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Effect Photodynamic therapy (PDT) And Single Walled Carbon Nanotube- OH on The Skin Cancer A431 Cell line

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ABSTRACT

The aim of this in vitro study was to analyze the effect of blue light (λ = 420 – 480 nm), a single-walled carbon nanotube -OH, and their combination on the viability (cytotoxic or enhancement) of the skin cancer A431 cell line after 24 hours of incubation periods .Cell culture is the process of removing cells from living tissues and growing them in a laboratory setting until they are ready to be tested using photodynamic therapy and nanoparticles, which is known as direct irradiation in vitro. Skin cell plates (A431 cell line) grown in culture medium supplemented with 10% fetal bovine serum (FBS) were irradiated with blue light, treated with the nanoparticle, and treated with the combination of them, and plates were incubated for 24 hours . In all the experiments, a crystal violet assay was used to determine the viability of the cells, and the intensity of color was measured by a plate reader. The cells were kept at 37°C. Blue light results showed a considerable decrease ($p \leq 0.001$) at 240 seconds after 24 hours of incubation time in the viability percent; nanoparticle results showed a considerable decrease in the viability percent ($p \leq 0.001$) for all concentrations; the most effective concentration was 200 µg/ml; and the combination results showed a significant decrease in cell viability percent ($p \leq 0.001$) for all concentrations as compared with the control group. The single-walled carbon nanotube- OH with a concentration of 200 µg/ml produced the greatest results when combined with a light exposure period of 240 seconds.

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تأثير العلاج الضوئي الديناميكي (PDT) وأنابيب نانوية كربونية أحادية الجدار- OH على خط خلايا سرطان الجلد A431

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الـــخُـــلاصـــة

كان الهدف من هذه الدراسة في المختبر هو تحليل تأثير الضوء الأزرق - $\lambda = 420$ - *480* نانومتر) ، وأنابيب الكربون النانوية أحادية الجدار -*OH* ، ومزيجها على قابلية الحياة (سامة للخلايا أو تعزيز) لسرطان الجلد . الزراعة الخلوية هي عملية إزالة الخلايا من الأنسجة الحية وزراعتها في بيئة معملية حتى تصبح جاهزة للاختبار باستخدام العلاج الضوئي والجسيمات النانوية ، وهو ما يعرف بالإشعاع المباشر في المختبر. . تم تشعيع صفائح خلايا الجلد (خط خلية *A431*) المزروعة في وسط مزرعة مكمل بـ 10 ٪ من مصل بقري جنيني (*FBS*) بالضوء الأزرق ، وعولجت بالجسيمات النانوية ، وعولجت بمزيج منها ، وحضنت الأطباق لمدة 24 ساعة. في جميع التجارب ، تم استخدام اختبار البنفسجي البلوري لتحديد صلاحية الخلايا ، وتم قياس شدة اللون بواسطة قارئ لوحة. تم حفظ الخلايا عند 37 درجة مئوية. أظهرت نتائج الضوء الأزرق انخفاضًا كبيرًا ($p\leq0.001$) عند 240 ثانية بعد 24 ساعة من وقت الحضانة في نسبة الصلاحية ؛ أظهرت نتائج الجسيمات النانوية انخفاضًا كبيرًا في نسبة الصلاحية ($p\leq0.001$) لجميع التركيزات ؛ كان التركيز الأكثر فعالية 200 جم / مل. وأظهرت نتائج المجموعة انخفاضًا معنويًا في نسبة بقاء الخلية ($p\leq0.001$) لجميع التركيزات مقارنة بمجموعة التحكم. أنتج الأنبوب النانوي الكربوني أحادي الجدار- *OH* بتركيز 200 ميكروغرام / مل أعظم النتائج عندما يقترن بفترة تعرض للضوء تبلغ 240 ثانية.

الكلمات المفتاحية:

سرطان الجلد
*PDT*العلاج الضوئي
الضوء الأزرق ،
الصمام الثنائي الباعث للضوء ،
SWCNTs

1. INTRODUCTION

Cancer nanomedicine has received both positive and negative responses on the process of overcoming the challenges associated with Clinical Translational Science (CTS). In medicine delivery systems, the fundamentals of nanoparticle interaction with biological molecules are not extensively characterized [1]. The PDT is used extensively in the treatment of a broad range of cancer types, and its benefits include little or no invasive therapy, the absence of cumulative harm, and the absence of drug resistance. The PDT application faces some challenges in achieving completely side-effect-free treatment, including long-lasting photoactivities of PS 31 molecule that may cause photosensitization on skin. This requires that the patients remain in the dark, together with a lack of tumor selectivity and accumulation in normal tissues that results in toxic effects [2,3]. These challenges include the need for patients to remain in the dark for several weeks following treatment. According to Singh et al.'s research (2020) [4], nanomedicine has the potential to improve the bioavailability of pharmaceuticals, limit the degradation of drugs, better target cells, control drug release at particular places, and offer therapeutic effectiveness with fewer adverse effects. The results of combining nanomedicine with photodynamic therapy (PDT), on the other hand, are primarily focused on decreasing the adverse effects of cancer therapies. The PDT can partially or completely kill tumor cells directly, and this death is not the result of a single process [5]. It has the ability to lower the quantity of clonogenic tumor cells. After the light irradiation of tumors treated with photosensitizers has finished, it was claimed that the number of clonogenic cells might be reduced by up to 72% [6]. Additionally, the number of clonogenic cells continued to decline over time following PDT, suggesting that tumor cell death is a kinetic

process. On target tumor cells, the PDT directly causes a combination of apoptosis and necrosis [7]. The PDT has been shown by Kah G et al. to cause DNA fragmentation and promote apoptosis [8].Time and dose both had an impact on the DNA fragmentation. In addition, they noted that there was a damage to cytoplasmic structures and chromatin condensation towards the nucleus's outer edge had occurred[8]. In this work, we used a panel of LEDs with distinct wavelengths. ranging from (420 – 480 nm) in order to define wavelength-specific biological effects on cultured human skin cells. It was concluded that the higher concentration (200 µg/ml) of SWCNT- OH had a better anticancer effect on the A431 cell line . .

2. Materials and methods

2.1 Cell line and culture condition

A cell line derived from a non-melanoma skin cancer called the A431 cell line was employed. A431 cell lines were obtained from the American Type Culture Collection (ATCC). The cell lines were grown in a complete growth medium called RPMI-1640, which was made according to the Gibco manual with 10%

fetal bovine serum (FBS) and 1% penicillin/streptomycin antibiotics. The culture was carried out at a temperature of 37 degrees Celsius.

2.2 Exposure irradiation

Before the process of irradiation, cells were planted in 200 µl of media on sterile 96-well plates with a cover. After that, each well had an area of 0.282 cm^2. The emission tip was held in a perpendicular position above the culture medium, and the irradiation was carried out in a dark room after careful timing. An assessment made using an optical power meter. This revealed that the emitted light fully covered the irradiated field of each culture plate. The cells in the control group were not subjected to the light treatment, and the cells were allowed to remain at room temperature (RT). Light emitting diode and xenon lights were used in the production of this paper (figure 1).

Figure 1: Setup of Photodynamic Therapy (PDT) by blue Light emitting diode at 400 mW/cm^2 in this work.

2.3 MTT assay

The absorbance of -3(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT;

Sigma–Aldrich) in live cells was measured to evaluate the growth of the cells in accordance with the methodology outlined in the previous passage [9].

In brief, cells were seeded at a density of 50,000 cells per wall on 96-wall plates, and then they were irradiated with blue light for the amount of time that was specified. After exposing all 96 of the wall plates to blue light, the MTT solution, consisting of 5 mg/ml in PBS and 10 microliters, was added to each plate. After a further three to four hours of incubation at 37 degrees Celsius, The formazan crystals will be formed, the plates were pipetted to remove the medium from the wells, and then one hundred microliters of Dimethyl Sulfoxide (DMSO) were added to each well in order to dissolve the formazan crystals. The optical density was measured at 570 nm with a microplate reader made by(Molecular Devices in the United States). The findings were presented as a percentage compared to the values of the control group. Every measurement was taken three times to improve the precision.

Figure 2: The 96-well plates for the MTT assay test.

3. Results and discussion

3.1 LED irradiation with 420–480 nm at 400 mW/cm^2 is toxic for Squamous cell carcinoma A431 cell line

The CCK-8 determines viability. To evaluate the effects of irradiation with LED blue light of 420–480 nm wavelength on squamous cell carcinoma, there was a significant cytotoxic effect. The growth rate of the A431 cells decreased gradually at different irradiation times, and the effect of cell death became higher with increased exposure time; the viability of cells was reduced to 45.526 at 240 seconds. After exposing the cells for 240 seconds, It was observed.

that irradiation with 400 mW/cm^2 killed nearly half of the cells as shown in figure 3. Exposure to blue light was also associated with a considerable reduction in the incidence and number of cases of skin cancer. Because of this, one of the goals of blue light exposure treatment is to treat skin tumors, in particular when there are a large number of them and they are still in the early phases of growth, in particular when surgical excision is difficult but external light exposure is simple to obtain. In the present research, phototherapy was likely more effective than utilizing daylight due to the use of a small, high-intensity band of blue light. This band of light may match the absorption wavelength. The visible blue light spectrum (450–490 nm) and ultraviolet (UV–400 nm) are incompatible, and

the radiation spectrum (100–400 nm) was not extensively discussed in many papers [10].

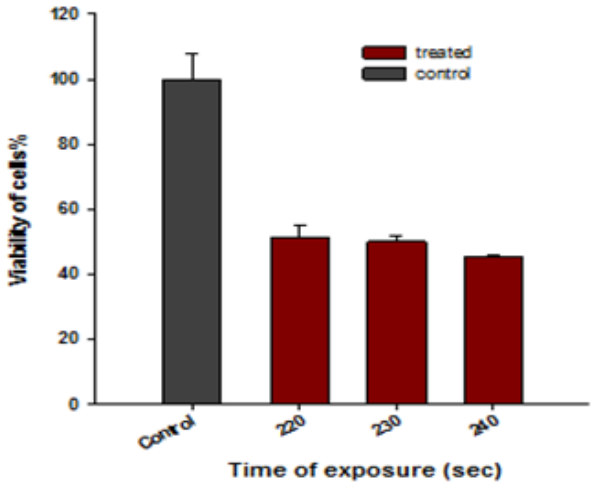

Figure 3: Effect of different irradiation times of Blue LED on A431 cell line after incubation for 24 hours.

3.2 Effect of xenon lamp on A431 non-melanoma cells

The A431 cell line were subjected to irradiation at wavelengths ranging 420-480 nm and a power of 40 W at 15,30 and 45 min. When we exposed the cells to direct light for different periods of time, the Xenon lamp had an effect of 82% in time of 45 minutes, but with the times of 15 and 30 minutes, as shown in figure 4, we observed an enhancement in cells rather than death, which i believe is due to the high direction and non-monochromatic nature of the xenon lamp.

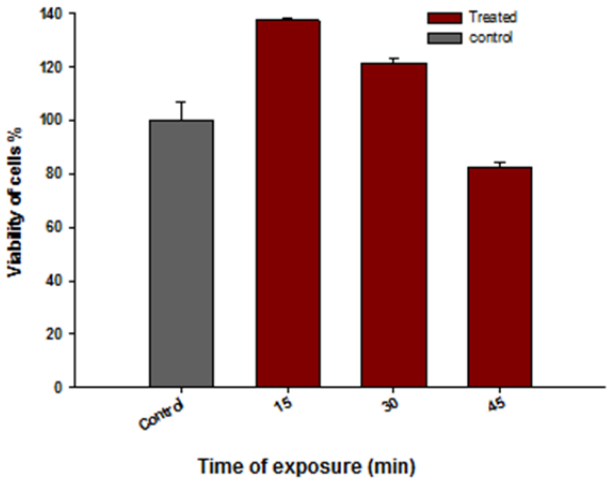

Figure 4: Effect of different irradiation times of Xenon lamp(420-480 nm-40 W) on A431 cell line after incubation for 24 hours.

3.3 Effect SWCNTs-OH nanoparticles on skin cancer cell line

The cytotoxicity behavior of SWCNTs-OH was analyzed using MTT assay kit. The A431 cell line were prepared with SWCNTs-OH, and then irradiated at 420-480 nm wavelength at 220,230,240 sec for LED and 15,30,45 min for xenon lamp. After the successful PDT treatment, the measured absorbance value of untreated cells was kept as a control. The assay results are shown in the Figures 5, 6 and 7 .

Table 1 and table 2 display the findings of the viability of cells assay. The results showed that the non-irradiated 0 J/cm^2 demonstrated a lower percentage of viability of cells behavior for SWCNTS-OH at concentration 200 µg/ml (48.140%). Figure 6, The viability of cells decreased with increasing time of exposure. The percentage viability of the cells treated with SWCNTs-OH at 220 seconds exhibited a viability of 30.421%, whereas the percentage viability of the cells at 230 and 240 seconds was 29.857% and 24.870%, respectively, as shown in Table 1. In Figure 7, it was observed that the percentage vitality of the SWCNTs-OH-treated cells was 82.691% after 15 minutes of xenon lamp exposure, compared to 80.260% and 74.985 after 30 and 45 minutes, respectively, as shown in Table 2.The exposure of blue LED 400 mw/cm^2 on plate significantly reduced the percentage of cell viability in 240 sec irradiation and SWCNTs-OH treated compared to cell alone.

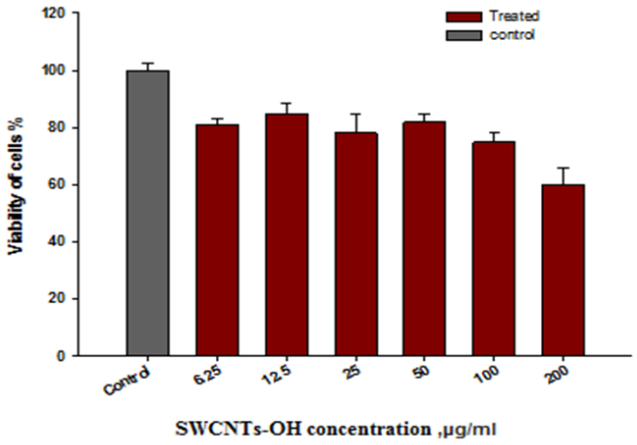

Figure 5: Effect of different concentrations of SWCNT-OH on A431 cell line after incubation for 24 hour

Table (1): Parameters of SWCNT-OH in combination with blue LED on A431 cell line.

Time (sec)	optical density of test well	Ave. optical density of control wells	Viability of cells %	Ave. Viability of cells %
220	0.297 0.368 0.305	1.062	27.944 34.624 28.696	30.421
230	0.355 0.307 0.29	1.062	33.401 28.885 27.285	29.857
240	0.339 0.165 0.289	1.062	31.895 15.524 27.191	24.870

Table (2): Parameters of SWCNT-OH in combination with xenon lamp on A431 cell line.

Time	optical density of test well	Ave. optical density of control wells	Viability of cells %	Ave. Viability of cells %
15 min	0.462 0.481 0.452	0.562	82.157 85.536 80.379	82.691
30 min	0.458 0.408 0.488	0.562	81.446 72.554 86.781	80.260
45 min	0.396 0.443 0.426	0.562	70.420 78.778 75.755	74.985

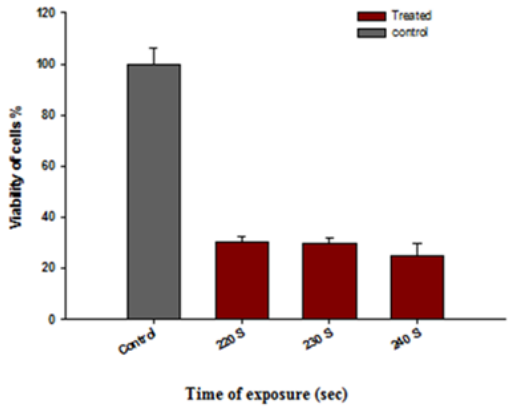

Figure 6: Effect of SWCNT-OH in combination with blue LED on A431 cell line after incubation for 24 hours.

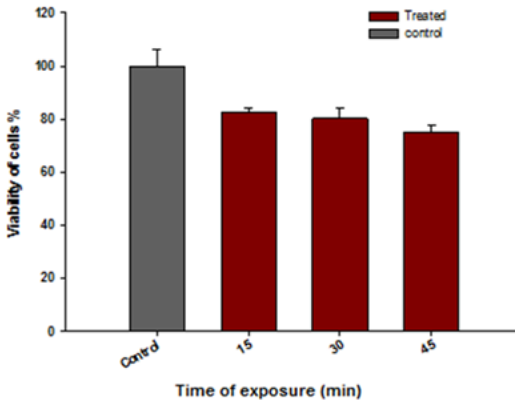

Figure 7: Effect of SWCNT-OH in combination with xenon lamp on A431 cell line after incubation for 24 hours.

3.4 XRD analysis of Single walled carbon nanotube-OH characterization

The crystallinity as well as the structural characteristics of the nano-sized particles, X-ray diffraction exams were used as a method of investigation. The XRD data that were collected were analyzed using the standards established by the Joint Committee on Power Diffraction (JCPDS). In order to carry out an XRD analysis, the SWCNTs-OH was positioned on a wafer that was fabricated from crystal glass. Figure 8 displays the XRD patterns of the SWCNTs. These patterns were determined using the JCPDS number 751621 reflecting graphite. These patterns suggest that reflections from hexagonal carbon atom layers and nanotube stacking layers that correspond to (002) planes were responsible for producing the distinctive peak at 24.2° and 25.6°. This peak was formed at these angles. The plane 002 in single-walled carbon nanotubes (SWCNTs) is an indicator of the presence of SP2-bonded carbon groups[11]. When seen in the plane, there was a discernible decrease in the peak's intensity, and the nanobiocomposite that was manufactured exhibited a minor shift at 25.8 degrees. The diameters of the crystallites in the study samples of SWCNT-OH ranged from 8 to 18 nanometers, which is rather small as shown in Table 3.

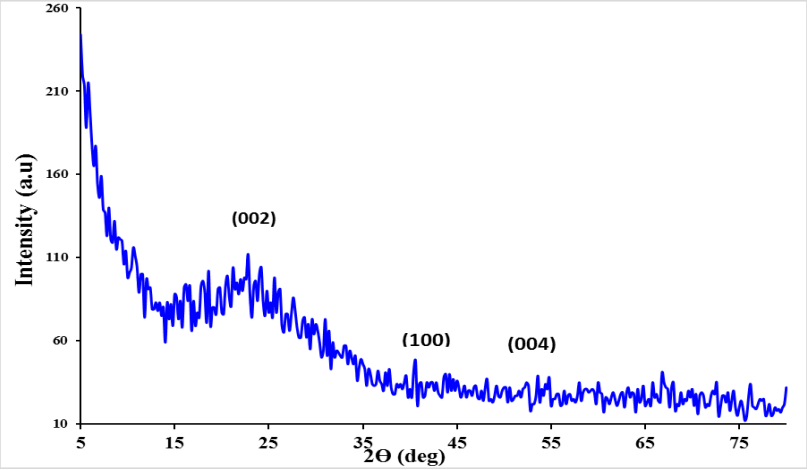

Figure 8: The XRD analysis of sample of SWCNTS-OH.

Table (3): The XRD data of SWCNT-OH.

Sample	2Θ	d (nm)	FWHM	Crystal size (nm)
SWCNT-OH/ glass as deposited	16.247	0.54511	0.694	12
	22.695	0.39148	0.714	12
	24.153	0.36817	1.067	8
	27.615	0.32276	0.968	9
	53.517	0.17109	0.528	18

According to Lei et al.[12], several researchers were focused their attention on the biological delivery of CNTs that have been combined with polymers, pharmaceuticals, DNA, and PS to specifically target cancer cells. According to Sajid et al. [13], the unique size, shape, and physicochemical features of SWCNTs contribute to their efficiency as nanocarriers. According to Anastasiadis SH et al. [14], endocytosis is the pathway via which 27 MWCNTs enter cells when SWCNTs circulate throughout the body and directly enter cells.According to Marangon et al. [15], single-wall carbon nanotubes are the best nanocarriers for encapsulating a wide variety of polymers, pharmaceuticals, and photomedicines. According to Alrushaid N. et el. [16], single-wall carbon nanotubes stand out among other nanocarriers used in drug delivery systems because they facilitate the functionalization of specific moieties in a manner that is both covalent and non-covalen, Gulati S et al. Diwan A, Singh P. Functionalized carbon nanotubes (FCNTs) as novel drug delivery systems: emergent perspectives from applications [17], the SWCNT-OH has great biocompatibility when used to the therapy of cancer.

4. Conclusion

The main presumption used in the production of this paper is the use of PDT in skin tumor treatment with a high radiation dose at various exposure times. After putting the cells through an exposure treatment for intervals of 220, 230, and 240 seconds for light-emitting diodes and 15, 30, and 45 minutes for xenon lamps, It was found that the irradiation dose with 400 mw/cm^2 killed over half of the cells at 240 seconds. According to the findings of this thesis, an increase in the number of cancer cells that were eliminated indicates that the work that is being given here improves photodynamic therapy as a treatment for cancer. The quantity of data that can be used to facilitate the planning of subsequent operations in vivo investigations and is sufficient to motivate investigation into the use of single walled carbon nanotube-OH with irradiation as light-emitting diode and adding nanoparticles into cell line and exposure to phototherapy them showed a 20% reduction in viability of cells, and this is a good result.

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