

REVIEW

by assoc. prof. **Maria D. Zhelyazkova-Savova**, MD, PhD

Department of pharmacology and clinical pharmacology and therapy,
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Regarding:

The dissertation work of **Mehmed Reizov Abtulov**, MD,
a self-study PhD student

at the Department of pharmacology and clinical pharmacology and therapy,
Medical University of Varna,

for awarding an educational and scientific degree "Doctor", in the area of Higher Education 7. Health care and sports, Professional direction 7.1. Medicine, Doctoral program "Pharmacology (including Pharmacokinetics and Chemotherapy)"

Topic of the PhD thesis:

PHARMACOLOGICAL EXAMINATION OF *ARONIA MELANOCARPA* FRUIT JUICE IN AN EXPERIMENTAL MODEL OF METABOLIC SYNDROME

By order No P-109-282/ July 08 2022, of the Rector of the MU of Varna, and with the decision of the Faculty Council of Medical Faculty at the MU of Varna (protocol No 1/ July 13, 2022), I have been designated as a Member of the Scientific Jury for the above procedure. Herewith, I submit my review of the dissertation work of **Mehmed Reizov Abtulov** entitled "**Pharmacological examination of *Aronia melanocarpa* fruit juice in an experimental model of metabolic syndrome**".

The documents presented by the applicant are in accordance with the requirements of the regulation for the acquisition of the educational and scientific degree "Doctor" and the Rules of the Medical University of Varna. All presented materials are duly arranged and described.

Education and professional development of the applicant

Mehmed Reizov graduated from the High school "Hristo Botev" in the town of Kubrat in 2011 and then in 2017 from the Medical University of Varna with distinction. He also finished a course in pedagogy and andragogy (2021).

He started working as an assistant-professor in 2018 in the Department of pharmacology and clinical pharmacology and therapy, Medical University of Varna. Currently he is involved in

teaching pharmacology and clinical pharmacology to medical students, including those from the English-language program. He is proficient in English (level C1). He works with the Office package and the statistical/ graphical software GraphPad Prism.

Relevance of the topic

The dissertation work of Mehmed Reizov is dedicated to an important current medical problem. Despite the high social price of the metabolic syndrome (MS) in terms of the multiple associated cardio-metabolic, liver, neuropsychiatric, etc. diseases, the adequate prevention and treatment of this common condition is still an unsolved issue. Modern knowledge of the pathogenic mechanisms linking the visceral adiposity with accumulation of free fatty acids, chronic low-grade inflammation and oxidative stress leading to insulin resistance, allows the search for new agents among the naturally existing plant-based substances with anti-inflammatory and anti-oxidant activity due to their high polyphenolic content. The present work is focused on the actions of *Aronia melanocarpa* fruit juice (AMFJ) on different aspects of diet-induced MS in rats.

A review of the PhD thesis and the results

The structure of the dissertation work

The dissertation work contains 158 pages. Its structure is in accordance with the accepted standards and includes the following sections: introduction – 3 pages; literature review – 42 pages; objective and tasks – 1 page; materials and methods – 10 pages; results with discussion – 50 pages; conclusions – 4 pages; contributions – 2 pages; list of publications and abstracts – 2 pages; references (409 in English; 1 in Bulgarian) – 34 pages. The structure is well balanced, except that a general discussion is not available.

Knowing the literature

The review of the literature is well structured and logically written, revealing a good knowledge of the applicant in the field. Among the references, 103 (~ 25%) are from the last five years, reflecting recent research.

The literature review is organized in two sections, dedicated correspondingly to the MS and the *Aronia melanocarpa*. In the first part, the MS is thoroughly discussed in terms of definitions, epidemiology, risk factors, pathophysiology, associated pathology, diagnosis, and treatment – including possibilities for intervention with biologically-active substances of plant origin, as well as existing experimental models. In table 5 (page 38), which summarizes the different diet-induced models of the MS, it would be appropriate to include the references that have been used, despite the fact that such information can be found in the text. The second part is dedicated to *Aronia melanocarpa*, with information related to botanical and phytochemical

aspects, polyphenolic content of the fruits, and the currently known pharmacological effects of the fruit juice and extracts, or of the polyphenols contained in them.

Summarizing the literature review, a transition from what is already known about the AMFJ and the still unexamined aspects of its activity is made, especially as far as MS is concerned. Based on this, the objective of the study is set: To examine the pharmacological effects of AMFJ in rats with experimental model of diet-induced MS, and the tasks are formulated in two directions: 1. To induce and verify the MS in rats by feeding them a high-fat high fructose diet; 2. To examine the AMFJ in terms of its psychopharmacological, biochemical, antioxidant, anti-inflammatory effects, as well as its actions on the fat indices, organ damage, markers of apoptosis and inflammation in this experimental model of MS.

Methods and design of the experiments

A variety of methods have been used, adequately selected to solve the tasks, including routine biochemical measures and common behavioral tests, as well as more or less original methods developed in the Department of pharmacology in Varna to induce MS, in addition to some advanced immunohistochemical approaches to characterize the MS-damaged organs.

Male Wistar rats have been allocated into 5 groups of 10 animals each. The AMFJ has been administered daily in 3 different doses by an intra-gastric probe for 10 weeks. All the procedures have been performed in accordance with the European directive 2010/63/EU and with the allowance issued by the Bulgarian agency of food safety (No 177/07.07.2017).

The chronic design of the experiments has been determined by the time needed for the rats to develop the typical signs of MS when fed a diet high in animal fat (lard) and fructose. This means that patience and dedication have been required for the daily routine of treating and feeding the animals. As an omission in describing the experimental design, I would note that it was not specified in what succession the behavioral tests and the manipulations were performed during the last two weeks. I would suggest that a chronologic scheme indicating when in time what experiment was carried out would give the reader a better idea about the sequence of the experiments, which is important, as that could have potentially affected each other's results.

To evaluate the locomotion, space memory, anxiety and depression-like states of the rats, classical behavioral tests have been used. As for the biochemistry – the glucose-tolerant test and serum triglycerides (TG) have been utilized to evaluate the impaired glucose and lipid metabolism. The standard test used to measure the TG level makes it useless to list the methodological details. The oxidative stress has been evaluated by the activity of the enzymes superoxide dismutase (SOD) and glutathione peroxidase. The fat tissue indices have been used as markers of visceral adiposity. The immunohistochemical methods are an essential addition to the histological examination of the organs susceptible to damage in MS. The semi-quantitative

determination of Bax and Bcl-2 in the fat and liver are indicative of the apoptosis status of the tissues and the macrophage factor MAC387 has been used as a marker of reactive inflammation.

The statistics utilized one-way and two-way ANOVA with Dunnett post-test, in addition to the Student's t-test. However, not always the specific analysis used has been mentioned when the results were reported, e.g., on page 86 it is stated that there is no significant difference in the expression of Bax between the control group and the two experimental groups MS+AMFJ_{2.5} and MS+AMFJ₁₀, although some differences can be seen, and if particular statistical data was available, there would have been no hesitation.

Analysis and evaluation of the results

The results are presented in seven parts according to the tasks. They are illustrated by 26 figures and 22 tables. Each part reports the specific experimental results and includes a discussion. The discussion of the current results allows for a concentrated analysis and focusing on the corresponding part of the work, where the applicant reveals his ability to look at the results in the context of the already existing information and to search and find in the literature data to support his findings or potentially to provide a mechanistic explanation.

Most of the effects of AMFJ established in this study are confirming previous data, but in the context of the experimental setting where they are found – the diet-induced experimental MS, they can be considered original. The metabolic parameters are improved with a trend of reducing the blood sugar in the glucose-tolerance test and with decreasing the TG; the visceral adiposity is reduced; there are signs of limiting the histopathological changes associated with MS in the fat tissue, the liver, the myocardium, and the blood vessels; the behavioral alterations in memory and the increased anxiety are reversed. AMFJ also tends to alleviate the carrageenan-induced paw edema at some doses and time intervals. Interesting are the results related to the apoptotic phenotype of the fat and liver. Although not all the changes in the expression of Bax and Bcl-2 are as expected, the author manages to look for and find a rational explanation of the findings, especially in the adipocytes. The effect of AMFJ on the macrophage factor MAC397 in the liver confirms the anti-inflammatory activity in a new light.

Reporting the results by sections with a corresponding discussion has its grounds and advantages. It is a general discussion, however, that can unite the separate parts, smooth potential discrepancies, encompass and unify the individual components.

The PhD thesis is easy to read, and the exposition is for the most part smooth, logical, and understandable, regardless of some mistakes or inaccuracies (see below).

Analysis and evaluation of the conclusions and contributions

Two main conclusions are drawn, corresponding to the tasks, each of which contains the summarized results of the MS modeling and the effects of AMFJ on the studied parameters and indicators.

The contributions of the present dissertation are summarized in seven points and follow the conclusions.

Both in the discussion and in the conclusions drawing and defining the contributions, I believe the author should be more careful and cautious regarding the statements he makes based on the obtained results. Thus, the glucose-lowering effect is statistically insignificant, regardless of the clear trend in this direction. As for the suppression of the oxidative stress by AMFJ, objectivity demands to note other publications as well that, contrary to the present results and the literature sources cited in this work, find the logical and expected decrease in SOD activity in MS, and, accordingly, the substances tested for antioxidant activity do increase this indicator – in other words, a certain flexibility in the interpretation of the results would be appropriate. The situation is analogous to the pro-apoptotic phenotype observed at the highest dose of AMFJ in the setting of MS, but in this case the applicant responsibly acknowledges the contradiction and subjects it to analysis instead of ignoring it.

I accept most of the contributions, including their originality.

Publication activity

In connection with his dissertation, Mehmed Reizov has published four full-text articles, one of which in an international scientific journal with an impact factor. In all publications, he is the first author, a fact that reflects his personal contribution. Four presentations related to the dissertation and reported at national or international scientific forums, are also listed.

Notes, omissions, recommendations

The notes and recommendations I address to the author do not underestimate the work and do not change my overall positive assessment. Rather, they aim to draw the attention of the young researcher, either to important details that should not be missed, or to principles that I think it is good to keep in mind. Along with the already noted, I would add the following:

In the “Materials and Methods” section

- In the place recognition test, there is no description of how the rats examine the objects and what the specific criteria are, that are accounted for.

In the "Results and Discussion" section

- Once again I would take the liberty of noting that the potential presence of a general final discussion would greatly increase the merits of the work.
- The work would benefit from a logical point of view, if the metabolic, antioxidant and anti-inflammatory results were presented first, and then the behavioral effects of AMFJ, following not the chronology of the experiments, but the nature of the MS – even more so that the explanation of the beneficial psychopharmacological effects of AMFJ includes precisely its action on the metabolic indicators, oxidative stress and inflammation.
- The results of the glucose tolerance test are considered only as glucose-lowering, but not in terms of improved glucose utilization.
- Most of the results are presented in text, figure and table, which is redundant.
- Some discrepancies are found between the cited publications in the text and those listed in the bibliography, e.g. Wang Z et al, 2020; Chambel et al, 2015; Piotrowska-Kempisty et al., 2020(p. 108) are omitted from the references and vice versa, sources from the list of references are not cited in the text, e.g. Dreiseitel et al, 2009; Figueira et al, 2017; Heimet al, 2002; Sathyapalan et al, 2010.

Language and style

- There have been some inappropriate terms used in describing the metrics in the social interaction test, (e.g., haircut?!), as well as some translated terms with a different meaning in Bulgarian language
- A mixture of grammatical tenses has been used in the exposition – e.g., against the background of the prevailing present tense, the immunohistochemical methods are described in the past tense.
- Some abbreviations have been introduced more than once (e.g. MS, PP) and others not at all (e.g. those in the description of immunohistochemical methods)
- On page 76, the sentence "... MS impairs glucose control and probably causes IR..." needs editing.

Evaluation of the abstract

The abstract, written on 78 pages, meets the regulatory requirements and correctly reflects the essential contents of the dissertation work.

Personal impressions of the candidate

I have known Mehmed Reizov since he joined the Department of Pharmacology at the MU of Varna. I have always been impressed by the zeal with which he embarks on every undertaking and task. He shows initiative, diligence and responsibility not only in his scientific work, but also in his teaching work with students. His presentations for the online seminars in pharmacology

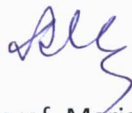
and clinical pharmacology were comprehensive, logical, and up-to-date. He shows respect for the opinion of his more experienced colleagues, he is not afraid of asking questions. With his persistence and hard work, and his interest in new developments in medical science and practice, Mehmed Reizov emerges as a promising young researcher.

In conclusion, I believe that Mehmed Reizov's dissertation represents an up-to-date scientific work with interesting results and original contributions. The author presents as a young specialist with good scientific and theoretical training and with attitude and desire for development and self-improvement. He demonstrates professional capabilities for conducting independent experimental research and analyzing the results. The scientific metrics satisfy the requirements of the Law for the Development of the Academic Staff in the Republic of Bulgaria and the Regulations for the Development of the Academic Staff at the MU-Varna for awarding the scientific and educational degree "DOCTOR". All this gives me a good reason to vote with conviction FOR awarding the scientific and educational degree "DOCTOR" to Mehmed Reizov Abtulov.

Varna

16.08.2022

Reviewer:



/assoc. prof. Maria D. Zhelyazkova-Savova, MD, PhD/