Original Article

# *In vitro* Effect of Low-level Lasers on Proteomic Concentration in Human Blood Plasma Using 375 nm and 650 nm Lasers

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## Abstract

**Background:** 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. **Methods:** Blood samples were collected through venipuncture into tubes containing, ethylenediaminetetraacidic as an anticoagulant from healthy adult donors, and plasma was separated from blood components. Blood plasma samples were irradiated for varying periods (5, 10, 15, and20) min. 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. **Results:** At the (375 and 650) nm laser wavelength and exposure durations of (5, 10, 15, and 20) min, it was observed that the total protein concentration had significant differences between pre- and postirradiation probate value (P = 0.05, P = 0.05, P = 0.05, and P = 0.05, respectively). It was observed that the total protein and albumin concentrations had significant differences between pre- and postirradiation. In addition, the results demonstrate that the concentration of total protein and albumin decreases more significantly at a laser wavelength of 650 nm compared to a laser wavelength of 375 nm at times of (5 and 10) min. **Conclusions:** Our results clearly indicate that low-level lasers with different wavelengths of ( 375 , 630) nm both affect the concentration of total protein and albumin in human blood plasma, which can contribute to the treatment of many pathological conditions in the future.

**Keywords:** Albumin, exposure time, human blood plasma, laser, low-level laser irradiation, total protein

## Introduction

When therapeutic lasers were utilized in dermatology for wound healing more than 20 years ago, the idea of biostimulation 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.[6,7] The effects of an *in vitro* 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 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 *et al*., their experimental research had shown that irradiation with laser light-induced processes that evidently lead to several changes in the charge of the blood proteins. The resultant of these effects depends on several factors such as the dose, incubation time after irradiation, and the cellular properties of the tissues.[10] Furthermore, research by Al Musawi Al-Gailani and 2020 concluded that the sample's protein concentrations did not significantly change after being exposed to laser light at a wavelength of 589 nm, but mechanisms that resulted in slower serum protein migration did.[11] Moreover, Hawkins and Abrahamse, in 2005, discovered that low-level

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Quick Response Code:

Website: http://www.mmjonweb.org

DOI: 10.4103/mj.mj_53_23

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**Submitted:** 20-Aug-2023 **Revised:** 11-Sep-2023 **Accepted:** 14-Sep-2023 **Published:** 29-Oct-2024



**How to cite this article:** Al-Kaabi AA, Al-Mosawi M, Al-Bayati AA. *In vitro* effect of low-level lasers on proteomic concentration in human blood plasma using 375 nm and 650 nm lasers. Mustansiriya Med J 2024;23:73-7.

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 utilized in this investigation were 650 nm and 375 nm.

## Materials and Methods

### Sample preparation

Adults who were in good health and had no prior history of major diseases or serious illnesses had their blood drawn. It was done to confirm the confidentiality of the study's confidential data and to notify him of the study's goals and objectives. All participants provided written agreement before the experiment, which involved choosing and examining healthy volunteers, were carried out in compliance with the local ethics committee of the Department of Physiology, College of Medicine, Mustansiriyah University in the year (2023). Each participant's 6 mL of blood was drawn through venipuncture. Blood samples from the venous donor were augmented with an ethylenediaminetetraacidic as an anticoagulant (EDTA) 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 was divided into four parts for each part exposed to the 650 nm red laser, and the third group was divided into four parts, each exposed to the 375 nm laser light.

### Laser irradiation

When using LLL, the fixed power density is 30 mW/cm$^2$ (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, whereas 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) min 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) As shown in Figure 1, the irradiation was performed at room temperature (23°C ± 2°C).

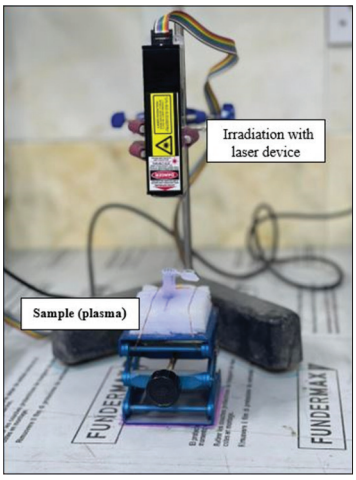


**Figure 1:** Shows the process of irradiating a plasma sample

### Total protein and albumin measurement

Total protein colorimetric determination is 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. The fact shows that the 630 nm wavelength laser had a greater effect, leading to a decrease in total protein and albumin than the 375 laser, which has a beneficial effect in determining the best wavelength to use in treating cases that require reducing the concentrations of total protein and albumin.

### Statistical analysis

For statistical analysis, *t*-test at $P \leq 0.05$. The statistical data of this study was performed using the SPSS IBM Corp. Released 2021. IBM SPSS Statistics for Windows, Version 28.0. Armonk, NY: IBM Corp. was used. To see the effect Low level laser, on the concentration of total protein and albumin in human blood plasma. The sign indicates To the construction the charts /* there is a significant difference, NS: not significant.

## Results

Figure 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) min, 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) min, all of which were significantly more significant.

Figure 4 presents a comparative analysis of the impact of 375 nm and 650 nm lasers on the overall protein concentration in human blood plasma. Statistical analysis revealed that there exists a discernible distinction in the impact of the two laser types for 5 min, with a statistically significant $P = 0.0194$. Furthermore, this distinction becomes even more pronounced when the duration is extended to 10 min, as indicated by a more significant $P = 0.0045$. Conversely, no statistically significant difference was detected for durations beyond 10 min. Based on statistical analysis, there is no discernible difference observed at the specific time interval of (15, 20).

However, Figure 5 demonstrates that the difference between the control and irradiation conditions demonstrates that the 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) min, 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 $P = 0.000293$. 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) min, all of which were significant.

Figure 7 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 for 5 min, with a statistically significant $P = 0.03783$. Furthermore, this distinction becomes even more pronounced when the duration is extended to 10 min, as indicated by a more significant $P = 0.00159$. Conversely, no statistically significant difference was detected for durations beyond 10 min. Based on statistical analysis, there is no discernible difference observed at the specific time interval of (15, 20) min.

## Discussion

The goal of this study was to demonstrate the impact of LLL 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

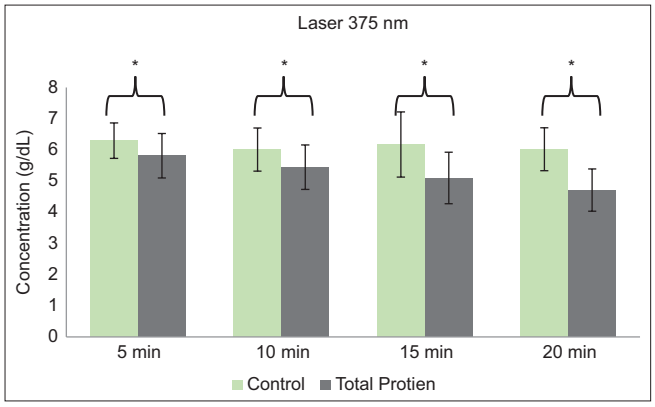


**Figure 2:** The change in total protein concentration during different irradiation times of 375 nm laser

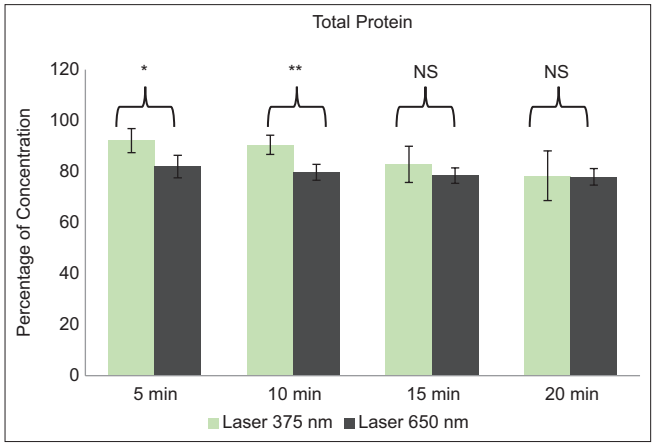


**Figure 4:** Comparison between the effect of laser 375 nm and 650 nm on the change in percentage of the total protein concentration during different irradiation times

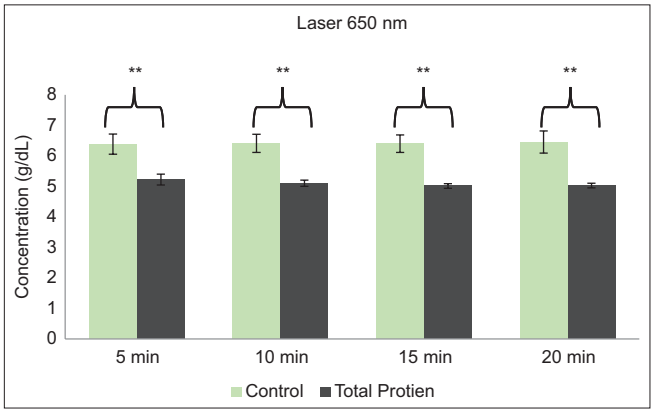


**Figure 3:** The change in total protein concentration during different irradiation times of 650 nm laser

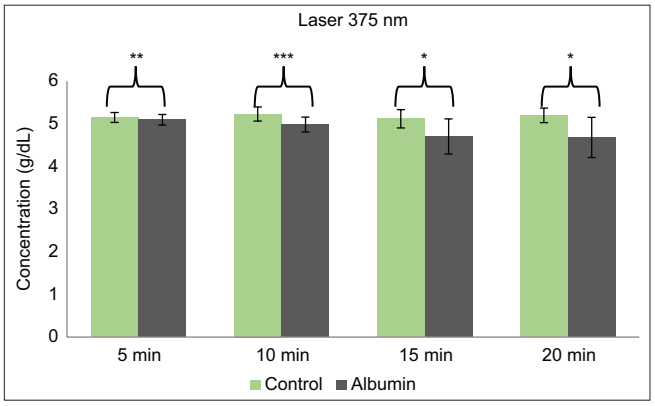


**Figure 5:** The change in albumin concentration during different irradiation times of 375 nm laser

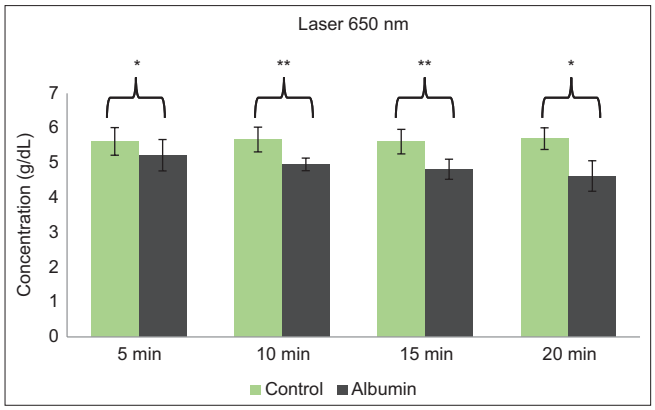


**Figure 6:** The change in albumin concentration during different irradiation times of 650 nm laser

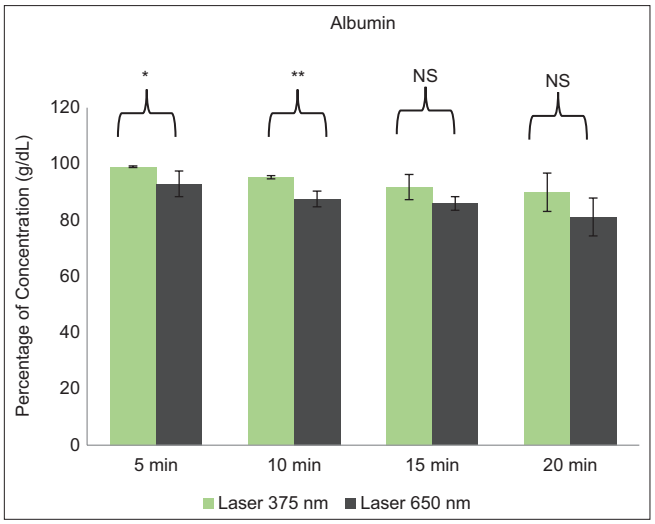


**Figure 7:** Comparison between the effect of laser 375 nm and 650 nm on the change in percentage of the albumin concentration during different irradiation times

a wide and dynamic concentration range such as total protein and albumin.[14] da Fonseca Ade *et al*. 2012, have proven that exposure to an infrared laser at low intensity (1–500 mW) impacts 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-min exposure. This decrease was found to be statistically significant, with $P = 0.0361$ and $P = 0.00596$, respectively. Furthermore, the decrease in these parameters persisted even after 10, 15, and 20 min 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 UV 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 LLL on proteins in blood plasma. Figures 2 and 5 demonstrate that after 5 min of exposure, the total protein and albumin content in the blood plasma decreased by ($P = 0.0066$) and ($P = 0.0614$), and that this trend was maintained after (10, 15, and 20) min. This is in agreement with the data presented by Genkin *et al*., 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.[10]

After proving the effect of each type of laser on different changes, and on this basis, a comparison was made between the effect of (375 nm) and (650 nm) lasers. According to Tunér and Christensen, 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.[20]

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) min and there was no statistical significance in time (15 and 20) min. These results indicate that low-level laser therapy 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 and Al-Gailani that low-level laser changes some physical cellular properties of the blood, which leads to a decrease in protein concentration.[21]

## Conclusion

Low-level laser has a significant effect on the level of reducing the concentration of blood plasma protein content, especially total protein and albumin which have therapeutic benefits for diseases related to blood proteins.

In addition to the difference in the type of laser and its wavelength, its effects differ in the percentage of reducing the concentration of proteins. This indicates the need to consider and use the best wavelength and best laser parameters in the treatment of hematology, which will further improve treatment results.

### Financial support and sponsorship

Nil.

### Conflicts of interest

There are no conflicts of interest.

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