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Photobiomodulation efficacy of 808 nm Low Level Laser therapy on Lipopolysaccharide compromised Immune status: An Experimental study in Rats

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ABSTRACT

The current study aimed to assess the efficacy of two unlike laser wavelengths (655 nm and 808 nm) used as photobiomodulation or low-level laser therapy (LLLT) on the immune status in male rats. Sixty-five male Sprague–Dawley rats were considered in the experiments which were further divided into three groups. For all the three groups, LPS (lipopolysaccharide) was used as an immunostimulant and it was administered 5mg/kg via intraperitonially (ip) route for 3 consecutive days. In the first group, 655 nm LLLT (power 150 mW) was investigated against LPS induced immune response. Second group investigated 808 nm LLLT (power 150 mW) against LPS induced immune function. After finding out the better responsive LLLT, third group investigated 808 nm LLLT with different power ie 75 mW and 150 mW. The results indicated that 655 nm LLLT at 150 mW power was not restored LPS associated TNF-α and IL-1β content. While, LPS induced enhance IL-1β and TNF-α level was significantly decreased in 808 nm LLLT group. Further, comparison was studied in 808 nm LLLT at different power ie 150 mW and 75 mW and pro-inflammatory cytokines estimation was quantified. The outcome of the study concluded that 808 nm LLLT has anti-inflammatory activity at 150 mW power. Hence, the further research work is required in this direction to advise that 808 nm LLLT at 150 mW could be considered as a treatment to protect inflammatory diseases.

Keywords: Photobiomodulation; low-level laser therapy (LLLT); immune status; 655nm; 808nm; in vivo

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INTRODUCTION

Inflammation is a common characterized symptom of numerous chronic diseases, including cardiovascular and bowel diseases, auto immune diseases, glomerulonephritis, coeliac disease, inflammatory bowel disease, hepatitis, allergy, pre perfusion injury, asthma, transplant rejection, diabetes and cancer[1] (Libby 2007). Even though, inflammatory response depends on location of body and the nature of initial stimulus. As an inflammatory response mechanism, different inflammatory pathways activated which resultant into release of cytokines from respective inflammatory cell[2].

Over the years, various approaches developed to overcome with inflammation but their effectiveness are limited[3,4]. To consider the seriousness of the problem, recently the extensive research focused on non-pharmacological intervention in which one of them is photobiomodulation (PBM) or low-level laser therapy (LLLT). The effect of LLLT is photochemical and mechanically, LLLT shows its beneficial effects via triggering biochemical changes within cells.

Many studies globally reported that low level laser therapy employed as an anti-inflammatory treatment against numerous disease conditions like cancers, infectious diseases and wounds. Hentschke et al.,[5] aimed to investigate the effect of LLLT against the inflammatory status of rats in reference to heart failure and depicted that LLLT has systemic and anti-inflammatory effects in rats. Tumilty et al.,[6] also reported the beneficial effect of LLLT for treating tendinopathy. Basford et al.,[7] reported that treatment with low-intensity 1.06 microm laser irradiation has ability to reduce moderate pain and improve function in musculoskeletal low back pain suffering patients. Oliveira et al.,[8] also reported the immunomodulatory effect of LLLT on delayed type hypersensitivity (DTH) to ovalbumin in Balb/C mice. Albertini et al.,[9] reported anti-inflammatory efficacy of low-level laser therapy (LLLT) with two red wavelengths (660nm and 684nm) in carrageenan-induced rat paw edema. He-Ne laser therapy also have beneficial efficacy as it can reduce the severity of oral mucositis, pain and functional impairment[10]. Hegde et al.,[11] reported the beneficial effect of laser dose of 3 Jcm^{-2} when applied immediately after the wounding in diabetic mice. Ganju et al.,[12] also reported the immunomodulatory effect of laser in male rats. Hence, the current study engaged to investigate the immunomodulatory activity of LLLT on whole body laser exposure in rats.

MATERIALS AND METHOD

Chemicals and Reagents

TNF-α, IL-1β, IL-6 and IFN-γ ELISA kits were purchased from Cayman Chemicals, New Orleans, Louisiana, USA, Lipopolysaccraide (LPS), were purchased from Sigma, USA.

Study design

Ethical clearance for animal study

Male Sprague-Dawley rats, weighing 220±10 g, bred in the animal facility of Defence Institute of Physiology and Allied Sciences (DIPAS), Delhi. Animals were managed on rice husk bedding in polypropylene cages. The controlled environment was maintained in the Institute's animal house ie 55±10 % humidity, 25±1°C temperature and 12-h light-dark cycle. Animals were freely access to standard rodent pellet feed and water ad libitum. Animal Ethical Committee of the Institute in accordance with Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), of the Government of India has approved the study (IAEC No. DIPAS/IAEC/2017/5b). The ARRIVE (Animal Research: Reporting of in vivo Experiments) guidelines for reporting animal research was followed Kilkenny et al.,[13] (2010).

Induction of immunostimulant state

LPS derived from *E coli* O111:B4 serotype was purchased from Sigma-Aldrich, Italy. The freshly prepared LPS solutions were administered with the doses of 5 mg/kg (ip; 2 mL/kg body weight) for 03 days continuous to produce immunostimulant state in rats.

Low-level-laser therapy (LLLT)

Whole body LLLT exposure on rats was performed using an Al-Ga-As diode laser with laser mode on CW (continuous wavelength) with power 75mW and 150mW (Figure 1). The complete laser parameters are given in table 1. Rats were exposed to LLLT once daily for 03 consecutive days simultaneously with LPS administration.

Table 1: Laser parameters of continuous wave

Laser Parameters	Specification
Experiment 1 (wavelength, power, energy)	655nm,150mW, 9J
Experiment 2 (wavelength, power, energy)	808nm,150mW, 9J
Experiment 3 (wavelength, power, energy)	Comparison between 808nm,150mW, 9J and 808nm, 75mW, 4.5J
Mode	Continuous
Spot size	Whole body exposure
Illumination Time (Exposure time)	60 sec
Illuminated area (cm^2)	270 cm^2
Irradiance (W/cm^2)	0.55 mW/cm^2 /1.11 mW/cm^2
Beam profile	Top-hat (height: 21 cm)
Manufacturer	TAPAN dual laser system, India

Figure 1: Positioning of the rat during photobiomodulation therapy with 808nm/655nm diode laser applied alone and on LPS treated rats

Experimental design

A total sixty-five male SD rats, weight of 220±10g, were divided into three experiments.

Experiment 1:

Experiment 1 consists of four sub-group with 5 rats each: a control group, a LLLT alone exposed group (655 nm, 150 mW), LPS administered group (5mg/Kg; immunostimulant group), LPS and LLLT simultaneously exposed group (655 nm, 150mW).

Experiment 2:

Experiment 2 consists of four subgroups with 5 animals each: a control group, a LLLT alone exposed group (808 nm; 150mW), LPS administered group (5mg/Kg; immunostimulant group), LPS and LLLT simultaneously exposed group (808 nm, 150mW).

Experiment 3:

Experiment 3 consists of five subgroups with 5 animals each: a control group, a LLLT exposed group (808 nm, 150mW), a LLLT alone exposed group (808 nm; 150mW), LPS administered

group (5mg/Kg; immunostimulant group), LPS and LLLT simultaneously exposed group (808 nm, 75mW), LPS and LLLT simultaneously exposed group (808 nm, 150mW).

After exposure, animals were sacrificed, blood was collected and serum was separated. The pro-inflammatory cytokines analysis was performed in serum.

Pro-inflammatory and anti-inflammatory cytokines evaluated

Pro-inflammatory and anti-inflammatory cytokines were estimated using ELISA as per manufacturer's instructions.

Statistical analysis

All the experiments were performed on a minimum of three different occasions. Data are depicted as mean±SEM. One-way analysis of variance with post hoc Bonferroni analysis was exploited to analyze statistical significance among groups. GraphPad Prism ver 8 software (GraphPad, CA, USA) was used for analysis. The p value of ≤0.05, with a 95% confidence interval was considered significant.

RESULTS AND DISCUSSION:

Effect of LLLT (655nm; 150mW) on pro-inflammatory cytokines:

Pro-inflammatory cytokines (TNF- α and IL-1β) production increased significantly in LPS treated rats with compared to control rats. TNF- α and IL-1β secretion was also increased non-significantly in LLLT alone exposed rats. The level of TNF- α and IL-1β decreased non-significantly in LPS and LLLT, simultaneously administered rats as compare to LPS treated rats (Figure 2).

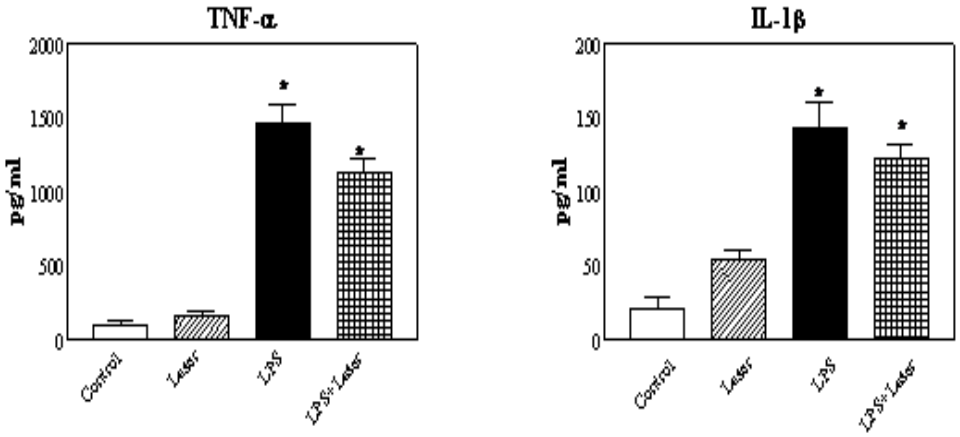

Figure 2: Effect of 655nm LLLT (power 150mW) for 3 days on pro-inflammaatory cytokines in immunostimulant rats. Data represented as mean ± SEM. n=5; *p<0.05 vs Control

Effect of LLLT (808nm; 150mW) on pro-inflammatory cytokines:

Pro-inflammatory cytokines (TNF- α and IL-1β) production increased significantly in LPS treated rats with comparison to control rats. The level of TNF- α and IL-1β was not changed in LLLT (808nm; 150mW) alone exposed rats. While, the level of IL-1β and TNF-α decreased significantly in co-exposed rats, LPS and LLLT (808nm; 150 mW) with compare to LPS treated rats (Figure 3).

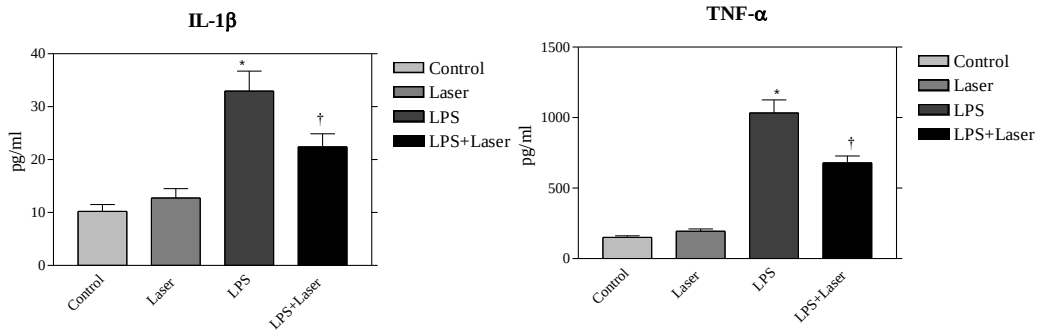

Figure 3: Effect of 808nm LLLT (power 150mW) for 3 days on pro-inflammaatory cytokines in immunostimulant rats. Data represented as mean ± SEM. n=5; *p<0.05 vs Control, †p<0.05 vs LPS

Effect of LLLT (808nm at 75mW and 150mW) on pro-inflammatory cytokines:

The level of pro-inflammatory cytokines, TNF-α, IL-6, IL-1β and IFN-γ increased significantly in LPS treated rats which recovered little but significantly in LLLT (808nm; 150 mW) exposed rats. On the other hand, LLLT (808nm; 75mW) exposed rats didn't show any significant changes in above pro-inflammatory cytokines as compare to LPS treated rats (Figure 4).

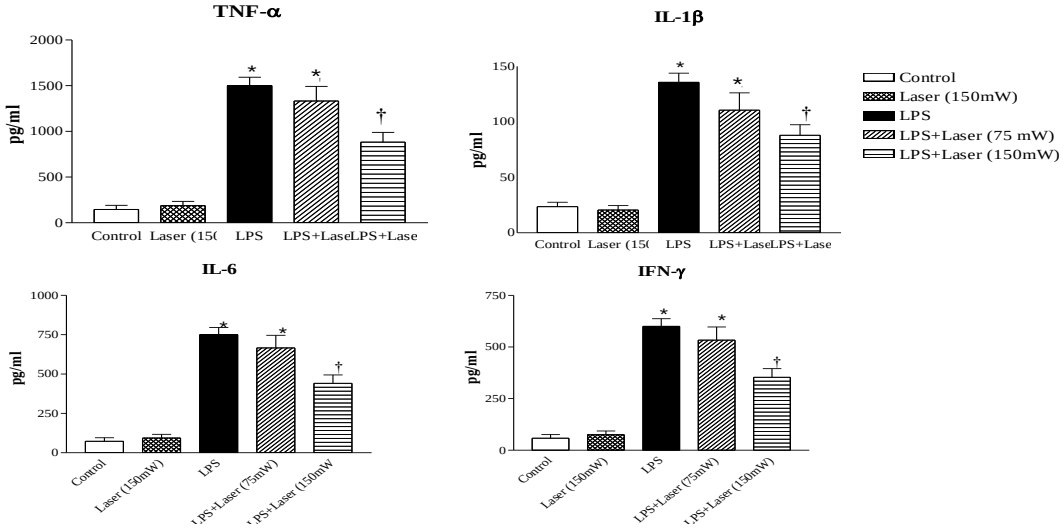

Figure 4: Effect of 808nm LLLT (different power, 75mW and 150mW) for 3 days on pro-inflammatory cytokines in immunostimulant rats. Data represented as mean ± SEM. n=5; *p<0.05 vs Control, †p<0.05 vs LPS

An overview of immunomodulatory activity of low level laser therapy (LLLT) with special context to pro-inflammatory cytokines secreted in serum was demonstrated in the present study. In detail, the first two experiments were conducted to compare two laser beams (viz. 655nm and 808nm) in rats with regard to immune response. First two experiments depicted that 808nm (with power 150mW) has anti-inflammatory activity as compared to 655nm LLLT. Further, Third experiment was designed to investigate the two power exposures with 808nm LLLT and demonstrated that 808nm LLLT with 150mW power showed anti-inflammatory activity while other was having no significant effect on LPS exposed rats.

Inflammation is the first response during any infection and in normal scenario it starts with the production of pro-inflammatory cytokines, TNF-α and IL-1β. A balance between the productions of pro- and anti-inflammatory cytokines determines the effectiveness of the inflammatory response[14] and once it disturbs that lead to inflammation. The agents that could suppress their secretion considered as anti-inflammators while the agents that could enhance their activity known as immunostimulators. In the initial phase of inflammation, TNF-α secrete rapidly and considered as the principal intermediary of inflammatory response[15]. In the further step of inflammation, TNF-α activates the production of other cytokines (IL-1 β, IL-6), eicosanoids and reactive oxygen species[16].

LLLT exposure at 655nm showed improvements in hair growth in males and females[17]. Few recent studies also reported the beneficial role of 655 nm LLLT in reducing oxidative stress and delay the fatigue of skeletal muscle[18, 19].

Assis et al.,[20] reported that 808nm LLLT (continuous wave, 808 nm, tip area of 0.00785 cm^2, power 30 mW, application time 47 seconds, fluence 180 J/cm^2; 3.8 mW/cm^2; and total energy 1.4 J) could be used as therapeutic approach for modulating oxidative and nitrative stress. Beside this, 808nm LLLT also reported to reduce inflammation in injured muscle after cryolesion. Another study also reported an increased muscle fatigue resistance activity at 808nm LLLT (100 mW, 4 J) in healthy young men and women[21- 23]. Beside this LLLT (808nm, 0.25 watts – during 1-minute cycle for 6 consecutive time) could be successfully used for the treatment of migrant glossitis[24].

The current study depicted that the level of TNF-α and IL-1β was not changed significantly after treatment of 655nm LLLT (power: 150mW). While, the level of TNF-α and IL-1β decreased significantly in 808nm (power: 150mW) LLLT treated rats. The comparison of these two wavelength LLLT treatment demonstrated that 808nm LLLT showed anti-inflammatory activity against LPS exposure. Ergo, a comparison between 808nm LLLT with different power was

established and the results described that 808nm LLLT with 150 mW power consists anti-inflammatory activity.

CONCLUSION

The findings of the present study depicted that low level laser therapy (LLLT) of 808nm wave length with 150 mW showed little anti-inflammatory activity as it decreased LPS induced pro-inflammatory cytokines. Hence, the further research work is required in this direction to advise that 808nm LLLT at 150mW could be clinically useful to protect inflammatory diseases.

ABBREVIATIONS:

LLLT: low-level laser therapy; LPS: Lipopolysaccraide

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Compliance with ethical standards

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