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Scientific review of the PhD thesis "*Protective Effects of Cannabidiol on Skin Keratinocytes in an Oxidative Microcellular Environment Induced by UVA/B Radiation or Exposure to Hydrogen Peroxide*" by Sinemyiz Atalay Ekiner.

One of the main driving forces of evolution is development of adaptation mechanisms in response to stressors. In the early biotic conditions, the Earth surface was exposed to wide range ultraviolet irradiation and that potentially destructive stressor had defined the chemistry of life we know: amino acids, nucleic acids, carbohydrates or phospholipids are building molecules with high UV photostability [Szabla R, Zdrowowicz M, Spisz P, Green NJ, Stadlbauer P, Kruse H, Šponer J, Rak *J.Nat Commun.* 2021;12:3018]. Soon after the life enriched the Earth atmosphere into free oxygen, the process reduced the amount of ultraviolet radiation reaching the ground; hence the UV stressor had been significantly extinguished in the early life era. Still however, UV and free oxygen exposure can impact potentially deleterious effects on biological organisms including humans. This might be potentially important in for example dermatology practice where UV and H₂O₂ agents can promote oxidative stress and inflammation of skin cells and there is a constant need for antioxidative cytoprotective compounds to protect skin cells from metabolic damage.

PhD dissertation by Sinemyiz Atalay Ekiner is an attempt to address the latter issue. Its main aim is to characterize the effect of cannabidiol (CBD), a non-psychoactive phytocannabinoid on keratinocyte metabolism under oxidative microenvironment conditions caused by exposure to H₂O₂ or UV radiation. CBD effects have been evaluated in relation to intracellular redox homeostasis and associated with cellular metabolism manifested by changes in phospholipid and protein profiles of cellular membrane. The scientific core of the thesis contains five publications devoted to that topic, they all combine into excellently designed and described story tackling the problem. The first publication "*Antioxidative and Anti-inflammatory Properties of Cannabidiol*" by Sinemyiz Atalay, Iwona Jaročka – Karpowicz and Elżbieta Skrzydlewska (P1) is a review article describing chemistry of CBD and its main derivatives, biological effects of CBD as well as its therapeutic potential evidenced in clinical and preclinical studies; particular attention has been paid to details of cellular, antioxidant, and antiinflammatory properties of CBD and its derivatives. The next article entitled "*Cannabidiol protects keratinocyte cell membranes following exposure to UVB and hydrogen peroxide*" by S. Atalay, I. Dobrzyńska, A. Gęgotek, E. Skrzydlewska (P2)



describes (using a set of bioanalytical LC-MS methods) that CBD is able to penetrate keratinocytes and accumulates in the cellular membrane, it reduces the shift of redox balance induced by UV or H₂O₂ estimated by analysis of lipidome (levels of polyunsaturated fatty acids, sialic acid and other peroxidation lipid products) what affects cell membrane integrity. In addition, CBD prevented UVB/ H₂O₂ induced reduction of keratinocyte size, zeta potential and activity of ABC transporters. The main findings of the next article "*Protective effects of cannabidiol on the membrane proteins of skin keratinocytes exposed to hydrogen peroxide via participation in the proteostasis network*" by Sinemyiz Atalay, Agnieszka Gęgotek, Pedro Domingues, Elżbieta Skrzydlewska (**P3**) are the changes in membrane proteome profile induced by H₂O₂ and how they can be reversed by CBD. Analysis of protein – protein interaction networks has shown that significant changes mainly involved proteins with important roles in proteasome activity, protein folding processes, protein biosynthesis and regulation of protein balance cell survival. The authors concluded that CBD combination could bring promising approach in the clinical use of H₂O₂ by preventing its pro-oxidative and pro-inflammatory effects through participation of CBD in membrane mediated molecular signaling. "*Protective Effects of Cannabidiol on the Membrane Proteome of UV-Irradiated Keratinocytes*" by Sinemyiz Atalay, Agnieszka Gęgotek and Elżbieta Skrzydlewska (**P4**) is a publication where short term and long term CBD application effects on proteomic profile of biological membranes in UVB irradiated keratinocytes were compared. The authors characterized UVB induced modifications of membrane proteins, in particular adducts with lipid peroxidation products which may alter protein folding, function and even modulation of various intra- and intercellular pathways. CBD exposure significantly reduced levels of those protein adducts, that was particularly notable among proteins related to cell proliferation and apoptosis. Moreover, CBD significantly increased UVB induced proliferation of proteins involved in protein translation and proliferation, inflammatory response and maintenance of redox balance. The long term (48 h) application of CBD reduced the severity of antioxidative response and suggests proapoptotic effects. The authors concluded the phytocannabinoid triggered cellular protective mechanisms by degradation of modified proteins or apoptosis of pathologically altered cells. The last article titled "*Therapeutic application of cannabidiol on UVA and UVB irradiated rat skin*" (**P5**) was published in JPBA by Sinemyiz Atalay, Agnieszka Gęgotek, Artur Wroński, Pedro Domingues and Elżbieta Skrzydlewska. The aim of that study was evaluation how CBD affected the metabolism of skin keratinocyte *in vivo* in nude rats exposed to UVA and UVB radiation. The results obtained with SDS-PAGE/nanoHPLC/QexactiveOrbiTrap show that exposure of rat skin to ultraviolet as well as CBD action significantly modified expression of proteins involved in inflammation redox balance and apoptosis. The article enlists an array of proteins with modified expression levels (the authors suggest Nrf2, superoxide dismutase as the most important) while cannabidiol apparently reversed most of those changes. The authors concluded that CBD maintained keratinocyte proteostasis and could be suggested as agent preventing UV induced metabolic changes in epidermal keratinocytes.



Therefore, presented collection of five articles comprises a systematic study of biochemical signatures; proteomic and lipidomic changes induced by ultraviolet irradiation or hydrogen peroxide on skin keratinocytes and specify how CBD co-administration may counter affect these changes. The research is an excellent example of well-designed and well documented study, which comprehensively addresses the topic employing whole array of bioanalytical methods, among others electron spin resonance spectroscopy, nano-HPLC QorbiTrap for LC-MS. All five articles have been published in peer-reviewed scientific journals well recognized in the field: *Antioxidants (Basel)* – **P1** (2019) and **P4** (2021), *Redox Biology* – **P2** (2020) and **P3** (2021) and the *Journal of Pharmaceutical and Biomedical Analysis* – **P5** (2021). It is worth noting that **P3**, **P4** and **P5** have been fortified with tabularized supplementary information, where very detailed lists of specific proteins and other markers altered by applied stressors or CBD application have been provided. Sinemyiz Atalay Ekiner is the first author of all five publications leaving no doubt on the leading role of the PhD candidate in design of each study, execution of key experiments, interpretation of the results and manuscript writing; all co-authors underlined that in their declarations accompanying each of the publications. The **P5** article is additionally followed by the Local Ethics Committee decision on nude rat experiments necessary for *in vivo* study of keratinocytes with UV light. According to the declaration the PhD candidate was not involved directly in those experiments.

The editorial layer of the PhD thesis needs additional attention. The author provides very illustrative commentary to presented set of articles with very clear introduction to the key cellular processes triggered by oxidative stressors, the definition of the aim and the outline of the whole thesis, followed by summary discussion concluding main findings of the whole story. It testifies to deep perception of complexity of the research problem and understanding of the perspective wider than presented in individual publications. Altogether, the thesis by Sinemyiz Atalay Ekiner belongs to one of the best I had a chance to review; it stands as very interesting and novel piece of science that is based on solid set of research publications. All articles underwent peer review procedures adhering to high standards of publishing journals; I found it hard to identify an important and noteworthy weakness.

In conclusion, I give my unequivocally positive assessment of scientific value and research novelty of doctoral thesis by Sinemyiz Atalay Ekiner. It meets the highest standards of biomedical research as well as proves the candidate's excellent knowledge in the field of the molecular toxicology and modern bioanalytics. In the opinion of the reviewer, the PhD thesis entitled "***Protective Effects of Cannabidiol on Skin Keratinocytes in an Oxidative Microcellular Environment Induced by UVA/B Radiation or Exposure to Hydrogen Peroxide***" is clearly presented, properly designed and documented, it addresses important and timely issues of biochemical consequences of CBD treatment on keratinocytes signatures after exposure to the most common oxidative stressors. Thus, it meets the substantive and formal requirements defined for doctoral dissertations by relevant legal acts



as well as customary standards of international community in the field of pharmaceutical sciences. Therefore, I am applying to the Senate of the Medical University of Białystok for admission of Sinemyiz Atalay Ekiner to the further stages of the doctoral dissertation.

In addition, taking into account high scientific value and novelty of the results and the fact that entire research has been previously published in recognized peer-review journals, I request for distinction of the thesis, if that adheres with regulations of the Medical University of Białystok.

Sincerely,

Krzysztof Józwiak, PhD DSc.