclimatized for two weeks before starting the experiment.

The rats were randomly separated to six experimental groups each group have eight rats (N=8): group I (negative control), group II (RA positive control), group III [RA treated with LLLT 650nm], group IV [RA treated with LLLT 910nm], group V [RA treated with LLLT 1064nm], and group VI [RA treated with methotrexate (RA+MTX)]. Figure 1 shows block diagram of this study.

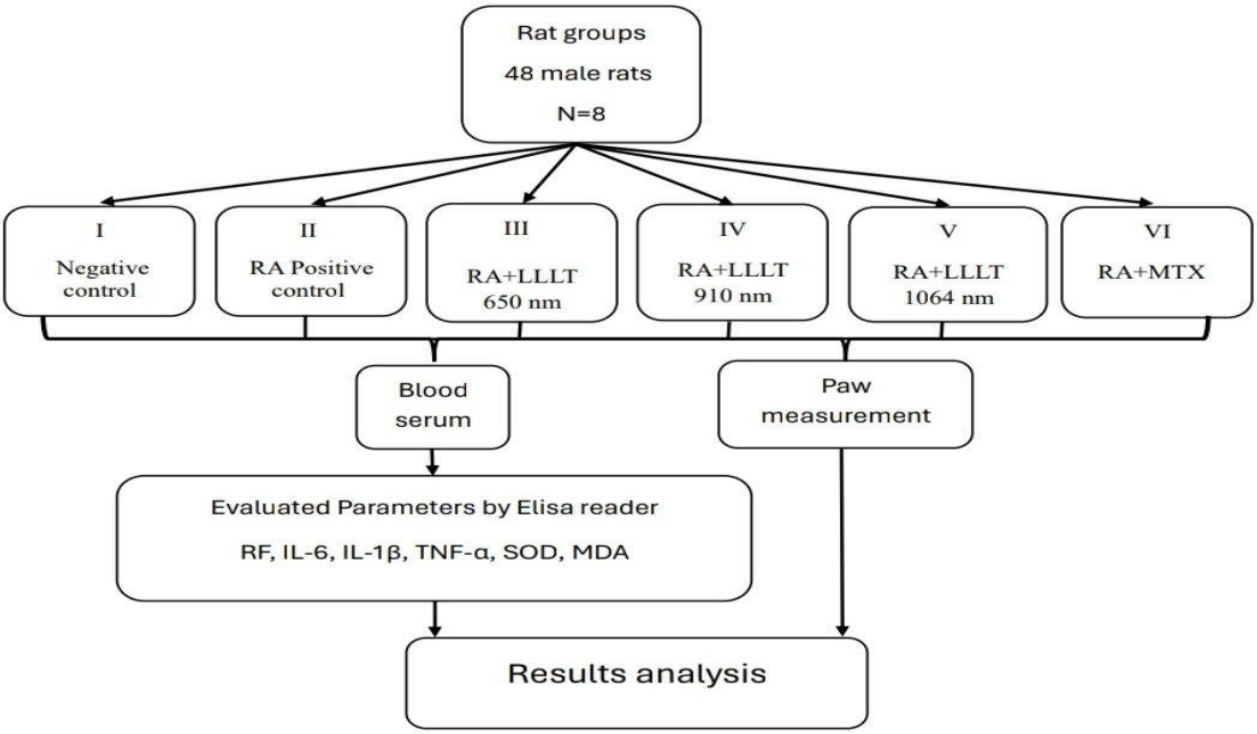


Figure 1: block diagram of the Study

Rheumatoid arthritis model by using complete Freund's adjuvant (CFA) induce.

To establish rheumatoid arthritis in the rat groups, Complete Freund's adjuvant (CFA) were procured from Sigma chemical company (St Louis, MO, USA), was induced at the plantar surface of the right hind paw of each rat with amount of 0.1 µL in RA positive control group, RA+LLLT groups and RA+MTX group. After seven days of injection acute inflammation and swell were produced in all induced groups accept the negative control group.

Treatment protocol

In this study two treatment protocols were used which are LLLT using three different wavelengths and parameters and drug therapy using methotrexate. As shown in figure 2.

Low level laser therapy

One week after complete Freund's adjuvant CFA induction, low level laser therapy was performed on the three laser groups (RA+LLLT). group III treated with Diode laser of wavelength 650nm (HOLMARC OPTO-MECHATRONICS PVT.LED) power 5mW, spot size 0.6cm2, irradiation time 180 s) continuous mode, group IV treated with diode laser of wavelength 910nm (PHS Laser therapy) , power 150mW, energy 2J, spot size 0.6cm2 , irradiation time 120 s, continuous mode, and group V treated with Nd:YAG laser of wavelength 1064nm, Laser pulse equipment (portable Nd:YAG laser,

model: AL-JH 800B), spot size 1 cm2, energy 25 J, frequency 5 Hz, pulses 200 pulse, in 40 second duration, continuously applied in a single point, pulsed mode laser. Calibration of the devices power was done by using (genetec-e Model: MAESTRO S/N: 216079). The LLLT procedure was done under general anesthesia with an intramuscular injection of a 10% ketamine solution and 2% xylazine solution at a proportion of 2:1 (0.2 mL per 100 g), rats were laid down on a table and the laser was applied 2 cm above the skin of the right paw of the LLLT group. The treatment consisted of twelve sessions, three sessions per week. All sessions were at the same time of the day, with the same devices, accessories, and laser parameters with 650nm, 910nm, and 1064nm wavelengths. Figure 4 shows the experimental setup of low-level laser therapy system for rheumatoid- arthritis rat model.

Methotrexate Drug therapy

Group VI (RA+MTX) is treated with methotrexate drug therapy for rheumatoid arthritis. Methotrexate was orally administered 3 times per week to rats, and for total 4 weeks. Preparation of methotrexate was dissolved in sodium bicarbonate. The concentrations of dosing were 1.165 mg/ml (2.33 mg/kg per time) (7 mg/kg per week). Rats were given MTX perorally (p.o.) via gavage at 2 ml/kg[13]. In this study methotrexate was used to compare its results with the laser therapy and see the difference is efficiency and which is better.

24 h after the last treatment session and five weeks after CFA rheumatoid arthritis induce all six rat groups were euthanized with deepening anesthesia which is over dose of anesthetic mix of xylazine and ketamine. blood serum was taken to evaluate RA parameters which are RF detected quantitatively in the rat serum by Elisa kit which was supplied from (SunLong Biotech Co.,LTD , Catalogue Number:SL1665Ra), IL-6 detected quantitatively in the rat serum by Elisa kit which was supplied from (SunLong Biotech Co.,LTD , Catalog Number: EL0034Ra , Assay range:31.25 pg/mL - 2000 pg/mL , Sensitivity:1.43 pg/mL), IL-1B detected quantitatively in the rat serum by Elisa kit which was supplied from (SunLong Biotech Co.,LTD , Catalog Number: HS-EL0039Ra, Assay range:7.81 pg/mL - 500 , Sensitivity:0.71 pg/mL), TNF-alpha detected quantitatively in the rat serum by Elisa kit which was supplied from(SunLong Biotech Co.,LTD, Catalog Number: EL0013Ra, Assay range:4.69 pg/mL - 300 pg/mL, Sensitivity:0.43 pg/mL), SOD detected quantitatively in the rat serum by Elisa kit which was supplied from (SunLong Biotech Co.,LTD, Catalogue Number: SL0664Ra) , and MDA detected quantitatively in the rat serum by Elisa kit which was supplied from (SunLong Biotech Co.,LTD, Catalogue Number: SL0475Ra) by using Elisa reader.

Results analysis

The collected data were coded, entered, presented, and analyzed by computer using the available data base software program statistical package of IBM SPSS-29 (IBM Statistical Packages for Social Sciences- version 29, Chicago, IL, USA). The data were displayed using basic frequency, percentage, mean, standard deviation, and range (lowest and maximum values) measurements. The significance of the differences between the means (quantitative data) was assessed using the Paired-t-test for differences between two dependent means or paired observations, the Students-t-test for differences between two independent means, and the ANOVA test for differences between more than two independent means. P values equal to or less than 0.05 were taken into consideration for statistical significance. # Represent Significant difference between two independent means using Students-t-test at 0.05 level and [NS] represent non-significant difference between two independent means using Students- t-test at 0.05 level

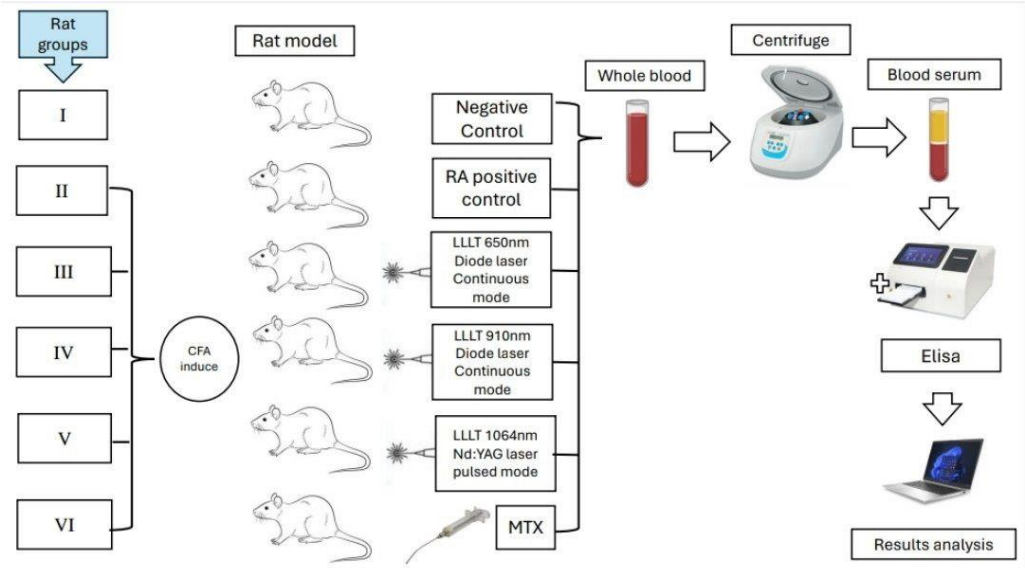


Figure 2: schematic diagram of low-level laser therapy system for rheumatoid-arthritis rat model

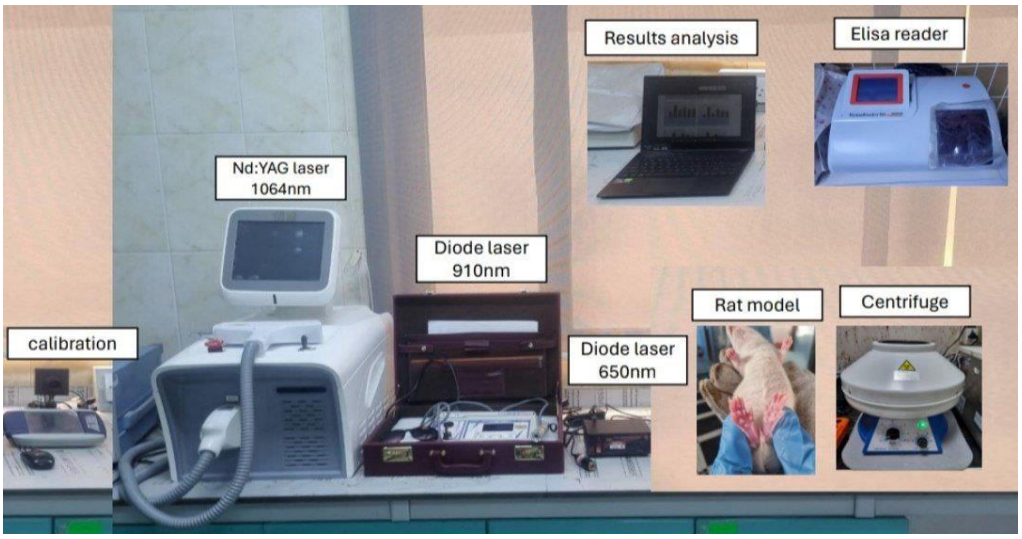


Figure 3: experimental setup of low-level laser therapy system for rheumatoid-arthritis rat model

RESULTS

The result was considered negative for rheumatoid factor in group I (negative control) and positive for groups II (RA positive control), while the results for the remaining four treated groups with low level laser therapy and methotrexate (III,1V, V,VI) were negative after four weeks of therapy.

In the present study, group II (RA positive control) showed a significant increase in the means serum levels of (IL-6, IL-1B, TNF and MDA). While there was a significant decrease in the mean serum level of (SOD) after complete Freund's adjuvant CFA arthritis injection (30 days) as compared with group I. After treated the RA+LLLT groups (III,1V,V) with low level laser in different wavelengths (650, 910,1064)nm respectively, the result showed a significant decrease in the means serum levels of (IL-6, IL-1B, TNF and MDA). While there was a significant increase in the mean serum level of (SOD) as compared with group II. Also when treated the RA+MTX group (VI) with methotrexate, the results

showed a significant decrease in the mean serum level of (IL-6, IL-1B, TNF and MDA). While there was a significant increase in the mean serum level of (SOD) as compared with group II, at $P < 0.05$ tables (1,2,3,4,5) respectively and figure (4) shows bar Chart of the Difference in mean of the evaluated biochemical parameters.

Table 1: The Effect of LLLT (650nm, 910nm, 1064nm) and MTX on IL-6 in rats with Rheumatoid Arthritis

Groups	IL-6 (pg/ml)		P- value compared to				
	Mean ±SD	Range	I	II	III	IV	V
I Negative control	27.531±5.505	(17.792-35.708)	-	-	-	-	-
II RA positive control	95.031±8.240	(81.542-104.875)	0.0001#	-	-	-	-
III RA+LLLT 650 nm	77.635±8.574	(66.542-92.375)	0.0001#	0.0001#	-	-	-
IV RA+LLLT 910 nm	59.510±7.907	(50.292-70.292)	0.0001#	0.0001#	0.0001#	-	-
V RA+LLLT 1064 nm	49.979±6.769	(43.208-58.625)	0.0001#	0.0001#	0.0001#	0.142[NS]	-
VI RA+MTX	38.208±6.922	(31.125-50.708)	0.067[NS]	0.0001#	0.0001#	0.0001#	0.030#

Table 2: The Effect of LLLT (650nm, 910nm, 1064 nm) and MTX on IL-1B in rats with Rheumatoid Arthritis

Groups	IL-1B (pg/ml)		P- value compared to				
	Mean ±SD	Range	I	II	III	IV	V
I Negative control	24.042±4.173	(18.625-30.292)	-	-	-	-	-
II RA positive control	67.635±4.476	(61.958-76.125)	0.0001#	-	-	-	-
III RA+LLLT 650 nm	61.594±5.592	(52.375-71.125)	0.0001#	0.363[NS]	-	-	-
IV RA+LLLT 910 nm	53.208±7.533	(40.708-64.458)	0.0001#	0.0001#	0.062[NS]	-	-

V RA+LLLT 1064 nm	47.844±7.321	(35.292-56.542)	0.0001#	0.0001#	0.0001#	0.517[NS]	-
VI RA+MTX	45.760±4.390	(40.292-54.458)	0.0001#	0.0001#	0.137[NS]	0.0001#	0.994[NS]

Table 3: The Effect of LLLT (650nm, 910nm, 1064 nm) and MTX on TNF- Alpha in rats with Rheumatoid Arthritis

Groups	TNF-alpha (pg/ml)		P- value compared to				
	Mean ±SD	Range	I	II	III	IV	V
I Negative control	37.147±7.223	(23.176-45.529)	-	-	-	-	-
II RA positive control	94.059±8.419	(80.824-107.882)	0.0001#	-	-	-	-
III RA+LLLT 650 nm	77.515±9.085	(64.353-92.588)	0.0001#	0.001#	-	-	-
IV RA+LLLT 910 nm	63.397±8.987	(50.235-80.824)	0.0001#	0.009#	0.0001#	-	-
V RA+LLLT 1064 nm	58.103±7.552	(45.529-69.059)	0.0001#	0.0001#	0.851[NS]	0.0001#	-
VI RA+MTX	52.956±6.351	(41.412-60.235)	0.002#	0.0001#	0.123[NS]	0.868[NS]	0.0001#

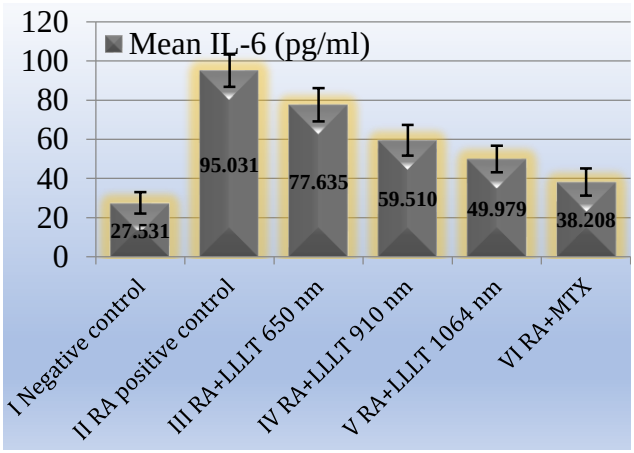
Table 4: The Effect of LLLT (650nm, 910nm, 1064 nm) and MTX on SOD in rats with Rheumatoid Arthritis

Groups	SOD (pg/ml)		P- value compared to				
	Mean ±SD	Range	I	II	III	IV	V
I Negative control	1919.417±194.352	(1545.667-2095.667)	-	-	-	-	-
II RA positive control	1078.583±151.232	(945.667-1299.012)	0.0001#	-	-	-	-
III RA+LLLT 650 nm	1278.583±174.696	(1082.333-1549.020)	0.0001#	0.331[NS]	-	-	-

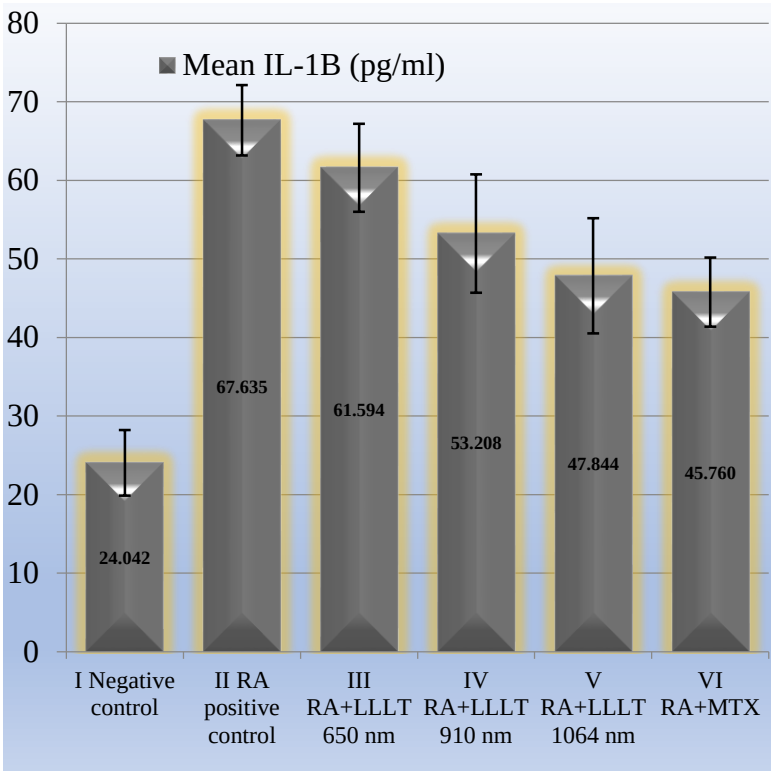
IV RA+LLLT 910 nm	1647.333±187.667	(1402.333-1939.001)	0.059[NS]	0.0001#	0.002#	-	-
V RA+LLLT 1064 nm	1783.583±196.452	(1545.667-2112.333)	0.783[NS]	0.0001#	0.0001#	0.781[NS]	-
VI RA+MTX	1841.917±166.737	(1682.333-2119.012)	0.987[NS]	0.0001#	0.0001#	0.365[NS]	0.998[NS]

Table 5: The Effect of LLLT (650nm, 910nm, 1064 nm) and MTX on MDA in rats with Rheumatoid Arthritis

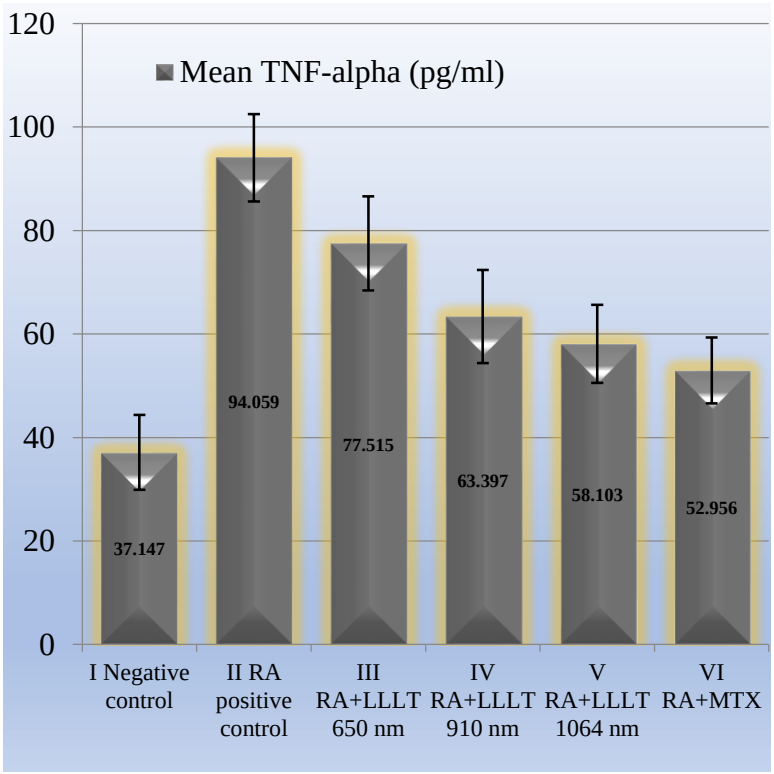
Groups	MDA (ng/ml)		P- value compared to				
	Mean ±SD	Range	I	II	III	IV	V
I Negative control	61.750±6.517	(53.041-69.522)	-	-	-	-	-
II RA positive control	94.304±6.609	(82.565-103.040)	0.0001#	-	-	-	-
III RA+LLLT 650 nm	88.245±8.372	(74.087-99.522)	0.0001#	0.717[NS]	-	-	-
IV RA+LLLT 910 nm	79.359±8.320	(64.087-89.522)	0.0001#	0.003#	0.251[NS]	-	-
V RA+LLLT 1064 nm	71.315±9.369	(55.391-86.043)	0.175[NS]	0.0001#	0.001#	0.372[NS]	-
VI RA+MTX	70.908±7.628	(56.261-79.522)	0.219[NS]	0.0001#	0.0001#	0.310[NS]	-



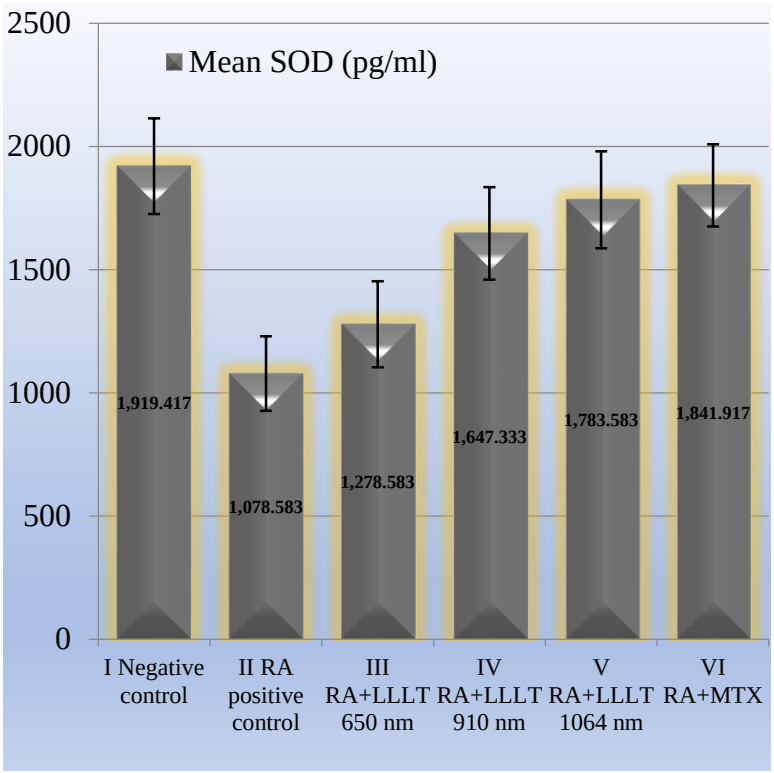
(a)


Mean IL-1B (pg/ml)
80
70
60
50
40
30
20
10
0
24.042
67.635
61.594
53.208
47.844
45.760
I Negative control
II RA positive control
III RA+LLLT 650 nm
IV RA+LLLT 910 nm
V RA+LLLT 1064 nm
VI RA+MTX

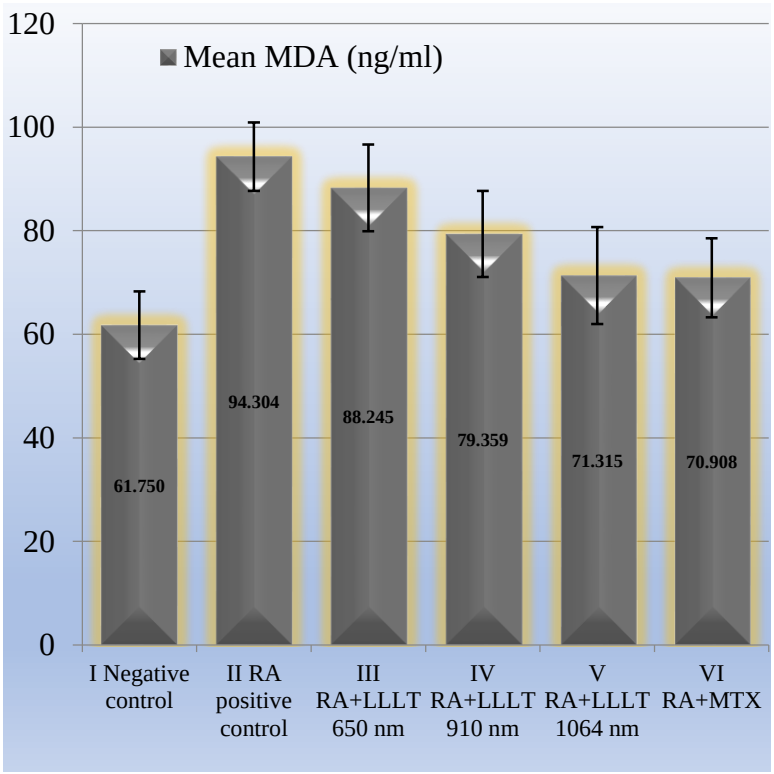
(b)


Mean TNF-alpha (pg/ml)
120
100
80
60
40
20
0
37.147
94.059
77.515
63.397
58.103
52.956
I Negative control
II RA positive control
III RA+LLLT 650 nm
IV RA+LLLT 910 nm
V RA+LLLT 1064 nm
VI RA+MTX

(c)



(d)



(e)

Figure 4: Bar Chart showing the Difference in mean of (a) IL-6, (b) IL-1B, (c) TNF-alpha, (d) SOD and (e) MDA among Groups under the Study

In this study, paw measurement (mm) were measured using Vernier digital caliper for all experimental groups in week 1 and week4 significant increase in the first week after CFA induced in rat groups paw measurement showed significant increase as compared to the group I (negative control). After four weeks the mean of the rat paw measurement for the treated groups was significantly decreased as compared to group II (RA positive control) (P< 0.05). Table 1 shows the P value and figure 4 shows bar Chart Showing the Difference in Mean of Paw measurement among Groups under the Study.

Table 6: The probability of reducing the swelling of paw measurement in the treated groups with LLLT (650nm, 910nm, 1064nm) and MTX

Paw thickness	I Negative control	II RA positive control	III RA+LLLT 650 nm	IV RA+LLLT 910 nm	V RA+LLLT 1064 nm	VI RA +MTX
Week1 compared to week4	0.010^	0.058	0.005^	0.0001^	0.0001^	0.001^

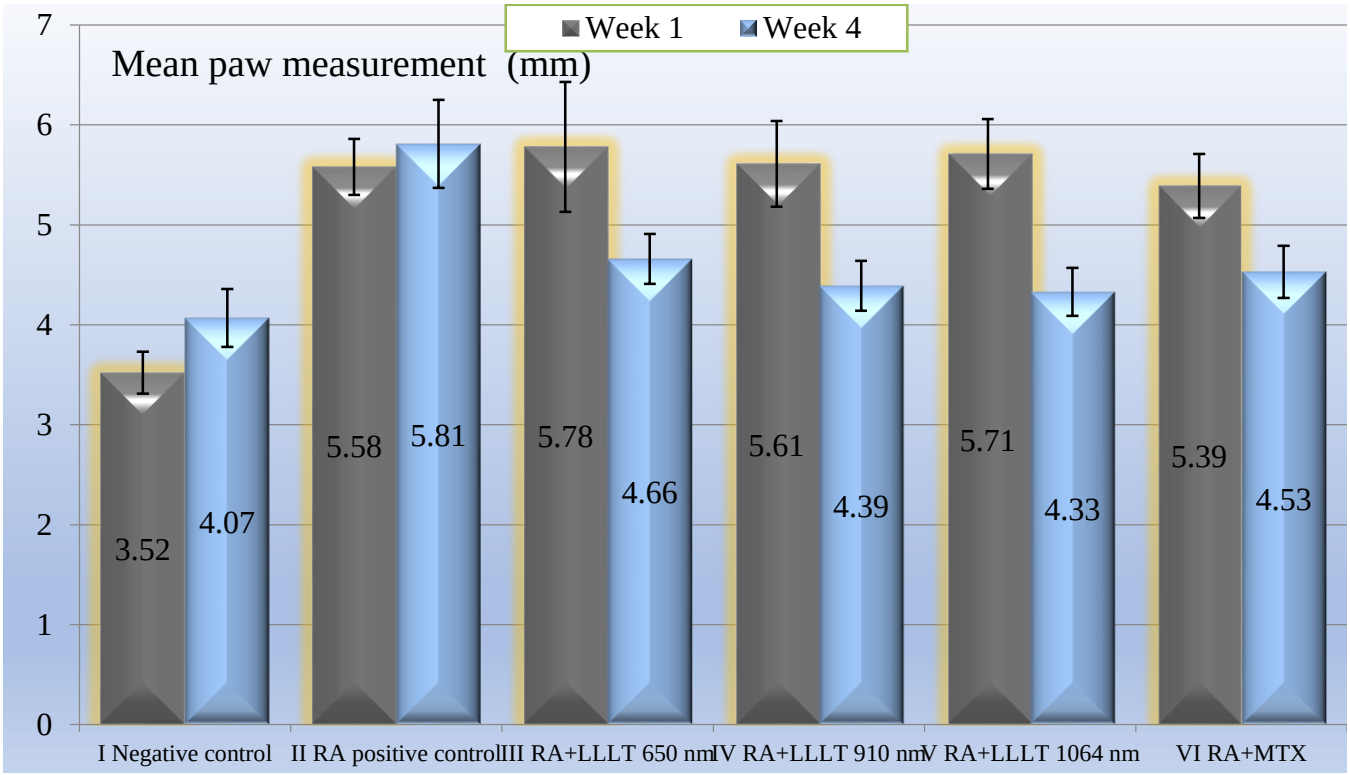


Figure 5: Bar Chart Showing the Difference in Mean of Paw measurement among Groups under the Study

DISCUSSION

One of the most common forms of inflammatory arthritis is rheumatoid arthritis. It is a joint-specific degenerative inflammatory disease that progresses over time. One popular experimental model of RA in rats is CFA-induced arthritis, which causes pathological RA that histologically resembles human arthritis.

Group III, which is treated with continuous mode diode laser with 650 nm wavelength was the least efficient treatment protocol because the laser parameters were not very effective for reducing rheumatoid arthritis anti-inflammatory biochemical parameters. Group IV that is treated with continuous mode diode laser with 910 nm wavelength reduces rheumatoid arthritis anti-inflammatory biochemical parameters in acceptable values. In group V which is treated with pulsed mode Nd:YAG laser with 1064 wavelength we can see rat serum biochemical parameters RF, TNF-α, IL-1β, IL-6, MDA, and SOD levels are very close to the normal level of group I. Treatment with LLLT is showing the best result in RA+LLLT 1064nm, average results can be seen in the RA+LLLT 910nm and less effective treatment was with the RA+LLLT 650nm as shown in figure 4.

In comparing the results between LLLT group with 1064nm wavelength and MTX group considering having similar results it could be concluded that low-level laser therapy recommended as first-line therapy. And it is possible to say LLLT is better than methotrexate in controlling RA symptoms with no associated side effects many research articles about the clinical performance and treatment efficacy of LLLT in RA revealed inconsistent and contentious results because the proper therapeutic parameters of laser, such as wavelength, energy dosage, and treatment duration, are still not well defined. In line with what we discovered, Fernando et al [14] assessed the effectiveness of using low-level laser treatment at 660 nm to treat acute arthritis caused by zymosan. The outcomes demonstrated that leukocyte infiltration, the release of IL-1 and IL-6 into the joint cavity, and inflammation at the sites of injury were all significantly decreased by the laser treatment. Faezeh et al [15] Similar to this, their study compared the early and late anti-inflammatory effects of betamethasone medication therapy and wavelength 810 nm with LLLT in RA. In comparison to the positive control group, the LLLT and steroid groups demonstrated considerably reduced mean ratings for inflammation in both the early and late stages of the evaluation. Nonetheless, the steroid group's early phase and the LLLT group's late phase produced the best results. They came to the conclusion that, like betamethasone in the early phases, the LLLT approach has a long-term hopeful effect on lowering the severity of TMJ inflammation. Bianca et al [16] Their study's goal was to assess the effectiveness of three separate wavelengths of low intensity laser treatment (LILT) for acute inflammatory models: 660 nm, 808 nm, and 905 nm. When administered once, LILT had anti-inflammatory benefits; however, after two sessions, LILT encouraged increased resorptive area, suggesting that a regulated LILT protocol is necessary to achieve therapeutic results. Also Morgana et al [17] In their research Exercise regimens and LLLT with a wavelength of 660 nm utilized to manage the inflammatory process and maintain physical function for joint disability evaluations in the treatment of rheumatoid arthritis.In a rat model of CFA-induced rheumatoid arthritis, LLLT, resistance training, or a combination of therapies enhanced the control of the inflammatory process, reducing joint dysfunction and leukocyte migration while also assisting in the restoration of peripheral functions. Similar findings were reported by Sıtkıcan et al [18] employing Wavelength 905 nm, where the treated groups displayed minor cartilage fibrillation, chondrocyte degradation, and cartilage erosion as well as mononuclear cell infiltration of the synovial membrane and successful LLLT-induced inflammation reduction.

One of the key variables to ascertain the relationship between the laser and tissue is laser fluence, which is dependent upon the wavelength, output power, mode, irradiation period, and power density. The primary constraint on this research is the challenge of verifying the association between every laser parameter and joint inflammation in rats with arthritic conditions caused by CFA. This study found that modifying factors such as RF, IL-6, IL-1β, TNF-alpha, SOD, and MDA can effectively control inflammation by low intensity laser therapy. Further research is therefore required in the future as it is yet unclear whether same effects occur for other fluences or modes when the same or different laser doses are applied.

CONCLUSION

After taking in count three different wavelengths, parameters and modes between continuous mode diode laser with 650nm and 910nm wavelengths, also pulsed mode Nd:YAG laser with 1064nm wavelength, the results of this study indicate that LLLT reduces inflammatory signs in rat model of CFA but in different quantities, making LLLT effective treatment for synovitis that is associated with acute inflammation in RA. The anti-inflammatory effects of LLLT appear mostly in Nd:YAG laser 1064 nm wavelength, intermediate effect in 910 nm wavelength, and less effective in wavelength diode laser 650nm. Comparing the results to methotrexate drug therapy the closest efficiency can be seen in the 1064 nm low level laser therapy and can be very good alternative to treatment without side effects such as toxicity, injection , allergic ,etc. The study's findings also point to a deeper comprehension of the function of laser settings, which may serve as the foundation for next medical procedures. More research should be done to study the laser wavelength differences, power, influence, energy and time of radiation.

ETHICAL APPROVAL

The study was approved by the ethics of Biomedical engineering department, Al-Nahrain University, Baghdad, Iraq.

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