Recognizing the effectiveness of the diode laser 850nm on stimulate the proliferation and viability of mice mesenchymal stem cells derived from bone marrow and adipose tissue

Z. AL-Timimi[1] and F. H. Mustafa[2]

[1]Laser Physics-College of Science for Women, Babylon University, [2] Medical Physics-College of Pharmacy, Hawler Medical University, Iraq. [1]Email: dr. altimimizahra@gmail. com, zahraja2007@yahoo. com, [2]Email farhad978@gmail. com

(Received February 25, 2018; Accepted June 22, 2018)

Abstract

Low Power Laser Irradiation therapy raises the levels of proliferation for the variety of cells. Although despite very little is recognized about its effect on the proliferation levels and possible nuclear morphological changes of mice mesenchymal stem cells, which derived from bone marrow and adipose tissue. This study examined the effects of the different fluence of 1, 2, 3 and 4 j/cm² with different dose durations on promoting proliferation, viability and possible nuclear morphological changes of mice mesenchymal stem cells. The growth curves have been used to analyse the proliferation of all the applications. Fluorescent stain (DAPI) (4'-6-diamidino-2-phenylindole) staining used to check the nuclear alterations at 72 hours, while the trypan blue assay used to test the cell viability. Mesenchymal stem cells that derived from bone marrow responded to LPLI in a dose-dependent manner. The higher cell growth determined once the cells irradiated with a fluence of 4 j/cm², especially after 24 hours (P<0. 01). Whereas adipose tissue-derived stem cells responded better to a fluence of 2 j/cm² after 48 hours (P<0. 05). There was no significant change in nuclear alterations and in cell viability detected in the studied groups. LPLI permits a significant increase in the proliferation of stem cells, without causing nuclear alterations, therefore, increasing the number of differentiating cells for tissue engineering, regenerative, and healing processes.

Keywords: Cell proliferation, low-level laser therapy, stem cells, adipose tissue, bone marrow
Available online at http://www. vetmedmosul. com

التعرف على فعالية الدايود ليزر 850 nm على تحفيز الانتشار والبقاء للخلايا الجذعية الوسيطة للفئران المستمدة من نخاع العظام والنسيج الدهني

زهراء جاسم محمد التميمي [1] و فرهاد حامد مصطفى[2]

[1] فيزياء الليزر، كلية العلوم للبنات، جامعة بابل، [2] الفيزياء الطبية، كلية الصيدلة، جامعة هولير الطبية، العراق

الخلاصة

الليزر منخفض الطاقة يرفع مستويات الانتشار لمجموعة متنوعة من الخلايا. على الرغم من أنه لم يتم التعرف إلا على القليل جدا حول تأثيره على مستويات الانتشار والتغيرات المورفولوجية النووية المحتملة للخلايا الجذعية الوسيطة في الفئران، والتي تستمد من نخاع العظام والنسيج الدهني. بحثت الدراسة التأثيرات لكثافات طاقة مختلفة 1, 2, 3 and 4 j/cm² في فترات تعرض مختلفة على تعزيز انتشار، وإبقاء الخلايا على قيد الحياة بالإضافة الى التغيرات المورفولوجية النووية المحتملة للخلايا الجذعية الوسيطة الفئران. تم استخدام منحنيات النمو لتحليل قابلية الانتشار، والصبغة الفلورية (4'-6-diamidino-2-phenylindole) (DAPI)للتحقق من التغيرات النووية في خلال 72 ساعة ، بينما تم استخدام التريبان الازرق لاختبار قابلية الخلية. لوحظ ان استجابة الخلايا الجذعية الوسيطة التي تستمد من نخاع العظم إلى اشعة الليزر منخفض الطاقة بطريقة تعتمد على معدل الجرعة. وجد ان اعلى معدل زيادة في نمو الخلايا تم في حالة تعرض الخلايا الى كثافة طاقة 4 j/cm² خاصة بعد مرور 24 ساعة وبزيادة معنوية ((P<0. 01، في حين أن الخلايا الجذعية المستمدة من الأنسجة الدهنية

استجابت بشكل أفضل عند كثافة طاقة $2j/cm^2$ بعد مرور 48 ساعة وزيادة معنوية ($P<0.05$). لم يكن هناك تغيير كبير في التعديلات النووية وفي حيوية الخلية المكتشفة في المجموعات المدروسة. الليزر منخفض الطاقة يتيح الفرصة لزيادة ذات اهمية كبيرة وبالغة في تكاثر الخلايا الجذعية ، دون التسبب في تغيرات نووية، وبالتالي زيادة عدد خلايا التمايز والتي تستخدم لأغراض هندسة الأنسجة، وعمليات اصلاح الانسجة المتضررة، ولالتئام الجروح.

Introduction

The photochemical interaction stems from empirical observation which shall induce chemical effects and reaction inside macromolecules or tissue among the foremost in style created by the evolution if self the energy unharnessed as a result of photosynthesis (1,2). In the most significant application of photochemical interaction are biostimulation wound healing and anti-inflammatory properties of red or near-infra-red light source as diode laser (3). Biostimulation believed to work out a low irradiance. It's associated with enhanced sure metabolic pathways within the living cells, for instance, healing of skin lesions and relief of pain (4,5). Numerous analysis studies have incontestable that Low-Power Laser Irradiation (LPLI) is effective for a few species (5). A tangle in dissecting this literature is that the variation in method and dosimetry between completely different studies. Not solely have a spread of various wavelengths have been examined, however, exposure times and the frequency of treatments also vary (5,6). However, the molecular mechanisms of LPLI process are still not completely understood (7,8). It suggested that the laser energy absorbed by intracellular chromospheres which, converted into metabolic energy and then used by a mitochondrial respiratory chain to produce the ATP and reproducible the DNA and synthesis of the RNA and proteins (9,10).

Mesenchymal stem cells (MSCs) are multipotent, undifferentiated and important potential for applications that are being clinically explored in cell therapy, as a matter of fact, new therapies for treating a variety of immune diseases. MSCs as a result of regenerative therapy for tissue repair shown to modulate the immune cells and alter some of the endogenous (11,12).

Pre-clinical studies of mechanism action suggest that the therapeutic effects given by the MSC transplantation are short live, related to dynamic and paracrine interactions within the MSCs and host cells (13,14). MSCs have traditionally been isolated from bone marrow, However, reports have detailed the isolation of cells with MSC characteristics from a variety of tissues, including cord blood, peripheral blood, fetal liver and lung, adipose tissue, skeletal muscle, amniotic fluid, synovium and the circulatory system (15,16).

Adipose tissue or fatty tissue is a connective tissue composed of fat cells (adipose cells, or adipocytes) that contain large globules of fat (17,18). The brown adipose tissue, that derives its colour from vascularization and densely packed mitochondria, was found in different locations, depending on the species and the age of the animals (19).

Approbating to many kinds of literature reported the higher production of fibroblast cell growth factor when irradiated with (660, 632. 8 and 670) nm laser (20,21). Furthermore, LPLI found to increase the proliferation and the differentiation of many different types of cells in culture such as keratinocyte, fibroblasts, stimulating the growth factor, higher proliferation (22).

In this study, LPLI with the wavelength of (850nm) is proposed in order to assess the influence of low power laser irradiation on promoting proliferation, viability and possible nuclear morphological changes of mice MSCs which derived from (bone marrow and adipose tissue).

Materials and method

The study has been approved by the Committee on the Ethics of Animal Experiments of Veterinary Medicine College, Bagdad University, Iraq, with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

Depending on the protocol of Soleimani and Nadri (23) The bone marrow stromal cells (BMSCs) and adipose-derived stem cells (ADSCS) obtained from three mice with a mean weight of (27 gm), and 60 days age used in this study. They housed in suitable cages and kept in a conditioned room (28-32 °C), nutrition, and day–night cycle to avoid physiological changes. All animals were left for two weeks for adaptation.

The BMSCs were extracted briefly; femurs and tibias dissected, and the BM cavities were washed with minimum essential media alpha -MEMA supplemented with 10% FBS - Fetal Bovine Serum.

For the isolation of the ADSCs (23), an adipose tissue fragment removed from the inguinal region of mice and washed 3 times with MEMA supplemented with antibiotics and antifungal agents. After that, the tissue fragment digested enzymatically with collagenase for an 1 h at 37°C (23).

BMSCs and ADSCs were cultured in plates containing MEMA supplemented with 10% FBS within a humid atmosphere by 5% CO_2 at 37°C. Culture medium changed at the intervals of four days until the cells reached in 80 to

95% confluence. For analysing the multipotent nature of the cells before LPLI, the two types of cells cultured in osteogenic and adipogenic AdipoMAX differentiation medium (24) for up to three weeks. The analysis of the cells after this time by using light microscopy revealed the morphology of osteoblastic and adipose cells (Figure 1). Third passage cell cultures were irradiated with diode laser 850 nm, in continuous mode using a power of 90 mW and fluency of 1, 2, 3 and 4 j/cm² and the cells analyzed at 0, 24, 48, 72 hours after the first laser irradiation. For LPLI, the probe of irradiation was directed perpendicular to all the plates at a distance of 2 cm from the cells. The cells plated in one well and between each seeded wells were left empty to prevent any unintentional dispersion of light between the wells during the LPLI. For an analysis of the effect of LPLI on the cell proliferation and viability, the trypan blue assay used in order to evaluate the viability during the experimental period, and to obtain the growth curves used to establish proliferation of cells that are submitted to LPLI (23). The cell culture was done in 26 well plates at a density of ($6x10^5$) cells per well. The trypan blue stained cells counted in a neubauer chamber at 0,24,48,72 hours. DAPI (4'-6-diamidino-2-phenylindole) was used to identify the appearance of pyknotic nuclei and nuclear fragmentation within 72 hours.

Statistical analysis

The results were submitted for non-parametric analysis (25). The differences between groups at each period (zero, 24, 48, and 72 hours) analysed by using two way ANOVA. A level of significance of 5% was espoused (P<0. 05).

Results

Figure 1 illustrates the growth curve number of BMSCs in different groups. The lowest proliferation was observed in the control group at all time periods when compared to the irradiated groups. The comparison of the four laser fluency show a tendency towards higher cell proliferation within the group treated with laser fluence 4 j/cm², but the difference was only significant after 72 hours.

A significant difference in proliferation of the cell was observed between a control group and the groups treated with laser fluence after 24 hours, with among fluence stimulating cell growth. There was no significant difference in the viability of the cells observed by using the trypan blue exclusion method (Figure 2).

The proliferation of ADSCs increase in all groups as shown in (Figure 3). A tendency towards higher proliferation observed in the irradiated group with the fluence of 4 j/cm² after 24 hours when compared it with the control group and the irradiated group with 3 j/cm².

Comparison of the proliferation of the cells of which irradiated groups were showed a significant difference only after 24 hours. A fluence of 4 j/cm² seemed to positively impact on ADSCs proliferation and a distinct behaviour observed over time when the mean number of the cell proliferation compared between LPLI groups and control group.

The Trypan blue exclusion assay has been shown that no difference in the viability of the cells in all groups. No nuclear changes like (nuclear fragmentation or even pyknotic nuclei) detected in BMSCs and ADSCs (Figure 4).

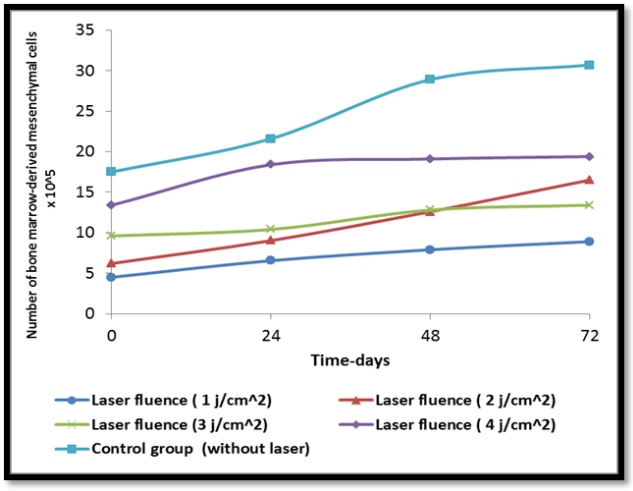

Figure 1. (BMSCs) submitted to different laser fluence and without to laser fluence (control group) over time periods.

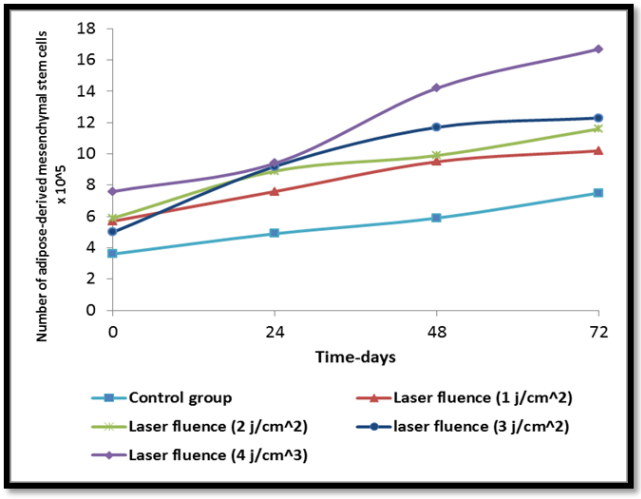

Figure 3. ADMSCs submitted to different laser fluence and not submitted to laser fluence (control group) over time periods.

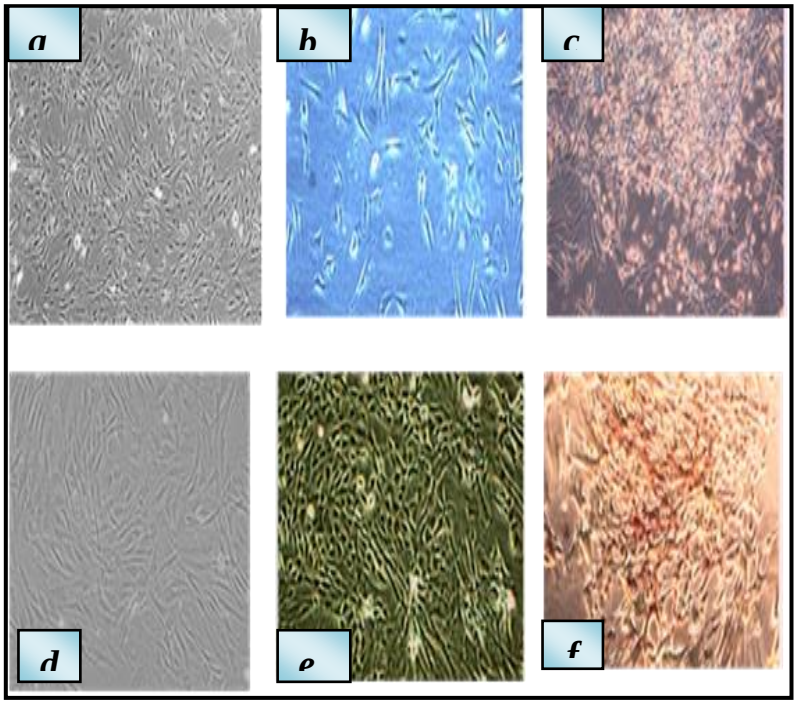

Figure 2. Morphological characterization of bone marrow (a - c) and adipose tissue (d -f), undifferentiated MSC (a,d), osteogenic cell (b and e) and adipogenic cell differentiation (c, f).

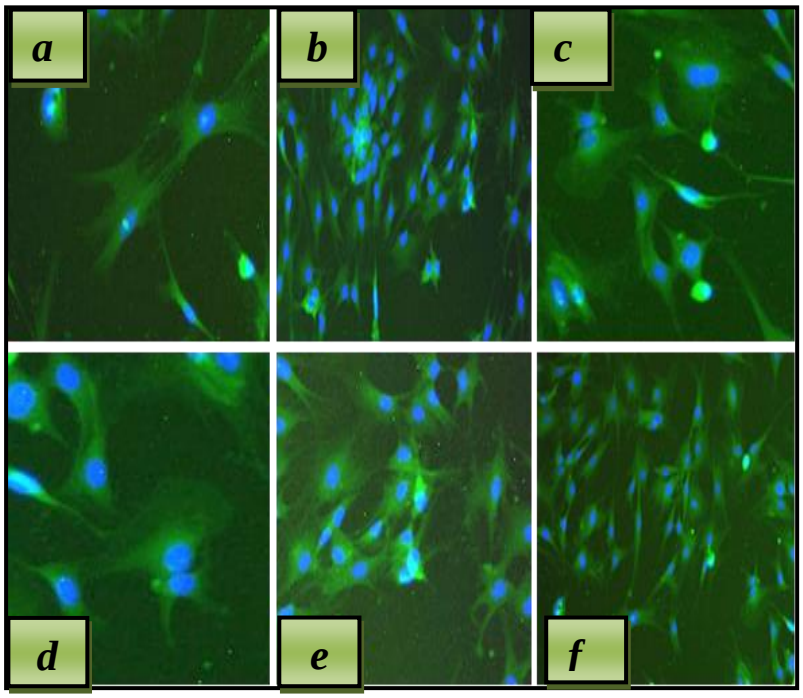

Figure 4. No nuclear morphological changes appear on a light micrograph of BMSCc (a - c) and ADMSCs (d to f) stained by using DAPI, (a) Control; (b) with laser fluence 1j/cm², (c) with laser fluence 2j/cm², (d) with laser fluence 3j/cm², (e and f) with laser fluence 4j/cm².

Discussion

LPLI recommended to stimulate the differentiation and proliferation of different types of cells since some studies, perform to use lower fluences for irradiation of the mucosa and wounds because of the absorption and spreading of their light wavelengths are greater as a result of lack an optical barrier so indicated in the case of biostimulation (26,27). However, biostimulation wasn't always observed because the variety of many factors affecting this process (1,28). LPLI parameters as power, fluence, time of irradiation, wavelength, types and physiological characteristics of cells (5,29). As a consequence of all these factors, the interaction between the laser wavelength with cells or tissues can stimulate or inhibit the cell proliferation.

On the basis of this hypothesis, fluences of 1, 2, 3 and 4 j/cm² used in the present study since the objective demonstrating that LPLI the proliferation of ADSCs and BMSCs. The fluences increase seemed to damages photoreceptors and exert some of negative impacting on biological process, that reduces the biomodulatory effects of the LPLI as a result of the inhibition of the metabolism and cell death.

In addition, some studies demonstrated that irradiation of human ADSCs uses 635nm laser wavelength positively impacting cell proliferation with viability, an expression of proteins, growth factor suggesting that the operation of LPLI interfered with the cell response (27,30). The results agree with present findings, which show increasing the number of cells (10).

We believe that lower fluences can reduce the risk of cell damage and allowed to promote the stem cell proliferation, that can keep their initial characteristics intact (2,31). As though, LPLI showed a fluences dependent effects in this study, that indicated higher proliferation rate of BMSCs and ADSCs that irradiated with a fluence of 4 j/cm² compared with 1, 2, and 3 j/cm². However, a higher proliferation rate was observed in cells irradiated with 4 j/cm^2. ADSCs responded better to the irradiation fluence of 4 j/cm^2,with the higher cell proliferation being observed after 72 hours of culturing. These results correspond with the suggestion of some investigators of the action of the LPLI is directly related to irradiation fluence and physiological cell state (7,32).

The utilized fluence induced that no adverse effects on cells which also observed in this study in which, no alteration appropriate with the Deoxyribonucleic acid (DNA) damage like pyknotic or fragmented nuclei. However, any other alterations as chromosomes deletions and translocations might not be excluded from this experiment so as, karyotyping is essential for further studies.

Conclusion

This study showed that LPLI promoted proliferation of BMSCs and ADSCs. Since the stem cells isolated from different sources and usually present as a low yield and low rate of proliferation.

LPLI may useful tool for some tissue engineering by using stem cells. LPLI can permit a significantly increase in stem cell number before differentiation, therefore increasing the number of differentiated cells for regenerative and healing processes.

Acknowledgments

We would like to thank the laboratory staff in the department of the Laser Physics in the College of Science for Women / Babylon University for their help.

References

1. Zahra AT. Clinical Evaluation of Scalpel Er: YAG Laser 2940 nm and Conventional Surgery Incisions Wound after Oral Soft Tissue Biopsy. Bangladesh Med Res Counc Bull. 2018;43(3):149.
2. Zahra'a AT. Assessment of the impacts of 830 nm Low Power Laser on Triiodothyronine (T3), Thyroxine (T4) and the Thyroid Stimulating Hormone (TSH) in the Rabbits. J Med Sci Clin Res. 2014;2(11):2902–2910.
3. Mustafa FH, Jaafar MS, Ismail AH, Omar AF, Timimi ZA, Houssein HAA. Red Diode Laser in the Treatment of Epidermal Diseases in PDT. World Acad Sci Eng Technol. 2010;4(10):677–680.
4. Morgan J, Oseroff AR. Mitochondria-based photodynamic anti-cancer therapy. Vol. 49, Advanced Drug Delivery Reviews. 2001;pp:71–86.
5. Zahra Al Timimi, Jaafar MS, Mohd Zubir MJ, Hend A, Houssein A, Farhad H, Mutafa GAA-D. The-Influence-of-low-power-laser-Energy-on-Red-blood-cell-and-platelets-In-vitro. In: International Conference on Bioscience. Biochem Pharmaceut Sci. (ICBBPS'2012) Penang, Malaysia. Penang, Malaysia; 2012. p.12–4.
6. Dąbrowski JM, Arnaut LG. Photodynamic therapy (PDT) of cancer: from local to systemic treatment. Photochem Photobiol Sci. 2015;14(10):1765–1780.
7. Zahra Al Timimi, Jaafar M, Zubir Mat Jafri M. Photodynamic therapy and Green Laser blood Therapy. Glob J Med Res. 2011;11 (5):22–8.
8. Zahra AT. Exploration of Additional Mechanical Phenomena of Laser-Tissue Interaction Contributes to the Damage and Elimination of the Small Blood Vessels. Int J Sci Eng Res. 2014;5 (12):672–675.
9. Azma E, Safavi N. Diode laser application in soft tissue oral surgery. J Lasers Med Sci. 2013;4(4):206–211.
10. Eduardo F de P, Bueno DF, de Freitas PM, Marques MM, Passos-Bueno MR, Eduardo C de P, et al. Stem cell proliferation under low intensity laser irradiation: A preliminary study. Lasers Surg Med. 2008;40(6):433-438.
11. Caplan AI. MSCs as therapeutics. In: Mesenchymal Stromal Cells: Biology and Clinical Applications. 2013.
12. Conner DA. Mouse Embryo Fibroblast (MEF) Feeder Cell Preparation. In: Current Protocols in Molecular Biology. 2001.
13. Prockop DJ, Youn Oh J. Mesenchymal stem/stromal cells (MSCs): Role as guardians of inflammation. Mol Ther. 2012;20(1):14-20.
14. Deans RJ, Moseley AB. Mesenchymal stem cells. Exp Hematol. 2000;28(8):875–884.
15. Minguell JJ, Erices A, Conget P. Mesenchymal Stem Cells. Exp Biol Med. 2001;226(6):507–20.

16. Waters JC. Live-cell fluorescence imaging. Methods Cell Biol. 2013;114:125–150.
17. Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. J Clin Endocrinol Metab. 2004;89(6):2548–2556.
18. Shen W, Wang ZM, Punyanita M, Lei J, Sinav A, Kral JG, et al. Adipose tissue quantification by imaging methods: A proposed classification. Vol. 11, Obesity Research. 2003. p. 5–16.
19. AL-TimimiZahra. Biological Effects of Yellow Laser-Induced of Cell Survival: Structural DNA Damage Comparison is Undergoing Ultraviolet Radiation Photocoagulation. Int J Eng Res Gen Sci. 2014;2(5):544–548.
20. Kashiwagi M, Saitoh K, Takenaga K, Tanigawa S, Matsuo S, Fujimaki M. Low bending loss and effectively single-mode all-solid photonic bandgap fiber with an effective area of 650 μm^2. Opt Lett. 2012;37(8):1292.
21. Zahra AT. Investigating the Effects of Green Laser Irradiation on Red Blood Cells: Green Laser Blood Therapy. Int J Appl Res Stud. 2014;3(10):1–5.
22. Hamad F, Jaafar M, Hamid A, Omar A, Houssein H. Influences of different low level laser power at wavelength 635 nm for two types of skin ; dark and light. Proc 7th IMT-GT UNINET 3rd Int PSU-UNS Conf Biosci Influ. 2009;7(3):130–135.
23. Soleimani M, Nadri S. A protocol for isolation and culture of mesenchymal stem cells from mouse bone marrow. Nat Protoc. 2009;4(1):102-106.
24. Wu Y, Chen L, Scott PG, Tredget EE. Mesenchymal stem cells enhance wound healing through differentiation and angiogenesis. Stem Cells. 2007; Stem Cells. 2007;25(10):2648-2659.
25. Anderson MJ. A new method for non-parametric multivariate analysis of variance. Austral Ecol. 2001;26(1):32-46.
26. Alshammari AM, Salman MI, Almzaien AK, Alwan LH, Al-Atabi SM, Ajeel AK, et al. Embryonic Markers association in Mice Bearing Mammary Adenocarcinoma (AN3) transplantable tumor model. Iraqi J Cancer Med Genet. 2015;8:152-156.
27. Gao X, Xing D. Molecular mechanisms of cell proliferation induced by low power laser irradiation. J Biomed Sci. 2009;16(1):4.
28. AL-Timimi Zahra FHM, Hend A. A. Houssein. Characterization of Cancer Photodynamic Therapy: Exploring the Effects of Hematoporphyrin Derivative Photosensitizer and Low Intensity Laser Irradiation. Int J Curr Microbiol App Sci. 2017;6(10):1-8.
29. MJ Suhaimi JZ. Therapeutic Laser for Chronic Low Back Pain. Bangladesh J Med Sci. 2009;8(4):118-128.
30. Lam E, Kato N, Lawton M. Programmed cell death, mitochondria and the plant hypersensitive response. Nature. 2001;411:848–853.
31. Medrado AR a P, Pugliese LS, Reis SR a, Andrade Z a. Influence of low level laser therapy on wound healing and its biological action upon myofibroblasts. Lasers Surg Med. 2003;32(3):239–944.
32. Enwemeka CS, Parker JC, Dowdy DS, Harkness EE, Harkness LE, Woodruff LD. The efficacy of low-power lasers in tissue repair and pain control: a meta-analysis study. Photomed Laser Surg. 2004;22(4):323-329.