

RESEARCH ARTICLE

Effect of 532nm and 589nm Diode-Pumped Solid-State (DPSS) Lasers with Different Doses on Concentration of Total Protein and Albumin in Vitro

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Abstract

Objective: To test the effects of Low-level laser irradiation at 532nm and 589nm with different doses and exposure times on total protein and albumin in blood plasma.

Method: This experimental study was conducted from November 2021 to April 2022 at Mustansiriyah University, Baghdad, Iraq, and comprised blood samples from healthy adults. The samples were stored in tubes containing anticoagulant ethylenediaminetetraacetic acid. Photoradiation was managed using green laser 532nm and yellow laser 589nm diode-pumped solid-state lasers with different doses. For total protein test and albumin test, each sample was divided into 3 equal aliquots. The first was control without radiation, the second was irradiated by a laser at 532nm, and the third was irradiated at 589nm with 30J/cm², 50J/cm², 70J/cm² and 90 J/cm² doses of irradiation. All samples were examined by using a spectrophotometer device. Data was analysed using SPSS 25.

Results: There were 24 samples from healthy adults in all. The most significant difference in total protein concentration occurred at dose 70J/cm² for both wavelengths compared to the controls (p<0.05). When this optimal dose was applied to the albumin test, a clear decrease in the albumin concentration was found for both 532nm and 589nm lasers (p<0.05).

Conclusion: At wavelengths of 532nm and 589nm, the laser light induced processes that caused a reduction in total protein and albumin.

Key Words: Edetic Acid, Lasers, Plasma, Light, Anticoagulants, Albumins

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Introduction

Over the last three decades, several experimental studies have been conducted to demonstrate that low-level laser therapy (LLLT) has a beneficial impact on the treatment of a variety of conditions¹. The effects of laser radiation interaction with tissues of biological origin and blood are of increasing interest that has received a lot of attention². Blood is an excellent model for studying an organ in the human body since it allows one to study the interaction of its components with laser light irradiation at various wavelengths. The cellular components of blood can be isolated and reconstituted relatively easily, allowing for the irradiation of specific components or the measurement of various factors after whole blood irradiation³. Because blood is found in almost all living tissues, it is chosen as an object for studying the influence of laser radiation on blood's rheological characteristics⁴. The reaction of human blood to low-level laser irradiation (LLLI) reveals important details about how lasers interact with biological tissues⁵. Intravenous (IV) LLLI has been used clinically in China to help treat acute cerebral

infarction, and the results have been encouraging⁶, and are regarded as the most effective method of biostimulation of an organism for therapeutic purposes. On the other hand, the mechanics underlying the potentially harmful effects of laser light are still being debated⁷. Currently, there is no general conclusion about the stimulating influence of laser on cells, as several research in vitro have revealed that biostimulation depends on a number of factors, in addition to parameters of laser irradiation, such as wavelength, output power, dose, beam area, polarisation and irradiance, as well as the kind of cells being subjected to laser irradiation^{8,9}. Human blood proteins have an impact on a variety of recovery body tissue processes, particularly those of patients suffering from various treatment options¹⁰. LLLI is now broadly used in various fields of medicine as a result of experimental advances and medical evidence of powerful and effective treatment efficacy as well as the absence of problems and negative consequences¹¹.

According to a study, laser light irradiation leads to numerous alterations in blood protein charge. The result of these effects is determined by many factors, including the dose, the time for incubation following irradiation, and characteristics of the tissue's cells¹⁰. Reserchers also

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discovered that LLLs can change a variety of biological processes, and that these changes are mediated by cellular functional proteins in specific enzymes. Albumin accounts for around 60% of whole plasma proteins, and it is responsible for the control of several important biological functions, such as acid-base balance, osmotic pressure, and hormone and metal transport¹².

The current study was planned to test the effects of LLLi at 532nm and 589nm with different doses and exposure times on total protein and albumin in blood plasma.

Materials and Methods

This experimental study was conducted from November 2021 to April 2022 at the Postgraduate Medical Physics Laboratory, Mustansiriyah University, Baghdad, Iraq, and comprised blood samples from 24 healthy adults who had no medical history of illness and who were not using any drugs for chronic conditions. The blood samples were drawn after taking informed consent form the subjects.

A total of 6ml of blood was directly transferred into ethylenediaminetetraacetic acid (EDTA) tube before being centrifuged at 4000rpm for 10 min to separate plasma from the red blood cell (RBC) complex. The plasma was divided into 3 equal parts. The first part was labeled as non-irradiated plasma control, the second part was irradiated by green laser 532nm laser with power density 0.03W/cm², and the third part was irradiated by yellow laser 589nm with power density 0.03W/cm² for 20, 30, 40 and 50 min with 30J/cm², 50J/cm², 70J/cm² and 90 J/cm² doses of irradiation.

Diode-pumped solid-state (DPSS) LLL (F Series, Changchun Dragon Lasers Co.China) with wavelength 589nm was used for irradiation in continuous wave mode, while green light 532nm laser (LD Didactic GmbH Class 3R.China) was used for tubes containing 2ml blood

sample. During the experiment, the blood samples were kept at a temperature of 23±2°C. Without any isolation of heat, the power output of the laser was measured using an optical power meter (Gentec-E, Maestro, Canada).

Total protein colorimetric determination was based on the biuret reaction principle¹³ (copper salt in an alkaline medium), according to which, when protein in plasma or serum is treated with cupric ions in alkaline solution, it produces a blue-coloured complex. The intensity of the blue colour is proportional to the content of protein. Total protein concentrations were determined using spectrophotometric analysis (Agappe, Switzerland).

The reaction of albumin from serum or plasma with the dye bromocresolgreen results in a colour shift proportional to albumin concentration. Serum albumin concentrations were determined using spectrophotometric analysis (Agappe Switzerland).

Data was analysed using SPSS 25. A paired t-test was used to evaluate plasma parameter changes before and after irradiation. P<0.05 was considered statistically significant.

Results

There were 24 samples from healthy adults in all. The reduction in total protein concentration at dose 30J/cm² was significantly influenced by 532nm (p<0.0152), but not with 589nm. At dose 50J/cm² there was a significant difference between pre- and post-irradiated plasma samples with green and yellow lasers (p<0.0077 and p<0.0107, respectively). The most significant difference (p<0.0012, p<0.0090, respectively) for the two wave length between (pre- and post-irradiated plasma was at dose 70J/cm². The 90J/cm² dose had significant differences in pre- and post-irradiated samples (p<0.0243 and p<0.0342, respectively) (Figure 1).

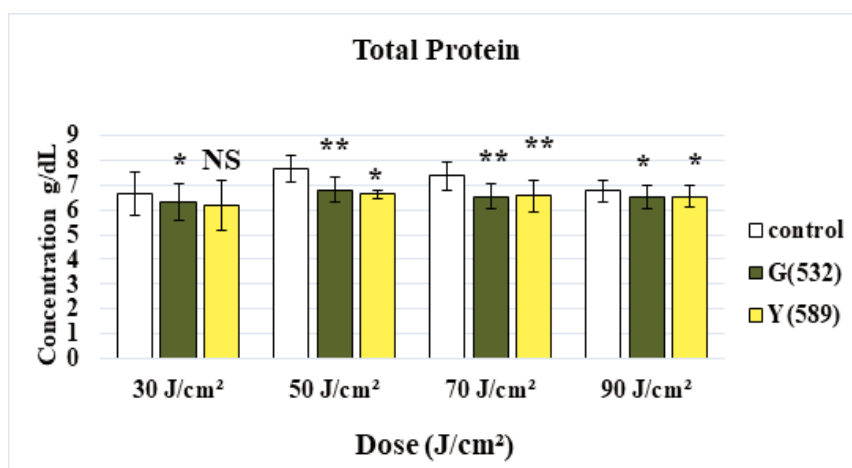


Figure-1: Decrease in total protein at 4 lasers doses of 589nm and 532nm wavelength (number of samples: 24)..

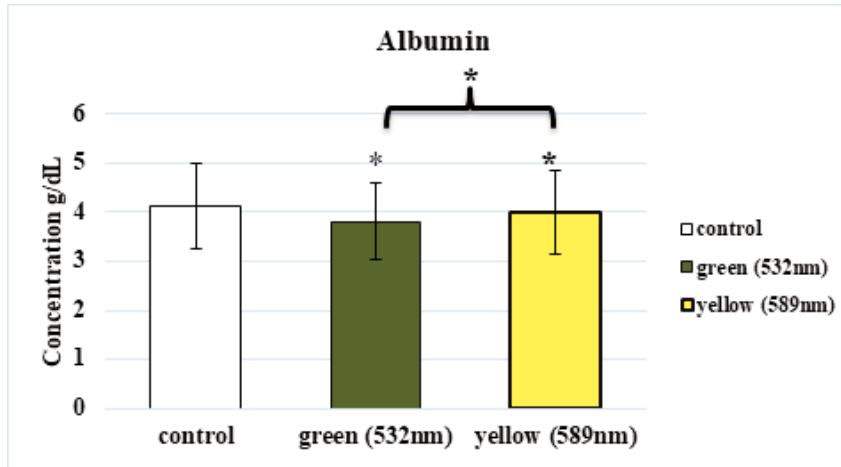


Figure-2: Decrease in albumin by one dose for 532nm ($p < 0.0119$) and 589nm wavelength laser ($p < 0.0142$).

When the optimal $70\text{J}/\text{cm}^2$ dose was applied to the albumin test, a clear decrease in the albumin concentration was found for both 532nm and 589nm lasers ($p < 0.05$) (Figure 2).

Discussion

The current results, compared to other studies¹⁴ showed that a certain threshold was necessary for effective use of LLLI. The results are consistent with the theory of biphasic dosage response. When differentiating "the threshold dose," the biphasic curve was significant¹⁵.

The use of LLLI for treatment is still a contentious issue. However, this approach is being increasingly used in therapeutic settings. The understanding of the early interaction between tissue and light is very limited. A study by Al-Kaabi suggests that this form of therapy can alter biological processes dependent on radiation criteria.¹⁵ It is understandable that laser energy affects many biological molecules, and these effects are noticeable when the energy of laser is absorbed by the molecules, particularly the molecules that contain chromophores and bonds capable of excitation and absorption of energy. To explain how LLLs affect live cells, a variety of hypotheses have been presented, starting from photon absorption by flavins and mitochondrial cytochromes to electron transfer to atoms' new energy levels^{16,17}. More than 35 structural alterations have been identified as a result of protein modification due to oxidative stress impacting protein molecules, including oxidation of amino acids, particularly methionine and cysteine¹⁸. Albumin accounts for 60% of plasma protein mass, and is involved in the regulation of osmotic pressure, the binding and transport of various biological substances, such as hormones, and medicines and blood buffering¹⁶.

Radiation of light absorption by living cells can lead to atom disturbance, resulting in a variety of chemical and biological changes. Radiation may also be influenced directly on live cells by radiolysis of water molecules, which produces reactive oxygen species (ROS), which destroys macromolecules, such as nucleic acids, lipids, acids and proteins¹².

The current study established the initiation of oxidative reactions in protein solution after a brief time of laser beam exposure, and identified oxidation of amino acids in the chains of polypeptide. The current evidence-based study suggests that laser photons facilitate the production of covalent bonds among molecules, resulting in cross-linking between them¹⁹. An experimental study confirmed that when subjected to oxidation and decrease caused by light radiation, a reaction can happen on the side chain of a polypeptide²⁰.

Limitation: The current study has limitations as the sample size was not calculated which could have affected the power of the study.

Conclusion

At wavelengths of 532nm and 589nm, the laser light induced processes that caused reduction in total protein and albumin.

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Conflict of Interest: None.

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