REVIEW

by Assoc. Prof. Maria Kazakova, PhD Department of Medical Biology, Medical University - Plovdiv

on the dissertation work for awarding educational and scientific degree "Doctor" professional field 4.3. "Biological sciences", specialty "Medical biology"

Doctoral candidate: Tsonka Slavova Dimitrova Department of Biology, Faculty of Farmacy, Medical University "Prof. Paraskev Stoyanov" - Varna

Topic: "Investigations and screening methods for early diagnosis of patients with gastrointestinal disorders due to mushroom consumption"

Scientific Supervisors: Prof. Dobri Ivanov, PhD and Prof. Petko Marinov, MD, PhD

By decision of the Chairman of the Scientific Jury (protocol $N^2/10.08.2021$) and by order of the Rector of Medical University – Varna ($N^2/10.08.2021$) I have been appointed to present review on the dissertation work: "Investigations and screening methods for early diagnosis of patients with gastrointestinal disorders because of mushroom consumption" for awarding educational and scientific degree "Doctor" in specialty of "Medical biology"

Brief biographical and professional data

Tsonka Dimitrova was born in 1972. She graduated with a master's degree in biology, Plovdiv University "P. Hilendarski". Dimitrova has been appointed as an Assistant Professor in the Department of Biology, Medical University – Varna. She is responsible for practical and lecture course for students in medicine, dental medicine, and pharmacy. She has been enrolled in a doctoral program in the same department on 01.08.2019. She acquired the specialty "Medical Biology" in 2016.

General characteristics of the dissertation work

The dissertation presented by Tsonka Dimitrova covers 146 pages. It includes 21 tables, 71 figures and one appendix. The reference list consists of 219 items of which 13 in Cyrillic and 206 in Latin. The work has been structured in accordance with the dissertation writing rules. The main sections are presented as follows: Introduction - 1 page; Literature review - 39 pages; Objective, tasks and working hypothesis - 1 page; Material and methods - 8 pages; Own results - 40 pages; Discussion - 23 pages; Concluding remarks and Conclusions - 2 pages; References - 19 pages; Contributions - 1 page; List of publications related to the dissertation work -1 page, and Appendix - 3 pages.

The doctoral student is well acquainted with the topic, which is evident from informative and well-written literature review. It presents information about the morphology, classification, and distribution of the wild and toxic mushrooms in Bulgaria and worldwide. The main toxic substances such as amatoxins, phallotoxins, muscarine, orellanine, gyromitrin etc. are described in detail. Data about morbidity and mortality rates are presented. The clinical peculiarities of mushroom poisonings are systematized.

The literature review concludes with a reasoned based section: "A critical estimation of the literature". The reasons to conduct such a study are presented.

The aim of the dissertation is to analyze the intoxications with wild mushrooms among the population in the North Black Sea coast of Bulgaria, to approve easily feasible methods for their diagnosis as well as to investigate the degree of awareness of these mushrooms and their usage as a basis for prevention. To achieve this goal, the doctoral student has formulated five tasks that can be summarized as:

• a retrospective analysis of the documentation about the wild mushroom poisonings of the patients who have been hospitalized in the city of Varna between 1991 and 2015.

- an application of Meixner's test in the patients with gastrointestinal symptoms suspected of mushroom poisoning.
- an application of ELISA for the presence of amatoxins in the patients following toxic wild mushroom consumption.
- a performance of an inquiry investigation of the degree of awareness of the population about the wild mushrooms on the territory of the Northern Black Sea coast of Bulgaria as well as of their usage by the population in this region.

The formulated tasks are precisely formulated and sufficient for the implementation of the defined purpose.

The section of Material and methods is clearly written. The dissertation analyzed retrospectively for a 24-year period (1991 – 2015) 147 patients hospitalized in the Clinic for Intensive Treatment of Acute Intoxications and Toxic Allergies at Naval Hospital of Varna, Military Medical Academy.

During the period between May and July 2021, 21 patients, 15 males and six females, are admitted to hospital with gastrointestinal complaints associated with wild mushroom poisoning. The presence of amatoxins was determined by Meixner's test (in stomach content) and ELISA (urine). The statistical methods for data processing include descriptive, variation and correlation analysis.

The section of ,Own results' is comprehensively presented. In the Discussion section, the doctoral student analyzes the obtained own results, discusses them with those of other groups.

Critical remarks and questions: I have no critical remarks concerning the essence of the dissertation thesis except for some of the included figures (Fig. 4.2.3.1; 5.3.1; 5.2.2 etc.) which are, in fact, photographic pictures. Their labeling as microphotographs could be more precise. In the section of 'Methods', the description of ELISA technique is shown in excessive details, and it is presented as a particular laboratory protocol.

I have two questions to the doctoral student:

- Whether and how she imagines an idea for the continuation of this scientific work?
- Has she any proposals (algorithm) concerning the education/information acquisition of the population about undesired mushroom poisonings?

The six conclusions made fit well to the defined tasks and goal. Six contributions are presented of which three are of original and three are of confirmatory nature.

Part of the results are published in five articles, two of which are *in press*. The doctoral student is the first author in two of the articles. I think that the doctoral candidate should promote the results of her research by preparing and publishing scientific articles in journals with Impact factor

The dissertation abstract reflects the most essential aspects of the dissertation work. It is illustrated with enough figures and tables.

My personal impressions of Dimitrova relate to the National conferences in Medical Biology. She has demonstrated an engagement and competent participation. The scientific work presented here is interesting. Doctoral student's skill to plan and perform experimental work, to generalize and present scientific information becomes evident.

CONCLUSION

In conclusion, I consider that the dissertation work of Tsonka Dimitrova comply with the requirements of the Academic staff development Act of the Republic of Bulgaria, and the Regulations for Academic development at MU-Varna. I give a *positive assessment* and *propose* to the Scientific jury to award the educational-scientific degree "Doctor" in Medical Biology to Tsonka Slavova Dimitrova.

15.09.2021

Reviewer:

Assoc. Prof. Maria Kazakova, PhD