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Comparison of The Low-Level Laser Irradiation Effects On Skeletal Muscle Fatigue Using Different Wavelengths and Doses

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----------ABSTRACT----------
The aim of this study was to evaluate the influence of pre-exercise muscle irradiation with various low-level laser therapy (LLLT) wavelengths and doses on skeletal muscle performance and fatigue after muscle contractions in in-situ mouse model using gastrocnemius muscle. Thirty-nine Swiss mice were arbitrarily allocated into control group and twelve LLLT irradiated groups receiving one of four different laser doses in the range 0.25-3.0 J from one of three wavelengths (637, 785 and 1064 nm) at one point on the gastrocnemius muscle before the fatigue protocol induced by electrical stimulation. Skeletal muscle fatigue was defined by fatigue index, half-relaxation time and force-time integration for all the 140 muscle contractions. At the 70th contraction, five laser irradiated groups (637 nm 0.5 J, 785 nm 0.5 J, 785 nm 0.75 J, 1064 nm 0.5 J and 1064 nm 1.5 J) had a significant difference ($P<0.05$) in terms of fatigue index. While, at the 140th contraction, only 637 nm 0.5 J and 1064 nm 0.5 J groups significantly differ ($P<0.05$) from control group. In term of half-relaxation time and force-time integration, there was no significant difference. These results indicate that LLLI has both wavelength and dose dependent effects on the gastrocnemius muscle and LLLI at appropriate wavelengths and dosage can enhance skeletal muscle performance and delays muscle fatigue.

Keywords: electrical stimulation, low-level laser therapy, muscle contraction, muscle fatigue, skeletal muscle.

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I. INTRODUCTION

The excessive use of muscles through high-intensity exercise or repetitive muscle contractions leads to a reduce in muscle performance and appearance of peripheral muscle fatigue [1, 2]. Muscle fatigue is a complicated phenomenon tried to be described by several hypothesis and scientific evidence among which are the energy sources depletion such as glycogen, phosphocreatine; dropped sensitivity level of myofibrils to Ca2+; increased level of adenosine diphosphate (ADP), phosphate inorganic (Pi), Mg2+, H+, Ca2+ and lactate; also the reactive nitrogen species (RNS) and reactive oxygen species (ROS) deposition during exercises [1, 2, 3]. Various factors such as the type and intensity of exercise, the muscle groups involved and environment influence muscle fatigue development [4]. Muscle fatigue are characterized by reduction in muscle strength, affected motor control and consequently muscular pain. In order to reveal the explanation of its process, various studies were made trying to examine the development of muscle fatigue among different exercises in human body [5, 6, 7].

Avoiding muscle fatigue in both healthy subjects as well as subjects with pathological conditions has been suggested by many preventive methodologies such as fluid reposition [8], creatine [9], nutritional supplements [10], arginine [11] and vitamin C [12]. Also, several methods have been used in order to speed up muscle restitution, such as active recovery, cryotherapy, massage, contrast heat therapy, hydrotherapy, stretching, and electrical stimulation [13]. However, medical evidence the efficiency of these techniques remains limited.

Low-level laser therapy (LLLT) is the use of laser device light for therapeutic purposes with an output range of 5–500 mW. There are evidence that LLLT stimulates tissue regeneration, relieves pain and reduces inflammation [14, 15, 16]. The laser light is typically of limit spectral width in the red and near-infrared (NIR) spectrum [17]. Cellular energy is influenced by the bio-energetic effect of the LLLT [17, 18, 19, 20]. It was reported by several studies that LLLT induces structural and metabolic alterations to the subcellular level of the biological tissues such as enlarged mitochondria effect which is assume to increase adenosine triphosphate (ATP) delivery to the cell [18]. Additionally, systematic reviews have shown that LLLT increases activity of antioxidant enzymes and attenuates the muscle's inflammatory mediators prior or after the exercise [18, 21]. Therefore, it is thought that due to such physiological alterations the muscular activity can be enhanced and the fatigue can be reduced when exercising [18, 22, 23].

In phototherapy, skeletal muscle fatigue is a novel area of research. Many researchers experimented the LLLT effect on rats and noticed the decrease in the weakness arises from the local fatigue on the muscle force and

potentially reduce the muscular tissue damage after aggressive exercises [24, 25, 26]. Additionally, skeletal muscle performance was significantly improved and muscle tissue was protected from injury by LLLT when applied immediately [26]. LLLT also leads to a dose- and a wavelength-dependent raise in cytochrome c oxidase expression in intact skeletal muscle tissue, showing that muscular metabolic rate can be improved by photo-therapy [24]. Photo-therapy also decreased the predicted development in Creatine kinase (CK) activity following exercises in pre-irradiated muscles [19, 25, 27, 28] and also in post-exercise irradiated muscles [29], which claim that phototherapy appears to have a protecting effect in skeletal muscles. In species other than rats, a small knowledge of LLLT responses, although various reviews have confirmed the advantages of LLLT in enhancing muscle function in dystrophic mice [27, 28, 29].

In this perspective, an experimental model of electrical nerve stimulation was used to induce fatigue by muscle contraction in mice and assess the influence of low-level laser therapy irradiation with various doses and wavelengths on development of skeletal muscle fatigue which could be relevant to rehabilitation and sports medicine [30].

II. MATERIALS AND METHODS

2.1 Experimental Animals and Groups

An animal experiment carried out with thirty-nine Swiss mice weighing between 20 and 25 g. They were acquired from the animal house of The Institute of Embryo Research and Infertility Treatment, Al-Nahrain University, Baghdad. The mice were maintained under controlled light and temperature, and with free access to food and water.

The mice were arbitrarily distributed into four experimental groups as follows:

I. Control group which did not receive laser irradiation (n = 3).
II. Experimental group receiving LLLT of 637 nm (n = 12).
III. Experimental group receiving LLLT of 785 nm (n = 12).
IV. Experimental group receiving LLLT of 1064 nm (n = 12).

Animals in the LLLT treated groups were further distributed according to laser doses administered (three animals each).

2.2 Experimental Procedures

A. Animal Preparation

Mice were anesthetized with an intraperitoneal hypodermic injection of pentobarbitone sodium (40 mg/kg) [31] and re-dosed as required throughout the experiment, and mounted on an experimental table, after which the gastrocnemius muscle and its distal tendon were dissected and the sciatic nerve was exposed with the proximal part of the muscle remained intact. The gastrocnemius muscle was hooked up through the distal tendon with a non-elastic thread to a force transducer (MLT500/A, ADInstruments) and the sciatic nerve was attached to a bipolar stimulating electrode. All experiments were conducted at room temperature (25° C). During the procedure, the muscle was kept wet and heated by continuously adding warm (37° C) Ringer's solution.

B. Low-Level Laser Application

Photobiostimulation was carried out using three lasers of wavelengths 637 nm (59-088, Edmund Optics), 785 nm (59-086, Edmund Optics), and 1064 nm (ISF064-100P, MeshTel/Intelite), a complete description of the parameters of the laser are presented in Table 1. The output power of the laser devices was tested using Sanwa Mobiken laser power meter LP1 with 400-1100 nm resolution. The laser illumination spot was placed in contact with the belly of the gastrocnemius muscle during irradiation. The irradiation was done once before the fatigue protocol and lasted for different time periods to achieve the desired dose delivered to the muscle as listed in Table 1.

The reason for such was to determine “therapeutic windows”. Doses utilized were selected based on former studies where LLLT successfully postponed skeletal muscle fatigue and improved biochemical markers relevant to post-exercise recovery in rats [25, 26, 32].

Table 1. Laser parameters.

Parameters	637 nm Laser	785 nm Laser	1064 nm Laser
Laser type	Diode laser	Diode laser	DPSS Nd:YAG laser
Operation mode	C.W.	C.W.	C.W.
Power [mW]	8.5	7	105
Spot size [cm^2]	0.188	0.188	0.045
Power density [mW/cm^2]	45.213	37.234	2333.3
Irradiation time [s]	30, 59, 118, 176	36, 72, 107, 143	5, 10, 15, 30
Delivered energy [J]	0.25, 0.5, 1.0, 1.5	0.25, 0.5, 0.75, 1.0	0.5, 1.0, 1.5, 3.0
Delivered energy density [J/cm^2]	1.33, 2.66, 5.32, 7.98	1.33, 2.66, 3.99, 5.32	11.1, 22.2, 33.3, 66.7

C. Electrical Stimulation for Induction of Muscular Fatigue

The electrical stimulation was applied with an intensity of 5 V, 50 Hz frequency and 300 ms stimulation duration to the sciatic nerve with an interval of 3 s between each stimulation (duty cycle of 10%) to induce muscle contraction during the following 10 min. The muscle contractions were recorded via an isometric force transducer (MLT500/A, ADInstruments) and Bridge Pod (ML301, ADInstruments) coupled to a data-recording unit (PowerLab 26T, ADInstruments). The muscle responses were acquired, digitized and saved for analysis offline. After finishing the records, the anesthetized mice were killed with an overdose of anesthetic given intraperitoneally.

2.3 Outcomes

The twitch curve induced by each stimulus was recorded (Fig. 1). The relaxation time couldn't be calculated accurately; for that reason, relaxation time was taken as the time period from peak twitch amplitude to 50 % of the amplitude (half-relaxation time). Muscular fatigue was defined by three parameters: (1) the fatigue index calculated at each of the 140th electrically induced muscle contractions; (2) half-relaxation time; and (3) the force-time integration for each contraction (area under the curve). The fatigue index was reported as a mean contraction force at a point divided by mean contraction force of the first contraction for each group. The other two parameters were reported as a percentage of the mean of the first contraction.

III. STATISTICAL ANALYSIS

The final results were shown as the mean ± standard error of the mean (SEM). Statistical evaluations of the control and laser-treated groups were performed by using analysis of variance (Two-way ANOVA), and Tukey test was used to compare individual groups. Values of $P < 0.05$ were considered significant. All data analyses were performed using GraphPad Prism version 6.05.

IV. RESULTS

Typical illustration of alteration in the muscle contraction force waveforms are shown in Fig. 1.

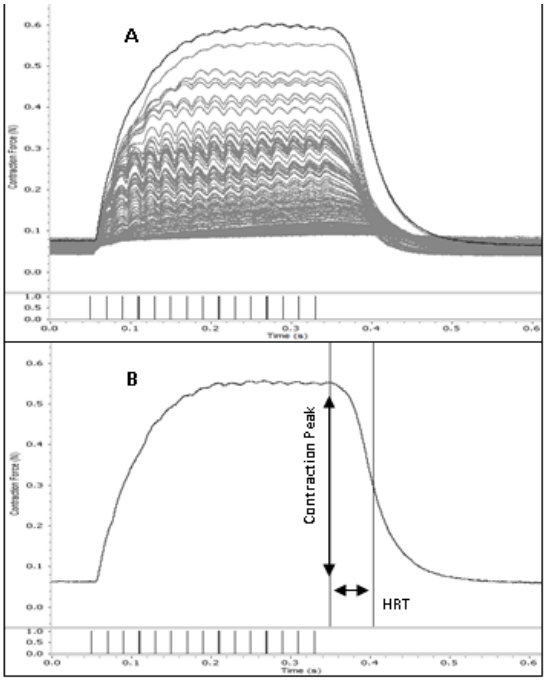

Figure 1. (A) A common example of muscle contractions (upper) induced by each stimulation (lower), records were superimposed. (B) Common record of mouse gastrocnemius skeletal muscle contraction. A, Peak force of contraction; B, Half-relaxation time (HRT).

In all the fatigue index curves, a typical non-linear decrease in the muscle contraction force during 140 contractions (about 10 minutes of stimulation) are shown in Fig. 2. In the control group, the fatigue index at the 70th and 140th contractions are 0.176 and 0.078. For the groups irradiated with 637 nm wavelength, the 70th and 140th contractions fatigue indexes are as follows: 0.376 and 0.259 in 0.25 J LLLT dose, and 0.461 and 0.39 in 0.5 J LLLT dose, and 0.404 and 0.103 in 1.0 J LLLT dose, and 0.373 and 0.298 in 1.5 J LLLT dose, respectively. For the groups irradiated with 785 nm wavelength, the 70th and 140th contractions fatigue indexes are as follows: 0.354 and 0.128 in 0.25 J LLLT dose, and 0.503 and 0.354 in 0.5 J LLLT dose, and 0.448 and 0.321 in 0.75 J LLLT dose, and 0.284 and 0.179 in 1.0 J LLLT dose, respectively. For the groups irradiated with

1064 nm wavelength, the 70th and 140th contractions fatigue indexes are as follows: 0.593 and 0.418 in 0.5 J LLLT dose, and 0.437 and 0.212 in 1.0 J LLLT dose, and 0.443 and 0.366 in 1.5 J LLLT dose, and 0.308 and 0.157 in 3.0 J LLLT dose, respectively.

Regarding the half-relaxation time (Fig. 3), in the control group, the half-relaxation time at the 70th and 140th contractions are 307% and 282%. For the groups irradiated with 637 nm wavelength, the 70th and 140th contractions half-relaxation time percentages are as follows: 150% and 199% in 0.25 J LLLT dose, and 130% and 102% in 0.5 J LLLT dose, and 258% and 411% in 1.0 J LLLT dose, and 100% and 110% in 1.5 J LLLT dose, respectively. For the groups irradiated with 785 nm wavelength, the 70th and 140th contractions half-relaxation times are as follows: 150% and 276% in 0.25 J LLLT dose, and 139% and 163% in 0.5 J LLLT dose, and 228% and 249% in 0.75 J LLLT dose, and 152% and 125% in 1.0 J LLLT dose, respectively. For the groups irradiated with 1064 nm wavelength, the 70th and 140th contractions half-relaxation times are as follows: 144% and 192% in 0.5 J LLLT dose, and 177% and 311% in 1.0 J LLLT dose, and 106% and 121% in 1.5 J LLLT dose, and 153% and 207% in 3.0 J LLLT dose, respectively.

Whereas the force-time integration (Fig. 4), in the control group, the force-time integration percentages at the 70th and 140th contractions are 28% and 12%. For the groups irradiated with 637 nm wavelength, the 70th and 140th contractions force-time integration results are as follows: 40% and 28% in 0.25 J LLLT dose, and 48% and 39% in 0.5 J LLLT dose, and 55% and 16% in 1.0 J LLLT dose, and 39% and 30% in 1.5 J LLLT dose, respectively. For the groups irradiated with 785 nm wavelength, the 70th and 140th contractions force-time integration results are as follows: 40% and 16% in 0.25 J LLLT dose, and 58% and 41% in 0.5 J LLLT dose, and 49% and 34% in 0.75 J LLLT dose, and 35% and 19% in 1.0 J LLLT dose, respectively. For the groups irradiated with 1064 nm wavelength, the 70th and 140th contractions force-time integration results are as follows: 64% and 48% in 0.5 J LLLT dose, and 52% and 25% in 1.0 J LLLT dose, and 45% and 37% in 1.5 J LLLT dose, and 36% and 20% in 3.0 J LLLT dose, respectively.

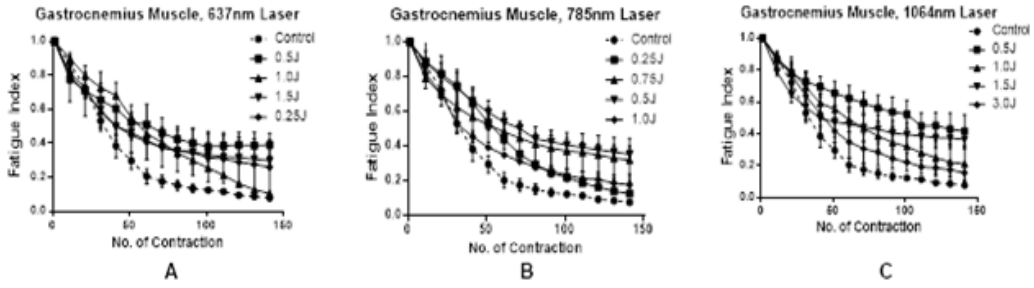

Figure 2. Fatigue index calculated at each contraction for control and laser-irradiated groups. A, 637 nm Laser irradiated group; B, 785 nm Laser irradiated group; C, 1064 nm Laser irradiated group. The data are expressed as the mean ± SEM.

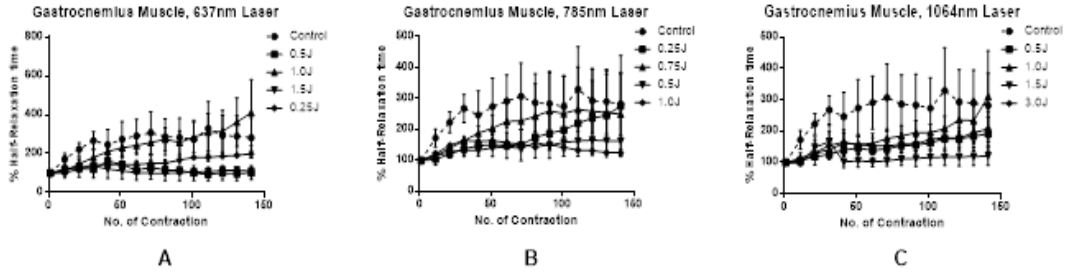

Figure 3. Half-relaxation time at each contraction for control and laser-irradiated groups. A, 637 nm Laser irradiated group; B, 785 nm Laser irradiated group; C, 1064 nm Laser irradiated group. The data are expressed as the mean ± SEM.

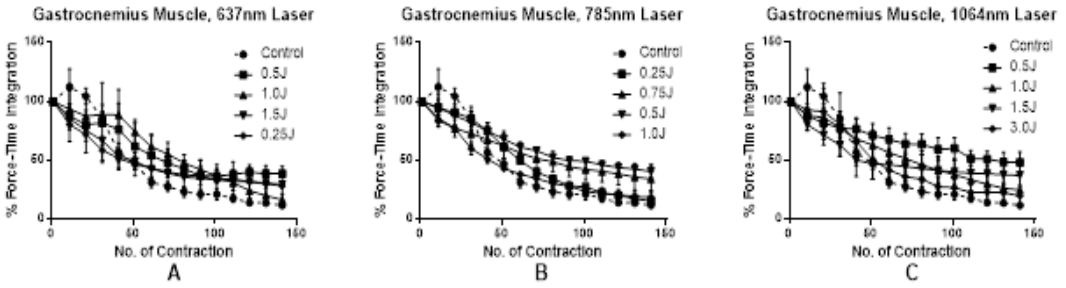

Figure 4. The force-time integration at each contraction for control and laser-irradiated groups. A, 637 nm Laser irradiated group; B, 785 nm Laser irradiated group; C, 1064 nm Laser irradiated group. The data are expressed as the mean ± SEM.

Regarding fatigue index alterations (Fig. 5), at the 70th contraction, the 637 nm 0.5 J group, the 785 nm 0.5 J and 0.75 J groups and the 1064 nm 0.5 J and 1.5 J groups showed a significant effect ($P<0.05$) compared to the control group. Also the 1064 nm 0.5 J was significant ($P<0.05$) compared to the both 785 nm 0.75 J and 1064 nm 3.0 J groups. While at the 140th contraction, only the 637 nm 0.5 J group and the 1064 nm 0.5 J group showed a significant effect ($P<0.05$) compared to the control group and the 1064 nm 0.5 J group was significant ($P<0.05$) compared to the 637 nm 1.0 J group.

In contrast, there were no significant differences at the 70th and 140th contractions and among all the groups for alterations in half-relaxation time (Fig. 6). In force-time integration alterations (Fig. 7), although an increase in the force-time integration results of the laser irradiated groups was shown at the 70th and 140th contractions. But, no significant difference was found among the three groups compared with the control group.

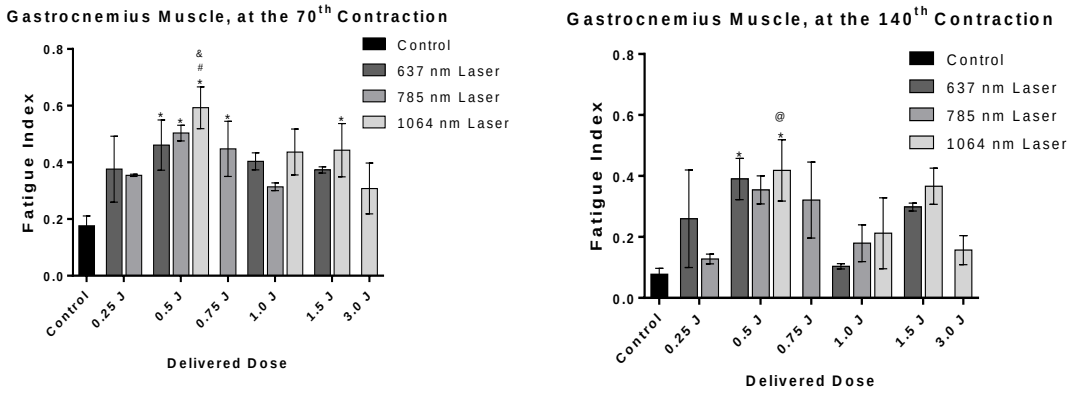

Figure 5. Changes in the averaged fatigue index values at 70th and 140th contractions during the fatigue protocol in the control and the laser-irradiated groups (n = 3 per group). * indicates significant difference compared to control group; # indicates significant difference compared to 785 nm 0.75J group; & indicates significant difference compared to 1064 nm 3.0J group; @ indicates significant difference compared to 637 nm 1.0J group ($P < 0.05$). Error bars indicate SEM.

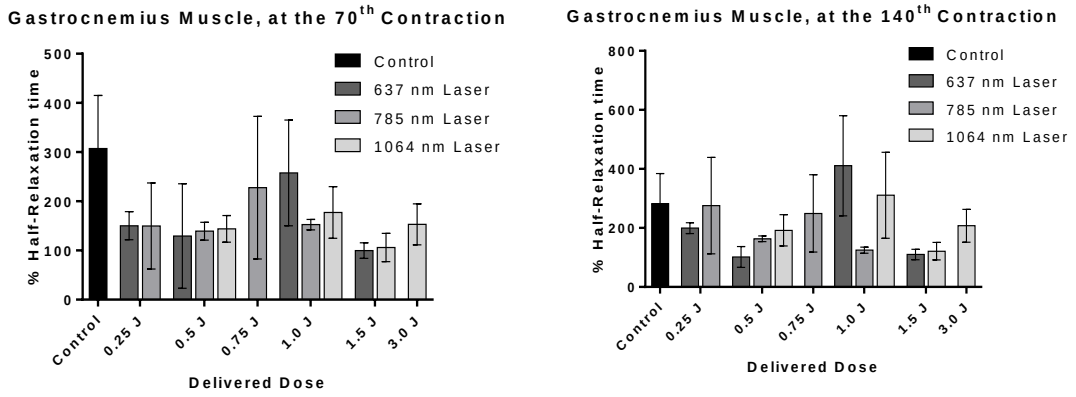

Figure 6. Changes in the averaged half-relaxation time values at 70th and 140th contractions during the fatigue protocol in the control and the laser-irradiated groups (n = 3 per group). Error bars indicate SEM.

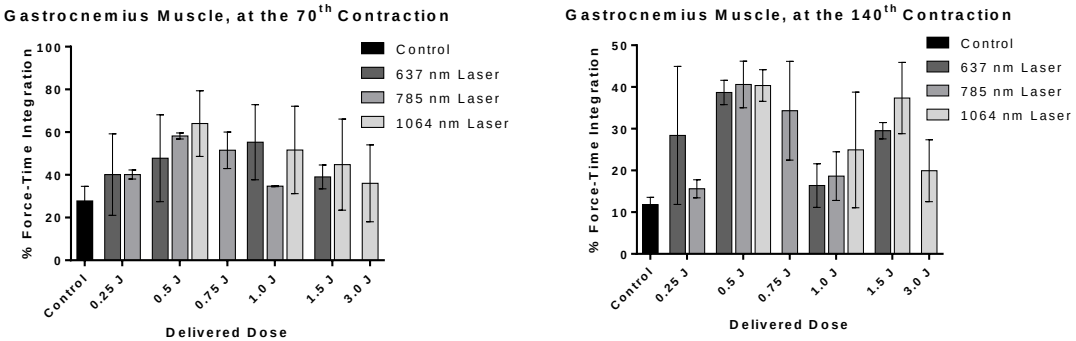

Figure 7. Changes in the averaged force-time integration values at 70th and 140th contractions during the fatigue protocol in the control and the laser-irradiated groups (n = 3 per group). Error bars indicate SEM.

V. DISCUSSION

This study confirmed that three LLLI wavelengths with four different delivered doses in the same experiment had diverse influences on fatigue development skeletal muscle in mice, a species commonly used for evaluating drug treatments and toxic compounds. It was hypothesized that laser beam irradiation could improve muscle performance in the irradiated mice. The main results have shown that mice with LLLT irradiation revealed a significant increase in fatigue index when compared to the control group but there was no significant difference in the half-relaxation time. Furthermore, laser irradiation produced a non-significant increase in force-time integration results during the 140 muscle contractions.

Regarding the skeletal muscle performance, the present study displays strong results favoring both 637 and 1064 nm wavelengths with a delivered dose of 0.5 J. Which means that when the objective of LLLT irradiation is to improve strength and delaying fatigue development, the above-mentioned dose and wavelengths appears to be optimal. The extended time needed for the onset of muscle fatigue predetermined with clinical reports describing increased torque in athletes treated with laser radiation with different wavelengths [33].

The criteria of muscle enhanced strength for sports and everyday activities without causing damage to the muscle fibers and without effecting muscle functionality is one of the privileges of the use of LLLT in skeletal muscle [25, 34, 35, 36]. Consideration of specific LLLT doses and forms were addressed to enhanced cellular activity [26]. The selected laser wavelengths are associated to the electromagnetic spectrum which is absorbed in the respiratory cycle [37]. These wavelengths provide simulation of the respiratory chain which result in raising the mitochondrial ATP generation in order to provide the required energy to the cell throughout the chemical bonds of cytochrome c [25].

As a consequence of combination of both enhanced ATP production and increased blood supply, the outcomes reveled that cell activity was elevated. Generally, troponins in phosphorylation utilize the ATP to promote the essential cross-bridges necessary for muscle contraction [38].

The major photoacceptors include cytochrome c oxidase and their cofactors are assumed to play a role in the production of ATP. Consequently, LLLI effects weren't caused by the thermal effect of laser energy. The utilization of LLLI to illustrate a therapeutic process, in fact, precludes any developing photothermal effects, as the energy is transferred to the cell atraumatically and athermally [39]. However, the influence is realized through a cascade of photophysical and photochemical effects through the triggering of proper photoreceptors in the respiratory chain of the mitochondria and other cellular targets [40].

A phototherapy dose- dependent pattern has been found in many recent studies which is differ from the conventional dose–response curves noticed for drugs. Referring to laser radiation, many research proposes the probability of ‘‘therapeutic windows”. A mid-level doses in these therapeutic windows cause stimulatory effects while high or low doses lead to no effects with a repeated pattern along the energy scale. This might be also the rationale for the deficiency of impact for the 1.0 J, 1.5 J, 3.0 J groups which probably resulted in too much-irradiated dose. In all the outcomes of muscle performance presented in this study, a dose–response pattern was noticed.

Nevertheless, the results of the present study pointed out the effectiveness of the laser parameters applied in the stimulation of the exercised muscle tissue and corroborate with latest scientific evidence in the use of pre-exercise phototherapy in the improvement of skeletal muscle performance and enhancement of skeletal muscle healing [41]. However, a few things to consider for optimizing clinical use. First, the doses used are “in-situ” doses, due to the fact that the skin was removed and the muscle was exposed. In a clinical environment, higher doses are necessary to penetrate human skin [42]. Second, the muscle in the experiment was less than 5 mm thick only, therefore most of the mouse gastrocnemius muscle received a sufficient LLLT dose.

VI. CONCLUSION

It is concluded that LLLI effects on the gastrocnemius muscle are wavelength dependent and LLLI at suitable wavelengths and doses has potential in improvement of fatigue index and thus increases the resistance of the muscle to fatigue. This model should be valuable for examining a wide range of pathological conditions in mice. The present finding may have significances for the use of LLLT in recovery after high-intensity exercises and in muscle pain relives in a dose-dependent manner. Further investigations are required to elucidate the exact laser mechanisms responsible for the delayed onset of skeletal muscle fatigue and analysis the morphologic aspects of muscle tissue to examine the protective effects of phototherapy in pre-exercise irradiated muscle tissue. These kinds of future studies will definitely play a role to a better understanding of the efficacy and safety of LLLT.

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