The effects of laser 532 nm with 4 mW and 650 nm with 135 mW on physical properties of human serum proteins in vitro

1Sadiq Hassan Lefta, 2Wajeha Abdle Diam, 3Zeman Hameed, 4Dakhel ghani omran, 5Gufran Sabar

1,2Department of laser physics, college of science for women, Babylon university, Iraq.
3Department of physics, college of science, Karbalaa university, Iraq.
4Department of biology, college of science for women, Babylon university, Iraq.

Email: wajehaab@yahoo.com

Abstract. The present study was undertaken to investigate the effects of two type of laser (532 nm with energy 4 mW and 650 nm with energy 135) on physical characteristics of serum proteins. All subjects of study were 15 men and their ages ranged between 18 to 25 years old. The blood samples were left to coagulate and centrifuged to obtained sera. Sera samples were divided in to three groups, the first group left without irradiation to serve as a control group, the second group was irradiated with laser 532 nm with 4mW for 10 minutes, and the third group was irradiated with laser 650 nm with 135 mW energy for 10 minutes. After irradiation, all samples were analyzed by using HPLC instrument. The results were indicated there were a significant changes among peaks of curves of irradiated samples compared to control group. Also, there were many obvious differences were noted among irradiated groups. From results mentioned above can be concluded that laser energy may be cause and create bonds inter and intra molecules of proteins across oxidation processes that finally change morphological and modifications structures of proteins molecules.

Keywords: Laser, Proteins, Blood

1. Introduction

In biological systems, the laser effects depend upon length of wave, power, and time of exposure, it is well known that energy of laser causing damage to living tissues especially blood cells. 1. Photo-biological stimulation is depend on intensity, dose, and time of exposure. Together, laser radiation and chemotherapy were used and pointed out the best therapeutic effects more than chemotherapy alone in treatment of many diseases 2.
From physics point view, laser undergoes many changes during penetration of tissues, according to difference of refraction constant of different surfaces for example laser transports from air to tissues or from air to liquids.

If the direction of light on surface is vertical, thus, the air is less density than tissues and liquids so that the refraction has been occurred at the same angle of dropped light on the surface according to Snell law. Within tissues, the light is absorbed by respiratory chains causing different alterations of mitochondria and cytoplasm of cells.

It is found clearly that low level laser intensity (LLLl) induces transport additional amount of calcium amount (Ca^{2+}) across cotransport mechanism or at the same time it triggers various biological process such as DNA replication and cell proliferation, and on the other hand, a high lase intensity leads to increase liberation excessive amount of calcium ions that in turn elevates ATPase activity and exhaustion of ATP pool of cells.

There are two types of laser, the first one is called low level laser that is applied commonly in many medical practices such as photobiostimulation of cells, the second type is called high level laser and employed in surgical operations causing heating, coagulation, and necrosis of affected tissues.

2. Design of experiment:
The present study was carried out in biology and laser physic departments of college of science for women in Babylon university from the period ranged between October 2016 to April 2017. A total number of subjects of the present study was 15 men and their ages ranged between 18 to 25 years old and they were free from chronic diseases.

The blood samples were collected from antecubital vein after cleaning the site of collection with alcohol (70%). Blood was drawn by needles with gages 23. Blood was directly transferred in to gel plain tubes and then left for 15 minutes to complete coagulation and then transferred to centrifugation at 3000 rpm for 10 minutes.

Sera were aspirated by micropipettes and kept in eppendorf tubes. The sera were divided in to three groups, the first group was left without irradiation to serve as a control group, the second group was exposed to 532nm with 4 mW laser (Danger company, China) for 10 minutes, and third group was irradiated with laser 650 nm with 135 mW for 10 minutes. After complete irradiation, the serum samples were directly loaded in to high performance liquid – chromatography (HPLC, Knuaer company, Germany) to analyzed results and changes occurring in proteins of sera.

3. Results:
Results which were illustrated in figures 1 and 2 showed that curves of proteins obtained from HPLC system were clearly decrease in apexes (peaks) of protein bands after exposure of samples to laser 650 nm with energy 4mW compared to non-irradiated samples (control group). At the same time, it had been found that irradiated samples with 532 nm with energy 135 mW, their curves of peaks were more drop than of samples exposed to laser 650 nm. The tables 1 and 2 revealed the numerical values of results that are taken from HPLC system.
First group of analyses

Figure 1. represents the effects of laser on protein groups; the red color showed group without irradiation with laser, the blue color represents group exposed to laser 532 nm and green color refer to group irradiated with laser 650 nm.

Table 1. A showed values of protein peaks before irradiation (control group).

| Reten. Time [min] | Area [mAU.s] | Height [mAU] | Area [%] | Height [%] | W05 [min] | Compound Name |
|-------------------|-------------|--------------|----------|------------|-----------|---------------|
| 1                 | 4.950       | 152.276      | 10.776   | 1.5        | 1.3       | 0.30          |
| 2                 | 5.233       | 681.612      | 68.925   | 5.2        | 8.3       | 0.18          |
| 3                 | 5.537       | 1069.821     | 664.031  | 82.1       | 80.2      | 0.25          |
| 4                 | 5.850       | 1452.786     | 94.335   | 11.2       | 10.2      | 0.20          |
| Total             | 1301.495    | 828.071      | 100.0    | 100.0      |           |               |

Table 1.B showed values of protein peaks after exposure to laser 532nm.

| Reten. Time [min] | Area [mAU.s] | Height [mAU] | Area [%] | Height [%] | W05 [min] | Compound Name |
|-------------------|-------------|--------------|----------|------------|-----------|---------------|
| 1                 | 4.933       | 151.079      | 8.703    | 1.3        | 1.2       | 0.28          |
| 2                 | 5.250       | 627.514      | 61.450   | 5.4        | 8.4       | 0.18          |
| 3                 | 5.533       | 963.529      | 495.943  | 83.0       | 81.0      | 0.25          |
| 4                 | 5.883       | 1197.418     | 69.685   | 10.3       | 9.5       | 0.18          |
| Total             | 11607.539   | 735.681      | 100.0    | 100.0      |           |               |
Table 1-C showed values of protein peaks after exposure to laser 650 nm

| Reten. Time [min] | Area [mAU.s] | Height [mAU] | Area [%] | Height [%] | W05 [min] | Compound Name |
|-------------------|-------------|-------------|----------|-----------|-----------|--------------|
| 1                 | 4.833       | 147.499     | 8.810    | 1.2       | 1.2       | 0.28         |
| 2                 | 5.233       | 626.611     | 61.945   | 5.3       | 8.1       | 0.18         |
| 3                 | 5.533       | 980.625     | 613.981  | 83.1      | 81.0      | 0.25         |
| 4                 | 5.850       | 1218.045    | 73.359   | 10.3      | 9.7       | 0.20         |
| Total             | 11822.770   | 757.695     | 100.0    | 100.0     |           |              |

Second group of analyses

Figure 2 showed the effects of laser on protein groups; the red color refers to samples without irradiation (control group), the blue color refers to serum sample group irradiated with laser 532 nm and green color represents to serum sample group irradiated with laser 650 nm.
Table 2-A showed values of protein peaks of control group (without irradiation)

| Reten. Time [min] | Area [mAU] | Height [mAU] | Area [%] | Height [%] | W05 [min] | Compound Name |
|-------------------|------------|--------------|----------|------------|-----------|---------------|
| 1                 | 4.833      | 178.550      | 9.755    | 1.5        | 1.3       | 0.28          |
| 2                 | 5.217      | 476.511      | 48.346   | 3.9        | 6.3       | 0.17          |
| 3                 | 5.533      | 10094.604    | 614.988  | 82.2       | 79.7      | 0.25          |
| 4                 | 5.883      | 15278.816    | 98.267   | 12.4       | 12.7      | 0.18          |
| Total             |            | 12277.482    | 771.355  | 100.0      | 100.0     |               |

Table 2-B give values of peak proteins after exposure to laser 532 nm.

| Reten. Time [min] | Area [mAU] | Height [mAU] | Area [%] | Height [%] | W05 [min] | Compound Name |
|-------------------|------------|--------------|----------|------------|-----------|---------------|
| 1                 | 4.850      | 162.774      | 8.899    | 1.4        | 1.2       | 0.30          |
| 2                 | 5.233      | 470.425      | 49.010   | 4.1        | 6.8       | 0.17          |
| 3                 | 5.533      | 9321.847     | 567.937  | 81.6       | 79.1      | 0.25          |
| 4                 | 5.900      | 1473.864     | 92.594   | 12.9       | 12.9      | 0.22          |
| Total             |            | 14282.210    | 718.430  | 100.0      | 100.0     |               |

Table 2-C showed values of peak proteins after exposure to laser 650 nm

| Reten. Time [min] | Area [mAU] | Height [mAU] | Area [%] | Height [%] | W05 [min] | Compound Name |
|-------------------|------------|--------------|----------|------------|-----------|---------------|
| 1                 | 4.967      | 188.745      | 9.627    | 1.7        | 1.4       | 0.33          |
| 2                 | 5.200      | 379.200      | 42.510   | 3.3        | 6.0       | 0.17          |
| 3                 | 5.517      | 9240.174     | 561.355  | 80.9       | 79.3      | 0.25          |
| 4                 | 5.883      | 1607.250     | 94.535   | 14.1       | 13.4      | 0.22          |
| Total             |            | 11415.370    | 708.028  | 100.0      | 100.0     |               |

4. Discussion

It is not surprising that laser energy can to affect many biological molecules and these effects are appeared clearly when laser energy are absorbed by those biological molecules in particular those who have chromophores and bonds capable to excite and absorbed energy. Study of Hawkin and Brahame * found that low level laser can to alter many biological activities and these alterations are mediated through affecting activities of cellular functional proteins in particular enzymes. Previous study showed when serum albumin exposed to 1000 HZ with energy 9 J causes conformational changes of albumin especially secondary structure of albumin and seem the alpha helix become down regulated but beta helix tend to elevate.
in the same context, when samples exposed to 2000 HZ do not affected. It is well documented that albumin constituents about 60% of total plasma proteins and responsible for regulation several vital biological functions such as osmotic pressure, acid–base balance, and transport of hormones and metals.

Many hypothesized mechanisms are suggested to explain how low level laser influence on living cells, from those effects absorption of photons by flavins and cytochromes of mitochondria leading to transport of electrons to new energy levels of atoms. It is documented that there are more than 35 structural changes resulted from modification of proteins because of oxidative stress affecting protein molecules that include oxidation of amino acids in particularly cysteine and methionine, in addition, oxidative damage of polypeptide chains or side chain modifications and carbonylation.

Absorption of light radiation by living cells can cause disruption of atoms causing multiple biological and chemical alterations, light radiation may be influenced in direct manner on living cells through radiolysis of water molecules generating reactive oxygen species that in turns damage macromolecules such as lipids, nucleic acids, and proteins. Previous study was carried out by Varma et al. confirmed onset oxidative processes in protein solution during a short period after exposure to laser beam and pointed out oxidation of amino acids of polypeptide chains. The present study supported by the fact suggest photons of laser radiations mediate induction of covalent bonds within inter and intra molecules leading to create cross–linking among molecules, and these findings can represented the basis to study and investigate complex proteins and nucleic acids structures.

Previous research was performed to explore how heat emitted from laser light on blood constituents especially those emitted from Nd: YAG laser and found that heat causes split of oxygen from hemoglobin forming deoxy–hemoglobin and denaturation of proteins. It is noted that LLLT acts to increase oxidation – reduction potential toward high level of oxidation activities that are accompanied with liberation of reactive oxygen species (ROS) causing morphological changes of protein molecules. Researchers found that irradiation with infra-red and electron are catalyzed in production of hydroxyl radicals rendering them have more deleterious effects on bio–macro molecules especially proteins. Another experimental study confirmed that reaction can occur on side chain of poly peptide when exposed to oxidation and reduction because of light radiation, and it is well found that oxidation processes are happened on N–terminal amino acids in particularly methionine because this amino acids has more reactivity level with hydroxyl free radicals than of other amino acids.

Furthermore, other amino acids in particular aromatic amino acids including tyrosine and tryptophan have high reactivity level because of light oxidation, so that, protein molecules are more targeted when exposed to light because of presence of these amino acids in back bone of protein structures. Aye et al. who investigated that irradiation with laser beam yield multiple oxidation changes on surface of protein molecules and he noted that UV – laser produced hydroxyl radicals through photo- dissociation to H2O2 and then hydroxyl radicals themselves mediate oxidation of amino acids located on the surfaces of proteins causing stable covalent modifications. Thermal and chemical effects are characterized that exert more effects on macromolecules, the chemical effects of light are mostly irreversible because of molecular modifications, photons give its effects according to wave length and mediates break down chemical bonds exactly located among aromatic amino acids such as tyrosine, tryptophan, and phenylalanine. Proteins have conductor and semiconductor properties because of transport of electrons through back bone of protein molecules and cross different energy levels of terminal amino acids creating enough conditions from electromagnetic absorption.

In conclusion, results mentioned above revealed changing occurring in physical and morphological properties of proteins molecules when exposed to two different laser can be returned that laser light create new inter and intra molecular bonds or as a result production of reactive oxygen species that attack sensitive amino acids.
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