Validation of a Light Source for Phototoxicity in *in vitro* Conditions

Ivan Iliev[1,2], Inna Sulikovska[1], Elena Ivanova[1], Mashenka Dimitrova[1], Biliana Nikolova[2], Christina Andreeva[3,4*]

[1]*Institute of Experimental Morphology, Pathology and Anthropology with Museum*
Bulgarian Academy of Sciences
Acad. G. Bonchev Str., Bl. 25, 1113 Sofia, Bulgaria
E-mails: taparsky@abv.bg, inna_sulikovska@ukr.net,
elena9512@abv.bg, mashadim@abv.bg

[2]*Institute of Biophysics and Biomedical Engineering*
Bulgarian Academy of Sciences
Acad. G. Bonchev Str., Bl. 21, 1113 Sofia, Bulgaria
E-mail: nikolova@bio21.bas.bg

[3]*Institute of Electronics*
Bulgarian Academy of Sciences
72 Tsarigradsko shosse Bulv., 1784 Sofia, Bulgaria
E-mail: c.andreeva@ie.bas.bg

[4]*Faculty of Physics,*
Sofia University "St. Kliment Ohridski"
5 James Bourchier Bulv., 1164 Sofia, Bulgaria

[*]*Corresponding author*

Received: June 06, 2021 **Accepted: December 10, 2021**

Published: June 30, 2022

Abstract: *Phototoxicity is a chemically induced skin irritation in the presence of sunlight. Phototoxic substances after irradiation with sunlight absorb and convert light energy into chemical energy, leading to the formation of highly reactive oxygen species with toxic effects. Recently LED technologies made possible their application for novel effective solar simulators, to be used in biology and medicine. We study the possibilities of application of LED sunlight simulator (Helios-iO, model LE-9ND55-H – 5500K) for in vitro testing of synthetic and natural products for phototoxicity. Cytotoxicity/phototoxicity was assessed by validated BALB/3T3 clone A31 Neutral Red Uptake Assay. The morphological alterations in BALB/3T3 cells induced by the Radachlorin® and irradiated with dose 2.4 J, were analyzed by fluorescent microscopy. The physical characteristics of the lamp were determined and the intensity distribution of the LED light over a model of a 96-well plate at a distance of 25 cm from the lamp diode matrix center was estimated. The obtained results show that the light emitted by the solar simulator used is similar to the natural sun light. The biological testing results reveal the high efficiency of the solar simulator in an in vitro experimental system for phototoxicity testing.*

Keywords: *LED Solar Simulator, Phototoxicity, in vitro.*

Introduction

Phototoxicity is a chemically induced skin irritation in the presence of sunlight [26]. Phototoxic compounds can be applied topically to the skin or by systemic circulation when administered orally or parenterally [5, 17]. In both cases of application in the presence of light, the symptoms are similar to sunburn [25]. Phototoxic substances, when irradiated with sunlight, absorb light energy and convert it into chemical energy, resulting in the formation of

highly reactive oxygen species (ROS) that cause toxic effects [21]. Many synthetic substances, including drugs such as tetracyclines, sulfonamides, photosensitizers and others may cause phototoxic effects [16]. In addition, many natural products of plant origin (some essential oils, plant extracts and pigments) are known to cause photodermatitis [23]. Recently, newly synthesized substances and natural products are increasingly used in pharmacy, medicine and cosmetics, and they must be tested for side effects, including phototoxicity. Due to the unstable characteristics of sunlight related to geographical location, seasonality, daily cycles, etc., it is necessary to use artificial light sources that simulate the physical characteristics of natural sunlight. There is a wide variety of artificial light sources (solar simulators) that can be successfully used to test new substances for phototoxicity, both *in vitro* and *in vivo*. The most commonly used light sources are: carbon arc lamp, halogen arc lamp, quartz tungsten halogen lamp, xenon arc lamp, mercury xenon lamp, argon arc lamp and diode or LED lamp (light-emitting diode lamp) [2, 3, 18]. Sunlight simulators must meet a number of requirements: light spectrum, lighting homogeneity, light flux stability and power. With the advancement and improvement of diode technology, diode simulators of sunlight are increasingly used [13]. Diode simulators have a number of advantages: easily portable with small size and weight, widely available (low cost), low heat radiation, low energy consumption, stable spectrum during operation, etc. [4, 14].

The aim of the present study was to investigate the possibilities of application of a LED sunlight simulator (Helios-iO, model LE-9ND55-H – 5500K) for *in vitro* testing of synthetic and natural products for phototoxicity.

To test the effectiveness of the solar simulator we used reference chemicals (phototoxic/non-phototoxic drugs). As a model of a highly phototoxic, synthetic substance, we used acridine orange, which is used as fluorochrome in fluorescence microscopy [15]. As a model of a weakly phototoxic compound with natural origin, we used the photosensitizer Radachlorine, often used in photodynamic therapy of cancer, psoriasis and others diseases [20, 22]. As a negative control (not-phototoxic substance with natural origin) we used geranium essential oil, which is widely used in cosmetics, pharmacy, medicine and others [7].

Materials and methods

Light source

The light source used is a light emitting diode (LED) matrix – an artificial solar light simulator Helios-iO, model LE-9ND55-H – 5500K (SERIC Ltd., Tokyo, Japan). The lamp spectrum was registered using an Ocean Optics spectrometer, model HR4000 (Ocean Optics Inc., Florida, USA) with optical spectral resolution 0.03 nm (full width at half maximum, FWHM). The radiation flux power of the lamp was measured by a pyranometer Hukseflux (thermosensors model SR05-A1) whose sensitivity is 14 $\mu V/(W/m^2)$. An optical power meter PM 100D with sensor S120VC (Thorlabs Inc., North Newton, Kansas, USA) was used to determine the output light power distribution of the lamp. Its operating range in wavelength is 200-1100 nm, and in power from 50 nW to 50 mW. The linearity of the sensor is ± 0.5% and the measurement uncertainty for the operating optical range (280-980 nm) is less than ± 5%. The diameter of the sensor aperture is 9.5 mm.

Chemicals

Reference chemicals (phototoxic/non-phototoxic drugs): acridine orange (Loba chemie Ltd., Fischamend, Austria), radachlorin® (Rada-Pharma Ltd., Moscow, Russia) and geranium essential oil (provided by Assoc. Prof. Ilian Kolev, Ph.D., Medical University of Varna, Bulgaria). Dyes for cell staining: Mayer's hematoxylin, Eosin Y and Propidium iodide, were

purchased from Sigma-Aldrich, Schnelldorf, Germany. Cell culture reagents: Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), antibiotics (penicillin and streptomycin), the disposable consumables were supplied by Orange Scientific, Braine-l'Alleud, Belgium.

Cell line

The BALB/3T3 clone A31 (mouse embryo fibroblasts) cell line was obtained from American Type Cultures Collection (ATCC, Manassas, Virginia, USA).

Methods for determining the physical characteristics of the emitted light

The pyranometer was centered below the lamp, at the operating distance of 25 cm. Although the diffuse natural solar light in the room was rather low (around 1.16 W/m^2), the measurements were performed in dark conditions.

The method for estimating the optical power distribution over the cell plate requires pre-setting of the measured wavelength in the power meter. For this reason, we used a green filter whose transmission spectrum was also registered with the spectrometer and compared with the lamp spectrum. During the power measurements, the filter was placed in front of the power meter sensor, and the center of the filter transmission spectrum was set as the operating wavelength for the power meter. This makes it possible to obtain reliable results in terms of power reading for the green light through the filter. To obtain the value of the total white light intensity, we integrated the area under the filter transmission spectrum and compared it with the integral area under the lamp emission spectrum.

Cytotoxicity and phototoxicity testing

BALB/3T3 cells were cultured in 75 cm^2 tissue culture flasks in DMEM, 10% FBS, 2 mM glutamine and antibiotics (penicillin 100 U/ml and streptomycin 100 µg/ml) at 37 °C, 5% CO_2 and 90% relative humidity. Cytotoxicity/phototoxicity was assessed by validated BALB/3T3 clone A31 Neutral Red Uptake Assay (3T3 NRU test) [9, 11]. Briefly, cells were plated in a 96-well microtiter plate at a density of 1 ×10^4 cells/100 µl/well and were incubated for 24 h. A wide concentration range of the test compounds was applied. In phototoxicity tests, 96-well plates were irradiated with dose 2.4 J/cm^2 and the cells were incubated for additional 24 h. After treatment with Neutral Red medium, washing and treatment with the Ethanol/Acetic acid solution, the absorption was measured on a TECAN microplate reader (TECAN, Grödig, Austria) at wavelength 540 nm.

Cytotoxicity/phototoxicity were expressed as CC_{50} / PC_{50} values (concentrations required for 50% cytotoxicity/phototoxicity), calculated using non-linear regression analysis (GraphPad Software, San Diego, California, USA). The CC_{50} values can be used to calculate the PIF (Photo-Irritancy Factor) for each test substance, according to the following formula:

$$PIF = \text{Cytotoxicity } (CC_{50}) / \text{Phototoxicity } (PC_{50})$$

The statistical analysis included application of One-way ANOVA followed by Bonferroni's post hoc test. $p < 0.05$ was accepted as the lowest level of statistical significance. All results are presented as mean ± SD.

Fluorescent microscopy

The morphological alterations in BALB/3T3 cells induced by the Radachlorin® and irradiated with dose 0.65 J, were analyzed by fluorescent microscopy. BALB/3T3 cells were cultured on

13 mm diameter cover glasses in 24-well plates and were treated with Radachlorin® in concentrations approximating the PC_{90}, PC_{50} and PC_{10} value established by the NRU test. 24-well plates were irradiated with dose 2.4 J/cm^2 and the cells were incubated for additional 24 h.

Acridine orange (AO) and Propidium iodide (PI) (live/dead) staining was performed as previously described [27]. The cells were stained with the fluorescent dyes AO (5 µg/ml) and PI (5 µg/ml) in PBS and mounted on microscope slides. Stained cells were visualized and examined under a fluorescence microscope (Leica DM 5000B, Wetzlar, Germany).

Results

Determining the characteristics of the solar simulator

The radiation flux power was measured 6 times and the average value obtained was 40.98 W/m^2.

The solar simulator spectrum, together with the natural solar spectrum, measured with the spectrometer, is presented in Fig. 1. As can be seen, the two spectra are very similar, except for the near UV-range, which is practically absent in the artificial solar light.

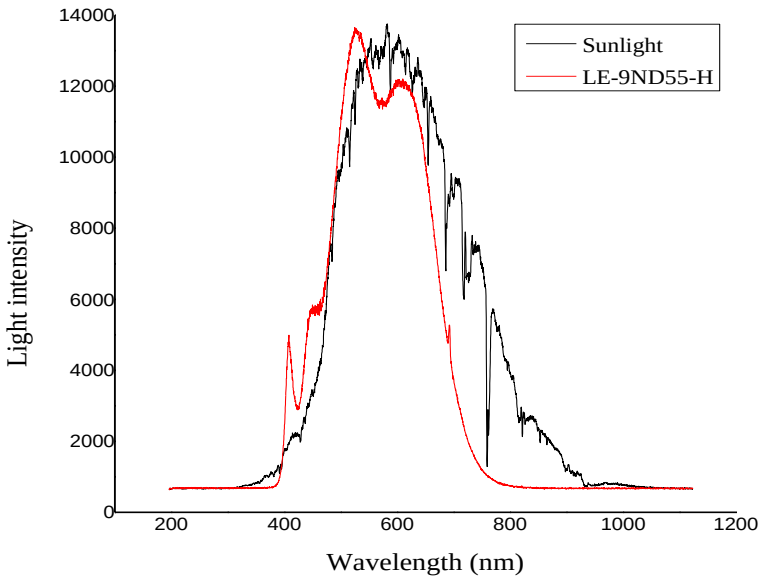

Fig. 1 Spectral comparison
of the solar simulator LE-9ND55-H and natural solar light

As mentioned, the measurement of the optical power requires pre-setting of the measured wavelength in the power meter. For this reason, we used a green filter whose transmission spectrum was also registered with the spectrometer and compared with the lamp spectrum. The results from this measurement are presented in Fig. 2.

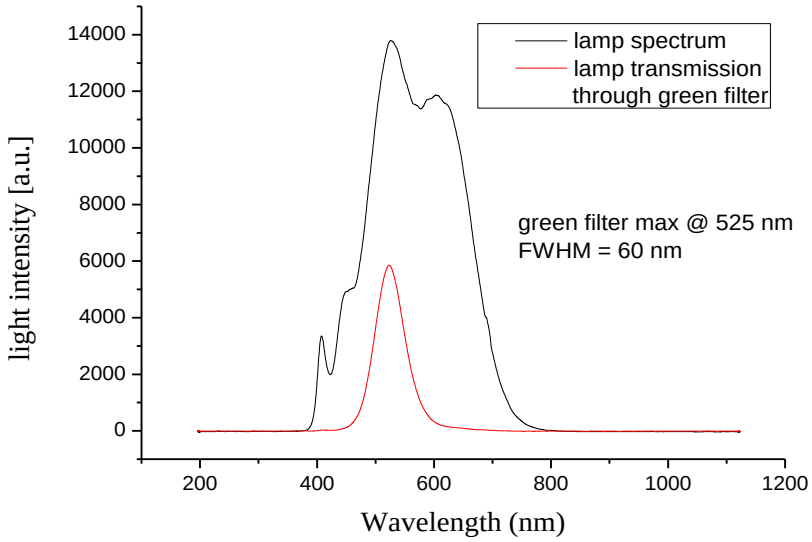

Fig. 2 Spectral comparison of the emission of the solar simulator LE-9ND55-H (black curve) and its transmission through the green filter (red curve)

The analysis of the transmission profile shows that it has a Lorentzian shape with a central wavelength of 525 nm and full line width at half maximum (FWHM) of 60 nm. As can be seen, besides acting as a band-pass filter, the green filter also decreases the total intensity within the transmission band. For the central wavelength of 525 nm, this decrease in the input light power is 2.36 times. We performed a numerical integration of the filter transmission profile and compared it with the area integration of a Lorentzian profile with the same linewidth but with amplitude corresponding to the amplitude of the solar simulator light (i.e., 2.36 times higher). The area ratio is 2.35 times, which shows that the Lorentzian lineshape of the filter transmission profile is a good approximation.

In order to check the homogeneity of the power distribution, we measured the light power at a vertical distance 25 cm from a 96-well plate (i.e. between the center of the diode matrix and the point between wells 6-7 and D-E). The power density was calculated using the value for the aperture of the power meter sensor. The area of the curve under the total LED spectrum is 6.84 times larger than the area under the Lorentzian transmission profile of the green filter, and this value was used as a correction factor for the green-to-total power density conversion.

The total power density distribution over the plate wells is presented in Fig. 3. The irradiation of the edge wells is ca. 30% lower than that of the central well, namely 4.04 mW/cm^2 for the central plates and 2.9 mW/cm^2 for the edges. To decrease this difference, we use the central (2-11) columns of the place, yielding a variation of ca. 20% in the irradiation dose. We should point out the excellent correspondence of the power density values obtained with the pyranometer and using the method of numerical integration of the spectral curves.

According to this distribution, for exposition times of 10 min, the obtained dose is 2.4 J/cm^2 in the plate center and 1.7 J/cm^2 at the edges.

Testing of the solar simulator in in vitro experiments

In order to be validated, the solar simulator had to be tested in laboratory conditions to determine phototoxicity according to a standard protocol [19]. The tests were performed on

known and well-characterized substances (acridine orange, radachlorine and geranium essential oil) under *in vitro* conditions. In the *in vitro* experiments, we used as a model of healthy tissue, mouse embryonic fibroblasts (BALB/3T3), as a standard for testing of phototoxic substances. The results of the cytotoxicity and phototoxicity tests show that the observed effects are of the dose-dependent type (Fig. 4).

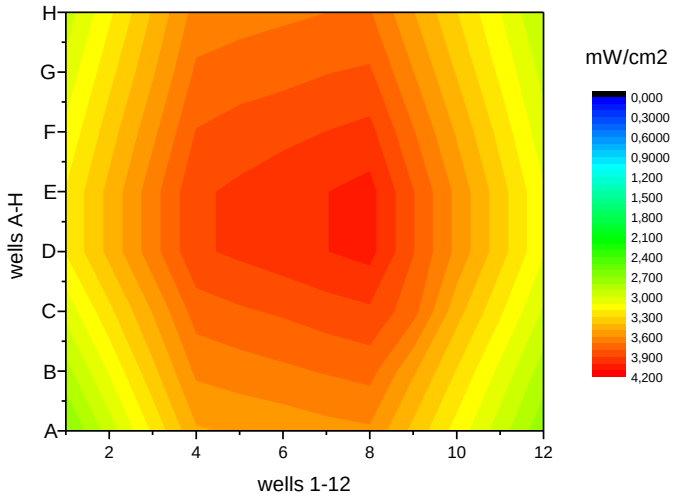

Fig. 3 Distribution of the light power density over a 2D model of a 96-well plate for a distance between the center of the diode matrix and the plate center equal to 25 cm

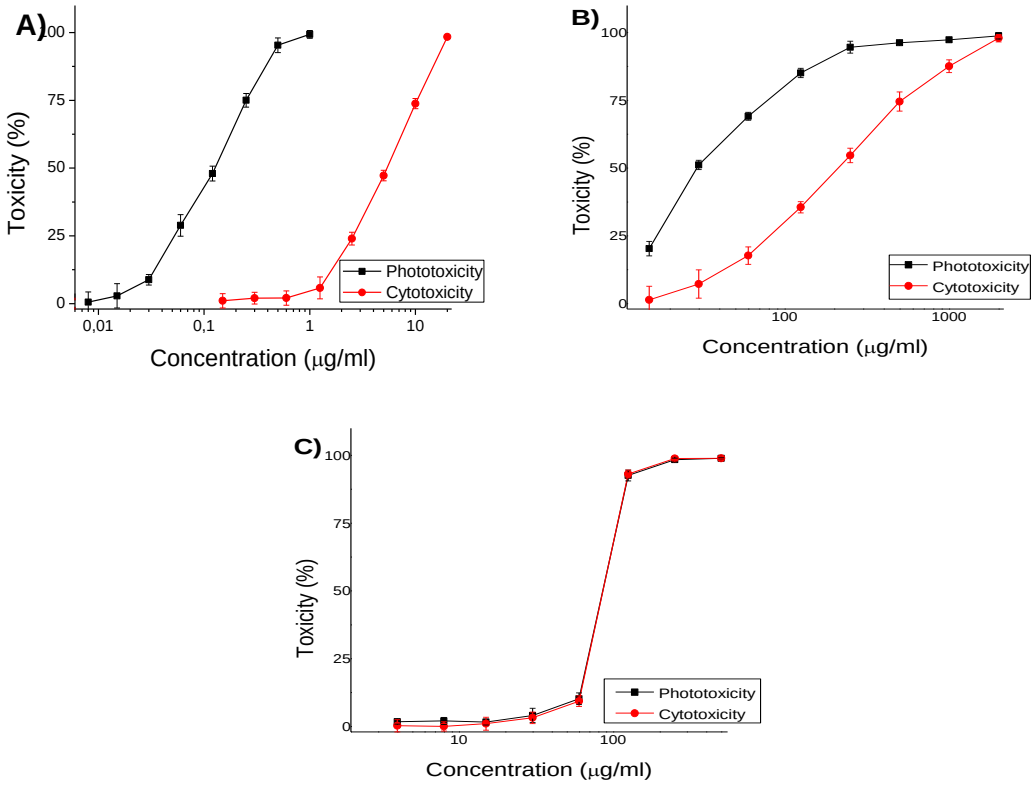

Fig. 4 Cito- and phototoxicity determined in the BALB/3T3 cell line: A) acridine orange; B) radachlorine, and C) geranium essential oil.

Based on the obtained sigmoidal, dose-response curves, the mean IC_{50} values for the cytotoxic and PC_{50} for the phototoxic effects caused by the test substances on the BALB/3T3 cell line were calculated (Table 1).

Table 1. IC_{50}/PC_{50} values of mean and PIF

Cell Line	Compounds	CC_{50} (PC_{50}) ± SD (µg/ml)		PIF*
		Cytotoxicity	Phototoxicity	
BALB 3T3	Acridine orange	5.51 ± 0.33	0.14 ± 0.06	**39.36**
	Radachlorine	220.8 ± 16.21	29.79 ± 1.43	**7.41**
	Geranium essential oil	91.54 ± 1.1	91.4 ± 1.43	**1**

*PIF – photo-irritancy factor, PIF < 2 – not phototoxic,
5 > PIF ≥ 2 – probable phototoxicity, PIF ≥ 5 – phototoxic.

Extremely strong phototoxic effect is observed with AO (PC_{50} = 0.14 ± 0.06 µg/ml). In the case of radachlorine, the phototoxic effect is significantly weaker (PC_{50} = 29.79 ± 1.43 µg/ml), while in the case of geranium oil such effect is practically not observed ($CC_{50}(PC_{50})$ = 91 µg/ml). The PIF evaluates the phototoxicity of the test substance (Table 1). In addition, the PIF can be used to compare the phototoxicity of different substances. The PIF for geranium oil is a 1, which is characteristic of non-phototoxic substances. The other two substances AO and radachlorine had a stronger phototoxic effect with PIF = 39 and 7, respectively.

Fluorescence analysis

We used fluorescence microscopy to more accurately determine the processes of apoptosis and cell death that occurred as a result of treatment with radachlorine and subsequent illumination with the solar simulator. Cells were visualized by double staining with acridine orange and propidium iodite (Fig. 5).

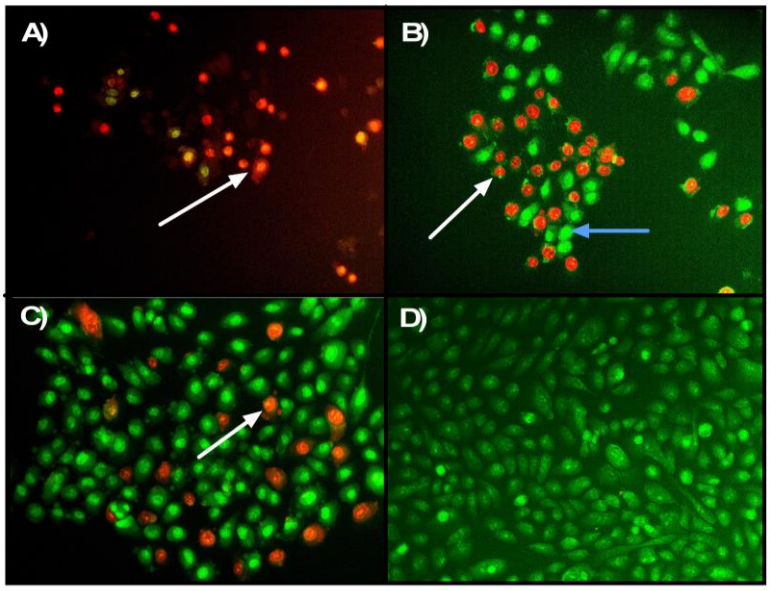

Fig. 5 Fluorescence analysis of radachlorine-treated BALB/3T3 cell culture at concentrations:
A) PC_{90}, B) PC_{50}, C) PC_{10}, and D) negative control (untreated cells).
The cell culture was illuminated for 10 minutes with the solar simulator.
Double staining with acridine orange/propidium iodide;
white arrows – cells in final stages of apoptosis;
blue arrow – cells in initial stages of apoptosis.

Through this method the different stages of apoptosis can be evaluated: cells with light green fragmented nuclei – initial stages of apoptosis, cells with orange-red nuclei – advanced stages of apoptosis, cells with condensed dark red nuclei – final stages of apoptosis. At a concentration of radachlorin PC_{90} (Fig. 5A) we observe many small dark red fragments of cell nuclei (white arrow) and several green cells in the initial stages of apoptosis. At a concentration of PC_{50} (Fig. 5B) we observe cells in the initial and advanced stages of apoptosis (blue and white arrow, respectively). At a concentration of PC_{10} (Fig. 5C), apoptotic cells and cells with normal morphology were observed.

Discussion

In order to be validated, the solar simulator had to be tested in laboratory conditions to determine phototoxicity according to a standard protocol. The tests were performed on known and well-characterized substances under *in vitro* conditions. The test substances were selected to cover a wide range of synthetic and natural products used in science, cosmetics, pharmacy and medicine. Acridine orange is a synthetic compound with very high phototoxicity [24]. AO used in fluorescence microscopy serves as a nucleic acid-selective fluorescent dye [6]. The essential oil of geranium, on the other hand, is a natural product that is not phototoxic. For this reason, this oil is a valuable component of many cosmetic and pharmaceutical products. Radachlorine is a second-generation photosensitizer with low phototoxicity and high photodynamic activity [8]. Radachlorine was isolated from microalgae and is widely used in photodynamic therapy of various diseases [10, 12]. In *in vitro* experiments, as a model of healthy tissue we used mouse embryonic fibroblasts (BALB 3T3), as a standard for testing phototoxic substances. The results of the cytotoxicity and phototoxicity tests show that the observed effects are of the dose-dependent type. Extremely strong phototoxic effect is observed with acridine orange. In the case of radachlorine, the phototoxic effect is significantly weaker, while in the case of geranium oil such effect is practically not observed. This confirms in practice the literature data on the phototoxicity of the substances acridine orange and radachlorine. From this we can conclude that the tested light source is suitable for phototoxicity testing *in vitro*. Due to its extremely high phototoxicity, acridine orange can be used as a positive control, which is an indicator of the correct implementation of the phototoxicity determination protocol.

We used fluorescence microscopy to more accurately determine the processes of apoptosis and cell death that occurred as a result of treatment with radachlorine and subsequent illumination with the solar simulator. At high concentrations of radachlorine we observed cells in advanced stages of apoptosis and fragments of dead cells. With a decrease in the concentration of radachlorine we observed a decrease in apoptotic cells. Similar results were observed in other radachlorine-treated cell lines [1, 19]. These results show that irradiation with the solar simulator leads to a significant increase in the toxicity of radachlorine. This is a direct evidence of the ability of the solar simulator to cause a phototoxic effect in an *in vitro* model system.

Conclusion

The recorded spectrum of the emitted light from the solar simulator Helios-iO, model LE-9ND55-H – 5500K has a great similarity with the spectrum of natural sunlight. In addition, the power of the emitted light is quite sufficient to cause a phototoxic reaction in photosensitive compounds. The power density distribution at 25 cm from the center of the diode array is relatively uniform, with the difference between the center and edge of the plate being reduced to about 20% by utilizing the central 10 columns of wells. The inhomogeneity could be further reduced to 10% by placing the plate at a distance of 35 cm from the

LED matrix center and excluding the end columns. The decrease in power in this case is less than 3 times, yielding about 30 min of irradiation for the same dose as used in our experiments. These data indicate that this light source can be used in phototoxicity tests using standard platelets for cell culture.

The second part of the results obtained from real biological experiments under *in vitro* conditions qualitatively prove the suitability and effectiveness of the tested light source for performing phototoxicity tests. Moreover, the observed mechanism of cell death (apoptosis and nonspecific necrosis) at different concentrations of the phototoxic substance radachlorine is fully consistent with the literature data. All this shows a high reproducibility of the results described in the literature for other, often used in practice light sources.

Acknowledgements

This work was financially supported by the Bulgarian National Science Fund, Grant No. KP-06-N31/1 "Selective Natural Inhibitors of Post-proline Degrading Peptidases from Bulgarian Medicinal Plants as Potential Agents for the Prevention and Therapy of Oncological Diseases".

References

1. Bae S., Y. Kim, J. Lee, S. Namkoong, S. Han, J. Kim, C. Lee, H. Chun, H. Jin, W. Ahn (2004). Photodynamic Effects of Radachlorin on Cervical Cancer Cells, Cancer Research and Treatment, 36(6), 389-394.
2. Beeson E. (1978). The CSI Lamp as a Source of Radiation for Solar Simulation, Lighting Research and Technology, 10, 164-166.
3. Bickler D. (1962). The Simulation of Solar Radiation, Solar Energy, 6, 64-68.
4. Bliss M., T. Betts, P. Gottschalg (2008). Advantages in Using LEDs as the Main Light Source in Solar Simulators for Measuring PV Device Characteristics, Proceedings of Reliability of Photovoltaic Cells, Modules, Components, and Systems, 7048, 704807, https://doi.org/10.1117/12.795428.
5. Boudon M., U. Plappert-Helbig, A. Odermatt, D. Bauer (2014). Characterization of Vemurafenib Phototoxicity in a Mouse Model, Toxicology Sciences, 137, 259-267.
6. Darzynkiewicz Z. (1990). Differential Staining of DNA and RNA in Intact Cells and Isolated Cell Nuclei with Acridine Orange, Methods in Cell Biology, 33, 285-298.
7. Dijoux N., Y. Guingand, C. Bourgeois, S. Duran, C. Fromageot, C. Combe, P.-J. Ferret (2006). Assessment of the Phototoxic Hazard of Some Essential Oils Using Modified 3T3 Neutral Red Uptake Assay, Toxicology *in vitro*, 20(4), 480-489.
8. Douillard S., D. Olivier, T. Patrice (2009). *In vitro* and *in vivo* Evaluation of Radachlorin Sensitizer for Photodynamic Therapy, Photochemical and Photobiological Sciences, 8, 405-413.
9. ESAC (1998a), Statement on the Scientific Validity of the 3T3 NRU PT Test (An *in vitro* Test for Phototoxic Potential), 9th Meeting of ECVAM Scientific Advisory Committee, 1-2 October 1997.
10. Filonenko E., V. Sokolov, V. Chissov, E. Lukyanets, G. Vorozhtsov (2008). Photodynamic Therapy of Early Esophageal Cancer, Photodiagnosis and Photodynamic Therapy, 5, 187-190.
11. Halle W. (2003). The Registry of Cytotoxicity: Toxicity Testing in Cell Cultures to Predict Acute Toxicity (LD50) and to Reduce Testing in Animals, Alternatives to Laboratory Animals, 31(2), 89-198.
12. Kochneva E., E. Filonenko, E. Vakulovskaya, E. Scherbakova, O. Seliverstov, N. Markichev, A. Reshetnickov (2010). Photosensitizer Radachlorin: Skin Cancer PDT

Phase II Clinical Trials, Photodiagnosis and Photodynimic Therapy, 7, 258-267.
13. Kohraku S., K. Kurokawa (2003). New Methods for Solar Cells Measurement by LED Solar Simulator, Proceedings of 3rd World Conference on Photovoltaic Energy Conversion, 2, 1977-1980.
14. Kolberg D., F. Schubert, N. Lontke (2011). Development of Tunable Close Match LED Solar Simulator with Extended Spectral Range to UV and IR, Energy Procedia, 8, 100-105.
15. Kusuzaki K., H. Murata, H. Takeshita, S. Hashiguchi, T. Nozaki, K. Emoto, T. Ashihara, Y. Hirasawa (2000). Intracellular Binding Sites of Acridine Orange in Living Osteosarcoma Cells, Anticancer Research, 20, 971-975.
16. Lee Y., J. Yi, H. Lim, T. Kim, Y. Ahn, K. Ko, JH. Kim, H.-K. Park, S. Sohn, J. Lee (2017). Phototoxicity Evaluation of Pharmaceutical Substances with a Reactive Oxygen Species Assay Using Ultraviolet A, Toxicology Research, 33(1), 43-48.
17. Lugovic L., M. Situm, S. Ozanic-Bulic, I. Sjerobabski-Masnec (2007). Phototoxic and Photoallergic Skin Reactions, Collegium Antropologicum, 31(1), 63-67.
18. Matson P., K. Emery, P. Bird (1984). Terrestrial Solar Spectra, Solar Simulation and Solar Cell Short-circuit Current Calibration: A Review, Solar Cells, 11, 105-145.
19. Mirzaei H., G. Djavid, M. Hadizadeh, M. Jahanshiri-Moghadam, P. Hajian (2015). The Efficacy of Radachlorin-mediated Photodynamic Therapy in Human Hepatocellular Carcinoma Cells, Journal of Photochemistry and Photobiology B: Biology, 142, 86-91.
20. Neginskaya A., V. Berezhnaya, V. Rudkovskii, B. Uzdensky (2015). Radachlorin as a Photosensitizer, Proceedings of Saratov Fall Meeting 2014: Optical Technologies in Biophysics and Medicine XVI; Laser Physics and Photonics XVI; and Computational Biophysics, 9448, 94480O, https://doi.org/10.1117/12.2179990.
21. Peter P., X. Qingsu, S. Xin, Y. Hongtao (2012). Phototoxicity and Environmental Transformation of Polycyclic Aromatic Hydrocarbons (PAHs) – Light-induced Reactive Oxygen Species, Lipid Peroxidation, and DNA Damage, Journal of Environmental Science and Health, Part C, 30(1), 1-41.
22. Privalov V., A. Lappa, O. Seliverstov, A. Faizrakhmanov, N. Yarovoy, E. Kochneva, M. Evnevich, A. Anikina, A. Reshetnicov, I. Zalevsky, Y. Kemov (2002). Clinical Trials of a New Chlorin Photosensitizer for Photodynamic Therapy of Malignant Tumors, Proceedings of Optical Methods for Tumor Treatment and Detection: Mechanisms and Techniques in Photodynamic Therapy XI, 4612, https://doi.org/10.1117/12.469355.
23. Quinn J., A. Kessell, L. Weston (2014). Secondary Plant Products Causing Photosensitization in Grazing Herbivores: Their Structure, Activity and Regulation, International Journal of Molecular Sciences, 15(1), 1441-1465.
24. Saetzler R., J. Jallo, H. Lehr, C. Philips, U. Vasthare, K. Arfors, R. Tuma (1997). Intravital Fluorescence Microscopy: Impact of Light-induced Phototoxicity on Adhesion of Fluorescently Labeled Leukocytes, The Journal of Histochemistry and Cytochemistry, 45(4), 505-513.
25. Schauder S. (2005). Phototoxische Reaktionen der Haut durch Medikamente, Deutsches Arzteblatt International, 102, 2314-2319 (in German).
26. Schuch A., H. Moreno, H. Schuch, C. Menck, C. Garcia (2017). Sunlight Damage to Cellular DNA: Focus on Oxidatively Generated Lesions, Free Radical Biology and Medicine, 107, 110-124.
27. Wahab I., A. Abdul, A. Alzubairi, M. Elhassan, S. Mohan (2009). *In vitro* Ultramorphological Assessment of Apoptosis Induced by Zerumbone on (HeLa), Journal of Biomedicine and Biotechnology, 2009, 769568.

Assoc. Prof. Ivan Iliev, Ph.D.
E-mail: taparsky@abv.bg

Ivan Iliev is an Associate Professor at the Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, where he received Ph.D. Degree in area of Immunology. His interests are in the field of immunology, biochemistry, and cell biology.

Inna Sulikovska, M.Sc.
E-mail: inna_sulikovska@ukr.net

Inna Sulikovska works at the Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences. She received M.Sc. Degree in area of Biomedical Engineering at the National Technical University of Ukraine "Igor Sikorskyi Kyiv Polytechnic Institute". Her areas of interests are in the field of biomedical engineering, nanomaterials, stem cells differentiation, pathology, cancer cell biology.

Elena Ivanova, M.Sc. Student
E-mail: elena9512@abv.bg

Elena Ivanova graduated from Sofia University "St. Kliment Ohridski" where she received a B.Sc. Degree in Molecular Biology and currently studies for her M.Sc. in Biotechnology at the University of Chemical Technology and Metallurgy. She works at the Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, under the guidance of Assoc. Prof. Ivan Iliev. Her interests are in the field of immunology, biochemistry, cell biology, biotechnology, and bioinformatics.

Prof. Mashenka Dimitrova, Ph.D.
E-mail: mashadim@abv.bg

Mashenka Dimitrova is a Professor in the Institute of Experimental Morphology, Pathology and Anthropology with Museum at the Bulgarian Academy of Sciences. She has a Ph.D. Degree in Morphology. Her research interests are in the field of enzymology, medicinal plants biochemistry, identification of novel markers or targets for cancer therapies.

Prof. Biliana Nikolova, Ph.D.
E-mail: nikolova@bio21.bas.bg

Biliana Nikolova graduated Sofia University, Faculty of Biology, specialization Biochemistry and Microbiology in 1992. Since 1992 she is working in Institute of Biophysics, now Institute of Biophysics and Biomedical Engineering Bulgarian Academy of Sciences. She received her Ph.D. Degree in 2001 in the same institute. Since 2022 she is a Professor in the Institute of Biophysics and Biomedical Engineering. Her scientific interests are in the field of electroporation, electroloading, drug delivery systems, and electrochemotherapy.

Assoc. Prof. Christina Andreeva, Ph.D.
E-mail: c.andreeva@ie.bas.bg

Christina Andreeva is an Associate Professor at the Institute of Electronics at the Bulgarian Academy of Sciences. She works also at the Faculty of Physics at the Sofia University "St. Kliment Ohridski". She received her Ph.D. Degree in the area of Atomic Spectroscopy. Her main field of work is related to coherent atomic spectroscopy with application to optical magnetometry and atomic clocks.