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*Zakład Patofizjologii Katedry Przedklinicznych Nauk Weterynaryjnych
Akademii Rolniczej w Lublinie
**Katedra Higieny Żywności Zwierzęcego Pochodzenia Akademii Rolniczej w Lublinie
***Dipartimento di Anatomia Biochimica e Fisiologia Veterinaria,
Facoltà di Medicina Veterinaria, Università di Pisa, Italia

RYSZARD BOBOWIEC*, ELŻBIETA TUSIŃSKA*,
KRZYSZTOF SZKUCIK**, FRANCO MARTELLI***,
URSZULA KOSIOR-KORZECKA*

Response of rats with alloxan-induced diabetes to diet supplemented with buckwheat

Odpowiedź szczurów z cukrzycą alloksanową na pokarm z dodatkiem gryki

SUMMARY

To investigate the improvement of the course of alloxan-induced diabetes in rats by buckwheat (BW) we prepared a diet enriched with BW and fed rats with diabetic for 5 weeks. To evaluate the effects of BW the following parameters have been appreciated: body weight gain, concentration of glycated hemoglobin (gHb), the level of malondialdehyde (MDA) in the plasma and glucose tolerance test (GTT). Both values of body weight gain and GTT were successively ameliorated together with progressive supplementation of diet with BW in diabetic rats. In contrast, concentration of gHb and MDA levels were found to be significantly increased in diabetic rats fed the diet supplemented with BW. Taking into consideration all these experimental findings, we have established that the beneficial effects of BW is not uniform and apart from some gain in body weight and improvement in GTT the BW exerts unfavorable effects on gHB and the level of MDA. Such equivocal response of our diabetic rats to diet supplemented with BW may be the result of low level of antioxidants (rutin, quercetin) and trace elements such manganese in our BW seeds, which, if present, exert beneficial effects on the course of diabetes.

Key words: buckwheat, MDA, glicated haemoglobin, glucose tolerance test

INTRODUCTION

Diabetes mellitus (DM) as a common endocrine disorder is defined as a state of chronic hyperglycaemia which may be due either to the absolute lack of insulin or to factors that oppose its action [Nishikawa *et al*. 2000]. Alloxan induced DM in rats, with destruction of insulin-producing β-cells, is widely used as a model of type I, insulin dependent DM [Oi *et al*. 1997, Murata *et al*. 1998, Palomar-Morales et al. 1998, Dhandapani *et al.* 2002, Visser *et al*. 2002]. Several metabolic disorders in this kind of diabetes are brought about by increased oxidative stress (OS), defects in antioxidant protection [Martinez-Cayuela 1995] and nonenzymatic glycation of proteins including Hb [Jensen 1995]. It is known that reactive oxygen species (ROS) are able to attack unsaturated fatty acids (UFA) of membrane phospholipids and, by this way, to initiate lipid peroxidation and further severe damages of the membrane structure with sequential variation in its fluidity and ability to function correctly [Nishikawa *et al*. 2000]. In addition, malondialdehyde (MDA), a by-product resulting from peroxidation of fatty acids and the main indicator of lipid peroxidation has also been found to cause membrane and DNA damage leading to the change in the cell permeability and ultimately to the cell death [Murata *et al*. 1998]. Furthermore, the measurement of glycated hemoglobin (gHb) is a reliable index in the development and progression variety of diabetic disturbances [Dutton *et al.* 2003].

Thanks to contained flavonoids and proteins, buckwheat (BW) may exert antioxidant and hypocholesterolemic effect, respectively [Xin *et al*. 2004]. According to some authors [Holasowa *et al*. 2002, Fabjan *et al*. 2003, Kawa *et. al*. 2003], BW could be used for the treatment of metabolic diseases like diabetes mellitus (DM). Therefore, it would be important to know if BW can influence glycation of Hb, production of MDA or shape the curve of glucose tolerance in the course of alloxan-induced DM in rats. Henceforth, the objective of this research is to assess if diet supplemented with BW may ameliorate the diabetic parameters.

MATERIAL AND METHODS

Male Wistar rats (n = 20) weighting 250–300 g, were acclimated for 7 days to the laboratory conditions and fed on a standard granulated diet and water *ad libitum* until the beginning of the experiments. Subsequently, the animals were divided into four groups:

1) control group (K) without any treatment;
2) control group fed with 40% of BW (K + G 40%)
3) alloxan induced diabetic group fed with 20% of BW (A + G 20%)
4) alloxan induced diabetic group fed with 40% of BW (A + G 40%)

Diabetic state was induced by a single *i.p.* injection of alloxan in the dose of 150 mg/kg b.w. During the development of diabetic state the animals were protected from unexpected death by injection of 6 ml 20% glucose *i.p*. together with 5% glucose used as a drinking solution. Food consumption and body weight of each rat were recorded every day. After 4 weeks of treatments, the diabetic state of the rats was confirmed by the presence of sugar in urine (glucosuria).

Analytical procedures

Determination of glycated hemoglobin (gHb)

The term glycated hemoglobin (gHb) usually refers to the series of minor glycated fractions of hemoglobin (Hb). The erythrocyte is freely permeable to glucose, and gHb is formed at a rate dependent on the average glucose concentration and increases in animals with diabetes mellitus.

Red blood cells were separated from 4 ml of blood with EDTA addition by centrifugation at 1400 × g for 15 min. After triple washing with saline, 1 ml of RBC was hemolised using distilled

water and 0.4 ml toluene. Following mixing, toluene and the stromal debris were removed by centrifugation (1400 × g for 20 min), filtration and a 12 h dialysis of the hemolysate. Hexose bound to Hb was hydrolyzed by 4 h heating at 100°C in the presence of oxalic acid (1 mol of oxalic acid in 2 mol HCL 1:2). After cooling, 4 ml 40% TCA was added to the mixture for protein precipitation and the whole was centrifuged at 2000 × g for 10 min. Acid was added to the 6 ml of obtained supernatant 2 ml of 0,05 mol 2-thiobarbituric and after 40-min incubation at 40°C, the absorbance of the samples was read at 443 nm [Saibene V., *et al*., 1979] against a reagent blank. Results were expressed as 5-hydroxymethylfurfural (5-HMF) absorbance per 10 g of total Hb (oxalic acid hydrolysis of hexoses bound to Hb releases 5-HMF). Total Hb concentration was assessed by the cyanmethemoglobin method.

Determination of malondialdehyde (MDA)

After addition of 2.5 ml 1.22 mol TCA (in 0.6 mol HCl) to the 0.5 ml of plasma and 15 min incubation 1.5 ml of thiobarbituric acid (TBA) solution (500 mg TBA/6 ml 1mol NaOH + 69 ml H_2O) was added [Wójcik *et al*. in press]. The mixture was heated in boiling water per 30 min. The tubes were then cooled to room temperature and to each tube 4 ml n-butanol was added and vortexed for 3 minutes. After centrifugation at 1500 × g for 20 minutes absorbance of supernatants was measured at 532 nm and the plasma concentration of MDA was expressed as μmol/l.

Blood glucose and glucose tolerance test

Blood glucose was determined by using blood glucose monitoring system. A glucose tolerance test (GTT) was performed on anesthetized rats at the sacrificing time. At the beginning, 1 g glucose as a 60% solution was administrated by gavage to rats that had been fasted for 18 hours. Samples of blood were collected before administration of glucose and 30, 60, and 90 minutes after glucose loading.

The results were analyzed using ANOVA test. The data are given as mean ±S.E for 5 rats in each group. The difference was considered significant when $P<0.05$.

RESULTS

In the course of the experiment, average control daily food intake (3.10 ±0.56 g) significantly ($p<0.05$) dropped to the value 2.05 ±0.71 g in the group of diabetic rats supplemented with 20% of BW.

The weight of the rats fed with BW diet increased. Fig. 1 shows that alloxan treatment induced a significant drop in body weight gains in the group supplemented with 20% of buckwheat (BW). This was accompanied by remarkable less food consumption successfully after alloxan injection. When the quantity of BW was doubled, the diabetic rats started to improvet their growth rate (Fig. 1).

It was surprising that the content of MDA was most increased in the control group supplemented with 40% of BW (Fig. 2). The elevated levels of MDA, especially when 40% of BW was introduced to the diet, were also seen in the diabetic groups. No suppressive effect of BW was found on the MDA level in plasma of control and alloxan induced diabetic rats.

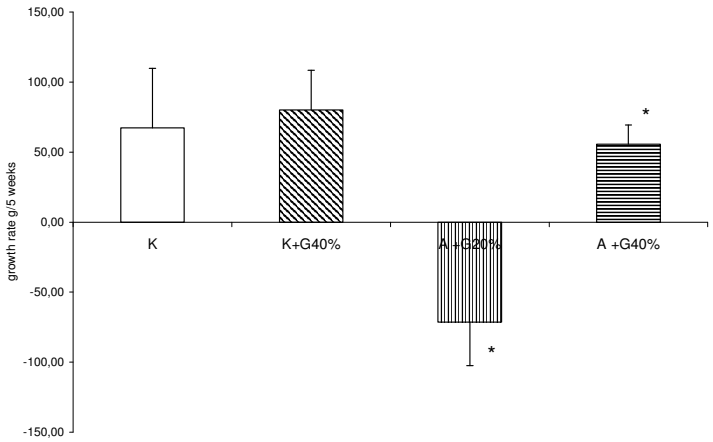

Fig. 1. Changes in growth rate of diabetic rats subjected to the diet supplemented with buckwheat (n = 5 in each group, ±SE, * P< 0.05 vs. control group)

Rys. 1. Zmiany przyrostu masy ciała szczurów w poszczególnych grupach (n = 5 w każdej grupie, ±SE, * P< 0,05 w stosunku do grupy kontrolnej)

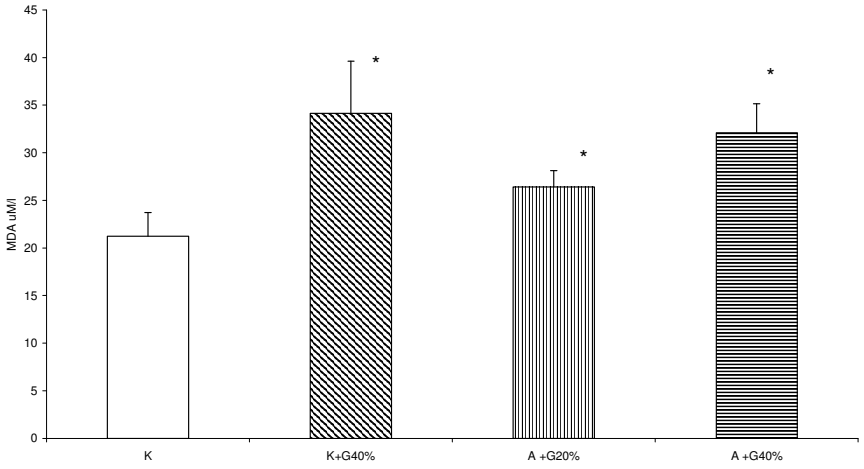

Fig. 2. Plasma MDA levels in four groups of rats (n = 5 in each group, ±SE, * P< 0.05 vs. control group)

Rys. 2. Poziom osoczowego MDA u szczurów w poszczególnych grupach (n = 5 w każdej grupie, ±SE, * P< 0,05 w stosunku do grupy kontrolnej)

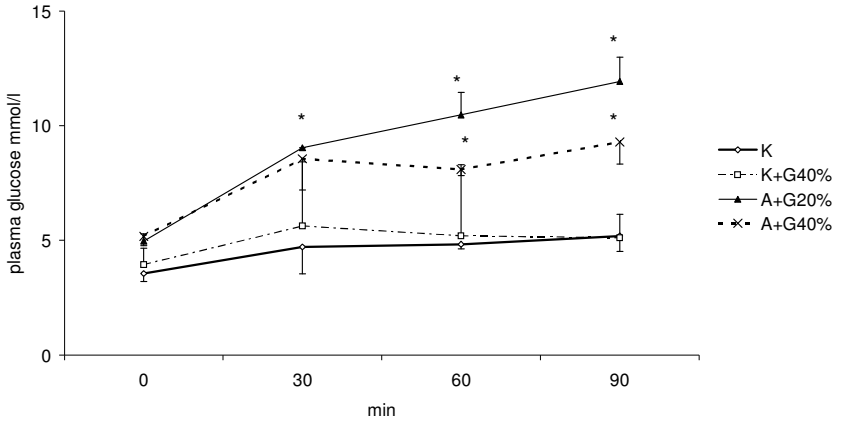

Fig. 3. Glucose tolerance test in the four groups of rats (n = 5 in each group, ±SE, * P< 0.05 vs. control group)

Rys. 3. Test tolerancji glukozy u szczurów w poszczególnych grupach (n = 5 w każdej grupie, ±SE, * P< 0,05 w stosunku do grupy kontrolnej)

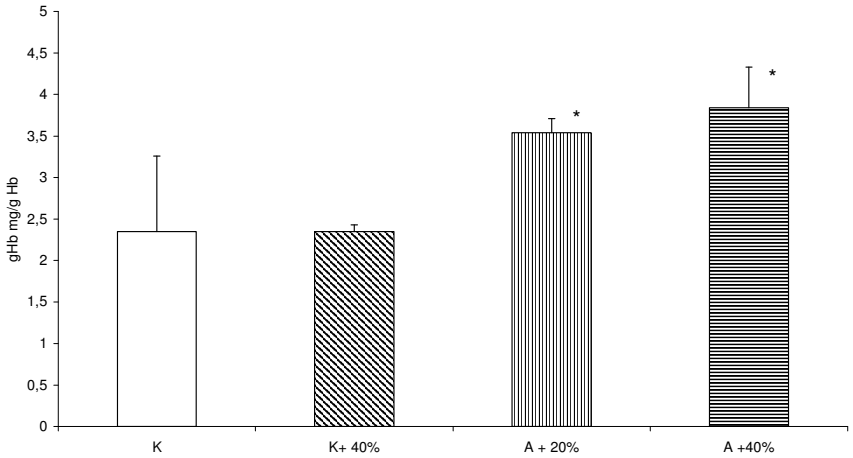

Fig. 4. Glycated hemoglobin (gHb) in four groups of rats (n = 5 in each group, ±SE, * P< 0.05 vs. control group)

Rys. 4. Glikozylowana hemoglobina u szczurów w poszczególnych grupach (n = 5 w każdej grupie, ±SE, * P< 0,05 w stosunku do grupy kontrolnej)

Considering the curve of glucose tolerance, we have not been able to reveal any significant differences between curves for control and control supplemented with BW groups. As is shown on the Fig. 3, independently of the level of supplementation with BW, the values of glucose tolerance test (GTT) were augmented after induction of diabetes. Furthermore, curves of GTT for diabetic rats supplemented with 40% of BW were beneath the values, which characterized the curve, for diabetic rats supplemented with 20% of BW. The values of GTT for diabetic rats, both supplemented with 20% and 40% of BW, were significantly ($p<0.05$) higher in comparison to control group. Although glycated hemoglobin (gHb) content in control rats supplemented with BW remains unchanged such a tendency was reversed in the case of diabetic rats (Fig. 4). The highest level of gHb was revealed in the group of diabetic rats treated with addition of 40% of BW.

Despite of the positive effect of daily treatment with BW on body weight and the curve of GTT the important parameters of diabetic disturbances including gHb and MDA remain unchanged or even worsened after exposition to BW. Because DM is associated with weakened cellular scavenging activity, which may increase vulnerability to oxidative stress [Nishikawa *et al*. 2000] and glycation of proteins including Hb [Dutton *et al*. 2003], we carried out experiments to investigate the effects of buckwheat on markers of OS, gHb and curve of glucose tolerance in alloxan-induced diabetic rats.

Buckwheat (BW) as a herbaceous plant is widely consumed in Central Europe and apart from beneficial effects on health [Gabrovska *et al*. 2002, Banafaccia *et al*. 2003, Fabjan *et al*. 2003], is actually regarded as a source of food allergen [Park *et al*. 2000, Park *et al*. 1997]. According to many authors [Gabrovska *et al*. 2002, Banafaccia *et al*. 2003, Fabjan *et al*. 2003, Xin *et al*. 2004] BW is rich in vitamins B_1 and B_2, many essential minerals (Zn, Mg, Mn) [Ikeda and Yamashita 1994], but first of all it contains rutin, vitexin, quercetin, isovitexin and isoorientin a flavonoids, which have antioxidant and anti-inflamatory properties. These features of BW suggest that an intake of BW products may reduce the signs of DM [Holasova *et al.* 2002, Sun and Ho 2005, Xin *et al*. 2004]. However, the obtained results only partly confirm the beneficial action of BW on the diabetic parameters in rats.

During the course of DM permanent hyperglycemia (HG) causes diverse microvascular and macrovascular pathologies by four different ways: increased polyol pathway flux, increased advanced glycation end-product (AGE) formation, activation of protein kinase C (PKC) isoforms, and increased hexosamine pathway flux. Common element linking the four above mentioned mechanisms of hyperglycemia-induced damage is overproduction of superoxides (SO) regarded as a principal cause of oxidative stress [Martinez-Cayuela 1995, Wójcik *et al*. 2003]. Hyperglycaemia increases the proton gradient as a result of overproduction of electron donors by TCA cycle. This, in turn, causes the marked increase in the production of SO. Signal generated by ROS is suppressed by mitochondrial activity of manganese superoxide dismutase (MnSOD). Overexpressed MnSOD corrects a variety of diabetic complications [Nishikawa *et al*. 2000]. It is well known that BW, depending on the source, contains an appreciable amount of manganese [Ikeda and Yamashita 1994] which improves insulin release and acts against oxidative stress [Bally *et al*. 1984] by correction of the MnSOD level. Because in our experiments the level of marker of OS i.e. MDA remains not only unchanged, but rises under the influence of BW, it is reasonable to think that either the level of Mn in our BW supplemented rats was low or the pancreas did not respond to this element. Similarly, the maintenance of high level of MDA under the influence of diet supplemented with buck-

wheat, both in control and diabetic rats points out that the level of flavonoids, at least in our seeds, was negligible. Such results are in accordance with those of Fabjan et. al [Fabjan *et al*. 2003]. They suggest that e.g. rutin and quercetin content in seeds of BW depends on a variety of growing conditions. Furthermore, we assume that increased glycation rate of Hb, under the influence of a growing dose of BW, reveals that our seeds were rich in glucose, but were poor both in flavonoids and important minerals. Moreover, the obtained results showed that any ingredient of BW was not in a position to lower the process of glycation of Hb during the course of alloxan-induced diabetes. Additionally, the elevated values of GTT together with the fact that the 1% change in the gHb concentration corresponds to the change in plasma glucose of approximately 1.8 mmol/l [Jensen 1995] permit to speculate that the used BW was, first of all, the source of carbohydrate [Link *et al*. 1997].

Summing up, we envisage that the influence of BW on diabetic state is not uniformly beneficial and that the data about improvement of diabetes by BW products [Kawa *et al*. 2003, Kim *et a*l. 2004, Peng *et al.* 2004] must be related to the presence of active compounds in the given source of BW seeds. Apart from some positive effects of BW, including improvement of the body weight lose during the course of diabetes and amelioration, the curve of the glucose tolerance test concurrently BW acts negatively on glycation of Hb and increases the accumulation of lipids peroxidation products in the form of MDA.

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STRESZCZENIE

Celem przeprowadzonych badań było określenie działania dodatku gryki na przebieg indukowanej alloksanem cukrzycy u szczurów. W przeprowadzonym układzie doświadczalnym, w którym szczury podzielono na 4 grupy (kontrolna, kontrolna z dodatkiem 40% gryki, i dwie cukrzycowe z dodatkiem 20 albo 40% gryki) oznaczano następujące parametry: przyrosty masy ciała, stężenie glikolizowanej hemoglobiny (gHb), poziom aldehydu malonowego (MDA) oraz wykreślono krzywe testu tolerancji glukozy (TTG). Stwierdzono, iż dodatek 40% gryki wywierał zarówno korzystne, zwiększające masę ciała, jak i obniżające krzywą TTG działanie u szczurów cukrzycowych. Przeciwnie, niekorzystnie wpływały stosowane dodatki gryki na zawartość gHb i MDA we krwi. Otrzymane wyniki pozwalają stwierdzić, że działanie gryki w przebiegu eksperymentalnej cukrzycy u szczurów nie jest jednolicie korzystne**.** Obok efektów poprawiających masę ciała i obniżających krzywą tolerancji glukozy, obserwuje się zwiększenie poziomu gHb i MDA. Taka dwuznaczna odpowiedź kontrastuje z wskazywanym przez innych autorów wyłącznie pozytywnym działaniem gryki. Wynikać to może z wahań w zawartości antyoksydantów (rutyna, kwercetyna), a także mikroelementów, w tym głównie manganu w różnych odmianach gryki, które łagodzą przebieg cukrzycy.

Słowa kluczowe: gryka, MDA, glikolizowana hemoglobina, test tolerancji glukozy