REVIEW

On: Dissertation submitted for the awarding of the educational and scientific degree "PhD" in the field of higher education 4. "Natural Sciences, Mathematics and Informatics", Professional Field 4.3 "Biological Sciences", Scientific Specialty "Biochemistry" with code 01.06.10 at the Institute of Experimental Morphology, Pathology and Anthropology with Museum - BAS

The review was prepared by: Assoc. Prof. Dr. Lyudmila Kabaivanova, Institute of Microbiology "Stefan Angelov" - BAS, Sofia

PhD student: Inna Sulikovska

General notes

In connection with the dissertation work I received for review on the topic: "Optimization and adaptation of the method for determining phototoxicity *in vitro 3T3 NRU phototoxicity test*, to the LED - solar simulator Helios-iO", where the results of the conducted research activity of PhD student Inna Sulikovska are presented. They are summarized in a complete work of 147 standard pages, which contains all the necessary sections of a dissertation - introduction, in-depth literature review on the topic of the dissertation, including 322 sources, aim and objectives, materials and methods, results and discussion, followed by conclusions and contributions arising from the results achieved. The obtained results are supported and illustrated with 60 figures and 16 tables.

The topic of the PhD thesisis is relevant, as based on the climatic changes that have occurred in recent years, there is an increase in the aggressiveness of the sun's rays, as well as an increase in the number of potentially photosensitive substances that are increasingly included in pharmaceutical and cosmetic products. This topic is of increasing interest and gaining great importance, and it is directed towards finding *in vitro* approaches to evaluate the safety of newly synthesized compounds and natural products in order to avoid some side reactions in the human body. This is necessary because the biological targets of photosensitization are cell membranes, cytoplasmic organelles and the nucleus of the cell. For this reason, photosafety tests and trials

begin, which requires the used solar simulator to resemble natural sunlight as much as possible in terms of physical parameters.

In the "**Literature review**" part, Ms Sulikovska shows a very good knowledge of the issues related to light-induced biological effects - phototoxicity and photodynamic effect and the study of the antitumor activity of newly synthesized compounds and natural products under *in vitro* conditions. A good knowledge of the available literature allows to clearly mark the PhD thesis of the present research.

In the material submitted for review, the main **Goal** of the PhD thesis is formulated, which is the optimization and adaptation of methods for determining phototoxicity (*in vitro* 3T3 NRU phototoxicity test) to a LED - solar simulator (Helios-iO, model LE-9ND55-H - 5500K), for a faster and qualitative study of the phototoxicity of substances of synthetic and natural origin with potential antitumor activity. The search for new natural and synthetic substances with a high level of safe application in the field of antitumor therapy is the main line in the PhD thesis.

Tasks set for implementation are in a logical sequence and are directly related to the goal set. They include:

- measurement of the physical characteristics of the emitted light (light spectrum, power, homogeneity of illumination, etc.);

- optimization and adaptation of a protocol for determining phototoxicity (*in vitro* 3T3 NRU phototoxicity test) to the LED - solar simulator;

-determining the effectiveness of the LED - the solar simulator, when studying different types of compounds for phototoxicity;

-screening of the possibilities of using the described solar simulator in photosafety tests of substances of synthetic and natural origin with potential antitumor activity.

The "**Materials and methods**" part describes the methodology used, which includes determining the physical characteristics of the light emitted by the solar simulator used in the phototoxicity tests, optimizing the protocol for phototoxicity determination, and determining the antiproliferative effect in normal and tumor cell lines. The advantages of light and fluorescence microscopy were used. The safety evaluation and the use of normal and tumor cell lines to determine the antiproliferative effect of the investigated substances represents an important final phase of the experimental setup. The potential for the safe application and

effectiveness of substances with potential antitumor action was analyzed. The methods for evaluating the obtained results require a good command of approaches and methodology used in biochemical, biophysical and biomedical research. All this demonstrates the good laboratory preparation of Inna Sulikovska.

In the **"Results and discussion"** part, the specific research work reflected in the PhD thesis is presented, concerning the optimization of phototoxicity determination by *in vitro 3T3 NRU phototoxicity test*, to LED - solar simulator (Helios-iO, model LE-9ND55-H - 5500K). This is dictated by the fact that exposure of the skin to sunlight in combination with photoreactive xenobiotics can lead to an abnormal skin reaction – phototoxicity. Of particular importance is the section devoted to the rapid and qualitative study of the phototoxicity of substances of synthetic and natural origin, which are increasingly being imposed as an alternative, with potential antitumor activity. The analysis of the obtained results in the dissertation convinces us of the importance of the conducted research.

From the obtained results, the following **conclusions** were drawn:

1. The determined physical characteristics: light spectrum, power and power distribution density of the tested solar simulator (Helios-iO, model LE9ND55-H - 5500K) are similar to natural sunlight. Therefore, the described light source is suitable for performing phototoxicity tests.

2. The biological experiments carried out: *in vitro* phototoxicity test, light and fluorescence microscopy, clearly show the presence of a phototoxic effect in the cells treated with Acridine orange and Radachlorin. This is direct evidence that the tested solar simulator is effective and suitable for performing phototoxicity tests.

3. Phototoxicity tests on substances of natural and synthetic origin demonstrate their photosafety and the prospect of application in pharmaceutical and cosmetic products. It also shows the practical application of the validated light source.

4. The results obtained for the antiproliferative activity of the ethyl acetate extract isolated from *Cotinus coggygria* Scop. showed high selectivity (SI = 11.2) in the HeLa cell line, which is a prerequisite for the more detailed characterization and in-depth study of this extract as a potential antitumor agent.

5. The newly synthesized compounds, derivatives of thieno[2,3-d]pyrimidines 4, 5, show high selectivity SI = 19.3 and 15.8 in the MCF-7 cell line, respectively. This high selectivity is comparable to the selectivity of therapeutics routinely used in the treatment of tumor diseases.

Formulated conclusions outline the following original **contributions**:

1. LED - solar simulator Helios-iO, model LE-9ND55-H - 5500K, has been validated as a light source in an in vitro phototoxicity test (in vitro 3T3 NRU phototoxicity test). Defined are the physical characteristics of the solar simulator as well as the biological effects it induces upon irradiation of BALB 3T3 cell culture treated with phototoxic substances (acridine orange and chlorine e6).

2. The Helios-iO solar simulator has been successfully used in safety testing of natural products and synthetic compounds (4 plant extracts and 14 synthetic substances). No phototoxic effect was observed in any of the tested samples. This allows safe use of the tested substances in the field of cosmetics and pharmacy.

3. During the screening for antitumor activity, several substances with high selectivity were identified (CCA, Ethyl 4-Amino-5-methyl-2-(4-nitrobenzyl) Thieno [2,3-d] Pyrimidine-6-carboxylate and Ethyl 4 -Amino-2-benzyl-5-methyl-thieno[2,3-d]pyrimidine-6-carboxylate), which shows a potential for application in the therapy of oncological diseases with a low level of side effects.

The presented **abstract** represents a general characteristic of the PhD thesis and has been prepared according to all the rules for the preparation of such.

CONCLUSION

My dissertation work submitted for review contains scientific and scientifically-applied results representing significant scientific contributions.

The scientific research work of PhD student Inna Sulikovska is presented fully, clearly and it's substantiated in a PhD thesis, and the author has managed to publish an important part of the obtained results in scientific publications with a high impact among the scientific community – one publication with Q1 and an impact factor of 4.412 and the second - with Q3 and an impact factor of 0.198. Results of the experimental work were also presented at 4 scientific conferences. It can be seen that the PhD student fully fulfills the requirements for

receiving the educational and scientific degree " PhD " according to Law on the development of the academic staff in the Republic of Bulgaria and IEMPAM - BAS.

In connection with the above, I confidently give my positive opinion for awarding the educational and scientific degree " PhD " to Inna Sulikovska.

30.06.2022. г.

Prepared by:

/ Assoc. Prof. PhD Lyudmila Kabaivanova /