

**CHARLES UNIVERSITY  
FACULTY OF PHARMACY IN HRADEC KRALOVE**

Department of Pharmacology and Toxicology

Study program: Pharmacy

**Opinion of the Opponent of the Diploma Thesis**

Year of the defense: 2023

Student: **Eliška Sáňková**  
Thesis Tutor: Prof. PharmDr. František Štaud, Ph.D.  
Consultant: Dr. Ramon Portillo, Ph.D.  
Opponent: PharmDr. Ivan Vokřál, Ph.D.  
Thesis title: **Use of cannabis in pregnancy; effect on placental functions**

Scope of work, number of 47 pages, 17 figures, 0 tables, 119 citations

**Evaluation of the work:**

- |   |           |
|---|-----------|
| a) Processing of the theoretical part:                                  | Excellent |
| b) The complexity of the methods used:                                  | Excellent |
| c) Preparation of the methodological part (clarity, comprehensibility): | Very good |
| d) The quality of the experimental data obtained:                       | Excellent |
| e) Processing of results (clarity):                                     | Very good |
| f) Evaluation of results, including statistical analysis:               | Excellent |
| g) Discussion of results:   | Excellent |
| h) Clarity, conciseness, and adequacy of conclusions:                   | Excellent |
| i) Meeting the objectives of the work:                                  | Excellent |
| j) Quantity and up to date of references:                               | Excellent |
| k) Language level (stylistic and grammatical level):                    | Excellent |
| l) Formal level of the work (text structure, graphic design):           | Very good |

I recommend the thesis for recognition as a rigorous thesis

**Comments on the evaluation:**

The diploma thesis of Eliska Sankova deals with the topic of selected cannabinoids (CBD, THC) and their effect on placental response to inflammation. The thesis itself is well-written without grammatical or stylistic mistakes, and the theoretical part, discussion and conclusion are very easy to read. The presented results are very interesting and bring new important information to the topic. On the other hand, in some parts, the thesis is very brief, and I miss some important information (physiology, CB receptors, and experimental workflow details). Please see my comments and questions below.

**Questions and comments to student:**

**Comments:**

The thesis title is a little bit misleading and could be more specific.

The list of abbreviations is incomplete, and some abbreviations used in the thesis are missing.

On page 9 is written that marijuana varieties with up to 55.7 % exist. Based on the cited literature, I think this is a mistake, as the value of 55.7 % THC is connected with hash oil.

P 14 - I miss information about the CB receptors and their localisation in the human body with a focus on the placenta.

As there is a completely missing chapter about the used chemicals and reagents, their basic specification should be given in the text. E.g. several types of LPS exist, but there is no specification given. There is also no description of how the standard solutions and control samples were prepared.

Although figures 6 and 7 are just examples of the MTT methodology and LDH assay, the student could change them to be more connected to the currently used experimental workflow. Also, Figure 7 contains a reference to the detailed reaction in Figure 4, but Figure 4 doesn't contain any information about the Red Formazan production.

P - 24 - The LDH assay workflow should be better described.

P - 27 - In the description of the q-PCR workflow, I miss information about the TaqMan assays that were used for the experiments. Until the Results part, it is not known what genes were studied during the experimental part.

P - 27 - The Heatmapper and generation of the heat map are mentioned in the methodology, but the thesis doesn't contain any heatmap, which is confusing.

P - 28 - Even though the cytokine quantification is routinely processed in the laboratory and was published in a recent article, some brief workflow and list of antibodies should be given.

P - 31 - In all presented graphs on page 31, there is a significant difference between the Ctrl+ and Ctrl groups. Unfortunately, I wasn't able to find what the Ctrl+ description means.

P - 33 - Gene expression was normalized to the four housekeeping genes EIF4A2, TBP, TOP 1, and UBC. But in chapter 6.5, five housekeeping genes are mentioned.

P - 33 - Gene names in the aFigure 14 description should be italicized.

P - 38 - Figure 17 doesn't have the same style as previous graphs.

Questions:

From the method description, it is unclear why formazan production is measured using two wavelengths. Even Figure 6 is not helpful as it doesn't mention the 690 nm wavelength. Could you please briefly explain the process of formazan production measurement?

Have you also considered evaluating the effect of CBD and THC on the expression of pro-inflammatory cytokines without the LPS pretreatment? I miss these samples in your results part.

Is there any evidence in the literature that endocannabinoids have any role in pregnancy?

I miss the information about the cannabinoid receptors in the placenta and their physiological role. Could you please present this information?

Do you think that it would have any value to study CB1 and CB2 expression in your samples during the experiments?

**Evaluation of the thesis: Excellent**

**For the  
defense:**

**Recommend**

In Hradec Králové

14. září 2023

signature of the opponent