

REVIEW

In connection with a dissertation for awarding the educational and scientific degree "DOCTOR", field of higher education 4. Natural sciences, mathematics and informatics, professional field 4.3. Biological sciences on the topic:

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

Author of the dissertation: Tsonka Slavova Dimitrova, full-time PhD doctoral student, doctoral program "Medical Biology", Department of Biology, Faculty of Pharmacy, MU "Prof. Dr. Paraskev Stoyanov" Varna

Supervisors: Prof. Biol. Dobri Lazarov Ivanov, D. B. and Prof. Dr. Petko Penkov Marinov, Ph.D.

Member of the Scientific Jury, who prepared the review - Prof. Dr. Snezha Zlateva Zlateva, MD - Department of Pharmacology, Toxicology and Pharmacotherapy, Faculty of Pharmacy, Medical University "Prof. Dr. Paraskev Stoyanov" Varna

The review was prepared in accordance with the Academic Staff Development Act (ASDA), the Regulations for the Application of the Academic Staff (RAAS) and the Regulations on the Terms and Conditions for Acquisition of Scientific Degrees and Occupation of Academic Positions (RTCASDOAP) at MU-Varna. The scientific jury for the public defense of the dissertation was determined by an order of the Rector of the Medical University - Varna № P-109-334 / 6.08.21. d. The presented set of materials on paper / electronic media is in accordance with the procedure for acquisition of Educational and Scientific Degree "Doctor" and the regulations of MU - Varna.

Tsonka Slavova Dimitrova was born on October 19, 1972 in the town of Sliven. Higher education, Master's degree - biologist, specialization in plant biotechnology acquired at Plovdiv University "Paisii Hilendarski", in 2000. He acquired the specialty of Medical Biology at the Medical University "Prof. Dr. Paraskev Stoyanov" - Varna in 2016.

He has been working as an assistant in the Department of Biology at MU - Varna since 2010, leading seminars and lectures with students of medicine, dentistry and pharmacy. Fluent in English. Tsonka Slavova Dimitrova has completed in time all the tasks and activities set in the individual curriculum. She has successfully passed the doctoral minimum exam.

Structure of the dissertation: The dissertation of biologist Tsonka Slavova Dimitrova is written on 146 standard pages, of which:

1. Title page - 1
2. Contents - 2
3. Acronyms - 1
4. Introduction - 1
5. Literary review - 40

6. Purpose, tasks, methodology - 9
7. Own results - 41
8. Discussion - 25
9. Conclusions - 1
10. Literature - 19
11. Yields -1
12. List of publications and scientific participations related to the dissertation-1
13. Appendices - 3

The ratio overview: methodical: result-disassembly part is optimal, respectively 28: 6: 50%. The dissertation is illustrated with 21 tables, 71 figures and one appendix. The list of cited literature includes 219 titles, of which 13 in Cyrillic and 206 in Latin. All the cited titles are directly related to the studied problem.

On the topic of the dissertation the candidate has published five works, three of which were published in the journals „Aviation, Marine and Space Medicine“, 2017; „Scripta Scientifica Pharmaceutica“, 2018; „J IMAB“, 2018; and two more publications accepted for publication in a referenced English language journal „J IMAB“, 2021.

Characteristic features of the work: The topic of the dissertation "**Studies and screening methods for early diagnosis of patients with gastrointestinal disorders due to mushroom consumption**" is well chosen in terms of significance of the problem, current relevance and projection into the future. In our country there is a lack of systematic research on the social epidemiology and early diagnosis of poisoning with wild mushrooms, as well as on the level of knowledge of different groups of the population about them and the use of these mushrooms in everyday practice. This determines the need for the current work. Additionally, this study shows the need to build a multidisciplinary approach to diagnosis, monitoring, treatment and follow-up of patients, which is a modern approach to health care and the integration of professional knowledge in the family and social environment.

The literature review is 40 pages long. The classification and morphology of wild mushrooms, their distribution in the world and in Bulgaria are successively covered. A general description of poisonous mushrooms and their distribution in the world and in Bulgaria has been made. The various toxic substances contained in poisonous mushrooms are considered, and the most dangerous toxins such as amatoxins, phallotoxins, muscarine, orellanin and gyromitrin are more widely represented. The main representatives of poisonous mushrooms in Bulgaria, the morbidity and mortality caused by them are considered. Classifications of poisonings with wild mushrooms have been made, the clinical features of the poisonings have been considered, emphasizing the gastrointestinal disorders, liver and kidney failure, their accompanying cardiovascular, neuropsychiatric and muscular disorders. Modern laboratory diagnostics of poisoning with wild mushrooms such as routine enzyme tests and new hardware tests are presented. The modern treatment and prevention of poisoning with wild mushrooms is considered.

The aim of the study is formulated accurately and clearly and includes analysis of poisoning with wild mushrooms among the population on the North Black Sea coast of Bulgaria, testing of easily feasible methods for their diagnosis and study the awareness of these mushrooms and their use as a basis for prevention.

To achieve this goal, the dissertation Tsonka Dimitrova has defined **5 tasks**: 1 / conducting a retrospective analysis of the documentation for poisoning with wild mushrooms in patients hospitalized in Varna during the period between 1991 and 2015; 2 / application of the Meixner test in patients with gastrointestinal symptoms suspected of fungal poisoning; 3 / application of enzyme-linked immunosorbent assay ELISA for the presence of amatoxins in patients after consumption of poisonous wild mushrooms; 4 / conducting a survey on the level of awareness of the population about wild mushrooms on the territory of the Northern Black Sea coast of Bulgaria; 5 / conducting a survey on the culinary use of wild mushrooms by the population on the territory of the Northern Black Sea coast. The formation of the sequence of tasks is an example of structuring research - from epidemiological research on the frequency of pathology, through the application of new diagnostic methods to ethno-epidemiological studies among the population aimed at preventing the problem.

The material used includes a clinical part that is both retrospective and prospective. The retrospective documentary analysis included 147 patients with *Amanita phalloides* hospitalized in the Clinic for Intensive Care of Acute Poisoning and Toxicallergies of the Military Medical Academy-MHAT-Varna between 1991 and 2015. The prospective clinical analysis included 21 hospitalized patients with gastrointestinal complaints, related to poisoning with wild mushrooms, which were tested by the methods tested by the dissertation. The survey covered a total of 200 people - 100 men and 100 women living in several towns and villages in Varna District. They are selected at random. The interviews were conducted by specially trained students.

The dissertation applies several **methods** in its research: documentary analysis; Meixner test with concentrated hydrochloric acid; enzyme-linked immunosorbent assay (ELISA), a competitive immunoassay with the polyclonal antibody specific for alpha and gamma amanitin; survey method for studying two aspects - the level of awareness of the population on edible wild mushrooms and ways of their culinary use, as well as statistical methods.

The results of the study are demonstrated in five areas: 1 / Retrospective analysis of hospitalized in the Clinic for Intensive Treatment of Acute Poisoning and Toxic Allergies of MMA-MHAT-Varna with wild mushrooms *A. phalloides* during the period between 1991 and 2015, which is well illustrated graphically and tabularly with respect to *A. phalloides* intoxications in men, women and all patients, showing the proportion of age groups; the distribution of cases by gender; different annual dynamics; the most frequent months during the entire observation period. 2. The results of the study for amatoxins with the Meixner test in hospitalized patients are differentiated in tabular and graphical form, with patients divided by sex and age; professional status; marital status and place of residence. 3. The results of the ELISA test are presented graphically; 4. The results of the survey on awareness of wild mushrooms are presented graphically and are divided into two large groups, those that recognize and those that do not recognize wild mushrooms. Each of these groups is characterized by gender and age; education and residence; Respondents are divided by the number of wild mushrooms they recognize, from one to 13; 13 of the most popular wild

mushrooms with their popular and botanical names are tabulated; Respondents are distributed by gender according to their attitude to the need to acquire more knowledge about wild mushrooms, as well as the ways in which they prefer to be informed / relatives, books, internet, parents, relatives and acquaintances /; Respondents are divided by gender in terms of their self-esteem as connoisseurs of wild mushrooms, their trust in people who prepare wild mushrooms as food; their knowledge as an endangered species in the environment; their knowledge of difficult-to-detect rare mushrooms; their knowledge of cases of poisoning with wild mushrooms; their knowledge of training programs for wild mushrooms; their encounter with poisonous species of mushrooms; their knowledge of the symptoms of mushroom poisoning; their knowledge of the existence of the method called "Mycotherapy"; the distribution of the respondents by place of residence and education according to their attitude to the need to acquire new knowledge about wild mushrooms. The results of a survey on the culinary use of wild mushrooms are presented tabularly and graphically and cover the relationship between the different ways of culinary preparation of wild mushrooms by sex, age, level of education and place of residence; The attitude to wild mushrooms as a culinary food has been studied according to the frequency of annual consumption from several times to none, preference for their preparation by cooking, drying, canning or freezing; the collection of mushrooms for sale and delivery to mushroom points; distribution of respondents to those who buy from mushroom outlets; distribution according to the preferred season / spring, summer, autumn / for their collection; distribution, according to whether they use mushrooms as a remedy;

The discussion compares the results of the above five areas with similar epidemiological and ethno-anthropomorphic studies in different parts of the world with emphasis on cultural, economic and environmental differences.

The conclusion corresponds to the obtained results, and the conclusions made with the set goal and tasks.

The contributions of the dissertation are of emphasized scientific and applied nature, and the results of the research are well formulated. The proposal for the application in clinical practice of the easy-to-perform and inexpensive Meixner test for the detection of amatoxins in gastric contents and collected mushrooms, as well as the organization of a procedure for routine application of the ELISA immunoenzyme method for the detection of amatoxins in urine is original. The socio-epidemiological analysis is original and sheds light on the peculiarities in the information and culture of consumption of wild mushrooms by the population on the Northeast Black Sea coast. -good training of students and doctors of different specialties.

As a remark I would note some violations with the structure of the dissertation such as inserting some of the results in the methods section, repeating some of the presented results in the discussion, lack of description of the treatment methods used in the clinical part and omission in the discussion of some leading Bulgarian toxicological research, for example Aneta Hubenova, worked in the field of poisoning with *Amanita phalloides* in Bulgaria.

I appreciate the development of Tsonka Dimitrova in several areas:

- An overview of the biological classification and characterization of wild and poisonous mushrooms, as well as their distribution in Bulgaria, review of toxins causing various lesions, which is valuable from both biological and toxicological points of view. This part of the

dissertation can be used for training of both biologists and medical students and doctors for postgraduate training.

- Our own research confirms, clarifies, supplements and enriches the knowledge about mushrooms toxins, symptoms and mortality in acute poisoning with *Amanita phalloides*.
- An extensive report on the existing methods for early and reliable diagnosis of mushroom poisoning is presented, two of which the dissertation approves for routine use, which is important for toxicological practice, early diagnosis, adequate treatment and reduction of mortality.
- The socio-ethnomicological study is original and shows the need for a different approach to different population groups, according to age, sex and education in terms of presenting knowledge about wild mushrooms in general and poisonous in particular.
- I would recommend continuing the work of biologist Tsonka Dimitrova in the direction of other poisonous mushrooms in Bulgaria, outside *Amanita phalloides*, expanding socio-ethnomicological research in other areas of Bulgaria, because mushrooms in nature for centuries have served as human food, and scientific knowledge about edible, poisonous, toxins, their diagnosis and treatment will always be up to date.

Conclusion: In conclusion, the dissertation presented by biologist Tsonka Slavova Dimitrova contains scientific, scientific-applied and applied results, which are a contribution to science and meet the requirements for awarding educational and scientific degree "DOCTOR". The dissertation shows that the doctoral student has in-depth theoretical knowledge and professional skills in the scientific specialty, demonstrating qualities and skills for independent conduct and discussion of research.

On these grounds, I propose to the esteemed members of the Scientific Jury to vote positively and propose to the Rector of MU - Varna to award the scientific and educational degree "Doctor" in the field of higher education 4. Natural Sciences, Mathematics and Informatics, professional field 4.3. Biological sciences and scientific specialty Medical biology.

Medical University "" Professor Dr. Paraskev Stoyanov "- Varna

Department of Pharmacology, Toxicology and Pharmacotherapy

Prof. Dr. Snezha Zlateva, MD



17.09.2021

Varna