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# PREVENTIVE EFFECT OF BIOFLAVONOIDS ON THE ONSET OF *DIABETES MELLITUS* IN BB RATS

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## ABSTRACT

We investigated the effect of bioflavonoid treatment during pre-diabetic period on the prevention of diabetes in the diabetic-prone BB rats. Rats at the 3 week age were treated with Flavin 7® (0.2 mg bioflavonoids [mix of quercetin and rutin]/l of drinking water) for 150 days. We measured the body weight, food intake, drinking water intake, urine output, glycaemia, superoxid dismuthase, glutathione peroxidase, glutathione and total antioxidant status. The treatment with flavonoids had a significant effect ($P<0.001$) on the delaying the onset of diabetes, the survival of rats, and reducing the incidence of diabetes occurrence. The treated animals showed a lower glycaemia rate ($P<0.001$) and achieved a higher body weight ($P<0.05$). The parameters of antioxidant protection were significantly better ($P<0.001$). The treatment with flavonoids almost completely prevented the onset of diabetes and enhanced beta-cell function, what resulted in maintaining the islet cell changes and significant antioxidant effects.

Key words: *diabetes mellitus*; prevention; flavonoids; antioxidant status; BB rats

## INTRODUCTION

Type 1 diabetes comprises <10% of all *diabetes mellitus* and its prevalence is increasing (Green et al., 2001). Affected individuals require lifelong injections of insulin for survival (Rossini et al., 2003). The disease cause an inflammatory infiltration of the islets of Langerhans (insulitis) and selective destruction of insulin-producing β-cells (Atkinson and Eisenbarth 2001), which is mediated by an autoimmune mechanism and consequent inflammatory process. Abundant information is available on the involvement of various cellular and molecular mechanisms (e.g. oxidative stress) in β-cell injury usually by apoptosis (Pearl-Yafe et al., 2007). It is generally assumed that reactive oxygen species (ROS) contribute to autoimmune-mediated pancreatic β-cell destruction and that application of antioxidants would benefit β-cell survival in type 1 diabetes. Benefit effect of therapeutic application of antioxidants was documented on the model of spontaneously diabetic BB rats (Kaneto et al., 2007) and human (Geng et al., 2007).

The prevention of *diabetes mellitus* is an urgent necessity for medicine and the human society at the beginning of this millennium. Epidemic growth, devastating complications, huge costs and other arguments support the above assertion (Cheta, 1999). Prevention projects for type 1 diabetes have a strong experimental basis. There is a great interest in orally active insulin-mimetics, particularly vanadium (Cam et al., 2000) and zinc compounds (Vančo et al., 2009) and various bioflavonoids (Mehta et al., 2006; Ghosh and Konishi, 2007).

In this study, we have attempted to evaluate the presumed preventive effect of bioflavonoids in pre-diabetic BB-DP rats, which spontaneously develop autoimmune *diabetes mellitus*, closely resembling human type 1 diabetes.

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## MATERIAL AND METHODS

**Animals and experimental design:** We used 40 Bio-Breeding Diabetes Prone (BB-DP) male rats 21 days after birth, provide by fy Mřllegaard. They were bred in specific pathogen-free conditions at the Central Animal Laboratory of Medical Faculty in Košice, Slovak Republic. Experiments were performed in the Central Animal Laboratory of Medical Faculty with accreditation for laboratory animal-breeding and experimentation. The experiments were approved by the local Ethical Commission and the State Veterinary Agency (ŠVPS SR Č.k. Ro-2806/05-221/e). Animals were fed with standard Larsen chow and tap water (without or with bioflavonoids) *ad libitum* and were kept in a temperature-controlled (22±2 °C) and humidity-controlled (55±5 %) room with a 12 h – 12 h light-dark illumination cycle and were housed in individual whole-glass metabolic cages.

The animals were randomly divided into two groups at the start of the experiment:

1.Control group (C) of rats (n=20) received during the experiment untreated drinking water.

2.Experimental group (BF) of rats (n=20) received during the experiment drinking water treated with Flavin 7® (Vita Crystal, Hungary; nutritive additive, concentrate of flavonoids, extract from fruits, made with special molecular separation from seven fruit species: black currant, red currant, cherry, grape, plum, apple, and brambleberry, contained quercetin and rutin at high concentration 22 000 mg/l) at final concentration of 0.2 mg/l.

The solutions were replaced every day. The animals were allowed to drink the solution *ad libitum*. All the groups received the same standard nutrition and stayed at the same living conditions for the 150-day period (to the 171 day age).

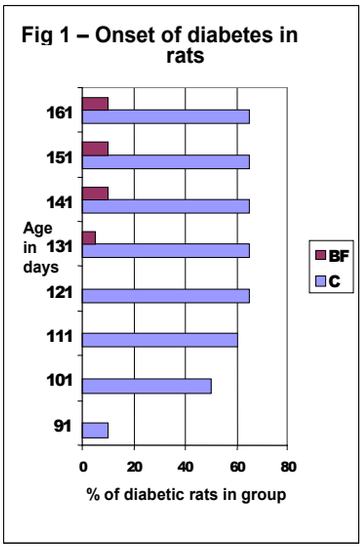
Intake of food and drinking water and urine output were daily measured from the start to the end of experiments. Weekly determined parameters were: the body weight, glycaemia, superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), glutathione (GSH) and total antioxidant status (TAS). Rats were diagnosed as diabetic on the basis of plasma glucose concentration (>12 mmol/l) measured at two consecutive occasions.

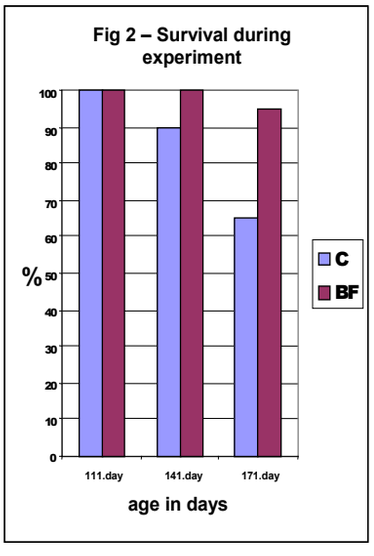
**Biochemical methods. The b**lood was collected from the tail vein at morning and then was centrifuged at 1 000 *g* for 10 min. Blood plasma was separated and buffy coat was removed by the aspiration. Erythrocytes were washed three times with cold ~~physiological~~ saline and stored at –20°C until analysis. SOD, GPx and CAT activities in erythrocytes, GSH and total antioxidant status (TAS) of blood plasma, respectively, were evaluated using commercially available kits (Randox Laboratory, UK).

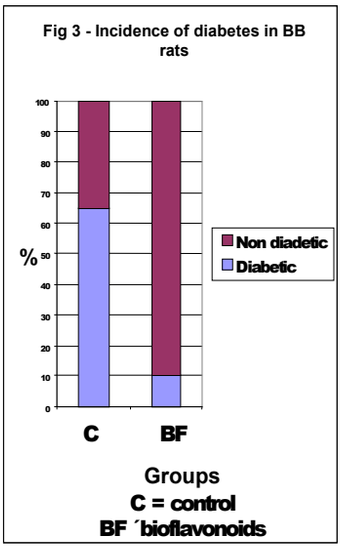
**Statistical analysis**. The data are presented as the mean ± SEM. Statistical comparisons were done using one-way analysis of variance (ANOVA) and followed by Student-Neuman-Keuls as the post-hoc test. Data were considered as statistically significant when P values were lower than 0.05.

## RESULTS AND DISCUSSION

The age of the diabetes onset was significantly elongated for the BF group (134±8.5 days compared to the control group, 98±9.0 days, P<0.001; Fig. 1). Mean age of the onset of overt clinical diabetes in BB rats ranged from 80 to 120 days, depending on sub line. Survival rate was higher (Fig. 2), and the incidence of diabetes was lower (Fig. 3) in the rats treated with bioflavonoids (10%), than in rats from the control group (65%).



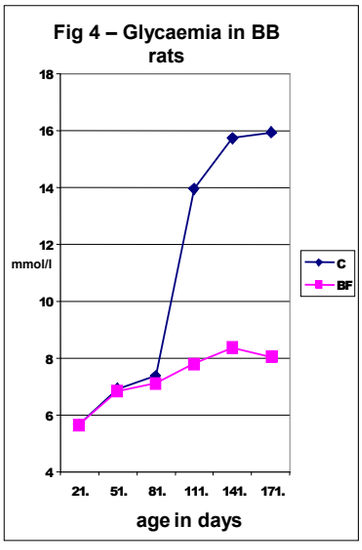



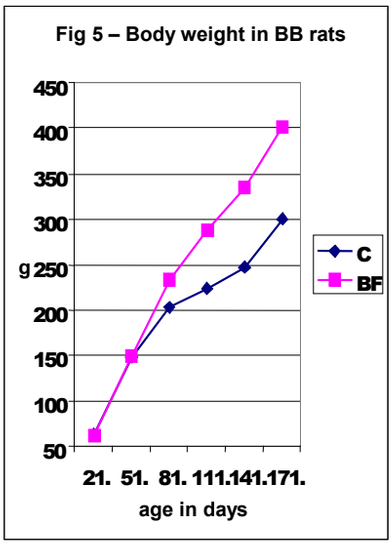



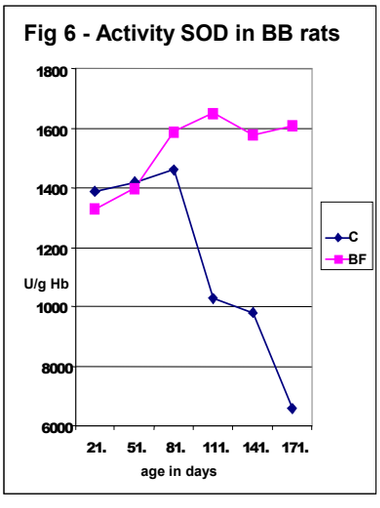
The levels of blood glucose in non-diabetic rats during the experiment increased slowly with age in the group treated with bioflavonoids (from 5.67±0.15 to 7.74±0.87 mmol/l), and this increase was lower ($P<0.05$) compared to the C group (from 5.58±0.07 mmol/l to 9.40±0.35 mmol/l). In the diabetic rats, the blood glucose at the onset was higher in the group C (17.1±4.4 mmol/l) than in the BF-treated group (10.5±0.9 mmol/l); the difference between BF and C was significant at $P<0.001$ (Fig. 4).

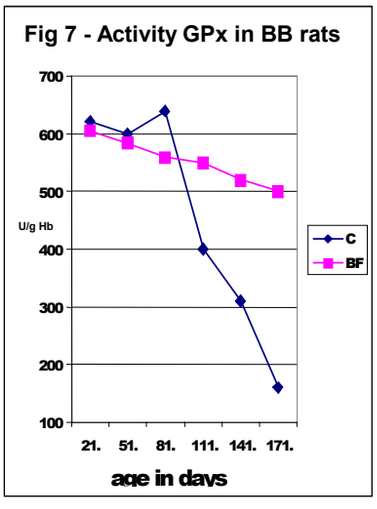
Body mass (Fig. 5) was affected already in pre-diabetic period depending on the time of onset of overt diabetes. Animals prior to the diabetes onset had a highest body mass at 51st week of the experiment. Within this period no differences between groups were observed. Within two weeks preceding the diabetes onset, the rats from both groups lost in body weight compared with non-diabetic animals. On the 81st day significantly higher body weights were recorded in BF group when compared to the control group ($P<0.05$). After 81st day, significant differences ($P<0.001$) were recorded between groups of diabetic and non-diabetic rats.

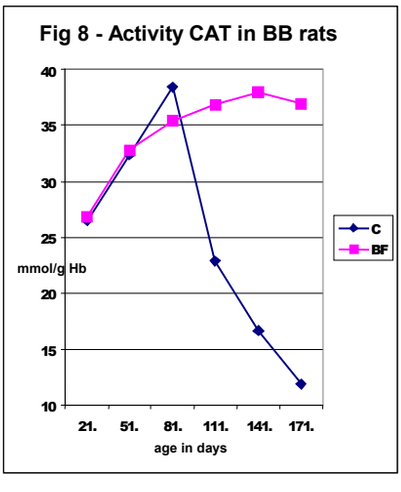
Parameters of antioxidant defense are presented on the Figs 6-10. Results demonstrated significant preventive effect after the treatment with bioflavonoids ($P<0.001$).

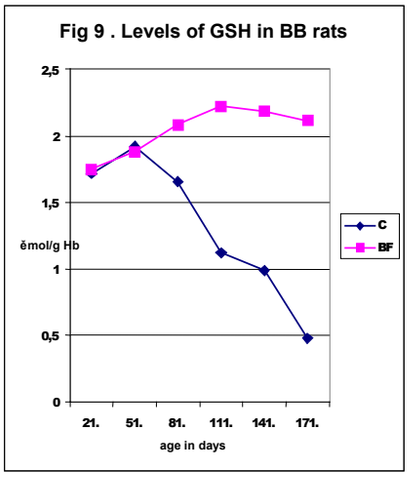


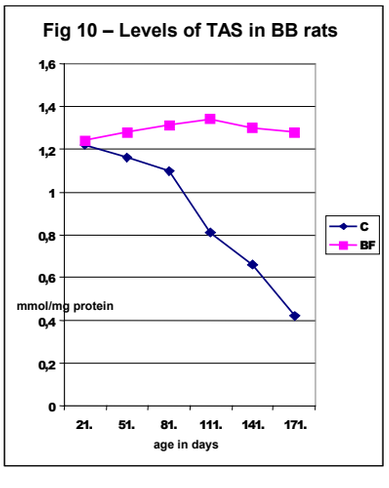




These results convincingly confirm preventive effect of application of bioflavonoids on the onset of spontaneous *diabetes mellitus* in BB rats. Pancreatic β-cell failure is the common characteristic of the type 1 and type 2 diabetes. The type 1 diabetes is induced by pancreatic â-cell destruction, which is mediated by autoimmune

mechanism and consequent inflammatory process. The Langerhans' islets are infiltrated by macrophages, natural killer cells and cytotoxic cells; various cytokines and oxidative stress produced by islet-infiltrating immune cells have been proposed to play an important role in mediating the destruction of â-cells (Kaneto et al., 2007). Cytokines, particularly the pro-inflammatory ones, such as TNF-α and IL-1, are cytotoxic for â-cells and induce the inflammatory cascade leading to necrosis and apoptosis of â-cell (Salden, 2000). The JNK pathway is also activated by such cytokines and oxidative stress and is involved in the â-cell destruction. Taken together, it is likely that oxidative stress and subsequent activation of the JNK pathway are involved in the pathogenesis of type 1 and type 2 diabetes (Kaneto et al., 2007).

In addition to the pathophysiological destructive actions of ROS, transient bursts of small amounts of cellular ROS have been recognized to play an important role in the action of various growth factors, cytokines, and hormones, including insulin-regulated signal transduction pathways (Kang et al., 2008).

Our study demonstrates that modulation of antioxidant defense mechanisms prior to onset of *diabetes mellitus* has a basic importance for delayed onset of diabetes and their moderate course.

We hypothesize that the basic cause of the diabetes induction is a disorder (disbalance) in a part of the genome encoding endogenous antioxidant enzymes and molecules, either through random mutations or deprivation of some growth factors. These imbalances may cause changes in intracellular function in term of "gain of function" or "loss of function", which is different than in normal cells.

During action of diabetes inductors and subsequent oxidative stress there is a metabolic reconfiguration in the sense of response to this stress (Grant, 2008). Modulation of this response could play a crucial role in the defense of the body. Protective substances should, above all, decrease blood glucose and, at the same time, they should increase the level of antioxidant defense (Surynarayana et al., 2007).

It is very important to establish the dose relationships of antidiabetogenic substances, especially when they are applied in combination to achieve an effective modulation of the gene expression in target cells. Moreover, also the chemical instability of some polyphenols (during storage, processing or analysis) together with the possibility of enzymatic hydrolysis and metabolite conversion can play a significant role in the process of identification of suitable bioactive forms of polyphenol compounds *in vivo* (Graf et al., 2006).

## CONCLUSIONS

In the present study, we aimed at expanding a knowledge related to the long-term treatment effects of bioflavonoids on pre-diabetic BB-DP rats (from 21 to 171 day of age). We found significant differences in the onset and incidence of spontaneous diabetes in rats treated with bioflavonoids (BF vs. C, $P<0.05$). In BB-DP rats a disease, resembling human type 1 diabetes, was developed.

It is known that the BB-DP rats become diabetic at a rate of 60-80% with the onset at about 80 days of age and the peak at about 110 days of age. We report that bioflavonoids delayed and prevented the development of *diabetes mellitus* in BBDP rats. Incidence of diabetes was lower in rats treated with bioflavonoids than in rats from Zn and control groups, and this difference was statistically significant. The age at the onset of diabetes was correlated with increase of some parameters of antioxidant status.

The results of this study prove the hypoglycemic and anti-diabetic activity of bioflavonoids. These activities are realized through increased peripheral glucose utilization and enhanced an antioxidant defense of animals, which agrees with the findings of Adaneye et al. (2007). Further research is required to understand the mechanism of action of these substances and to define their value in the prevention and management of human *diabetes mellitus*.

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