

OPINION

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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 Chaenomeles maulei fruit juice in an experimental model of 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 act on Development of the Academic Staff in the Republic of Bulgaria (ADASRB), the Regulations for its implementation and the Regulations of the Medical University of Varna.

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 ≥ 94 cm in men and ≥ 80 cm in women plus two of the following features: elevated triglyceride levels ≥ 1.7 mmol /l or specific treatment, due to this lipid disorder – low HDL-cholesterol (HDL-C) ≤ 1.04 mmol /l in men and ≤ 1.29 mmol /l in women or specific treatment due to this lipid disorder; increased arterial pressure (BP) – systolic BP ≥ 140 mm Hg or diastolic BP ≥ 90 mm Hg , or treatment of previously diagnosed hypertension ; fasting hyperglycemia - fasting blood glucose value ≥ 6.1 mmol /l or previously diagnosed type 2 diabetes; when measuring a value ≥ 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. CMCs 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 involvement 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 /MC/, 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 are conflicting opinions about the optimal ratio of carbohydrates to fat, but limiting high-glycemic index foods in the diet can 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 blood 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.

The Japanese quince, which is found as a wild shrub in many areas of Japan and China, can actually be grown in Bulgaria without any difficulties. In addition to being extremely unpretentious, it has a high decorative value because it blooms profusely in the spring, and its fruits, although they have nothing in common with the quince in taste, are extremely useful. Because of its beautiful colors, which can be dyed in all shades of red, the Japanese quince is grown in various parts of the world. The fruit of the Japanese quince is edible and has a very high vitamin C content, much higher than that found in the popular apples or lemons. In the fruits of the Japanese quince there is high content

of vitamins C, P, pectin, and the species acids, essential oils etc. Because of this the *Chaenomeles* everything used in Chinese and Japanese folk medicine for treatment of liver diseases, liver and digestive system, the fruits influence good immune nervous system and the general tone of the human body. The data on the useful properties of the plant are limited, and precisely because of this and **all the above-mentioned facts, I define the topic as extremely relevant.**

3. Knowing the problem

The doctoral student understands the state of the problem and creatively evaluates literary material. A bibliography of 187 literary sources is attached, 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

The dissertation contains a total of 123 standard typewritten pages and follows a classical structure (Introduction – 2 pages; Literature overview – 29 pages; Purpose and tasks – 2 pages; Material and method – 7 pages; Own results and discussion – 50 pages; Conclusions – 4 pages; Contributions – 3 pages; Bibliography – 16 pages). A list of the doctoral student's publications and scientific appearances (as well as supporting material) is duly attached. 30 figures and 13 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 models of metabolic syndrome are also presented.

2. The second section is devoted to *Chaenomeles maulei* and contains botanical data, chemical composition, fruit juice, biological activity, in vitro studies and in vivo animal studies.

3. The third part looks at effects of polyphenols in metabolic syndrome, effects on visceral obesity, insulin resistance, dyslipidemia, blood pressure, non-alcoholic fatty liver disease and effects on miRNA.

The purpose of the dissertation work is well formulated and sets 2 main tasks before the researcher, the first containing 3 and the second extremely well divided into 5 sub-tasks.

In material and method are excellently described: The experimental animals (all the highest European standards and protocols for ethical treatment were observed), substances used, *Chaenomeles maulei* juice, experimental model of metabolic syndrome, fractionation of tissues and organs, obtaining blood serum, isolation of organs for histopathological examination, biochemical

studies (Determining the levels of triglycerides , total cholesterol, glucose, determining the levels of superoxide dismutase , determining the levels of thiobarbiturate- reactive acid substances), determination of adipose tissue indices, TyG index and liver index, histological studies and behavioral methods - Method for evaluating motor activity - Open field test (Open field test , OFT), Anxiety research methods - Elevated crossed maze test (Elevated plus-maze test , EPM), social interaction research test (Social interaction test , SIT), a method for assessing spatial memory - Test for the location of objects (Object location test , OLT) and a method for evaluating depressive behavior - Forced swimming test (Forced swim test , FST). Statistical methods are well selected based on the selected methodologies. In general, I define the production 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, 3 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:

1. The effects of *Chaenomeles maulei* fruit juice in an experimental model of metabolic syndrome were investigated for the first time.

2. For the first time, the effects of *Chaenomeles maulei* fruit juice on energy metabolism were investigated in rats in a diet-induced metabolic syndrome model, and it was found that:

- 2.1. Increases food consumption;
- 2.2. Reduces the consumption of fructose solution;
- 2.3. Increases caloric intake.

3. For the first time, the effects of *Chaenomeles maulei* fruit juice on visceral adiposity were investigated in rats in a diet-induced metabolic syndrome model, and it was found that:

- 3.1. Reduces visceral fat tissue, even against the background of increased caloric intake;
- 3.2. Reduces mesenteric adipose tissue;
- 3.3. Reduces paranephric adipose tissue.

4. For the first time, data on stimulation of the body's antioxidant defenses and reduction of lipid peroxidation by *Chaenomeles maulei* fruit juice were obtained in rats in a model of diet-induced metabolic syndrome.

maulei fruit juice on the histology of myocardium, coronary vessels, liver and adipose tissue in rats in a model of diet-induced metabolic syndrome were investigated for the first time , and it was found that:

- 5.1. It exhibits a dose-dependent cardio- and vasoprotective effect;
- 5.2. Prevents high-fat induced high-fructose diet changes in adipose tissue and preserves the normal size of adipocytes in all doses used;
- 5.3. Leads to a dose-dependent inhibition of the high-fat induced high fructose diet liver damage.

6. For the first time, the effects of Chaenomeles maulei fruit juice on the behavior of rats in a diet-induced metabolic syndrome model were investigated, and it was found that:

- 6.1. Does not affect motor activity;
- 6.2. It exhibits a dose-dependent anxiolytic effect;
- 6.3. Shows a tendency to improve spatial memory;
- 6.4. It does not affect depressive symptoms.

7. The studies done with Chaenomeles maulei fruit juice contribute to a more complete understanding of the effects of the juice and its polyphenols in the context 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 - 1 in international refereed journal, 2 in national refereed journals and 1 in an international refereed journal with an impact factor (in press). All articles are in English. On all scientific works Dr. Clementina Moncheva Moneva-Marinova is the first author, which proves her personal participation in the conducted dissertation research, as well as that the formulated contributions and obtained results are her personal merit. She has 2 participations in scientific forums – in Bulgaria and 1 in Spain.

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

7. Abstract

The submitted abstract (79 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 Act on Development of the Academic Staff in the Republic of Bulgaria (ADASRB), the Regulations for its implementation and the Regulations of the Medical University of Varna. The presented materials and dissertation results **fully** correspond to the specific requirements adopted in connection with the Act on Development of the Academic Staff in the Republic of Bulgaria (ADASRB), the Regulations for its implementation and the Regulations of the Medical University of Varna. The dissertation shows that Dr. Clementina Moncheva Moneva-Marinova **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 research, presented by the above peer-reviewed dissertation, author's abstract, obtained results and scientific contributions, and would recommend to the honorable members of the scientific jury to award the educational and scientific degree "Doctor"** to Dr. Clementina Moncheva Moneva-Marinova in doctoral program Pharmacology (incl. Pharmacokinetics and chemotherapy).

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Prepared the opinion

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