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Review

of the habilitation thesis manuscript titled: "Antidiabetic and antioxidant potential of plant extracts in streptozotocin-induced diabetic mice",

Author: dr Agnieszka Greń, PhD

Diabetes is defined as metabolic disorder, characterized by chronic hyperglycaemia with disordered carbohydrate, lipid and protein metabolism resulting from defective secretion and/or effects of insulin. As a result of such deviations retinopathy, nephropathy, nervous system neuropathy and disorders of cardiovascular system often occur. It is estimated that nowadays, about 300 million people suffer from diabetes and its complications. According to some theories, a crucial role in both diabetes pathogenesis and chronic diabetes complications is played by oxidative stress. Oxidative stress may be considered as a result of diabetes, mainly of disturbed energy metabolism, but on the other hand it may also participate in diabetes ethiology. There is substantial evidence of the role of redox imbalance in diabetes pathogenesis, such as lipid peroxidation, oxidative damage to mitochondria, the increase in reactive oxygen species concentration, the decreased enzymatic antioxidants activity and non-enzymatic antioxidants decreased concentrations etc. Taking the above into consideration, it may be assumed that proper application of exogenous antioxidants in diet may prevent, at least to some extent, the diabetes development. What is more important, the supplementation with antioxidants may be efficiently used in the attenuation of some health complications in patients with diagnosed diabetes. In recent years, special attention has been paid to the potential use of herbs of anti-diabetic properties. It was shown that antidiabetic properties of plants and plants extracts are mainly related to their antioxidative properties. On the other hand, most of the plant preparations show low or moderate antioxidative and antidiabetic

potential. Thus, there is a need to indicate plants and/or plant extracts which may efficiently support antidiabetic therapies. The author of the manuscript aimed at the determination of antioxidative and antidiabetic potentials of several plant extracts, i.e. leaves extract of *Morus alba*, seeds extract of *Trigonella foenum-graecum*, common beans extract of *Phaseolus vulgaris*, the leaves extract of *Galega officinalis*, the leaves extract of *Vaccinium myrtillus*, in the animal model.

The manuscript is written on 111 pages and includes 15 figures and 18 tables. The outline of the manuscript is classical: abstract in English, abstract in Slovak, key words, a list of abbreviations and acronyms, introduction, material and methods, results, discussion, conclusions, resume, bibliography.

Bibliography contains 229 positions. Most of the cited literature was published 10 or less years ago. More than 75 cited articles were published during the last 5 years. This indicates that the author knows the newest scientific literature. This also indicates the significance of the analysed problem to the scientific community in recent years.

The introduction is a well composed and deliberative review of scientific literature in the field of oxidative stress, ethiology, prevention and medical treatment in diabetes type I. First, the author concentrates on the definition of diabetes, its clinical symptoms and the most common complications. Next, main factors responsible for diabetes occurrence are analysed, including autoimmune reactions and genetic predispositions of patients. Other factors that can take part in ethiology of diabetes type I, such as viral infections, food contamination or improper diet, are just mentioned here. The author focuses mainly on the role of oxidative stress in diabetes pathogenesis. Oxidative stress is defined here as the state of increased ROS activity, caused by their greater generation and/or impaired recycling by antioxidant systems of the organism. The author points out that mitochondrial dysfunction resulting from oxidative damage may have a significant role in pathogenesis of insulin resistance and diabetes and that the increased ROS generation was observed in muscles of diabetic patients. The intensity of ROS generation in diabetes depends on the degree of protein glycation, and on glyceamia. Yet, there are also other mechanisms participating in redox imbalance in diabetes. Hyperglycaemia weakens the efficiency of antioxidative systems in cells and leads to the increased formation of reactive nitrogen species, including peroxynitrite. The author also gives very interesting and actual information on ROS engagement in disturbances of signal transduction by JNK and p38 kinases, protein kinase C-theta, insulin receptor substrate 1 or phosphatidylinositide 3-kinases.

Next, the basic information on enzymatic and non-enzymatic antioxidants are given. This part resembles an academic teaching text. On the other hand, the general description of the major antioxidants' function could not be avoided in this work. More interesting is the description of antidiabetic herbal supplements, which contains some not commonly known information. The author analyses the strategies of potential therapies with plants or plants extracts and informs about the directions of scientific research in the field of herbalism. The therapeutic properties of several plants are described, including *Morus alba* L., *Trigonella foenum graecum* L., *Pericarpium Phaseolus vulgaris* L., *Galega officinalis* L., *Vaccinium myrtillus* L. The description is informative and mainly limited to the active substances and their physiological effects, which may be helpful in the treatment of diabetes.

The introduction section ends with research assumptions and aims. Here, the author summarizes the most important aspects of free radical engagement in diabetes pathogenesis and main directions of the scientific research. Some of the research studies consider the application of medicaments regulating glycolysis, gluconeogenesis and glucose absorption and/or the organism protection from the effects of free radicals. The research aims presented in this part of the work are based on the main hypothesis that plant extracts, having anti-diabetic effect, may stabilize the blood sugar concentration, increase cell sensitivity to insulin, alleviate inflammations or decrease the number of free radicals generated in diabetes. The aims resulting from the main study hypothesis are well formulated. Nonetheless, I would like to question the last one – aim number 5. I do not understand how the author was able to assess the importance of the used extracts for the improvement of a diabetic's quality of life using the animal model. **Here, I would like to ask the author to present advantages and disadvantages of different models applied in biomedical research during the habilitation colloquium.** Certainly, my critical comment on one of the formulated aims does not disqualify this part of the text.

The study methods applied for biochemical analyses are commonly used. The research was conducted on the total number of 240 male Swiss albino mice selected from inbred colony. Animals were segregated into 2 series differing with the applied dose of plant extract. Thanks to a very well planned research, the author was able to compare the effects of plant extracts on diabetic and non-diabetic mice. The study design gave also the possibility to test different doses of plant extracts and to point the extract with the highest antidiabetic effects. Diabetes was induced with streptozotocin injection. The occurrence of diabetes was evaluated by blood glucose concentration measurements. Experimental procedures were approved by the Local Ethical Committee. Statistical analysis was well performed. The distribution of

variables was estimated with the use of the Shapiro-Wilk test and the homogeneity of variances was estimated with the Levene's test. The main analysis was performed by multivariate analysis of variances MANOVA followed by the Tukey's test.

The results are presented in the form of text, tables and figures. Generally, this part of the text is informative and well written. The text describes essential results. Tables contain means, standard deviation ranges and results of the *post hoc* tests. Figures present the most interesting results. On the other hand, some parts of the Results section are not very clear. In my opinion, the text gives too detailed information, whereas it should describe general trends, because all the results are placed in the tables. Such a detailed description of the results makes the impression that some of them are contradictory. For instance, in one of the subsections the author writes: "After the administration of all extracts (in dose 100 $mg \cdot kg^{-1}$ b.w.) was found a decrease [MA-7.23%; TFG-12.16%; PVP-2.72; GO-0.17%; VM-1.02%] in the concentration of glucose in comparison to control values. Similarly, after the administration of all extracts (in dose 500 $mg \cdot kg^{-1}$ b.w.) was found a decrease (MA-14.85%; TFG-19.53%; PVP-18.18%) but also increase (GO-22.56%; VM-32.66%) in the concentration of glucose in comparison to control" [sic]. This information might be given more clearly, like – the application of extracts resulted in the decrease of glucose concentrations in most of the studied groups. Figures are also overloaded with information, moreover the legends to figures are complicated and have different group symbols than the tables. For instance, in the tables diabetic mice are indicated by STZ but in the figures diabetic mice are indicated by DM. It would be much easier to read the results chapter and understand the data presented here if the text and figures contained less information and the groups had homogeneous symbols. Nonetheless, my critical comments consider mainly technical disadvantages of the Results section which do not influence the scientific value of this work.

The discussion starts with the controversial statement that the most important achievement of the 20^{th} century is the consideration of the plant extracts as the source of biologically active components of particular spectrum of activity". The 20^{th} century had numerous important achievements, like antibiotics, immunization, insulin therapy and many, many other. Of course vitamins, alkaloids and other active compounds extracted from plants play significant role in different therapies but indicating them as the most important achievements of the 20^{th} century is just an overstatement. On the other hand, restrictions on the use of antibiotics in animal production makes the scientists study the alternative methods of animals health care. Thus, it is possible that the significance of plant extracts will increase in the future.

It must be stated here that this is the only controversial point of the discussion. The next parts of the discussion are well thought over and well-balanced. The discussion is divided into 3 subsections entitled as follows: influence of plant extracts on carbohydrate metabolism; influence of plant extracts on lipid metabolism; antioxidative influence of plant extracts. The author, using well-chosen literature, presents different possible mechanisms of active plant components' action on metabolism in diabetes. Hypoglycaemic effect is attributed to the inhibition of the activity of different enzymes decomposing polysaccharides; inhibition of the sorbitol synthesis; intensification of insulin release; stimulation of glycolyse enzymes; inhibition of gluconeogenesis; stimulation of glucose absorption by cells; inhibition of the intestine absorption of glucose; intensification of glycogen synthesis; stimulation of the immune system – mainly monocytes. The strongest hypoglycaemic effects were indicated after the application of *Trigonella foenum graecum* seeds extract which, according to the author, may be related to the presence of active compound 4-hydroxyisoleucine.

The tested extracts had also significant influence on the depletion of cholesterol and triglyceride concentrations in plant extracts. There are also different paths of fat metabolism which can be modified by active plant compounds. They include gene regulated expression of LDL receptors and the increase of the ability to absorb LDL by hepatic cells; decrease of the expression of genes coding triglyceride and cholesterol synthesis; decrease of the expression of adipogenic genes; decomposition of low density lipoproteins; lower lipid synthesis; stimulation of cholesterol metabolism and its transformation in liver to bile acids; decrease intestinal absorption of fatty acids.

A great achievement of the work is also the indication that plant extracts act as regulators on redox balance in diabetic animals. First, the author describes the mechanism mediating redox imbalance in diabetes as uncontrolled production of free radicals by polyol pathway stimulation and the decrease in enzymatic and non-enzymatic defence mechanisms. Next, the mechanism of protective action of plant extract is analysed. This mechanism is mainly related to large content of antioxidants like alkaloids, fatty acids, phenolic compounds, flavonoids, anthocyanins vitamins and microelements. There are also other mechanisms. For instance the author points the specific properties of trigonelline an active compound found in *Trigonella foenum graecum* seeds. Trigonelline has positive impact on the regeneration of pancreatic beta cells in the islets of Langerhans, stimulates insulin secretion, and activates enzymes engaged in glucose metabolism.

To summarize the assessment of the discussion it must be stated that the discussion chapter is well planned and well-written. Some parts of the discussion contain recurring

information. However, this was difficult to avoid because the studied plants contain similar groups of active compounds and only some of them, like trigonelline or 4-hydroxyisoleucine, have exceptional properties.

The discussion is followed by conclusions enclosed in separate section. Here, the author presents 6 detailed conclusions. Nonetheless, the general message is that plant extracts may be used in both the prevention of diabetes in patients of increased risk and attenuation of the complications occurring in diabetic patients. Among the tested supplements, the *Trigonella foenum graecum* seed extracts seem to be the most efficient in regulation of fat and carbohydrates metabolism and in the protection against oxidative stress.

The reviewer conclusion

The habilitation work titled "Antidiabetic and antioxidant potential of plant extracts in streptozotocin-induced diabetic mice" by dr Agnieszka Greń, PhD, concentrates on the important problem of potential use of herbal diet supplements in the prevention and treatment of diabetes. The author used appropriate methods and performed interesting discussion of the results which were followed by well formulated conclusions. The whole work presents high scientific quality, and fulfils the criteria for a habilitation thesis. I recommend the candidature of dr Agnieszka Greń, PhD, for further stages of the habilitation procedure.