Physiology and Pharmacology

Physiol Pharmacol 22 (2018) 11-18 **www.phypha.ir/ppj**

Original Article

Preventive effect of natural dietary supplement -Flavin7- on the onset of spontaneous diabetes mellitus in bio-breeding diabetes prone rats

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Abstract

Introduction: The aim of the present study was to evaluate the preventive effects of Flavin7 in prediabetic bio-breeding diabetes prone (BB-DP) rats.

Methods: Foutthy rats were divided into 2 equal groups: group C (untreated control group) and group F7 with Flavin7 (natural dietary supplement F7 with bioflavonoids, 0.2 mg/l) in drinking water from 21st day after birth to 171st day of their life, respectively. Blood glucose, superoxide dismutase, glutathione peroxidase, catalase, total antioxidant capacity, glutathione, body weight, food intake, water intake and urine output were determined.

Results: The age of diabetes onset was significantly higher for group F7 compared to group C (P<0.05). The incidence of diabetes was lower in group F7 than in group C. Blood glucose at the diabetes onset was higher in group C than in F7 group (P<0.05). Decrease of antioxidant status parameters, at the treatment onset as well as immediately after its termination showed a drop in the F7 group firstly, but increased progressively later, until the end of the experiment.

Conclusion: F7 delayed the development of diabetes in BB-DP rats and prevented its onset. The severity of diabetes mellitus was milder in rats treated with F7 than in control group.

Keywords:
Type 1 diabetes mellitus;
BB-DP rats;
Flavin7;
Prevention of diabetes

Received: 8 Jan 2018
Accepted: 24 Mar 2018

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Introduction

Type 1 diabetes represents 7-12% of all cases of diabetes mellitus and its prevalence is steadily rising (International Diabetes Federation, 2015). Affected individuals have to be insulin-injections treated for life (Greenbaum et al., 2017). This disorder is a consequence of an inflammatory infiltration of the pancreatic Langerhans islets with selective destruction of insulin-producing β-cells (Smith et al., 2017) by autoimmune mechanisms and subsequent inflammatory processes. There is a large amount of information on different mechanisms at cellular and molecular levels (e. g. oxidative stress) and β-cell apoptosis (Wagner, 2016). It is generally accepted that reactive oxygen species (ROS) contribute to autoimmune mediated pancreatic β-cell elimination and that a use of antioxidants could protect β-cells from damage leading to type 1 diabetes mellitus

(Lukačínová et al., 2008). However, there are contradictory views in the literature that consider antioxidants as compounds with no detectable benefits in autoimmune diabetes in NOD mice (Li et al., 2006). To avoid the ethical and logistical constraints resulting from the type 1 diabetes studies in the outbred human population exposed to chemical and microbiological agents (triggers) accidentaly, we have to rely on animal models that can easily be used for examination, biopsy and autopsy. These models are eligible for the study of heredity (including genetically modified animals) and for testing of their responses to environmental factors (Lukačínová et al., 2013). Therefore, bio-breeding (BB) rats are the most appropriate model for these studies (Mordes et al., 2004). Antioxidants used in systemic therapy delay the onset of type 1 diabetes (Asmat et al., 2016; Hoffman, 2014; Kaneto et al., 2007). Despite many studies, type 1 diabetes remains resistant to prevention (Skyler et al., 2002), except of unacceptable toxic immunosuppression (Parving et al., 1999). Prevention of diabetes mellitus is an urgent issue not only for medicine, but also for whole human society at the beginning of this millennium. Epidemic growth, devastating complications, enormous expenses on a healthcare related and other arguments confirm the necessity of prevention.

Many studies regarding hypoglycaemic and hypolipidemic effects of various flavonoids have been reported with administration of bioflavonoids to diabetic animals (Aarland et al., 2017; Cazarolli et al., 2006; Roslan et al., 2017), but also to humans (Geng et al., 2007; Williamson, 2017). The basic mechanism of antidiabetic effect of flavonoids is the suppression of oxidative stress (Ghosh and Konishi, 2007; Mehta et al., 2006). Increased oxidative stress and impaired nitric oxide synthesis are mechanism likely to play a major role in the pathogenesis of vascular complications of diabetes (Sudnikovich et al., 2007).

Various natural dietary bioflavonoid-based supplement are used in order to improve health support and to increase a quality of life in many countries currently. One of these natural-compound supplements is Flavin7 (F7) a bioflavonoid-containing dietary supplement with potential antioxidant activity, composed of the extracts from seven different fruits in which a suspected influence on glucose homeostasis regulation, chronic diseases, mainly oncologic is hypothesized (Kello et al., 2017). Besides that, F7 has also antiinflammatory and antivirotic effects.

Therefore, the aim of the present study was to verify the F7 preventative effect on autoimmune diabetes mellitus in pre-diabetic bio-breeding diabetes prone (BB-DP) rats, which is very similar to human type 1 diabetes.

Materials and methods

Animals

We used 40 rats of BB-DP, 21-day-old males (weighing 55-69 g) from fy Møllegaard (donated by this company for Professor Korec). The experiment protocol was designed to minimize pain and discomfort of animals. The rats were bred under specific pathogen-free conditions in Central Animal House of Medical Faculty at University of Pavol Jozef Šafárik. All aspects of animal care complied with the ethical guidelines, technical requirements and were approved by the Institutional Animal Ethics Committee and the State Veterinary and Food Administration of Slovak Republic (ŠVPS SR No. Ro-2806/05-221/e).

Animals were housed individually in glass-bottomed metabolic cages in an animal facility with controlled environment (22 ±2 °C, humidity 60 ±5%, 12 h light: 12 h dark cycle) with free access to a standard commercial diet and water *ad libitum*.

Experimental design and animal grouping

The subjects were randomly divided into two groups at the start of the experiment (21st day after birth): control group (C) of rats (n = 20) received clean drinking water and experimental group (F7) of rats (n = 20) received drinking water containing Flavin7® (Vita Crystal Ltd., Budapest, Hungary, dietary supplement, flavonoid concentrate, fruit extract made by special molecular separation of seven fruits: blackcurrant, redcurrant, black cherry, elderberry, plum and blackthorn at a high concentration of 22 mg/ml of polyphenols with the following flavonoids' composition: myricetin, quercetin, kaempferol, rutin, isorhamnetine, catechin, epicatechin, malvidin-3-glucoside, caffeic acid, chrysin, galangin, apigenin, fisetin, luteolin and morin, as well as several anthocyanidins and stilbene resveratrol /trans-form, cis-form and glycosides. Nutritional value to 10 ml of solution: 9.7 kJ, proteins 0.07 g, saccharides 0.16 g, lipids 0.02 g, flavonoids 170 mg and alcohol 0.8 %

v/v) at a final concentration of 0.2 mg/l.

The solution was prepared each day anew. Animals received drinking water (treated and untreated) *ad libitum*. Both groups were fed the same standard diet. Experiment took 150 days (until the 171st day of life of experimental animals).

From the beginning to the end of the experiment food and water intake as well as urine expenditure were monitored on a daily basis. Glycemia was assessed every other day. The weight of the animal was monitored once a week. Rats were considered diabetic when they had glycemia >12 mM in two follow-up studies (Ništiar et al., 1999).

Biochemical assays

Blood was collected from tail vein in the morning and centrifuged at 1000 g for 10 minutes. The erythrocytes were washed three times with ice saline and stored at -20 °C until assays were performed. The glucose level was determined by the glucose oxidase-peroxidase enzymatic method (Lab test Set for Glucose, BioLaTest, Lachema, Czech Republic).

The activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) in erythrocytes were determined in hemolysates using commercial kits (Randox Laboratory, UK). The erythrocytes were hemolyzed by addition of ice deionized water and homogenized. Determination of SOD (E.C.1.15.1.1) was based on the principle of superoxide radical formation (Duzguner a Kaya, 2007). The activity of erythrocyte GPx (E.C.1.11.1.9) was determined by Paglia and Valentine (1967). The activity of erythrocyte catalase (E.C.1.11.1.6) was determined by the Aebi method (Aebi, 1984).

The concentration of glutathione (GSH) in full blood was determined by the method of Beutler et al. (1963). GSH was assessed as a total plasma glutathion (GSH + 2 x GSSG) by enzymatic method regarding glutathion reductase catalysis and 5,5´-dithiobis-(2-nitrobenzoic acid) as an indicator (Sigma-Aldrich, Germany). Total antioxidant capacity (TAC) of plasma was determined according to Miller et al. (1993) on a Cobas Mira automatic analyzer (Roche, Switzerland) using a commercial kit Total Antioxidant Status (Randox Laboratories, UK).

Statistical analysis

The data are presented as mean±SD. Statistical comparisons were made by one-way analysis of variance (ANOVA) and followed by Student-Neuman-Keuls as the post hoc test. Data were considered statistically significant if *P* values were <0.05.

Results

Age in the diabetes mellitus onset

The age of animals at onset of diabetes was significantly higher in the F7 group (135±8 days) than in the C group (98±9 days; *P*<0.05).

Survival during the trial and diabetes mellitus incidence

The survival was higher in the F7 group (95%) versus C group (65%), while the incidence of diabetes mellitus was lower in the F7 group compared to the C group (10 % in the F7 group vs. 65% in the C group).

Levels of glycemia

Blood glucose levels in non-diabetic subjects (Table 1) increased slowly in the F7 group (from 5.67±0.15 to 7.74±0.87 mmol/l), similar to group C (from 5.58±0.07 mmol/l to 9.40±0.35 mmol/l), this difference was statistically significant at *P*<0.05 (Table 1). The difference in blood glucose between all rats in F7 and C groups (15.94±7.87 mmol/l vs. 8.03±1.51 mmol/l) was statistically significant (*P*<0.05). In diabetic individuals, the difference in levels of glycemia was not statistically significant between F7 and C groups.

Development of body weight, food intake, water intake and urine output

The body weight of animals that later developed diabetes was different already in the pre-diabetic period (Table 2). In both F7 and C groups, individuals who developed diabetes had a slightly higher body weight at 51st day of age. Until this time, significant differences in weight were not found between groups. Two weeks before the onset of diabetes, there was a significant weight loss in subjects who developed diabetes mellitus later, compared to animals that did not develop diabetes. From day 81, treated groups had significantly higher weights in comparison to untreated controls (*P*<0.05). From day 81 of the experiment, there were significant differences between the body weight of diabetic and non-diabetic subjects in both groups (*P*<0.05).

In the assessment of food intake, water intake and

Table 1: Glycemia (mmol/l) in BB-DP rats

GROUP		AGE (IN DAYS)					
		21	51	81	111	141	171
C	All	5.7±0.1	6.9±0.2	7.4±0.6	14.0±5.6	15.8±6.3	15.9±7.9
	D	5.7±0.1	7.0±0.2	7.6±0.6	17.1±4.4$^{\#}$	20.2±3.3$^{\#}$	23.6±4.3$^{\#}$
	ND	5.6±0.1	6.9±0.2	7.0±0.2	8.1±0.4	8.7±0.3	9.4±0.4
F7	All	5.7±0.1	6.9±0.2	7.1±0.5	7.8±1.2*	9.4±2.5*	8.0±1.5*
	D	5.6±0.1	6.9±0.2	7.6±0.9	10.5±0.9*$^{\#}$	15.1±2.0*$^{\#}$	13.2±5.1*$^{\#}$
	ND	5.7±0.2	6.9±0.2	7.1±0.4	7.5±0.8	7.6±0.9	7.7±0.9

Data are presented as mean±SD (n=20/group). D = diabetic, ND = non diabetic. *P< 0.05 *vs.* the corresponding subgroup of C group. $^{\#}P$< 0.05 compared to corresponding ND subgroup.

Table 2: Body weight (g) in BB-DP rats

GROUP		AGE (IN DAYS)					
		21	51	81	111	141	171
C	All	62±3	148±4	203±25	223±56	247±86	301±128
	D	62±2	150±2	187±16	185±22$^{\#}$	181±18$^{\#}$	167±007$^{\#}$
	ND	61±4	145±5	231±02	294±04	351±04	415±003
F7	All	62±3	149±4	232±04*	288±21*	335±43*	402±049*
	D	62±1	152±2	231±01*	226±06*$^{\#}$	210±14*$^{\#}$	200±002*$^{\#}$
	ND	62±3	148±4	232±04	294±03	349±03	413±006

Data are presented as mean±SD (n=20/group). D = diabetic, ND = non diabetic. * < 0.05 *vs.* the corresponding subgroup of C group. $^{\#}P$< 0.05 compared to corresponding ND subgroup.

Table 3: Activity of superoxide dismutase (U/mg of hemoglobin) BB-DP rats

GROUP	AGE (IN DAYS)					
	21	51	81	111	141	171
C	13.9 ±2.5	14.2 ±2.8	14.6 ±3.1	10.3 ±2.1	9.8 ±2.3	6.6 ±1.9
F7	13.3 ±1.8	14.0 ±2.9	15.9 ±3.3	16.6 ±3.3*	15.8 ±2.2*	16.2 ±3.0*

Data are presented as mean±SD (n=20/group). *P< 0.05 compared to C group.

Table 4: Activity of glutathione peroxidase (U/ml) in BB-DP rats

GROUP	AGE (IN DAYS)					
	21	51	81	111	141	171
C	26.4 ±4.0	25.5 ±3.2	27.1 ±2.8	17.0 ±1.7	13.1 ±1.3	6.8 ±0.5
F7	29.6 ±2.1*	24.8 ±1.9	23.7 ±1.8*	23.3 ±1.7*	22.9 ±1.8*	21.2 ±1.1*

Data are presented as mean±SD (n=20/group). *P< 0.05 compared to C group.

Table 5: Activity of catalase (U/ml) in BB-DP rats

GROUP	AGE (IN DAYS)					
	21	51	81	111	141	171
C	1.8 ±0.2	2.1 ±0.3	2.6 ±0.4	1.5 ±0.5	1.1 ±0.5	0.8 ±0.5
F7	1.8 ±0.2	2.2 ±0.4	2.4 ±0.3	2.5 ±0.3*	2.5 ±0.6*	2.5 ±0.4*

Data are presented as mean±SD (n=20/group). *P< 0.05 compared to C group.

Table 6: Levels of glutathione (μM) in BB-DP rats

GROUP	AGE (IN DAYS)					
	21	51	81	111	141	171
C	17.2 ±1.0	19.2 ±1.8	16.6 ±2.0	11.2 ±2.0	9.9 ±2.9	4.8 ±2.0
F7	17.2 ±1.0	18.8 ±1.9	20.8 ±2.4*	22.2 ±2.1*	21.8 ±1.9*	21.1 ±1.5*

Data are presented as mean±SD (n=20/group). *P< 0.05 compared to C group.

Table 7: Levels of TAC (mM) in BB-DP rats

GROUP	AGE (IN DAYS)					
	21	51	81	111	141	171
C	1.2 ±0.2	1.2 ±0.3	1.1 ±0.4	0.8 ±0.3	0.7 ±0.5	0.4 ±0.3
F7	1.2 ±0.2	1.3 ±0.2	1.3 ±0.2*	1.3 ±0.2*	1.3 ±0.2*	1.3 ±0.2*

Data are presented as mean±SD (n=20/group). *P< 0.05 compared to C group. TAC = Total Antioxidant Capacity

urine output, differences between groups were significant (P<0.05). All the variables were higher in group C than in F7 group. Similarly, there were significant differences between diabetic and non-diabetic animals within groups (P<0.05). Food and water intake, as well as urinary excretion were higher in diabetic subjects. In both F7 and C groups, the comparison of non-diabetic and diabetic subjects has shown statistically not significant differences.

Development of indicators of antioxidant status in BB rats

The antioxidant status parameters were significantly higher in the F7 group compared to C group (P<0.0001) especially because of differences between diabetic and non-diabetic animals (P<0.0001). The results are shown in Tables 3 – 7.

Discussion

In this work we demonstrated the effect of long-term (150 days) bioflavonoid administration on reduction of the incidence of diabetes mellitus (from 65% to 10%) and delay of the onset of diabetes (from 98±9 to 134±9 days) in BB-DP rats.

Preventive administration of F7 significantly reduced (P<0.05) gylcemia levels in diabetic BB-DP rats. Decrease of glycemia levels was detected also in non-diabetic rats after administration of F7, although it was not significant compare to untreated non-diabetic rats (Table 1). This fact points to finding that oral consumption of F7 is one of the most effective ways of affecting pancreatic β-cells and treating diabetes. Our results showed that F7 has high antioxidant activities by the ability to scavenge free radicals and chelate metals. Given the hypothesized relation between diabetes and inflammation and the potential for F7 to protect the body against free radicals and other pro-oxidative compounds, it is plausible that consumption of F7 or flavonoid-rich dietary supplements may reduce the risk of diabetes onset. Tables 3 to 7 confirm, that after F7 application

per os a significant increase (P<0.05) of all parameters assessed in this experiment occurs. New concepts appeared regarding this trend, such as nutritional therapy with nutraceuticals, and phytotherapy with phytonutrients. This functional foods and phytomedicines play positive roles in maintaining blood glucose levels, glucose uptake and insulin secretion, as well as modulating immune function to prevent of diabetes mellitus (Vinayagam and Xu, 2015).

Presented findings significantly confirm the preventive effect of bioflavonoids on spontaneous diabetes in BB-DP rats. Pancreatic β-cell failure is a major feature of both type 1 diabetes and the type 2 diabetes end stage. Type 1 diabetes is induced by pancreatic β-cell destruction due to the autoimmune mechanism and the inflammatory processes. Langerhans islets are infiltrated by macrophages, natural killer cells and cytotoxic T cells (In't Veld and Klöppel, 2016), which produce various cytokines leading to generation of ROS and oxidative stress, which is critical for β-cell destruction (Tomita, 2017). Cytokines, especially pro-inflammatory such as tumor necrosis factor-α and interleukin-1, are cytotoxic to β-cells and induce an inflammatory cascade leading to necrosis and β-cell apoptosis.

From the view of pathophysiology, the destructive effects of ROS play a significant role in a transient burst with the formation of small amounts of cellular ROSs, leading to the modulation of some cytokines and hormones, including insulin-regulated signal transduction pathways (Kang et al., 2008). In BB-DP rats, an onset of diabetes usually occurs between 60th and 120th days of age (Mordes et al., 2001). According to our results, the onset of diabetes mellitus shows a strong correlation with the decrease of antioxidant status parameters in BB-DP rats. These findings support the theory that modulation of the antioxidant status may have a significant preventive effect on the occurrence of autoimmune type diabetes not only in BB rats (Rajput and Sarkar, 2017; Redan et al., 2016; Strzyga-Lach and Czeczot, 2016). Numerous data available in the literature demonstrate the preventive effect of suppressing oxidative stress in various pathogenic processes (Steven et al., 2017).

In our opinion, the underlying cause of diabetes mellitus induction is the disruption in the expression of genes coding endogenous antioxidant enzymes and molecules, either due to random mutations, epigenetic mechanisms or lack of certain growth factors, resulting in a "gain of function" or "loss of function" changes of intracellular defense mechanisms which differ altered cells from unaltered. In the presence of a diabetes inducer and subsequent oxidative stress, metabolic reconfiguration represents a regulated response to oxidative stress (Grant, 2008) and the modulation of this reconfiguration may be crucial for organism protection. When assessing antioxidant stress parameters, the central position has a reduced glutathione (García-Giménez et al., 2017). Changes of individual parameters of antioxidant protection should always be assessed in accordance with the overall state of the organism. Particularly important is the fact that protectively used substances which modify glycemia simultaneously modulate mechanisms of antioxidant defense of the organism (Khan et al., 2009).

It will be very important to determine the dose portions of the individual antidiabetogenic agents, especially those used in the mixture regarding the effective modulation of the normal expression of the target cell genome. Similarly, the chemical instability of some polyphenols during storage, extraction or analysis as well as potential enzyme hydrolysis and metabolite conversion are important for identifying bioactive forms of polyphenol compounds *in vivo* (Williamson, 2017). In addition, it should be discussed about minerals and trace elements with the possible beneficial effects of such components, which could be contained in natural dietary supplements.

The molecular mechanisms underlying the glucose metabolism in diabetes would provide new insights in the field of drug development, continue to fuel excitement in this area of research and buoys the hope that future discoveries may one day yield therapeutic benefits (Vinayagam and Xu, 2015). With the rapidly increasing incidence of diabetes worldwide, there is a greater need for safe and effective functional biomaterials with antidiabetic activity and potential preventive effects against diabetes mellitus.

Conclusion

The results of this study demonstrated the hypoglycemic and antidiabetic efficacy of F7 that had

a significant effect on antioxidant status and onset of spontaneous diabetes mellitus, which can not be ignored. On the other hand, further studies are needed to clarify the possible mechanisms of their effect, to determine complete defense profiles as well as the potential values of these compounds in the management and prevention of diabetes mellitus in clinical practice. Hence, meticulously intended human studies are needed to further measure the likely connected with a number of nutritional flavonoids to treat diabetes and its complications.

Acknowledgments

This research was supported by the Grant Agency of the Ministry of Education, Slovak Republic (VEGA 1/3494/06). The authors express their thanks to Agnesa Ništiarová DVM for the animal care and Mr. Martin Kurpas for technical assistance.

Conflict of interest

The authors declare that none of them have any conflicts of interest with the contents of this article.

References

Aarland RC, Bañuelos-Hernández AE, Fragoso-Serrano M, Sierra-Palacios ED, Díaz de León-Sánchez F, Pérez-Flores LJ, et al. Studies on phytochical, antioxidant, anti-inflammatory, hypoglycaemic and antiproliferative activities of Echinacea purpurea and Echinacea angustifolia extracts. Pharm Biol 2017; 55: 649-56.

Aebi H. Catalase in vitro. Methods Enzymol 1984; 105: 121-6.

Asmat U, Abad K, Ismail K. Diabetes mellitus and oxidative stress – a concise review. Saudi Pharm J 2016; 24: 547-53.

Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Med 1963; 61: 882-8.

Cazarolli LH, Zanatta L, Jorge AP, de Sousa E, Horst H, Woehl VM, et al. Follow-up studies on glycosylated flavonoids and their complexes with vanadium: their anti-hyperglycemic potential role in diabetes. Chem Biol Interact 2006; 163: 177-91.

Duzguner V, Kaya S. Effect of zinc on the lipid peroxidation and the antioxidant defense systems of the alloxan-induced-diabetic rabbits. Free Radic Biol Med 2007; 42: 1481-6.

García-Giménez JL, Romá-Mateo C, Pérez-Machado G, Peiró-Chova L, Pallardó FV. Role of glutathione in the regulation of epigenetic mechanisms in disease. Free Radic Biol Med 2017; 112: 36-48.

Geng P, Yang Y, Gao Z, Yu Y, Shi Q, Bai G. Combined effect of total alkaloids from Feculae bombycis and natural flavonoids on diabetes. J Pharm Pharmacol 2007; 59: 1145-50.

Ghosh D, Konishi T. Anthocyanins and anthocyanins-rich extracts: role in diabetes and eye function. Asia Pac J Clin Nutr 2007; 16: 200-8.

Grant CM. Metabolic reconfiguration is a regulated response to oxidative stress. J Biol 2008; 7: 1.

Greenbaum C, Lord S, VanBuecken D. Emerging concepta on disease-modifying in type 1 diabetes. Curr Diab Rep 2017; 17: 119.

Hoffman RP. Vascular endothelial dysfunction and nutritional compounds in early type 1 diabetes. Curr Diabetes Rev 2014; 10: 201-7.

International Diabetes Federation: IDF Diabetes Atlas. 7th Edition, ISBN 978-2-930229-81-2, Brussels, International Diabetes Federation, 2015; p. 1-140.

In't Veld P, Klöppel G. Insulitis in type 1 diabetes. Pathologe 2016; 37: 245-52.

Kaneto H, Katamaki N, Kawamori D, Miyatsuka T, Sakamoto K, Matsuoka TA, et al. Involvement of oxidative stress in the pathogenesis of diabetes. Antioxid Redox Signal 2007; 9: 355-66.

Kang MA, Mott TM, Tapley EC, Lewis EE, Luckhart S. Insulin regulates aging and oxidative stress in Anopheles stephensi. J Exp Biol 2008; 211: 741-8.

Kello M, Kulikova L, Vaskova J, Nagyova A, Mojzis J. Fruit peel polyphenolic extract-induced apoptosis in human breast cancer cells is associated with ROS production and modulation of p38MAPK/Erk1/2 and the Akt signaling pathway. Nutr Cancer 2017; 69: 920-31.

Khan I, Ahmad A, Iqbal M. Modulation of antioxidant defence system for arsenic detoxification in Indian mustard. Ecotoxicol Environ Saf 2009; 72: 626-34.

Li X, Chen H, Epstein PN. Metallothionein and catalase senzitize to diabetes in nonobese diabetic mice. Reactive oxygen species may have a protective role in pancreatic β-cells. Diabetes 2006; 55: 1592-604.

Lukačínová A, Mojžiš J, Beňačka R, Keller J, Maguth T, Kurila P, et al. Preventive effects of flavonoids on alloxan-induced diabetes mellitus in rats. Acta Vet Brno 2008; 77: 175-82.

Lukačínová A, Hubková B, Rácz O, Ništiar F. Animal models for study of diabetes mellitus. In: Oguntibeju OO (Ed.): Diabetes mellitus - insights and perspectives. Chapter 13, ISBN 978-953-51-0939-6, Rijeka, InTech, 2013; p. 229-54.

Mehta JL, Rasouli N, Sinha AK, Molavi B. Oxidative stress in diabetes: a mechanistic overview of its effects on atherogenesis and myocardial dysfunction. Int J Biochem Cell Biol 2006; 38: 794-803.

Miller JK, Brzezinska-Slebodzinska E, Madsen FC. Oxidative stress, antioxidants, and animal function. J Dairy Sci 1993; 76: 2812-23.

Mordes JP, Bortell R, Groen H, Guberski DL, Rossini AA, Greiner DL. Autoimmune diabetes mellitus in the BB rat. In: Sima AAF, Shafrir E (Eds.): Animal models of diabetes: a primer. Warsaw: Harwood Academic Publishers, 2001; p 1-41.

Mordes JP, Bortell R, Blankenhorn EP, Rossini AA, Greiner DL. Rat models of type 1 diabetes: genetics, environment, and autoimmunity. ILAR J 2004; 45: 278-91.

Ništiar F, Kováč G, Rácz O, Seidel H, Ništiarová A. Effects of vanadium on glucose homeostasis in diabetic and non-diabetic BB rats. Czech J Anim Sci 1999; 44: 547-50.

Paglia DE, Valentine WN. Studies on quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967; 70: 158-69.

Parving HH, Tarnow L, Nielsen FS, Rossing P, Mandrup-Poulsen T, Osterby R, et al. Cyclosporine nephrotoxicity in type 1 diabetic patients: A 7-year follow-up study. Diabetes Care 1999; 22: 478-83.

Rajput MS, Sarkar PD. Modulation of neuro-inflammatory condition, acetylcholinesterase and antioxidant levels by genistein attenuates diabetes associated cognitive dechne in mice. Chem Biol Interact 2017; 268: 93-102.

Redan BW, Buhman KK, Novotny JA, Ferruzzi MG. Altered transport and metabolism of phenolic compounds in obesity and diabetes: Implications for functional food development and assessment. Adv Nutr 2016; 7: 1090-104.

Roslan J, Giribabu N, Karim K, Salleh N. Quercetin ameliorates oxidative stress, inflammation and apoptosis in the heart of streptozotocine-nicotinamide-induced adult male diabetic rats. Biomed Pharmacother 2017; 86: 570-82.

Skyler JS, Brown D, Chase HP, Collier E, Cowie C, Eisenbarth GS, et al. Effects of insulin in relatives of patients with type 1 diabetes mellitus. N Engl J Med 2002; 346: 1685-91.

Smith MJ, Simmons KM, Cambier JC. B cells in type 1 diabetes mellitus and diabetic kodney disease. Nat Rev Nephrol 2017; 13: 712-20.

Steven S, Daiber A, Dopheide JF, Münzel T, Espinola-Klein C. Peripheral artery dinase, redox signaling, oxidative stress – basic and clinical aspects. Redox Biol 2017; 12: 787-97.

Strzyga-Łach P, Czeczot H. The role of flavonoids in the modulation of inflammation. Pol Merkur Lekarski 2016; 40: 134-40.

Sudnikovich EJ, Maksimchik YZ, Zabrodskaya SV, Kubyshin VL, Lapshina EA, Bryszewska M, et al. Melatonin attenuates metabolic disorders due to streptozotocin-induced diabetes in rats. Eur J Pharmacol 2007; 569: 180-7.

Tomita T. Apoptosis of pancreatic β-cells in type 1 diabetes. Bosn J Basic Med Sci 2017; 17: 183-93.

Vinayagam R, Xu B. Antidiabetic properties of dietary flavonoids: a cellular mechanism review. Nutr Metab (Lond) 2015; 12: 60.

Wagner DH. Of the multiple mechanisms leading to type 1 diabetes, T cell receptor revision may play a prominent role (is type 1 diabetes more than a single disease?). Clin Exp Immunol 2016; 185: 271-80.

Williamson G. The role of polyphenolsin modern nutrition. Nutr Bull 2017; 42: 226-35.