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Effect of Low-Level Laser Therapy and Sinensetin (Combination therapy) on Tumor Cells (Hela) and Normal Cells (CHO)

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Abstract

Introduction: Cervical and ovarian cancers are well-known causes of death among women in developing countries. There are various technologies to treat cancer cells, but the polyphenolic compound is a natural one and has an anti-cancer effect. Sinensetin is one of them and is found in *Orthosiphon stamineus* and citrus fruits. Since combination therapy is more effective than drug treatment alone, in this study, we investigated combination therapy using sinensetin and low-level laser therapy (LLLT) to enhance treatment.

Methods: The cancer cells purchased from Pasteur Institute, Iran, were cultured.
The cells were treated with various concentrations of sinensetin (0.1-1-10-50,150 µg/mL for 24 hours), wavelengths of laser therapy (660 nm) and power density (3 J/cm2) for different times)30, 60, and 90 seconds) separately. Furthermore, sensitivity of cells to sinensetin, LLLT and combined therapy was determined by clonogenic assays. To measure DNA damage and repair at individual cell level used comet assay. To examine the intracellular generation of reactive oxygen species used 2′,7′-dichlorodihydrofluorescein (DCFH) as an intracellular probe. To analyze data we used SPSS software and comparison between groups was used (ANOVA) and *t* test statistical analyses were performed using SPSS 17 software. Data are presented as means – standard error of mean. The level of statistical significance was set at a two-tailed *P* value of 0.05. All tests were performed in triplicate.

Results: Our results demonstrated that the doubling time for CHO is more than Hella cells, with 20.7 and 27.7 h for each cell respectively. The pretreatments (first LLLT, then sinensetin) can decrease the viability of both cell lines more than the first treatment (sinensetin + LLLT). In the clonogenic assay, the pretreatment of cells with LLLT and Sinensetin significantly reduced the surviving fraction of both cell lines. MTT results showed that pretreatment with LLLT and Sinensetin can increase cell death compared to Sinensetin and LLLT alone. Production of ROS within the cell was enhanced with LLLT + sinensetin.

Conclusion: Our result indicated that combined therapy with LLLT and Sinensetin can treat CHO and Hela cells better than the other groups. Combination treatment with sinensetin-LLLT and the other treatment means, sinensetin and LLLT alone, did not change the cell viability significantly.

Keywords: Sinensetin; Low-level laser irradiation (LLLT); CHO; Hella; Anti-cancer effect; Combined therapy.

Introduction

Cervical cancer is one of the most frequently female cancers in developing countries. The young patients are forced to endure cervical cancer, and the pathological types of the malignancies change. The main risk factor for developing this carcinoma is HPV. HPV types are classed as low-risk or high-risk strains depending on their oncogenic potential.[1-4] Ovarian cancer is one of the most prevalent cancers in women. The texture type of ovarian malignancies contains germ cell (6%–40%), borderline (21%–35%), epithelium (28%–30%), and sex cord-stromal (9%–16%) origin.[5-7] In 2009, some researchers demonstrated that the survival gap for both ovarian and cervical cancers could be decreased by early diagnosis.[8] Phenolic compounds are secondary metabolites in plants that have become an emerging field in cancer, cardiovascular diseases, and neurodegenerative disorders.[9,10] Citrus fruits are noticed to be great sources of phytochemicals. Sinensetin is a polymethoxylated flavones (PMF) found in *Orthosiphon stamineus* and orange oil. This PMF acts against cancer with antioxidant effects.[11-13] Photobiomodulation (PBM), including low-level laser therapy (LLLT), is one type of non-invasion therapy.[14] LLLT could be useful in anti-inflammatory effects and tissue repair action.[15] LLLT can increase cell permeability for drugs due to enforced mitochondria

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transition mediated by reactive oxygen species.[16] LLLT is accepted with cytochrome c oxidase. In this way, ROS and nitric oxide are ephemerally produced.[17] The clonogenic assay is necessary for tests in cells for its ability to sustain unlimited division. The clonogenic assay is the method of assigning the cell death after treatment and shows the effectiveness of some cytotoxic factors.[18,19] The comet assay, which can demonstrate DNA damage and DNA repair kinetics, has been extensively used in radiation biology, toxicology, oncology, and molecular epidemiology.[20]

In the present survey, we examined sinensetin and LLLT cytotoxicity, ROS production, clonogenic potential, and the activity of the Comet test in two cell lines, Hella and CHO.

Materials and Methods

Cell Culture

Cervical cancer cells (Hella) and Chinese hamster ovary cell lines (CHO) were obtained from the National Cell Bank of Iran (Pasteur Institute, Iran).

These two cell lines were cultured in medium RPMI-1640 (Gibco: UK), which is a complement to 10 % heat-inactivated fetal bovine serum (FBS), 500 units/mL penicillin (Sigma, USA), and 200 mg/mL streptomycin (Jaberebn-Hayan, Tehran, Iran). The cells were incubated at 37°C in a humidified atmosphere of 5% CO_2.

Growth Curve and Doubling Time

In order to measure growth cure and doubling time (TD), Hella and CHO cell lines were seeded with efficiency cells in 24-well plates (SPL, Korea) for 7 days in triplicate (used Trypan blue dye exclusion method).

The doubling time of each category was defined with the Patterson formula.

Cell Viability Assay (Sinensetin and LLLT)

Succinctly, Hella and CHO cell lines were seeded in 96-well culture plates (SPL, Korea) beneath 5% CO2, at 37°C for 24 hours. The next day, the two groups were treated as follows: the first one was treated with 0.1-1-10-50,150 µg/mL of sinensetin (Cayman Chemicals, Germany) and the other one was exposed to a low-level laser (660 nm; power density: 3 J/cm^2) for different times (30, 60, and 90 seconds). These two groups of cells were incubated for 24 hours. The MTT reagent (Sigma Aldrich, USA) was added to each well and after that, the cells were incubated for 3 hours then added dimethyl sulfoxide (Merck, Germany) to dissolve the formed formazan crystals. The plates were rotated slowly to measured Absorbance at 570 and 630 nm wavelengths (BioTek, USA).

Treatment with Sinensetin + LLLT and LLLT + Sinensetin

The cells in the log phase were seeded in a 24-well plate and incubated for 24 hours. Thereafter, the cells were treated with 10, 50 µg/mL sinensetin for CHO and Hela respectively and exposed to LLLT (660 nm, 3 J/cm^2) for 90 seconds. The other treatments included LLLT followed by sinensetin. The control groups were not exposed to LLLT and sinensetin.

Colony-Forming Assay

The cells were seeded in 35 mm Petri dishes (SPL, Korea) and treated with sinensetin 10, 50 µg/mL for CHO and Hella, respectively, and they were exposed to LLLT (660 nm, 3 J/cm^2 for 90 seconds). Afterward, the cells were incubated for 7-10 days to give rise colony formation. Colonies were stabled with formaldehyde (Merck) and dye with a 2% crystal violet dilution (Merck). The plating efficiency was determined.

Alkaline Comet Assay

In order to comet assay, sinensetin-treated, LLLT-treated, and sinensetin + LLLT-treated cells were suspended in 1 % low-melting-point agarose in PBS, pH 7.4, and poured onto glass microscope slides pre-coated with a layer of 1% normal melting-point agarose. The agarose was allowed to be set at 4°C for 10 minutes. Then the slides were incubated in a lysis buffer (1% Triton X-100, 100 mM EDTA, 2.5 M NaCl, and 10 mM Tris/HCl at pH 10) at 4°C for 1 hour. The denaturation buffer (NaOH (300 mM), EDTA (1 mM); pH 13) was set on slides for 30 minutes. Electrophoresis was done in a fresh denaturation buffer at 1 V/cm for 30 minutes. The neutralization buffer (Tris-HCl (400 mM); pH 7.5) was poured on slides for 5 minutes. All points were done at 4°C. In each sample, 200 cells were analyzed by Cometscore software.

The Activity of Intracellular Reactive Oxygen Species

CHO and Hella were exposed to LLLT at 660 nm for 90 seconds for the first group; afterward, the cells were treated with sinensetin 10, 50 µg/mL for CHO and Hella respectively. The other groups were treated with LLLT and sinensetin. After 24 hours, the cell culture medium was picked up and the cells were incubated with 2µM 2, 7 dichlorodi hydrofluoresceindiacetate (DCFH-DA) (Sigma, USA) and incubated at 37°C for 45 minutes. Then, the cells were washed with PBS and read in 530 nm by a plate reader device (BioTek H4).

Statistical Analysis

All data were illustrated as the mean ± standard error of the mean (SEM). Data analysis was done using SPSS software. Standard analysis of variance (ANOVA) and *t* test were employed to evaluate the data between groups. All experiments were done in triplicate and a *P* value of 0.05 was considered to be significant.

Results

Determination of Potential Doubling for CHO and Hella Cells

Calculating doubling time is very useful to demonstrate

the change in the number of cells. These phases were divided into lag phase, exponential or log phase, linear phase, deceleration phase and stationary. The treated cells and colony assay required a growth curve. In this investigation, the duplication rate of CHO is more than Hella cells, 20.7 and 27.7 hours respectively (Figure 1).

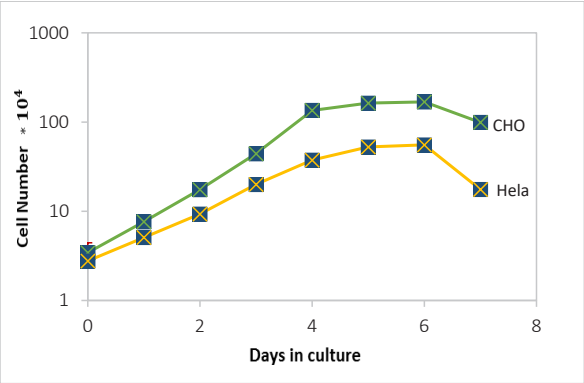

Figure 1. Evaluation of CHO and Hella Growth Curve. As it is obvious in the figure, CHO proliferation is more than Hella.

Cell Viability Affected by Sinensetin and Low-Level Laser Treatments

Due to the effect of sinensetin and LLLT on cell viability, we examined several experimental parameters. The MTT assay was accomplished to investigate the cytotoxic effects of sinensetin on CHO and Hela cells for 24 hours. As shown in Figures 2A and 2B, the viability of CHO and Hela cells decreased at high concentrations of sinensetin (IC_{50} concentrations were 10μM, 50μM for CHO and Hella respectively). Also, this test examined the effect of different wavelengths of LLLT (660 nm; power density: 3 J/cm^2) for different times, 30, 60, and 90 seconds separately. The cytotoxicity effect of LLLT was reduced over 30 to 90 Sierra Time Zone (Figure 2C, 2D). To compare these two treatments, sinensetin + LLLT and LLLT + Sinensetin used half maximal inhibitory concentration (IC50) of CHO (10μM) and Hela (50μM) for 90 seconds. As it can be seen in Figures 2E and 2F, the treatment with LLLT and sinensetin reduced the survival of CHO and Hela cells more than the other treatments (sinensetin + LLLT) ($P<0.05$).

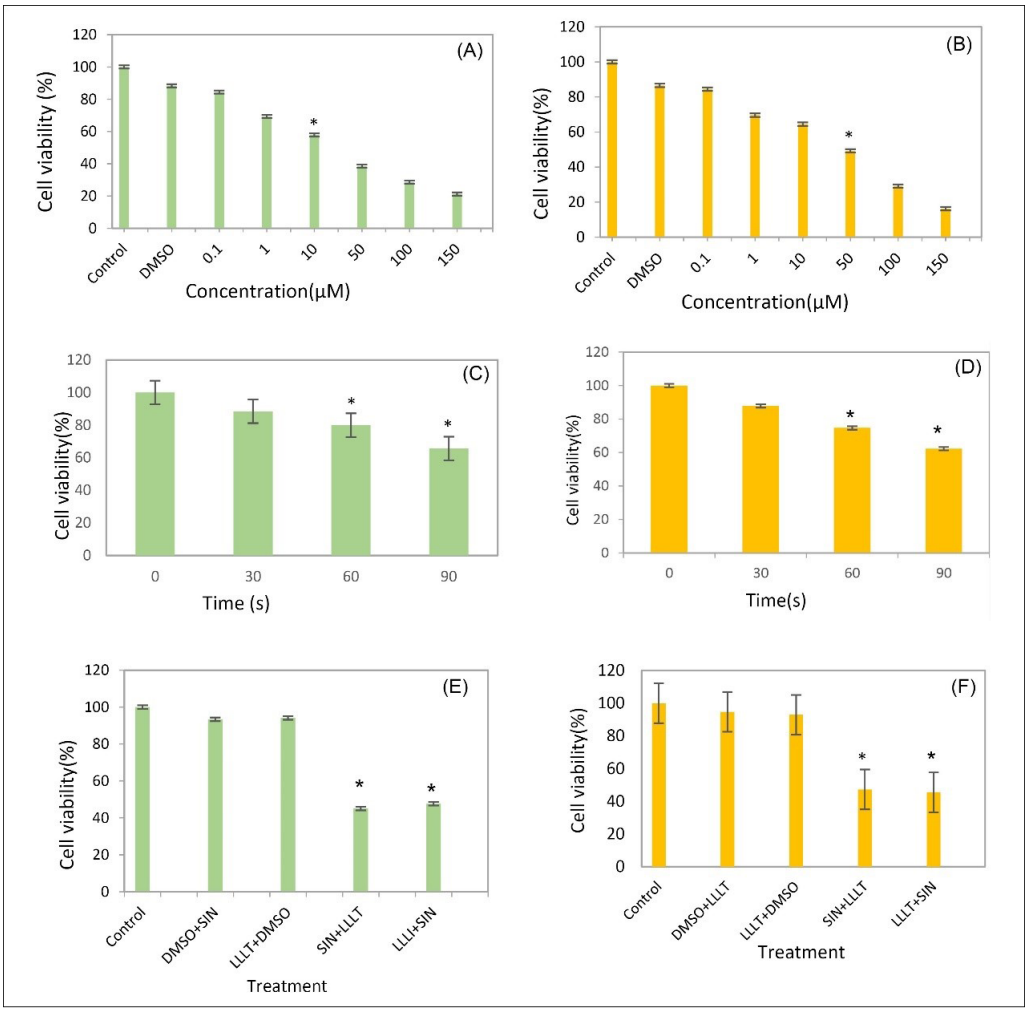

Figure 2. Effect of Various Concentrations of Sinensetin on the Viability of (A) CHO and (B) Hela Cells. The cells were treated with low-level laser irradiation with 3 J/cm^2 (C) CHO and (D) Hela. The cells were treated with sinensetin (IC50) and LLLT was used for 90 s (E) CHO and (F) Hela. The data represent the mean ± SEM of three independent experiments ($^*P<0.05$).

Effect of Sinensetin and LLLT on Cell Proliferation

In order to detect the effect of different concentrations of LLLT and sinensetin on the clonogenic potential of CHO and Hela cancer cells, both cell lines were irradiated with 3 J/cm^2 for 90 seconds, and then they were treated with 10, 50 µM sinensetin. LLLT and sinensetin slightly decreased cell proliferation on CHO and Hela cells. In comparison, this treatment may reduce the survival of CHO more than Hela cells (Figure 3). Each result is compared to the control group, $^*P<0.05$. This way of treatment significantly changed CHO and Hela morphology. The microscopic assessment of the cells before and after the treatment showed significant changes (Figure 4).

The Level of DNA damage

The Alkaline comet assay represented the level of DNA damage in each modality. The cells were treated with LLLT (90 seconds) and sinensetin (10, 50 µM) for CHO and Hela cells respectively. In the treated CHO and Hella cells with sinensetin only, there were not any significant changes in the level of DNA damages, whereas both cells that were treated with LLLT alone and LLLT + sinensetin showed slight changes in the DNA tail and DNA moment. The DNA level and migration were determined in Figures 5 and 6 ($P<0.05$).

Evaluated ROS in CHO and Hela Cancer Cells After Treatments

Here we hypothesized that combined therapy change ROS levels in Hela and CHO cells. In this direction, the intracellular ROS level was analyzed by the DCFH-DA probe. As it is evident in Figure 7, CHO and Hela

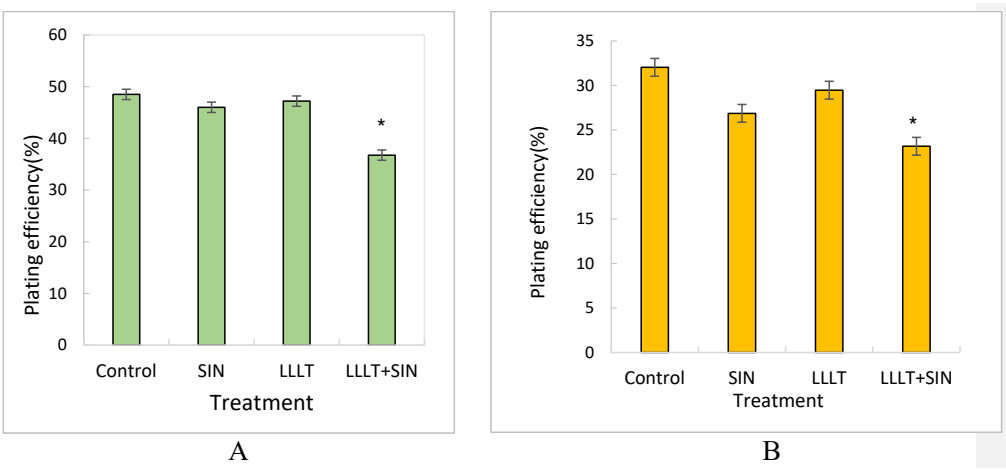

Figure 3. Colony Forming Ability of CHO and Hela Cells in the Presence of LLLT and Sinensetin IC50. The cells were exposed to LLLT and treated with 10, 50 µM Sinensetin, repeated, and incubated for the indicated number of days to form colonies. (A) CHO cells. (B) Hela cells (*$P<0.05$).

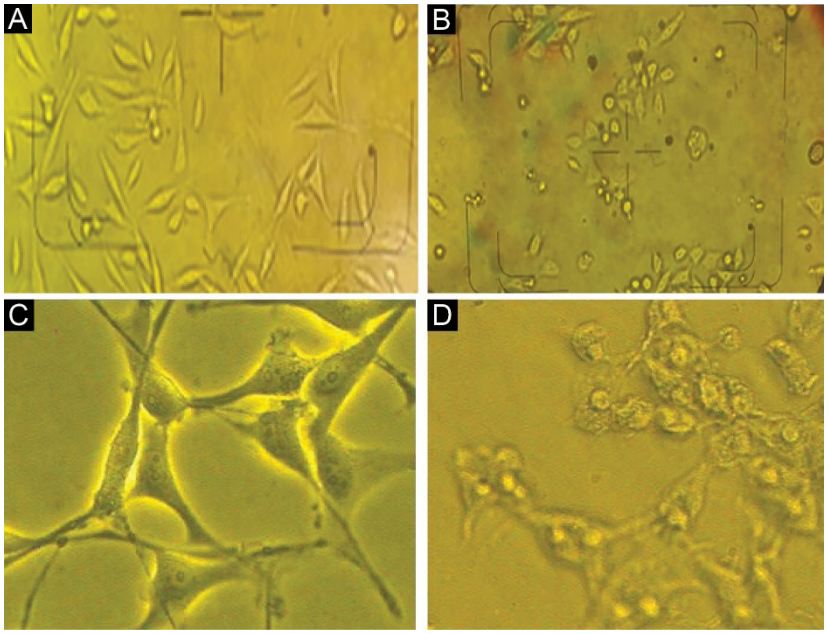

Figure 4. Light Microscopy Images of (A) CHO and (B) Hela Cells Before the Treatment; C and D were shown CHO and Hela after combine therapy (treated with 3 J/cm^2 for 90 s LLLT and 10, 50 µM sinensetin respectively.

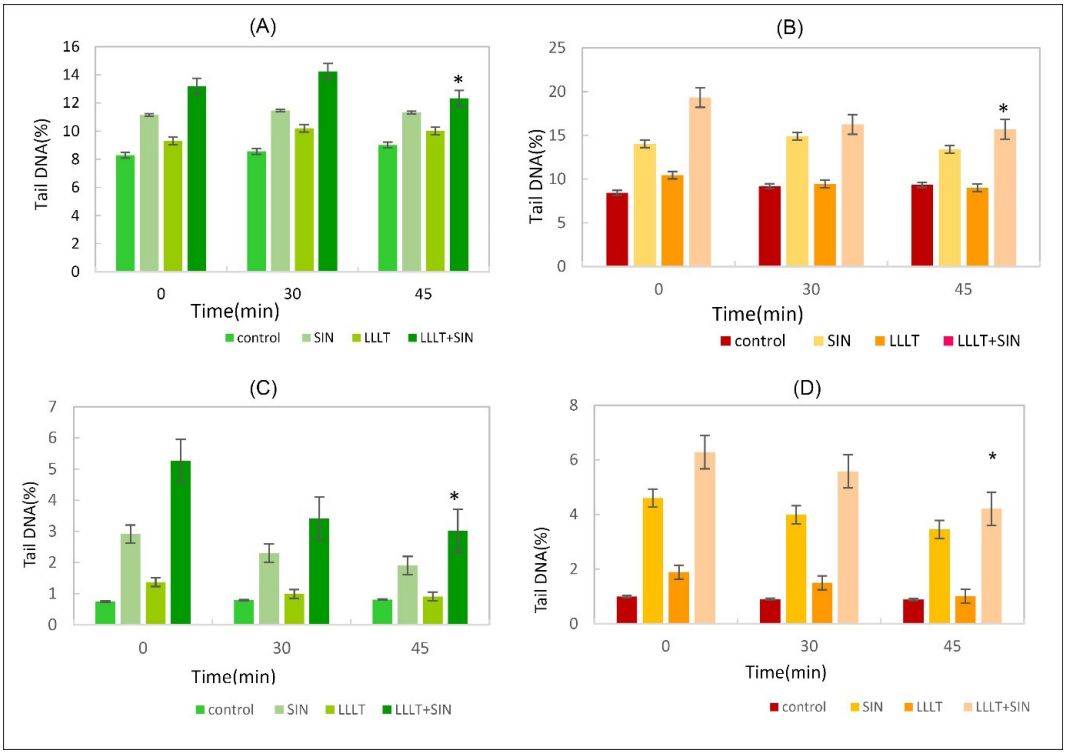

Figure 5. Effects of LLLT and Sinensetin-Induced DNA Damage Measured by Comet assay. LLLT induced breaks in the presence of Sinensetin. (A) %DNA in the tail in CHO, (B) %DNA in the tail in Hela. C) Tail moment in CHO, (D) Olive tail moment in Hela. The data are represented as the mean ± SD (n = 3). *$P < 0.05$.

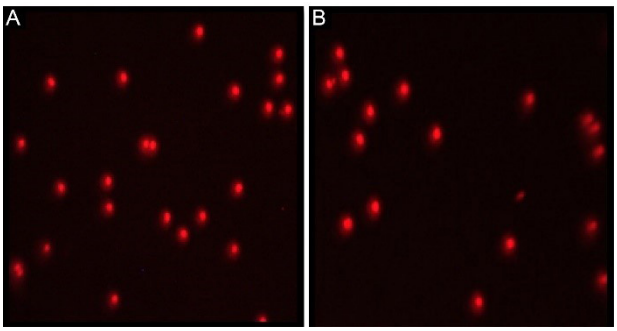

Figure 6. Comet assay images of CHO and Hela cells did not show any specific changes in DNA damage levels. The cells were treated with LLLT 660nm,3J/CM^2 for 90S and Sinensetin 10,50 µM sinensetin for A) CHO and B) Hela respectively.

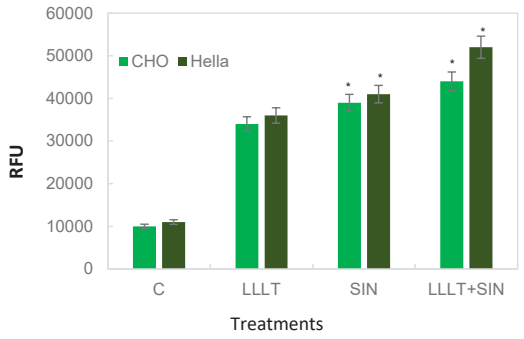

Figure 7. ROS Production Level. The cells were treated with LLLT, sinensetin and a combination of these treatments. The intracellular ROS was measured with DCFH-DA. All values are mean ± SEM; These data were shown in 3 independent samples. *$P < 0.05$. Abbreviation: RFU, Relative fluorescence unit.

cells demonstrated ROS production. The level of ROS substantially increased during combined treatment (LLLT + sinensetin), whereas treating with LLLT and sinensetin alone did not change significantly. This data may show that irradiation is more effective than sinensetin in the level of reactive oxygen molecules ($P < 0.05$).

Discussion

LLLT has been used in Eastern Europe and Asia since many years ago.[21] Laser treatment covers various fields in the clinic such as pain, tinnitus, and cancer cells.[22,23]

Low-level laser irradiation is a novel treatment, this way of treatment without any remarkable thermal effects on cells, which treats a wide range of diseases including wound healing and tissue repair, reduces inflammation and relieves pain.[24,25] The substantial mechanism of the low-level laser initiates mitochondria as the first cellular target for the photons to enhance cytochrome *c* oxidase activity.[26] For reducing cancer mortality with the use of the natural substance, this way of treatment can sensitize tumors.[27] Treated cancer cells with chemotherapy plant compounds are valuable.[28] It has been shown LLLT reduces oxidative stress and improves antioxidants levels in several clinical treatments.[29] It seems this way of treatment can reduce cancer.

Polyphenols are natural compounds detected in ubiquitous plants like fruits, vegetables, cereals, and drinks. Polyphenols are secondary metabolites and against insects, herbivores, and microorganisms. In

food, polyphenols have effects on bitterness, astringency, color, flavor, odor, and oxidative stability.[30,31] Polyphenols scavenge free radicals by H-atom transfer, therefore, decrease noxious effects due to oxidative stress.[32] LLLT provides oxidative stress (ROS) in biological systems with cellular antioxidant systems.[33] Sinensetin which is one of the significant groups of polyphenols (poly methylated flavone) is gained from *Orthosiphon aristatus* var. *aristatus* and various citrus fruits. These groups behaved as an anticancer activity.[34] The purpose of this research was to investigate the effect of combined therapy (LLLT and sinensetin) on the treatment of cancer cells. In the present study, we used LLLT and sinensetin to treat cancer cells. Our data demonstrated that sinensetin has cytotoxicity against CHO and Hela cell lines; this result is similar to other studies.[12] IC50 values for CHO and Hela cells were 10μM and 50μM respectively. Our results showed that sinensetin has cytotoxicity effect against CHO and Hela cell lines, and the half-maximal inhibitory concentration of the Hela cell line is more than the CHO other cell lines. However, another study demonstrated that sinensetin can against AML-2/D100 cells with a high concentration.[35] These researches illustrated sinensetin has the lowest toxicity for normal cells. This study showed that treated cancer cells with low-level laser alone could not be more effective than the use of low-level laser and sinensetin together. Treating the cells with LLLT and sinensetin also decreased the colony-forming ability of the cells. The clonogenic ability showed how cells can divide after the treatment.[36] Djavid et al, in 2015, demonstrated a possible radio sensitizing effect with colonogenic ability for 685 nm LLLI in HeLa cancer cells.[19] Based on a previous study in the comet assay, DNA damage was reduced in each treatment, whereas x-irradiation and polyphenols enhanced DNA damage significantly.[37,38] Tanaka et al introduced polyphenols sensitivity, and in this treatment, DNA could be repaired.[39] Our data revealed that treatment with low-level laser and sinensetin compared to the other treatments means LLLT and sinensetin increased the amount of ROS production in both cell lines and the fundamental absorption of low-level laser is cytochrome c oxidase.[40] One of the respiratory complexes, complex IV or cytochrome c oxidase, catalyzes the transfer of electrons from reduced cytochrome c.[41] Our results demonstrated that sinensetin can act as an anti-cancer effect in CHO and Hela cell lines. Treated CHO and Hela cells with LLLT and sinensetin could be impressive treatment compared to sinensetin and LLLT.

Conclusion

Combined treatments (LLLT + sinensetin) inhibited clonogenicity in the two cell lines, conforming with the previous results. LLLT + sinensetin compared to the inverse treatment cannot change DNA breaks significantly. The use of LLLT and sinensetin may improve cellular penetration. To attain better results, these data needed to detect the molecular mechanism.

Acknowledgements

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Conflict of Interest

The authors declare that they have no conflict of interest.

Ethical Considerations

Not applicable.

References

1. Bedell SL, Goldstein LS, Goldstein AR, Goldstein AT. Cervical Cancer Screening: Past, Present, and Future. *Sex Med Rev*. 2020;8(1):28-37. doi: 10.1016/j.sxmr.2019.09.005.
2. Lopez-Charcas O, Espinosa AM, Alfaro A, Herrera-Carrillo Z, Ramirez-Cordero BE, Cortes-Reynosa P, et al. The invasiveness of human cervical cancer associated to the function of $Na_V1.6$ channels is mediated by MMP-2 activity. *Sci Rep*. 2018;8:12995. doi: 10.1038/s41598-018-31364-y.
3. Stark A, Gregoire L, Pilarski R, Zarbo A, Gaba A, Lancaster WD. Human papillomavirus, cervical cancer and women's knowledge. *Cancer Detect Prev*. 2008;32(1):15-22. doi:10.1016/j.cdp.2008.02.002.
4. Vargas AN. Natural history of ovarian cancer. *Ecancermedicalscience*. 2014;8:465. doi:10.3332/ecancer.2014.465
5. Kwon YS, Mok JE, Lim KT, Lee IH, Kim TJ, Lee KH, et al. Ovarian cancer during pregnancy: clinical and pregnancy outcome. *J Korean Med Sci*. 2010;25(2):230-4. doi: 10.3346/jkms.2010.25.2.230.
6. Barut A, Arikan I, Barut F, Harma M, Harma MI, Payasli B. Ovarian cancer during pregnancy. *J Pak Med Assoc*. 2011;61(9):914-916.
7. Peto J, Gilham C, Fletcher O, Matthews FE. The cervical cancer epidemic that screening has prevented in the UK. *Lancet*. 2004;364(9430):249-56. doi: 10.1016/S0140-6736(04)16674-9.
8. Thomson CS, Forman D. Cancer survival in England and the influence of early diagnosis: what can we learn from recent EUROCARE results?. *Br J Cancer*. 2009;101(Suppl 2): S102-S109. doi:10.1038/sj.bjc.6605399
9. Johnson R, Shabalala S, Louw J, Kappo AP, Muller CJF. Aspalathin Reverts Doxorubicin-Induced Cardiotoxicity through Increased Autophagy and Decreased Expression of p53/mTOR/p62 Signaling. *Molecules*. 2017;22(10):1589. doi: 10.3390/molecules22101589.
10. Singh A, Holvoet S, Mercenier A. Dietary polyphenols in the prevention and treatment of allergic diseases. *Clin Exp Allergy*. 2011;41(10):1346-59. doi: 10.1111/j.1365-2222.2011.03773.x.
11. Pereira CV, Duarte M, Silva P, Bento da Silva A, Duarte CMM, Cifuentes A, et al. Polymethoxylated Flavones Target Cancer Stemness and Improve the Antiproliferative Effect of 5-Fluorouracil in a 3D Cell Model of Colorectal Cancer. *Nutrients*. 2019;11(2):326. doi: 10.3390/nu11020326.
12. Rezakhani N, Goliaei B, Parivar K, Nikoofar A. Effects of

X-irradiation and sinensetin on apoptosis induction in MDA-MB-231 human breast cancer cells. *Int J Radiat Res*. 2020; 18 (1) :75-82. doi:10.18869/acadpub.ijrr.18.1.75
13. Han Jie L, Jantan I, Yusoff SD, Jalil J, Husain K. Sinensetin: An Insight on Its Pharmacological Activities, Mechanisms of Action and Toxicity. *Front Pharmacol*. 2021;11:553404. doi:10.3389/fphar.2020.553404
14. Wang C. Complementary and Alternative Medicine and Osteoarthritis. *Int J Integr Med*. 2013;1:13. doi: 10.5772/56431.
15. Silva JC, Lacava ZG, Kuckelhaus S, Silva LP, Neto LF, Sauro EE, et al. Evaluation of the use of low level laser and photosensitizer drugs in healing. *Lasers Surg Med*. 2004;34(5):451-7. doi: 10.1002/lsm.20062.
16. Wu S, Xing D, Gao X, Chen WR. High fluence low-power laser irradiation induces mitochondrial permeability transition mediated by reactive oxygen species. *J Cell Physiol*. 2009;218(3):603-11. doi: 10.1002/jcp.21636.
17. Agrawal T, Gupta GK, Rai V, Carroll JD, Hamblin MR. Pre-conditioning with low-level laser (light) therapy: light before the storm. *Dose Response*. 2014;12(4):619-49. doi: 10.2203/dose-response.14-032.Agrawal.
18. Franken NA, Rodermond HM, Stap J, Haveman J, van Bree C. Clonogenic assay of cells in vitro. *Nat Protoc*. 2006;1(5):2315-2319. doi:10.1038/nprot.2006.339
19. Djavid GE, Goliaie B, Nikoofar A. Analysis of Radiomodulatory Effect of Low-Level Laser Irradiation by Clonogenic Survival Assay. *Photomed Laser Surg*. 2015;33(9):452-459. doi:10.1089/pho.2015.3893
20. Seidel C, Lautenschläger C, Dunst J, Müller AC. Factors influencing heterogeneity of radiation-induced DNA-damage measured by the alkaline comet assay. *Radiat Oncol*. 2012;7:61. doi: 10.1186/1748-717X-7-61.
21. Moshkovska T, Mayberry J. It is time to test low level laser therapy in Great Britain. *Postgrad Med J*. 2005;81(957):436-41. doi: 10.1136/pgmj.2004.027755.
22. Mansouri V, Arjmand B, Rezaei Tavirani M, Razzaghi M, Rostami-Nejad M, Hamdieh M. Evaluation of Efficacy of Low-Level Laser Therapy. *J Lasers Med Sci*. 2020;11(4):369-380. doi:10.34172/jlms.2020.60
23. da Silva JL, Silva-de-Oliveira AFS, Andraus RAC, Maia LP. Effects of low level laser therapy in cancer cells-a systematic review of the literature. *Lasers Med Sci*. 2020;35(3):523-529. doi: 10.1007/s10103-019-02824-2.
24. Wong-Riley MT, Liang HL, Eells JT, Chance B, Henry Salzman MM, Buchmann E, et al. Photobiomodulation directly benefits primary neurons functionally inactivated by toxins: role of cytochrome c oxidase. *J Biol Chem*. 2005;280(6):4761-4771. doi:10.1074/jbc.M409650200
25. Liang HL, Whelan HT, Eells JT, Wong-Riley MT. Near-infrared light via light-emitting diode treatment is therapeutic against rotenone- and 1-methyl-4-phenylpyridinium ion-induced neurotoxicity. *Neuroscience*. 2008;153(4):963-974. doi: 10.1016/j.neuroscience.2008.03.042
26. Huang YY, Nagata K, Tedford CE, McCarthy T, Hamblin MR. Low-level laser therapy (LLLT) reduces oxidative stress in primary cortical neurons in vitro. *J Biophotonics*. 2013;6(10):829-838. doi:10.1002/jbio.201200157
27. Tadjalli-Mehr K, Becker N, Rahu M, Stengrevics A, Kurtinaitis J, Hakama M. Randomized trial with fruits and vegetables in prevention of cancer. *Acta Oncol*. 2003;42(4):287-293. doi:10.1080/02841860310011852
28. Chen D, Dou QP. Tea polyphenols and their roles in cancer prevention and chemotherapy. *Int J Mol* Sci. 2008;9(7):1196-1206. doi:10.3390/ijms9071196
29. Palazzetti S, Richard MJ, Favier A, Margaritis I. Overloaded training increases exercise-induced oxidative stress and damage. *Can J Appl Physiol*. 2003;28(4):588-604. doi:10.1139/h03-045.
30. Pandey KB, Rizvi SI. Plant polyphenols as dietary antioxidants in human health and disease. *Oxid Med Cell Longev*. 2009;2(5):270-8. doi: 10.4161/oxim.2.5.9498.
31. Othman L, Sleiman A, Abdel-Massih RM. Antimicrobial Activity of Polyphenols and Alkaloids in Middle Eastern Plants. *Front Microbiol*. 2019; 10:911. doi: 10.3389/fmicb.2019.00911.
32. Di Meo F, Lemaur V, Cornil J, Lazzaroni R, Duroux JL, Olivier Y, et al. Free radical scavenging by natural polyphenols: atom versus electron transfer. *J Phys Chem A*. 2013;117(10):2082-92. doi: 10.1021/jp3116319.
33. Fife D, Rayhan DJ, Behnam S, Ortiz A, Elkeeb L, Aquino L, et al. A randomized, controlled, double-blind study of light emitting diode photomodulation for the prevention of radiation dermatitis in patients with breast cancer. *Dermatol Surg*. 2010;36(12):1921-7. doi: 10.1111/j.1524-4725.2010.01801.x.
34. Kim JA, Kim SM, Ha SE, Vetrivel P, Saralamma VVG, Kim EH, et al. Sinensetin regulates age-related sarcopenia in cultured primary thigh and calf muscle cells. *BMC Complement Altern Med*. 2019;19(1):287. doi: 10.1186/s12906-019-2714-2.
35. Kotarsky K, Nilsson NE, Flodgren E, Owman C, Olde B. A human cell surface receptor activated by free fatty acids and thiazolidinedione drugs. *Biochem Biophys Res Commun*. 2003;301(2):406-10. doi: 10.1016/s0006-291x (02)03064-4.
36. Ghavami L, Goliaei B, Taghizadeh B, Nikoofar A. Effects of barley β-glucan on radiation damage in the human hepatoma cell line HepG2. *Mutat Res Genet Toxicol Environ Mutagen*. 2014 Dec;775-776:1-6. doi: 10.1016/j.mrgentox.2014.09.005.
37. Bigdeli B, Goliaei B, Masoudi-Khoram N, Jooyan N, Nikoofar A, Rouhani M, et al. Enterolactone: A novel radiosensitizer for human breast cancer cell lines through impaired DNA repair and increased apoptosis. *Toxicol Appl Pharmacol*. 2016; 313:180-194. doi: 10.1016/j.taap.2016.10.021.
38. Azqueta A, Collins A. Polyphenols and DNA Damage: *A Mixed Blessing. Nutrients*. 2016;8(12):785. doi: 10.3390/nu8120785.
39. Tanaka T, Munshi A, Brooks C, Liu J, Hobbs ML, Meyn RE. Gefitinib radiosensitizes non-small cell lung cancer cells by suppressing cellular DNA repair capacity. *Clin Cancer Res*. 2008;14(4):1266-1273. doi: 10.1158/1078-0432.CCR-07-1606
40. Turrens JF. Mitochondrial formation of reactive oxygen species. *J Physiol*. 2003;552(Pt 2):335-44. doi: 10.1113/jphysiol.2003.049478.
41. Guerra-Castellano A, Díaz-Quintana A, Pérez-Mejías G, Elena-Real CA, González-Arzola K, García-Mauriño SM, et al. Oxidative stress is tightly regulated by cytochrome *c* phosphorylation and respirasome factors in mitochondria. *Proc Natl Acad Sci U S A*. 2018;115(31):7955-7960. doi: 10.1073/pnas.1806833115.