Oncology
and Radiotherapy ©

17 (12) 2023: 001-006 • RESEARCH ARTICLE

The effects of different laser irradiation techniques on the proteome composition of human blood plasma with breast cancer

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ABSTRACT

As a continuation of earlier laboratory research and its findings, we are studying the effects of biostimulation and alteration on human blood plasma to improve blood circulation in blood vessels, treat some infections, and treat various diseases, including blood protein-related ones. Blood samples with breast cancer were collected through venipuncture into tubes containing, ethylenediaminetetr acidic (EDTA) as an anticoagulant from healthy adult donors, and plasma was separated from blood components. Blood plasma samples were irradiated for varying periods of time (5,10,15, and 20) minutes. Before and after irradiation, total protein and albumin concentrations were calculated using 375 nm and 650 nm lasers. Using a spectrophotometer, the concentration of total protein and albumin was determined for each sample. At the (375 and 650) nm laser wavelength and exposure durations of (5,10,15, and20) minutes, it was observed that the total protein concentration had significant differences between pre- and post-irradiation probate value (p-value) (0.05, 0.05, 0.05, and 0.05, respectively). It was observed that the total protein and albumin concentrations had significant differences between pre- and post-irradiation. In addition, the results demonstrate that the concentration of total protein and albumin decreases more significantly at a laser wavelength of 650 nm as compared to a laser wavelength of 375 nm at times of (5 and 10) minutes. With preference given to the laser with a wavelength of 630 nm.

Keywords: laser irradiation, breast cancer, human blood plasma

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Word count: 2915 **Tables:** 0 **Figures:** 6 **References:** 22

Received: 06 October, 2023, Manuscript No. OAR-24-114073
Editor assigned: 18 October, 2023, Pre-QC No. OAR-24-114073(PQ)
Reviewed: 03 November, 2023, QC No. OAR-24-114073(Q)
Revised: 16 November, 2023, Manuscript No. OAR-24-114073(R)
Published: 10 January, 2024, Invoice No. J-114073

INTRODUCTION

When therapeutic lasers were utilized in dermatology for wound healing more than 20 years ago, the ideas of bio stimulation were first introduced [1]. Low-Level Lasers (LLL) are a unique class of laser that have an impact on biological processes without using heat [2]. Instead, it produces a chemical change by absorbing light, which is known as a photochemical effect [3]. The delivery of appropriate levels of energy density is low, which is why this technology is referred to as low-profile [4]. According to the first law of photobiology, chromophores, which are specific molecular photoreceptors, are required for low-energy visible light to have any impact on a living biological system [5]. It increases Reactive Oxygen Species (ROS) [6,7]. The effects of an in vitro Low-Level Laser (LLL) on the suspended rheology of irradiated plasmas are mainly due to the effects of LLL on the plasma composition which ultimately affects the whole blood. On this basis, human blood plasma was used to influence an important, including proteins found in plasma [8]. Human blood proteins have an impact on a variety of recovery processes in body tissues, particularly those of patients undergoing various medical treatments [9]. According to Genkin, their experimental research had shown that irradiation with laser light induces processes that evidently lead to several changes in the charge of the blood proteins. The resultant of these effects depends on several factors like the dose, incubation time after irradiation and the cellular properties of the tissues [10]. Also, research by Al Musawi 2020, concluded that the samples' protein concentrations did not significantly change after being exposed to laser light at a wavelength of 589 nm, but mechanisms that result in slower serum protein migration did [11]. Moreover, Hawkins and Abrahamse 2005, discovered that low-level laser can change a variety of biological processes, and that these changes are mediated by cellular functional proteins in specific enzymes [12]. This shows that laser light therapy procedures must take into account doses, wavelengths, and frequencies of the laser light before beginning treatment. In another study, exposure to an infrared diode laser beam at a wavelength of 810 nm increased plasma protein concentrations, which may depend on the doses of the laser used on blood samples [13]. The present study aimed to investigate the impact of laser irradiation on proteins and albumin in normal human blood plasma. Specifically, the concentrations of proteins and albumin were examined before and after laser irradiation at various exposure durations. The wavelengths of the laser light utilised in this investigation were 650 nm and 375 nm.

MATERIALS AND METHODS

Sample preparation

All participants provided written agreement before the experiment, which involved choosing and examining healthy volunteers, was carried out in compliance with the local ethics committee of the Department of Physiology, College of Medicine, Mustansiriyah University in year (2023). Each participant's 6 ml of blood were drawn via venipuncture. Blood samples from the venous donor were augmented with an EDIA anticoagulant tube (approximately 1.3 mg/mL) under aseptic conditions, and blood components were separated by a blood centrifuge, using a centrifuge at 4000 rpm for 10 min. Approximately 3 ml of plasma was aspirated and stored in normal tubes using micropipettes. The plasma was divided into three groups: the first group was left unirradiated as a control group, the second group divided into four parts for each part exposed to the 650 nm red laser, and the third group divided into four parts each exposed to the 375 nm laser light.

Laser irradiation

When using Low level laser (LLL), the fixed power density is 30 mW/cm2 (Product type F Series, Changchun Dragon Lasers Co., China), and the LLL wavelengths employed are 375 and 650 nm. Designated protective eyewear was worn during the irradiation process, which took place in a dimly lit room.

Sample irradiation

In this experiment, a volume of 20 μL was irradiated to determine the overall protein content, while a volume of 10 μL was irradiated to specifically measure the albumin content. This process was repeated for different time intervals (5, 10, 15, and 20) minutes for each portion of the sample. The administered dosage was assigned to each sequentially exposed cohort. The laser beam was directed normally into tubes containing blood samples, from top to bottom (only one point of the tube in the center of the tube). The irradiation was performed at room temperature (23 ± 2 °C).

Total protein and albumin measurement

Total protein colorimetric determination based on the principle of biuret reaction (copper salt in alkaline medium). When protein in plasma or serum is treated with copper ions in an alkylene solution, it produces a blue-colored compound. The intensity of the blue color is proportional to the protein content. Total protein concentrations were determined using Switzerland AGAPPE, and albumin concentrations were measured using the Spinreact spain kit. Principle of albumin,the reaction of albumin from serum or plasma with the dye bromocresolgreen results in a color shift proportional to albumin concentration.

Statistical analysis

For statistical analysis, T-Test at p–value is ≤ 0.05. SPSS Statistics (version 26) was used. In order to know the effect of low-level laser, on total bilirubin in human blood plasma. Indicate / * significant difference, NS: Non-Significant

RESULTS

Figure 1 and 2 demonstrates that the difference between the control and irradiation conditions demonstrates that the Ultraviolet (UV) laser with a wavelength of 375 nm had a statistically significant effect on the amount of total protein concentration in human blood plasma with differing exposure times of (5, 10, 15 and 20) minutes, all having a substantial effect on the amount of total protein concentration in human blood plasma. Moreover, according to Figure 3, the difference between the control and irradiation groups demonstrates that the laser (a red laser with a wavelength of 650 nm) statistically produced a clear influence on the level of total protein concentration that was found in human blood plasma at exposure times of (5, 10, 15, and 20) minutes, all of which were significantly more significant.

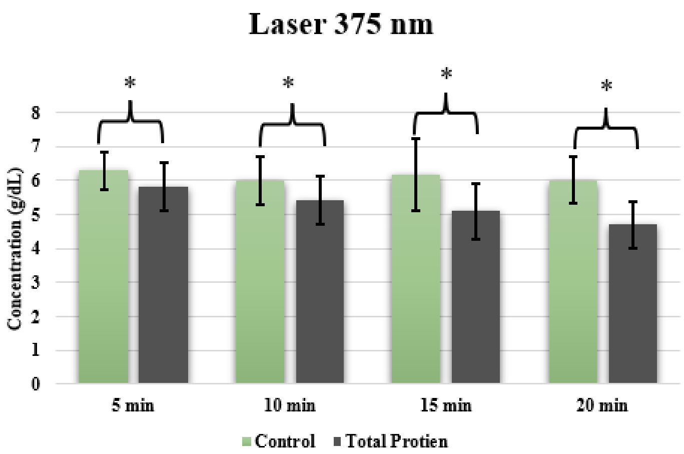

Fig. 1. The change in total protein concentration during different irradiation times of 375 nm laser

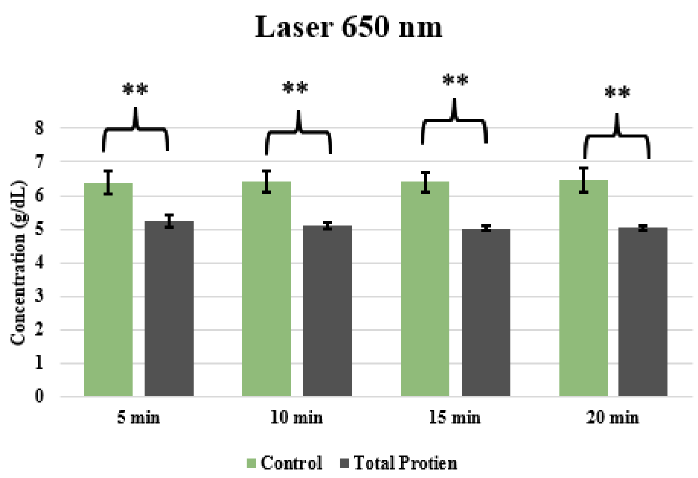

Fig. 2. The change in total protein concentration during different irradiation times of 650 nm laser

Figure 3 presents a comparative analysis of the impact of 375 nm and 650 nm lasers on the overall protein concentration in human blood plasma. A statistical analysis revealed that there exists a discernible distinction in the impact of the two laser types over duration of 5 minutes, with a statistically significant p-value of 0.0194. Furthermore, this distinction becomes even more pronounced when the duration is extended to 10 minutes, as indicated by a more significant p-value of 0.0045. Conversely, no statistically significant difference was detected for durations beyond 10 minutes. Based on statistical analysis, there is no discernible difference observed at the specific time interval of [15, 20].

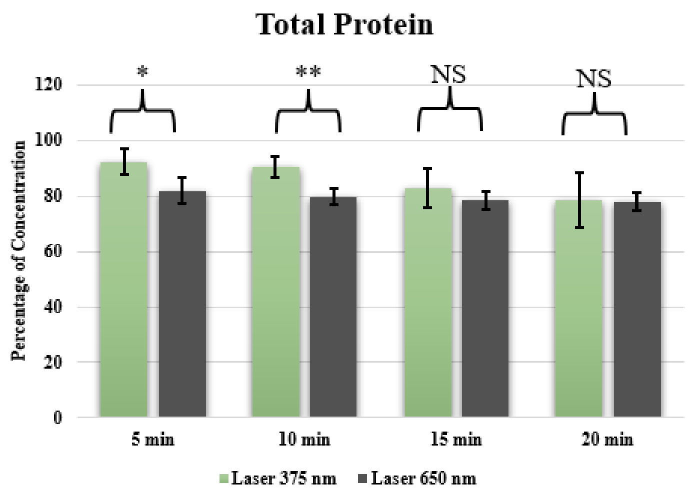

Fig. 3. Comparison between the effect of laser 375 nm and 650 nm on the change in percentage of the total protein concentration during different irradiation times

However, Figure 4 and 5 demonstrates that the difference between the control and irradiation conditions demonstrates that the Ultraviolet (UV) laser with a wavelength of 375 nm had a statistically significant effect on the amount of total albumin concentration in human blood plasma with differing exposure times of (5,10,15, and 20) minutes, all having a substantial effect on the amount of albumin concentration in human blood plasma and, the best effect was at a time of 10 min with a p-value of 0.000293. Moreover, according to Figure 6, the difference between the control and irradiation groups demonstrates that the laser (a red laser with a wavelength of 650 nm) statistically produced a clear influence on the level of albumin concentration that was found in human blood plasma at exposure times of (5,10,15, and 20) minutes, all of which were significantly.

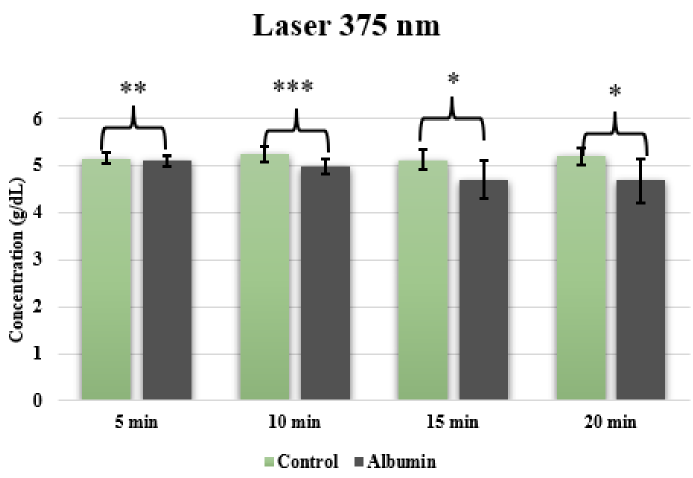

Fig. 4. The change in albumin concentration during different irradiation times of 375 nm laser

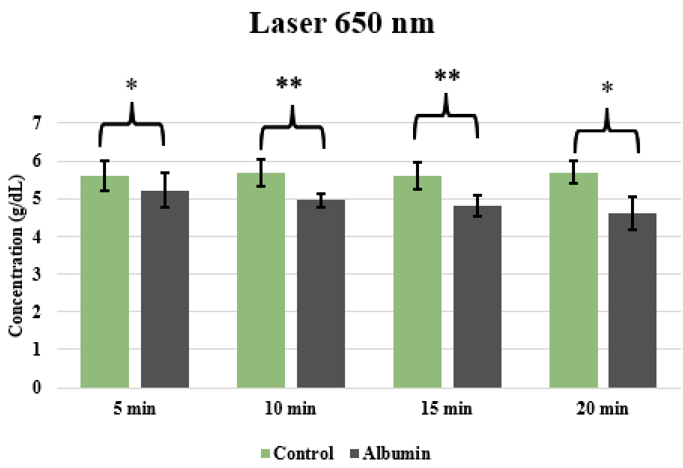

Fig. 5. The change in albumin concentration during different irradiation times of 650 nm laser

Figure 6 presents a comparative analysis of the impact of 375 nm and 650 nm lasers on the overall albumin concentration in human blood plasma. A statistical analysis revealed that there exists a discernible distinction in the impact of the two laser types over duration of 5 minutes, with a statistically significant p-value of 0.03783. Furthermore, this distinction becomes even more pronounced when the duration is extended to 10 minutes, as indicated by a more significant p-value of 0.00159. Conversely, no statistically significant difference was detected for durations beyond 10 minutes. Based on statistical analysis, there is no discernible difference observed at the specific time interval of (15, 20) minutes.

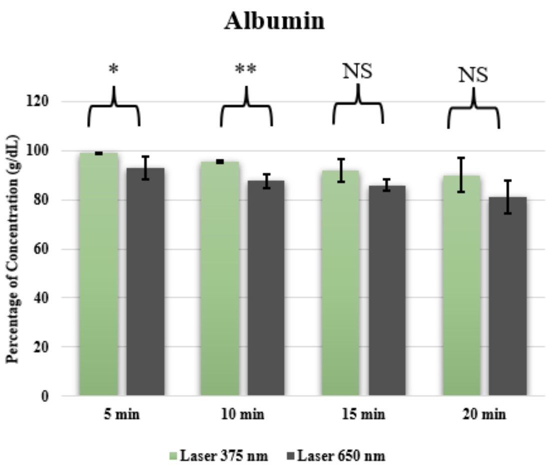

Fig. 6. comparison between the effect of laser 375 nm and 650 nm on the change in percentage of the albumin concentration during different irradiation times

DISCUSSION

The goal of this study was to demonstrate the impact of low-level lasers on proteomic in human blood plasma. In this study, a low-level-energy laser that is less damaging was applied to see how it affected the level of proteomic concentration. Proved in a previous study plasma contains a variety of proteins in a wide and dynamic concentration range such total protein and albumin [14]. De Souza Da Fonseca 2012, has proven that, exposure to an infrared laser at low intensity [1–500 mW] impact on protein in blood samples [13]. This study used laser irradiation in the violet spectral region because hemoglobin absorbs the lightest in this region compared to other spectral regions, allowing light to penetrate deeply into living tissues [15].

Based on the data presented in Figures 1 and 4, it can be observed that the levels of total protein and albumin concentration in blood plasma exhibited a decrease following a 5-minute exposure. This decrease was found to be statistically significant, with P-values of 0.0361 and 0.00596, respectively. Furthermore, the decrease in these parameters persisted even after 10, 15, and 20 minutes of exposure. This matches with prodouz, K.N, where he proved that the radiation caused unacceptable denaturation of plasma proteins. Hence, a multitude of physical and chemical factors, both individually and in conjunction, contribute to alterations and reductions [16]. This is explained by the fact reported in another study that ultraviolet radiation affects blood components and it leads to the loss of potassium ions and affects nucleotides in adenine metabolism, causing a decrease in total protein and albumin concentration [17].

The study of proteins allows us to conduct a broader investigation of LLL through sources [lasers] in which other spectral regions with longer wavelengths may be more suitable for phototherapy [18, 19]. Another type of laser, a red diode laser (650 nm), was investigated to confirm the effect induced by low-level lasers on proteins in blood plasma. Figures 2, and 5 demonstrates that after 5 minutes of exposure, the total protein, albumin content in the blood plasma decreased by P (0.0066) and (0.0614), and that this trend was maintained after (10, 15, and 20) minutes. This is in agreement with the data presented by Genkin VM, who found that incubation and irradiation with a He-Ne red laser (638.2 nm) resulted in a drop in the level of plasma proteins due to charge alterations [20]. After proving the effect of each type of laser on different changes, and on this basis a comparison was made between the effect of (35 nm) and (650 nm) lasers. according to Tunér J, It is necessary to choose the best parameters, to get the best laser type for treatment. Due to the different wavelengths and laser power, the penetration depth varies [21].

According to Figure 6, a comparison was made of the effect of 375 and 650 nm lasers on albumin concentration in human blood plasma. There was a significant difference between the effect of the two types of lasers in time (5 and 10) minutes and there was no statistical significance in time (15 and 20) minutes These results indicate that Low-Level Laser Therapy (LLLT) has an effect on the concentration of human blood plasma proteins, which leads to a significant decrease in the concentration of blood proteins, both for total protein and albumin. This is explained by It was proved Al Musawi & Al-Gailani, that low-level laser changes some physical cellular properties the blood which led to a decrease in protein concentration [22].

CONCLUSION

Plasma protein concentrations, including total protein and albumin, are significantly lowered by low-level lasers. This can be used to treat conditions associated with abnormal blood protein levels. In addition to variations in laser type and wavelength, variations in the extent to which proteins are depleted also exist. This highlights the significance of selecting and employing the optimal wavelength and laser settings for haematology treatment in order to optimise outcomes.

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