

## OPINION

by **Assoc. Dr. Delyan Penev Delev, PhD - Head of the Department of Pharmacology and Clinical Pharmacology, MU-Plovdiv, MF**

of a dissertation for awarding the educational and scientific degree '**doctor**'

professional direction *7.1. Medical*

Doctoral Program *in Pharmacology (incl. Pharmacokinetics and Chemotherapy)*

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**Form of doctoral studies:** full -time

**Department:** Pharmacology and clinical pharmacology and therapy, Medical University "prof. Dr. Paraskev Stoyanov" - Varna, Faculty of Medicine

**Topic** : *PHARMACOLOGICAL STUDY OF THE EFFECTS OF ARONIA MELANOCARPA FRUIT JUICE IN AN EXPERIMENTAL MODEL OF THE METABOLIC SYNDROME*

**Research supervisor** : *Prof. Dr. Stefka Vasileva Valcheva-Kuzmanova, MD*

### 1. General presentation of the procedure and the doctoral student

The presented set of materials on paper and electronic media **is** in accordance with the Procedure for the acquisition of the DEGREE "Doctor" at the MU - Varna; Regulations of MU-Varna and ZRASB.

The PhD student has attached 4 publications .

Notes and comments on the documents - I don't have any .

### 2. Relevance of the topic

Metabolic syndrome (MS), also called insulin resistance syndrome, is a complex symptom complex , and the most important risk factors for its development seem to be abdominal obesity and insulin resistance. The current diagnostic criteria for MS are central obesity (mandatory component) – for the Caucasian race - waist circumference  $\geq 94$  cm in men and  $\geq 80$  cm in women plus two of the following features: elevated triglyceride levels  $\geq 1.7$  mmol /l or specific treatment, due to this lipid disorder – low HDL-cholesterol (HDLC)  $\leq 1.04$  mmol /l in men and  $\leq 1.29$  mmol /l in women or specific treatment due to this lipid disorder; increased arterial pressure (BP) – systolic BP  $\geq 140$  mm Hg or diastolic BP  $\geq 90$  mm Hg , or treatment of previously diagnosed hypertension ; fasting hyperglycemia - fasting blood glucose value  $\geq 6.1$  mmol /l or previously diagnosed type 2 diabetes; when measuring a value  $\geq 6.1$  mmol /l, it is strongly recommended to conduct an oral glucose challenge and an immunoreactive insulin test (OGTT+IRI) to confirm the syndrome.

Although MS is considered a disease of adults, with the increase of obesity, hypercaloric diet and reduced physical activity among young people, components of the group of dysmetabolic disorders are beginning to appear even in children. The incidence of MS increases with obesity severity and affects 50% of severely obese young adults.

One of the most widespread hypotheses to describe MS is that of insulin resistance as a central key factor linking abdominal obesity to the other components of the syndrome. According to this glucocentric hypothesis, the responsible primary disorder is obesity (genetically determined or nutritionally induced), and IR and its subsequent chronic hyperinsulinemia are compensatory mechanisms to restore the energy balance and maintain the euglycemic state in the body. A major role in the development of IR is played by increased CMC, which are produced by lipolysis of stored triglycerides in adipose tissue . visceral and muscle tissue. CMC produced by intramuscular triglyceride molecules lead to reduced insulin- mediated clearance of circulating glucose and impaired oxidative and non-oxidative utilization of glucose by myocytes . As a result, relative postprandial hyperglycemia and compensatory postprandial hyperglycemia occur hyperinsulinemia .

There is a close relationship between metabolic syndrome and fertility in both women and men. The exact pathophysiological mechanisms have not been established, but the participation of hereditary factors, lifestyle, etc. is assumed.

The most frequently occurring clinical symptoms reflecting the relationship between metabolic disorders and the hypothalamic - pituitary -ovarian axis are:

Disturbance in the rhythmicity of menstrual cycles. Chronic anovulation . Most often, girls start with normal monthly cycles /MCs, but over the years MCs become irregular or stop for a certain period of time due to repeated anovulatory cycles.

Increase in adipose tissue mainly in the waist area - the symptom is closely related to insulin resistance and is the basis of the connection of gonadal disorders with the biochemical disorders characteristic of the metabolic syndrome.

Appearance of hirsutism and/or other clinical hyperandrogenic symptoms - acne, alopecia, seborrhea, etc. - mainly due to the disturbed hormonal regulation of ovarian hormone production, expressed in androgenic excess, stimulated by endogenous hyperinsulinemia , the changed ratio of LH/FSH, high levels of IGF 1, chronic anovulation and others.

Behavior and therapeutic approaches:

Lifestyle modification is the therapeutic method of first choice in patients at increased metabolic risk. Specific measures include a 5-10% reduction in body weight, increased physical activity, changes in eating habits and an anti- atherogenic diet. Quitting smoking is also important.

To reduce abdominal obesity, a reduction in caloric intake is necessary. An initial weight loss of 5-10% over a period of about 12 months is appropriate. This can be achieved with a moderate reduction of daily caloric intake by 500 to 1000 kcal , which implies a weight loss of 500 to 1100 g per week. Losing weight to such an extent is an effective strategy for lowering the risk of developing T2DM, as well as for reducing multiple cardiovascular risk factors. No less important is the retention of the achieved lower weight.

The role of physical activity is extremely large, as it contributes to weight reduction and can reduce overall cardiovascular risk. 30-60 minutes of moderate physical activity five times a week (eg

brisk walking) is recommended, with longer durations of exercise associated with greater benefit. It is also appropriate to increase daily routine physical activity. Patients at high risk of CVD should begin exercise under medical supervision after a functional assessment of the cardiovascular system.

Qualitative changes in the diet are necessary for patients with metabolic syndrome:

The diet should be low in saturated fat, cholesterol, salt and simple sugars, and rich in fruits, vegetables and fish.

Fiber, whole grains and unsaturated fatty acids should be in a higher percentage ratio in the menu.

There is conflicting opinion about the optimal ratio of carbohydrates to fat, but limiting high-glycemic index foods in the diet may lower metabolic risk.

Drug treatment is required as a next step in some patients in whom lifestyle change is impossible or ineffective.

Due to the unclear pathophysiology of MS, no specific intervention is currently possible. This requires treatment of the individual components of the syndrome.

Insulin resistance is a leading pathophysiological factor in MS, which is why there is great interest in medications that can improve insulin sensitivity.

The Diabetes Prevention Program has shown that treatment with metformin in patients with prediabetes prevents or delays the development of type 2 diabetes mellitus. Metformin reduces excessive hepatic glucose production and increases glycogen synthesis, stimulates glucose uptake into muscle cells, through an insulin-dependent mechanism and suppresses lipolysis (and accordingly CMC) without causing hypoglycemia. It improves the lipid profile, leads to weight loss, increases fibrinolytic activity, reduces platelet aggregation and has a beneficial effect on hypertension.

Arterial hypertension in patients with metabolic risk should be treated according to current recommendations and consensus . For patients with MS, target blood pressure values below 140/90 mmHg are recommended . A moderate increase in arterial pressure can be influenced by lifestyle changes (reduction in body weight, physical activity, restriction of salt, alcohol and fat, increased intake of fruits and vegetables). In the absence of a sufficient effect, the application of medication is appropriate. The most suitable is the use of ACE inhibitors, but in practice it is often necessary to combine antihypertensive agents.

Lifestyle modification is the most important and effective measure to reduce the development of MS and the progression of NGT to overt diabetes.

Aronia melanocarpa is a plant whose fruits are known to stimulate healing processes in humans and animals. Bulgaria is a country with wonderful climatic conditions and many areas with suitable soil and irrigation for growing Aronia melanocarpa . The homeland of the Aronia bushes is North America. Since ancient times, the fruits of this plant have been known for their medicinal properties.

They are used for various diseases and have always had a stimulating effect on healing processes. In the 20th century chokeberry became popular in the former Soviet Union and in the countries of Eastern Europe. It is grown in home gardens as a fruit from which juices, marmalades, fruit wine are prepared, used to influence hypertension and atherosclerosis. In folk medicine, chokeberry fruits are included in the treatment of achlorhydria, avitaminosis, in the period of convalescence in severe diseases, and last but not least – in hemorrhoids. By 1976, 17,800 hectares of cultivated Aronia were planted in the Soviet Union melanocarpa. In 1976 Aronia cultivation melanocarpa was introduced to Japan with the assistance of specialists from the former Soviet Union. In 1986, a large Aronia project was launched in Sweden with the aim of obtaining a highly efficient source of pigments. The idea is spreading in Poland, the Czech Republic, Slovakia and Ukraine.

Over the course of many years, chokeberry fruits have been accepted as a dietary supplement with specific medicinal properties. There are no known toxic effects when consuming any of the products made from or containing aronia. It is now known that the anthocyanin content is related to the health-enhancing properties of this plant. Today, chokeberry fruits are used for the prevention of the most widespread socially significant diseases on the basis of their powerful antimutagenic effect, lipid-modifying effect and reduction of the general risk of cardiovascular diseases. A certain difference in the results of the action of anthocyanins should be taken into account in in vitro and in vivo, noted in a number of studies. Anthocyanins, taken orally, found in the gastrointestinal system, are sensitive to the digestive juices of the pancreas, which are alkaline in nature. A modification in the bioavailability of anthocyanins is possible.

antioxidant activity of chokeberry anthocyanins has also been studied in humans - related to the phenomenon of oxidative stress, which accompanies various metabolic and vascular problems such as dyslipoproteinemia, diabetes mellitus, atherosclerosis. In patients with cardiovascular risk (hypertensives, smokers, with high cholesterol, with diabetes mellitus) by known metabolic pathways, called cardiometabolic, there is a change in the membrane receptors of platelets, as a result of increased production of free radicals in the body as a whole and activation of tissue adhesion factors, as well as those of the macrophage system. This increases the possibilities of platelets for adhesion and aggregation, which is related to arterial thrombus formation.

Despite the many data on the beneficial influence of Aronia melanocarpa are still lacking in-depth experimental and clinical studies on the problem of metabolic syndrome.

**Due to all the above-mentioned facts, I define the topic as extremely relevant.**

### **3. Knowing the problem**

The PhD student understands the state of the problem and creatively evaluates literary material. A bibliography of 407 literary sources (404 in Latin and 3 in Cyrillic), which are extremely well selected and up-to-date.

### **4. Research methodology**

The chosen research methodology allows to fully achieve the set goal and obtain an adequate answer to the tasks solved in the dissertation work.

### **5. Characterization and evaluation of the dissertation work and contributions**

dissertation contains a total of 159 standard typewritten pages and follows a classic structure (Introduction – 2 pages; Literature review – 41 page; Purpose and tasks - 1 page; Material and method – 9 pages; Own results and discussion – 46 pages; Conclusions – 4 pages; Contributions –

2 pages ; Bibliography – 35 pages). A list of the doctoral student's publications and scientific appearances ( as well as supporting material) is duly attached . 30 figures and 27 tables are presented, which are well constructed and undoubtedly contribute to a better presentation of the complex matter.

In the literature review for considered:

1. Metabolic syndrome , containing many subsections : definitions and criteria for metabolic syndrome, epidemiological data, risk factors for the development of metabolic syndrome, overweight, abdominal obesity, sedentary lifestyle, eating habits, hyperuricemia , age, genetic predisposition , medications, circadian rhythm, disturbed sleep quality, sleep disorders and metabolic syndrome, disturbed intestinal microflora, ethnicity and race, pathogenesis of metabolic syndrome, insulin resistance and metabolic syndrome, chronic inflammation and metabolic syndrome, metabolic syndrome and related conditions, cardiovascular disease , diabetes mellitus type 2, non-alcoholic fatty liver disease, polycystic ovary disease, obstructive sleep apnea syndrome ( hypopnea ) , neuropsychiatric diseases, oncological diseases, diagnosis of metabolic syndrome and treatment, pharmacological effects of biologically active substances of plant origin in metabolic syndrome , experimental splits of metabolic are also presented.

2. The second section is devoted to *Aronia melanocarpa* and contains: botanical data on the plant, origin, distribution and species, characteristics, fruit composition, polyphenolic content of *Aronia* berries *melanocarpa* , pharmacological effects of biologically active substances from *Aronia melanocarpa* . A particularly good impression is made by the last part of the review, which contains a summary of the literature data and defines the motivation for conducting a similar type of experimental study.

The objective of the dissertation work is well formulated and sets 2 main tasks before the researcher, the second objective being extremely well divided into 7 sub-tasks.

In material and method excellently described: The experimental animals (all the highest European standards and protocols for ethical treatment were observed), substances used, *Aronia* juice *melanocarpa* (PSAM), reagents and kits, as well as methods such as: induction of metabolic syndrome and treatment, induction of acute hindpaw inflammation, open field test ( Open field test , OFT), social interaction test ( Social interaction test , SIT), test for recognizing the place of objects ( Place recognition test , PRT), forced swimming test ( Forced swim test , FST), biochemical methods, obtaining blood serum, glucose-tolerance test (GTT), determination of triglycerides in blood serum, determination of the activity of antioxidant enzymes in serum, determination of indices of the mesenteric , paranephric , perigonadal , retroperitoneal and general adipose tissue, histological and immunohistochemical methods, histological methods, immunohistochemical methods and statistical methods, which I define as modern, sufficient and adequate.

The "own results and discussion" part presents in a wonderful way the work done and the analysis of the own data in the light of reputable sources from the international literature. It is logically constructed and presented in a highly scientific style and illustrated with appropriate figures and tables.

In the "conclusions" section, 2 main conclusions are formulated very concisely and comprehensibly, which are also a natural conclusion of the conducted research. They are presented in a structured way, in several sub-points, which makes it easier to perceive the enormous amount of work done by the doctoral student.

The author also presents 7 scientific and innovative contributions, namely:

*Aronia melanocarpa* fruit juice have been obtained for the first time on behavior in rats with an experimental model of diet-induced metabolic syndrome, finding:

- 1.1. Anxiolytic -like effect;
- 1.2. Spatial memory improvement.

2. For the first time, data on the metabolic effects of *Aronia melanocarpa* fruit juice have been obtained in rats with an experimental model of diet-induced metabolic syndrome, and found:

- 2.1. Glucose -lowering effect;
- 2.2. Antihypertriglyceridemic effect.

3. For the first time, data have been obtained on the suppression of oxidative stress by *Aronia melanocarpa* fruit juice in rats with an experimental model of diet-induced metabolic syndrome.

Visceral obesity reduction data obtained for the first time from *Aronia melanocarpa* fruit juice in rats with an experimental model of diet-induced metabolic syndrome.

protective effects of *Aronia melanocarpa* fruit juice have been obtained for the first time relative to histopathological changes in adipose tissue, myocardium, coronary vessels and liver in rats with an experimental model of diet-induced metabolic syndrome.

fruit juice from *Aronia melanocarpa* have been obtained for the first time on programmed cell death in adipose tissue and liver in rats with an experimental diet-induced metabolic syndrome model, finding that:

- 6.1. The juice suppresses programmed cell death in adipose tissue in doses of 2.5 and 5.0 ml /kg. It was found for the first time that the fruit juice of *Aronia melanocarpa* in a high dose (10 ml /kg) can induce apoptosis of adipocytes in adipose tissue. This calls for further research on the effect of the juice on adipocyte apoptosis at different doses and with different durations of treatment in order to identify the possible consequences of such an effect.
- 6.2 . It suppresses programmed cell death in the liver at all doses.

*Aronia melanocarpa* fruit juice has been established for the first time after carrageenan -induced acute hindpaw inflammation in rats with an experimental model of metabolic syndrome.

I strongly wish that they will be implemented in practice with prospects for future development.

## **6. Evaluation of the publications and personal contribution of the doctoral student**

The doctoral student presents 4 publications related to the dissertation - 3 nos. in international refereed o journal Scripta Scientifica Medica and 1 pc. in an international refereed journal with an impact factor (in press). All articles are in English. Dr. Mehmed Abtulov is the first author of all scientific works, which proves his personal participation in the conducted dissertation research, as well as that the formulated contributions and obtained results are his personal merit.

Critical remarks and recommendations ( to the conducted research and presented materials) - I have none.

## **7. Abstract**

My submitted abstract (78 pages) is made according to the requirements and reflects the main results achieved in the dissertation .

## CONCLUSION

The dissertation *contains scientific, scientific-applied and applied results, which represent an original contribution to science* and **meet all** the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (ZRASRB), the Regulations for the Implementation of ZRASRB and the Regulations of the MU - Varna. The presented materials and dissertation results **fully** correspond to the specific requirements adopted in connection with the Regulations of the Ministry of Education - Varna for the application of the ZRASRB.

The dissertation work shows that the doctoral student Dr. Mehmed Reizov Abtulov **has in - depth** theoretical knowledge and professional skills in the scientific specialty of Pharmacology (including Pharmacokinetics and Chemotherapy), **demonstrating** qualities and skills for independent conduct of scientific research.

Due to the above, I confidently give my *positive assessment* of the conducted research, presented by the above-reviewed dissertation work, abstract, achieved results and contributions, and *offer to the honorable scientific jury Yes awarded the educational and scientific degree "doctor"* to Dr. Mehmed Reizov Abtulov in doctoral program in Pharmacology (incl. Pharmacokinetics and chemotherapy).

20.09. 2022.

Prepared the opinion : 

(Assoc. Dr. Delyan Penev Delev, PhD)