TO
Assoc. Prof. Silvia Gancheva, MD, PhD
Chairman of the Scientific Jury
under the procedure for the acquisition of ESD "Doctor"
PhD program "Pharmacology (incl. pharmacokinetics and chemotherapy)"

REVIEW

by Prof. Galya Tsvetanova Stavreva-Marinova, MD, PhD
Department of Pharmacology and Toxicology
Medical University – Pleven
e-mail: drstavreva@yahoo.com

According to the procedure for awarding the **educational and scientific degree "Doctor"** in
Field of higher education: 7. "Health and sports"
Professional direction: 7.1. "Medicine"
PhD program: Pharmacology (incl. pharmacokinetics and chemotherapy)
Author: Dr. Klementina Moncheva Moneva-Marinova
Form of doctoral studies: full-time doctoral student
Dissertation title: "Pharmacological investigation of the effects of *Chaenomeles maulei* fruit juice in an experimental model of metabolic syndrome"
Research supervisor: Prof. Stefka Valcheva-Kuzmanova, MD, PhD, DSc

I present the review in my capacity as a member of the Scientific Jury, determined by the order of the Rector of the University of Varna (No. R-109-46/14.02.2024) and the decision of the 1st absent meeting of the Scientific Jury held on 23.02.2024. I declare that I have no conflict of interest, including co-authorship with the PhD student Dr. Klementina Moncheva Moneva-Marinova.

General presentation of the procedure

The presented set of materials on an electronic medium is in accordance with the requirements of Art. 69 of the Regulations for the Development of the Academic Staff at the Medical University - Varna. On the basis of the set of materials and documents, I declare that the rules and conditions for the defense of a dissertation work for the acquisition of the ESD "Doctor" have been complied with, in accordance with the Law on the Development of the Academic Staff in the Republic of Bulgaria, the Rules for its application and the Regulations for the Development of the Academic Staff at the Medical University – Varna.

Education and qualification

The dissertation candidate Dr. Clementina Moneva-Marinova is a Master of Medicine (diploma No. 005905/01.11.2018 from Medical University – Varna). From October 2018, she started working as a part-time assistant at the Department of "Pharmacology and Clinical Pharmacology and Therapy", and from April 2019, she is currently a full-time assistant. and from April 2019. By Order of the Rector of MU-Varna (No. P-109-64 of 31.01.2020) Dr. Moneva enrolled as a doctoral student in full-time study and the topic of the dissertation work "Pharmacological study of the effects of *Chaenomeles maulei* fruit juice in an experimental model of metabolic syndrome" and scientific supervisor Prof. Dr. Stefka Valcheva-Kuzmanova. The dissertation work was discussed at a Departmental Council of the Department of Pharmacology and Clinical Pharmacology and Therapy, held on 01.29.2024. and was referred for public defense. Dr. Moneva was dismissed with the right of defense by order of the Rector of MU-Varna No. R-109-46/14.02.2024.

She speaks English and German, has the skills to work with a computer and analytical equipment. Dr. Moneva presents a list of 9 full-text publications that have been cited 20 times and 15 participations in scientific forums.

The dissertation is written on 123 pages, illustrated with 30 figures and 13 tables. All the main elements of the generally accepted in our country structure for presenting a dissertation work are included: introduction – 2 pages; literature review – 30 pages; purpose and tasks - 2 pages; materials and methods – 8 pages; results and discussions – 51 pages; summary discussion – 4 pages, conclusions – 2 pages; contributions - 2 pages; list of publications and participation in scientific events; literature - 15 pages. The bibliography is extremely up-to-date, contains 187 titles in

Latin, of which 124 (66%) were published from 2019 to 2023. It is written in a clear, highly scientific and elegant language, which shows the excellent preparation of the dissertation student. An impression is made by the good technical execution of the dissertation work and the precision in referring to the used literary sources.

Relevance of the topic and appropriateness of the set goals and tasks

Metabolic syndrome (MS) is an increasingly common pathology: one in three adults have MS. Knowing the risk factors and following a healthy lifestyle can prevent or reduce the likelihood of its development and related complications. Nutrition is a modifiable factor, of particular importance in the course of chronic diseases. The consumption of fruit juices in healthy eating recommendations is a current and controversial issue. The intake of functional foods provokes great interest among the public due to its suitability and accessibility. *Chaenomeles maulei* fruit juice (CMFJ), which is the subject of Dr. Moneva's research, can also be considered as a functional food. The interest in this plant is fully justified, due to its potential positive effects on human health, its climate resistance and the ability to grow in different climatic conditions, which also makes it economically valuable.

The fruits of *Chaenomeles maulei* are rich in biologically active substances, such as polyphenolic compounds, organic acids, terpenoids, alcohols, pectins, carotenoids, ketones or aldehydes, high concentration of vitamin C. Parts of plants of the genus *Chaenomeles* have great potential for application as various medicines and/or nutritional supplements. For centuries, they have been used to prepare various food products and to treat anemia, rheumatism, gout and cardiovascular diseases. A hepatoprotective effect, anti-inflammatory properties, antioxidant action, antimicrobial and neuroprotective effect, as well as a beneficial effect on the intestinal microbiome have been proven. The fruits of the Japanese quince are characterized by a high content of potassium and a low content of sodium, which is an advantage for patients with cardiovascular diseases.

Based on the long-term experience of the department's team with *Chaenomeles maulei* and the data from the scientific literature, it is logical to hypothesize that the application of fruit juice from the plant will improve the disorders in the lipid profile, glucose metabolism, body weight and behavioral changes in rats with an experimental model of diet-induced MS.

The formulated objective "Pharmacological study of the effects of *Chaenomeles maulei* fruit juice in an experimental diet-induced model of metabolic syndrome in experimental animals" is adequate and logical, considering the known effects of plants of the genus *Chaenomeles*, as well as the established activities of polyphenols that are a major component in their composition.

To achieve the goal, two tasks are defined - the first with three subtasks, and the second with five. They are adequate to investigate and generalize the pharmacological effects of *Chaenomeles maulei* fruit juice in rats with an experimental model of MS.

Research methodology

The design of the conducted study corresponds to this research format. The set of experimental methods makes a good impression, they are described briefly and precisely. 50 mature male rats divided into five groups were used. MS was induced by a high-fat, high-fructose 10-week diet providing 405 kcal/100 g of food and 40 kcal/100 ml of fructose syrup. Biochemical metabolic indicators (triglycerides, total cholesterol, blood glucose), markers of oxidative stress and lipid peroxidation (superoxide dismutase, thiobarbituric acid-reactive substances), adipose tissue indices, TyG index, liver index were used. Histological examinations of liver, heart and visceral adipose tissue preparations were performed. A number of behavioral methods have been applied - to study motor activity (open field test), to study anxiety (elevated cross maze test, social interaction study), to assess spatial memory (for location of objects) and to assess depressive behavior (forced swim test). Methods for statistical processing and analysis of survey data are adequate. The selected, presented and applied methodical approaches allowed Dr. Moneva to fulfill the tasks of the research and achieve the set goal.

The presented results of the dissertation show that the study was not only well planned, but also successfully executed. The research data are described briefly and clearly, well illustrated with 13 tables and 22 figures. From the obtained results and the discussion presented after the results of each task, it can be concluded that the dissertation student knows the methods used, skillfully and comprehensibly presents the obtained data. The discussion of the results by tasks introduces some disproportionality into the overall structure of the thesis, but makes the results easier

to understand. The discussion is done in a good scientific style, calmly handling the data of other authors and comparing them with one's own results.

Formulated conclusions correspond to the set and completed tasks. Oral administration of CMFJ in three different doses (2.5 ml/kg, 5.0 ml/kg and 10 ml/kg) in rats during the induction of metabolic syndrome prevented a large part of the morphometric, biochemical, histopathological and behavioral disorders:

- Lowered the total, mesenterial and paranephral adipose tissue indices at a dose of 5 ml/kg.
- Provoked a weak tendency in a direction of lowering the triglycerides and total cholesterol levels.
- Did not influence glucose tolerance.
- Provoked a weak tendency in a direction of lowering the TyG index and insulin resistance, respectively.
- Improved endogenous antioxidant defense by opposing the high-fat high-fructose diet-induced decrease in serum superoxide dismutase levels, most pronounced at doses 2.5 and 5 ml/kg.
- Dose-dependently decreased the lipid peroxidation in the serum of the animals, demonstrated by lowering the thiobarbituric acid reactive substances levels, most pronounced at a dose of 10 ml/kg.
- At all doses used, histological changes typical of metabolic syndrome were decreased and with the highest dose of CMFJ used, and in the group receiving the highest dose of CMFJ, the structural damage of the cardiomyocytes in the myocardium is prevented, the degenerative and steatotic changes in the liver are reduced and there is no adipocyte hypertrophy.
- CMFJ did not influence locomotor activity when compared to high-fat high-fructose diet-fed animals that were not treated with the juice.
- CMFJ dose-dependently reduced the anxiety levels in high-fat high-fructose diet-fed experimental animals as demonstrated by the increase in time spent in the open arms and in the ratio of time spent in the open arms to total time spent in any of the arms of the elevated plus maze test apparatus.
- CMFJ prevented the high-fat high-fructose diet-induced tendency of spatial memory impairment.

- CMFJ did not influence depressive-like behaviour in Forced swim test.

Conclusions are a logical reflection of the results obtained. They are accurate and comprehensive.

Contributions and significance of the development for science and practice

I accept the contributions formulated by the doctoral student, which I qualify as scientific-theoretical and scientific-applied:

- For the first time, CMFJ effects in an experimental model of metabolic syndrome were evaluated.
- For the first time, CMFJ effects on energy metabolism in rats in a model of diet-induced metabolic syndrome were evaluated.
- For the first time, CMFJ effects on visceral adiposity in rats in a model of diet-induced metabolic syndrome were evaluated.
- For the first time, data was gathered about stimulation of the endogenous antioxidant defenses and inhibition of lipid peroxidation by CMFJ in rats in a model of diet-induced metabolic syndrome.
- For the first time, CMFJ effects on the histology of the myocardium, coronary vessels, liver and adipose tissue in rats in a model of diet-induced metabolic syndrome were evaluated.
- For the first time the effects of CMFJ on the behaviour of rats in a model of diet-induced metabolic syndrome were evaluated.
- The conducted research on CMFJ contributed to the better understanding of the effects of the juice and of the polyphenols in its content in the context of metabolic syndrome.

Thesis summary

The summary, presented in Bulgarian and English, meets the requirements both in terms of content and layout. It accurately and correctly reflects the essence of the dissertation work. It is illustrated with highly informative figures and tables. It reflects the methodology, the main results with their discussion and the relevant conclusions, contributions and publications related to the work.

Assessment of publications

The results of the dissertation work have been published in 3 real articles, with 1 more article accepted for publication. Two of the journals are indexed and referenced in WoS. In all published articles, Dr. Moneva is the first author. The presented articles reflect and are fully related to the subject matter of her research work. Results of the conducted research were presented in one scientific forum abroad and one in the country.

Critical remarks and recommendations

I highly appreciate the experimental work done, the dissertation and summary written. I indicate some notes, which mainly concern the reference and do not diminish the value of the work submitted for review.

The reference presents sources in Latin, but a team working on *Chaenomeles maulei* published articles in Bulgarian, e.g. in Journal of Mountain Agriculture on the Balkans, 2018, 21 (5), 193-206; Plant Breeding Sciences, 2019, 56(4), etc. Some of the sources appearing in the reference are not cited in the text (eg Zakłos-Szyda M, Pawlik N. Japanese quince (*Chaenomeles japonica L*.) fruit polyphenolic extract modulates carbohydrate metabolism in HepG2 cells via AMP-activated protein kinase. Acta Biochimica Polonica 2018; 65(1): 67-78). When there are two or more identical quotations, they are distinguished by adding the letters "a", "b", "c", etc. after the years and this marking is given to in-text citations, e.g. (Valcheva-Kuzmanova et al. 2018a) and (Valcheva-Kuzmanova et al. 2018b).

The name of the medicinal plant in the summary is not given in Italics as it should be written.

CONCLUSION

The dissertation entitled: "Pharmacological study of the effects of Chaenomeles maulei fruit juice in an experimental model of metabolic syndrome" contains scientific-theoretical and scientific-applied results that represent an original contribution to science. The work meets all the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria, the Regulations for its Implementation and the relevant Regulations of the Medical University "Prof. Dr. P. Stoyanov" - Varna. The presented materials and dissertation results fully correspond to the specific requirements of the University.

The dissertation shows that the PhD student Dr. Clementina Moncheva Moneva-Marinova has bibliographic culture at a scientific level, has mastered experimental models and methods, knows how to analyze, synthesize results and formulate conclusions, has in-depth theoretical knowledge and professional skills. From the above, it follows that the two qualification tasks of the doctorate have been fulfilled - educational and scientific, and the completed written work can receive a well-deserved positive evaluation.

The results of Dr. Clementina Moneva's dissertation enrich the long-term research of biologically active substances of plant origin conducted in the department. Convincing is the confirmation and new evidence of the beneficial effects of PSHM demonstrated in the experimental model of MS, probably due to the rich polyphenolic content of the juice. *Chaenomeles maulei* fruit juice can be considered as a functional food, and the results of the presented experiment demonstrated anti-obesity properties. Prevention of adipose tissue accumulation both in visceral depots and ectopically is a beneficial effect in the context of the increasing health, economic and social burden of diet-induced chronic diseases. In combination with the cardioprotective action and antioxidant properties, the intake of CMFJ emerges as a useful intervention to limit the adverse effects of an unbalanced diet on metabolic health. The combination of polyphenols in the CMFJ composition confirms the potential of these phytonutrients to regulate mood, showing an anxiolytic-like effect in animals with diet-induced MS.

These results and those of the team's previous research should lead to the creation/implementation of nutritional supplements/medications to prevent and influence MS.

Due to the above, I confidently give my positive assessment and propose to the respected members, the Scientific Jury to award the ESD "Doctor" to Dr. Klementina Moncheva Moneva-Marinova in the PhD program in Pharmacology (incl. pharmacokinetics and chemotherapy), Professional direction 7.1. Medicine.

18.03.2024 Signature:

Pleven /Prof. Galya Stavreva, MD, PhD/