Effect of oligosaccharides on the antioxidant, lipid and inflammatory profiles of rats with streptozotocin-induced diabetes mellitus

Abstract: Prebiotics, gut microbiota-fermentable substances, delay the development of type I diabetes. In the present study, we investigated the effect of two prebiotics (galacto-oligosaccharides and xylo-oligosaccharides) on the antioxidant protection, lipid profile, and inflammatory activity of rats with streptozotocin-induced diabetes. The following markers were studied – malondialdehyde, 8-hydroxy-2′-deoxyguanosine, ferric reducing ability of plasma (FRAP), triacylglycerols, total cholesterol (TC), high-density lipoproteins, C-reactive protein (CRP), and interleukin-6. Diabetes was induced in male Wistar experimental rats by streptozotocin injection, while the non-diabetic controls were injected with saline. Afterward the oligosaccharides were administered orally to the experimental animals. The blood collected following the decapitation was analyzed by ELISA. A modified protocol was used only for measuring the FRAP values. The galacto-oligosaccharides and xylo-oligosaccharides lowered the malondialdehyde levels in the diabetic rats ($p < 0.05$). The galacto-oligosaccharides decreased the serum levels of 8-hydroxy-2′-deoxyguanosine ($p = 0.01$), while the xylo-oligosaccharides increased the FRAP ($p < 0.05$) in the experimental animals. None of the oligosaccharides affected triacylglycerol and interleukin-6 concentrations, but the galacto-oligosaccharides decreased the TC and CRP levels in the diabetic animals. Both oligosaccharides exert a beneficial effect on the antioxidant protection of the diabetic rats, but have a minor effect on their lipid and inflammatory profiles.

Keywords: antioxidant protection; diabetes mellitus; galacto-oligosaccharides; rats; xylo-oligosaccharides.

1 Introduction

Diabetes mellitus is a group of metabolic disorders characterized by high blood glucose levels (hyperglycemia) resulting from either insufficient production or defects in the secretion of the pancreatic hormone insulin [1]. Apart from the hyperglycemia, other metabolic disorders, such as hyperlipidemia and oxidative stress play a substantial role in the pathogenesis of the disease and put people with diabetes at high risk of developing complications [2]. The non-enzymatic glycation of proteins, spontaneous oxidation of glucose, oxidative stress, and increased lipid peroxidation in diabetes can damage enzymes and cellular processes, which in turn could lead to the development of insulin resistance [1].

A number of human and animal studies have reported about specific prebiotic substrates that exert hypoglycemic effects and thus improve the health of type 1 diabetic patients. Prebiotics are substances that are selectively fermented by the gut microbiota and their products exert a beneficial effect on the health of the host [3]. The favorable effects of prebiotics include selective stimulation of the growth and activity of beneficial intestinal microbes that adequately modulate the immune system of the intestine [4]. Several studies have demonstrated that dietary fibers assist in maintaining microbiota homeostasis by exerting a beneficial effect on the intestinal permeability, thus delaying the development of type 1 diabetes [5–7].

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The aim of the present study was to investigate the effect of xylo-oligosaccharides (OS1) and galacto-oligosaccharides (OS2), both commercially available, on biomarkers of the oxidative stress and antioxidant protection, the lipid profile and inflammatory activity in rats with streptozotocin-induced diabetes mellitus. The oxidative stress biomarkers that were studied are malondialdehyde (MDA), 8-hydroxy-2’-deoxyguanosine (8-OHdG) and ferric reducing ability of plasma (FRAP); for the lipid profile – triacylglycerols (TAGs), total cholesterol (TC) and high density lipoproteins (HDL), and the inflammatory biomarkers were C-reactive protein (CRP) and interleukin-6 (IL-6).

2 Materials and methods

2.1 Ethics statement

This study was performed in strict accordance with the guidelines of the European Community Council directives 86/609/EEC.0.2010/63/EC. All animal experiments were carried out according to protocols approved by the Bulgarian Agency for Food Safety (BAFS resolution №150/09.04.2019) and are in accordance with the ethical standards of the Medical University of Plovdiv (resolution of the University Ethic Committee №2/13.06.2019).

2.2 Experimental animals

The study included 63 male Wistar albino rats (mean body weight – 195 ± 30 g, 8-weeks-old), provided by and bred in the vivarium of the Medical University of Plovdiv. They were randomly divided into six groups: three groups of 12 rats, each with induced diabetes and another three groups of nine rats each used as non-diabetic controls. The animals were housed in cages and kept under standard laboratory conditions: floor area 350 cm², humidity 55 ± 10%, temperature 22 ± 2°C, a 12-h light/dark cycle, and free access to food and water for the entire period of the experiment.

The experimental animals were divided into the following six groups:

- Test group DOS1 – diabetic rats, treated with xylo-oligosaccharides,
- Test group DOS2 – diabetic rats, treated with galacto-oligosaccharides,
- Positive control group DUT – diabetic rats, untreated (fed a standard diet),
- Test control group NDOS1 – non-diabetic rats, treated with xylo-oligosaccharides,
- Test control group NDOS2 – non-diabetic rats, treated with galacto-oligosaccharides,
- Negative control group NDUT – non-diabetic rats, untreated (fed a standard diet).

2.3 Diabetes induction

Type 1 diabetes was induced in 36 rats (groups DOS1, DOS2, and DUT) that were injected intraperitoneally with a single dose of 60 mg/kg body weight of streptozotocin (STZ) in freshly prepared citrate buffer (pH 4.5). The remaining 27 animals (control groups NDOS1, NDOS2, and NDUT) were injected with the same volume of saline. All animals were fed a standard diet from birth to the end of the experiment.

2.4 Treatment of the experimental animals

Six days after STZ administration, OS1 (xylo-oligosaccharides) was administered orally to 21 animals (experimental group DOS1 and control group NDOS1) and OS2 (galacto-oligosaccharides) – to 21 of the animals (experimental group DOS2 and control group NDOS2). The oligosaccharides were administered at a dose of 100 mg/kg every day for 9 weeks. Working concentrations of galacto-oligosaccharides and xylo-oligosaccharides were achieved by dilution in distilled water.

Dietary oligosaccharides are not pure products. They are mixtures of oligosaccharides with different degrees of polymerization (dp) [8]. DP is the number of monomeric units in a macromolecule [9]. The degree of polymerization of the carbohydrates for the two oligosaccharides was as follows: galacto-oligosaccharides (TOS-P from Yakult, Tokyo, Japan) contained 2% dp2, 48% dp3, 38% dp4, 12% dp5, and xylo-oligosaccharide powder, dp 2–10 (XOS dp 2–10 from Lenzing AG, Lenzing, Austria) contained 99% XOS – 13% dp2, 19% dp3, 11% dp4, 60% dp > 5.

At the end of week 9, the animals were decapitated after being anesthetised with ketamine/xylazine mixture (87.5/12.5 mg/kg, respectively). After the decapitation the blood was collected, centrifuged at 1500 x g for 5 min, and the separated serum was frozen at –18 °C until the biochemical analysis.

2.5 Biochemical analyses

An ELISA method based on standard purchased (commercial) kits was used to determine the biochemical parameters. The following markers were analyzed: malondialdehyde (Rat MDA (Malondialdehyde), ELISA Kit, Wuhan Fine Biotech Co.), 8-hydroxy-2’-deoxyguanosine (8-OHdG (8-hydroxydeoxyguanosine) ELISA Kit, Elabscience Biotechnology Inc.), high density lipoproteins (Rat HDL (High Density Lipoprotein) ELISA Kit, Elabscience Biotechnology Inc.), triglycerides (Triglyceride (Rat) ELISA Kit, BioVision Inc.), C-reactive protein (Rat hs-CRP (high-sensitivity C-Reactive Protein) ELISA Kit, Elabscience Biotechnology Inc.), and interleukin-6 (Rat IL-6 (Interleukin 6) ELISA Kit, Elabscience Biotechnology Inc.).

FRAP was determined using the method of Benzie & Strain [10]. FRAP reagent was added to the obtained serum, the mixture was incubated for 30 min in the dark at 37 °C and the absorbance was measured at 593 nm. The results were expressed as μmol Trolox equivalent used to prepare the standard curve.
2.6 Statistical analysis

The statistical analysis was performed with SPSS, v.17 (SPSS Inc., Chicago, IL, USA). The Mann–Whitney U-test was used for the comparison of the quantitative variables with non-Gaussian distribution between two independent groups. These variables were presented as median and 95% confidence interval. Only the FRAP values showed a Gaussian distribution and were therefore presented as mean and standard error of the mean (mean ± SEM). The comparison was performed with the Independent Samples t-Test.

3 Results

3.1 Antioxidant profile

3.1.1 MDA

The oral administration of OS1 to diabetic rats (test group DOS1) resulted in significantly lower levels of MDA compared with those of the positive (DUT) and negative controls (NDUT) by 65 and 64%, respectively. The most pronounced effect was observed versus the test control group NDOS1 – lower by 77% (Figure 1).

A similarly statistically significant effect of OS2 on the MDA levels was observed in the DOS2 test group. The MDA concentrations were 63 and 62% lower in comparison with the positive and negative control groups, respectively. OS2 caused a 52% decrease in the MDA levels of DOS2 group compared with NDOS2 group (Figure 1).

When comparing the effects of OS1 and OS2 given to non-diabetic rats of NDOS1 and NDOS2 groups we found that the MDA levels were 2-fold lower in OS2 versus the effect from OS1 administration (p < 0.05) – Figure 1.

3.1.2 8-OHdG

OS2 administration to the diabetic rats (test group DOS2) decreased the serum 8-OHdG levels by 31% in comparison with the OS2-fed non-diabetic rats (test control group NDOS2) – Table 1. No statistically significant differences

| Group    | 8-OHdG, ng/mL, median (95% CI) |
|----------|-------------------------------|
| DOS1     | 15.22 (4.19–26.09)a,b,c        |
| DOS2     | 16.12 (9.94–19.67)d,e,f        |
| DUT      | 16.97 (8.77–37.79)             |
| NDOS1    | 24.09 (16.48–36.51)f           |
| NDOS2    | 24.71 (18.75–38.12)            |
| NDUT     | 17.63 (14.12–29.83)            |

DOS1 versus DUT (p > 0.05), DOS1 versus NDOS1 (p > 0.05), DOS1 versus NDUT (p > 0.05), DOS2 versus NDOS2 (p = 0.01), DOS2 versus DUT (p > 0.05), NDOS1 versus NDOS2 (p > 0.05). 8-OHdG – 8-hydroxy-2′-deoxyguanosine, DOS1 – diabetic rats, treated with xylo-oligosaccharides, DOS2 – diabetic rats, treated with galacto-oligosaccharides, DUT – diabetic rats, untreated (fed a standard diet), NDOS1 – non-diabetic rats, treated with xylo-oligosaccharides, NDOS2 – non-diabetic rats, treated with galacto-oligosaccharides, NDUT – non-diabetic rats, untreated (fed a standard diet), CI – confidence interval.

Figure 1: Effects of OS1 and OS2 on serum MDA levels of streptozotocin-induced diabetic rats. Data are expressed as median and 95% confidential interval. *DOS1 versus NDOS1 (p < 0.01); **DOS1 versus DUT (p < 0.01); ***DOS1 versus NDUT (p = 0.025); #DOS2 versus NDOS2 (p = 0.01); #DOS2 versus NDUT (p < 0.05); #DOS2 versus DUT (p < 0.01). MDA – malondialdehyde, DOS1 – diabetic rats, treated with xylo-oligosaccharides, DOS2 – diabetic rats, treated with galacto-oligosaccharides, DUT – diabetic rats, untreated (fed a standard diet), NDOS1 – non-diabetic rats, treated with xylo-oligosaccharides, NDOS2 – non-diabetic rats, treated with galacto-oligosaccharides, NDUT – non-diabetic rats, untreated (fed a standard diet).
were found in the 8-OHdG levels in the other groups (Table 1).

3.1.3 FRAP

Administration of OS1 led to significantly higher FRAP levels in the DOS1 test group compared with the positive (DUT) and negative controls (NDUT) by 18 and 16%, respectively (Figure 2). Furthermore, OS2 tended to increase the FRAP levels in the diabetic rats (test group DOS2) compared to the non-diabetic rats (test control group NDOS2, \( p = 0.083 \)) – Figure 2. Higher levels of FRAP were found in the DOS1 test group compared with the NDOS1 test control group when OS1 was administered but the values failed to reach statistical significance (Figure 2). Similar results were observed when OS2 was supplied to the DOS2 test group in comparison with the positive (DUT) and negative (NDUT) control groups (Figure 2).

3.2 Lipid profile

3.2.1 TAGs and TC

The administration of OS1 to the diabetic rats (group DOS1) as well as OS2 to the diabetic and non-diabetic rats (groups DOS2 and NDOS2) significantly elevated (by 524, 492, and 500%, respectively) the serum TAG levels in comparison with the non-diabetic rats fed a standard diet (group NDUT) – Table 2. On the other hand, treatment of the diabetic rats with OS2 (group DOS2) lowered the levels of the total serum cholesterol by 15% compared with the levels of the non-diabetic controls (group NDUT) – Table 2. The values for the rest of the groups have no statistically significant differences (Table 2).

3.2.2 HDL

When comparing the results of the experiments we conducted with the non-diabetic control groups, we found that the OS1 treated rats had a 9% higher serum HDL concentration (NDOS1 group) than the rats treated with OS2 (NDOS2 group). Also, HDL tended to be 12% higher in the healthy rats, in the group that was not treated with oligosaccharides (group NDUT) when compared with the HDL concentration in OS2-treated non-diabetic rats (Table 2). The effect of the two oligosaccharides supplied to the diabetic animals failed to reach statistical significance when compared with that in the controls (Table 2).

3.3 Inflammatory markers

3.3.1 hs-CRP and IL-6

OS2 administered to the diabetic rats exerted a slightly more beneficial effect on the hs-CRP levels than OS1 did – it reduced hs-CRP by 15%. But hs-CRP concentrations were
even lower in both control groups treated with OS1 (NDOS1) and fed a standard diet (NDUT) compared with group DOS1 – 23 and 20%, respectively. On the other hand, OS1 tended to reduce the IL-6 levels by 48% in the diabetic rats compared to the non-diabetic ones fed a standard diet (Table 3). No statistically significant differences were observed among the rest of the groups (Table 3).

4 Discussion

Hyperglycemia in diabetes is known to cause an increased production of free radicals as a result of protein glycation or glucose autooxidation [11]. The reactive oxygen species (ROS) are directly associated with increased lipid peroxidation [12, 13]. Several studies have shown increased lipid peroxidation in untreated diabetic animals [14–16].

MDA is one of the end products of polyunsaturated fatty acids peroxidation in the cells. Overproduction of MDA is caused by the increase of free radicals [17], which is the reason why MDA is considered as one of the most reliable markers of oxidative stress in clinical practice [18]. MDA levels have been found to be higher in type 2 diabetic patients than those in healthy controls [19]. Furthermore, various authors have reported elevated levels of MDA both in plasma [20] and in some tissues of STZ-induced diabetic rats – in erythrocytes [21], in the pancreas [22], in hepatic tissues [23], in the testicular tissue [24], in the lens [25].

Our results showed that the oral administration of OS1 and OS2 to the diabetic rats (DOS1 and DOS2 groups) reduced the serum MDA levels when compared with the diabetic and non-diabetic groups of rats fed a standard diet (DUT and NDUT) to an almost the same extent. This suggests that galacto-oligosaccharides and xylo-oligosaccharides exert a similar lowering effect on MDA blood levels in the diabetic rats.

Comparing the effect of OS1 and OS2 in the diabetic versus non-diabetic rats, we established that OS2 did not allow a significant increase of the MDA levels in the non-diabetic group (NDOS2), as it was observed in the non-diabetic group treated with OS1 (NDOS1). This observation is also confirmed by the direct comparison of non-diabetic groups treated with OS1 and OS2.

The analysis of our results allowed us to conclude that galacto-oligosaccharides and xylo-oligosaccharides lower the MDA levels of the diabetic rats, with galacto-oligosaccharides being more effective than xylo-oligosaccharides in the non-diabetic rats.

ROS can directly oxidize both the double-stranded DNA and free bases in the cell pools of deoxynucleoside triphosphates [26]. Among all nitrogen bases, guanine is the

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**Table 2:** Serum levels of TAGs, TC and HDL.

| Group   | TAGs, µmol/L, median (95% CI) | TC, µmol/L, median (95% CI) | HDL, ng/mL, median (95% CI) |
|---------|-------------------------------|-------------------------------|-----------------------------|
| DOS1    | 1.56 (1.27–1.87)             | 1.63 (1.06–2.21)             | 5.43 (2.64–10.60)           |
| DOS2    | 1.48 (1.32–1.70)             | 1.75 (1.39–2.06)             | 6.18 (0.25 to 19.44)        |
| DUT     | 1.64 (1.51–1.73)             | 1.85 (1.53–2.30)             | 6.55 (5.96–7.47)            |
| NDOS1   | 1.51 (1.32–1.61)             | 1.68 (1.48–2.29)             | 5.45 (5.28–6.59)            |
| NDOS2   | 1.50 (1.36–1.60)             | 1.89 (1.63–2.21)             | 5.02 (4.87–5.15)            |
| NDUT    | 0.25 (0.10–1.08)             | 2.05 (1.85–2.54)             | 5.62 (4.82–6.59)            |

**Table 3:** Serurm levels of hs-CRP and IL-6.

| Group   | hs-CRP, mg/mL, median (95% CI) | IL-6, pg/mL, median (95% CI) |
|---------|-------------------------------|-------------------------------|
| DOS1    | 13.27 (10.84–15.75)           | 17.63 (3.92–32.43)           |
| DOS2    | 11.34 (9.27–12.76)            | 25.54 (6.26–45.36)           |
| DUT     | 11.56 (10.25–12.38)           | 33.98 (9.45–54.80)           |
| NDOS1   | 10.28 (9.12–11.01)            | 25.53 (17.80–32.73)          |
| NDOS2   | 9.30 (8.96–10.68)             | 21.44 (18.76–29.31)          |
| NDUT    | 10.58 (9.22–11.45)            | 33.98 (20.77–40.64)          |
most susceptible to oxidation by ROS [27]. Oxidation of 2'-deoxyguanosine produces 8-hydroxy-2'-deoxyguanosine (8-OHdG). Formation of 8-OHdG in DNA can cause mutations due to improper pairing as a result of the G:C to T:A transversion and these mutations are thought to be closely associated with tumor development and progression, cell aging, and some degenerative diseases [28].

8-OHdG is one of the products of DNA oxidation that can be easily quantified and is therefore used very often as a biomarker in the oxidative DNA damage assessment [29]. As a marker of oxidative stress, 8-OHdG appears to be in high concentrations in diabetes [30]. This is confirmed by several studies on STZ-induced diabetic rats in which high levels of 8-OHdG were found in the urine [31], in the liver and kidney [32] and in the renal tissues [33].

The results of our experiments show that OS2 treatment beneficially lowers the serum 8-OHdG levels in the diabetic rats compared to the non-diabetic rats. This indicates that the galacto-oligosaccharides not only exert a protective effect against the increasing oxidative stress associated with diabetes, but could also be used for prevention and protection of the cell from the adverse effects of 8-OHdG [28].

FRAP analysis was performed for the antioxidant capacity to be assessed [34]. Several studies have shown that FRAP values in STZ-induced diabetic rats were decreased both in plasma [35] and in various tissues compared to that in controls. The provision of substances that improve the antioxidant status is found to restore FRAP, e.g., curcumin in the liver [36], crab shell extract in the kidneys [37], and Royal jelly in testis [38].

The results demonstrate that OS1 administered to the diabetic rats increases the FRAP values in comparison with the diabetic and non-diabetic rats not treated with an oligosaccharide. The xylo-oligosaccharides are thus suggested to be able to increase the antioxidant capacity in diabetes just like the galacto-oligosaccharides do. The tendency for OS2 treatment to increase the FRAP values in the diabetic rats compared to the non-diabetic rats, further reinforces the effectiveness of the galacto-oligosaccharide as a substance with an antioxidant properties.

Diabetes mellitus is associated with significant abnormalities in the lipid metabolism. Several studies have shown changes in the lipid profile of STZ-induced diabetic rats: elevated serum triacylglycerols levels, total cholesterol and LDL-cholesterol, and decreased HDL levels [39–41].

Our results on serum HDL levels in the non-diabetic groups suggest that OS1 slightly increases the serum HDL levels compared to OS2. Our observation is that oligosaccharide intake has no beneficial effect on the levels of HDL, as a higher level of “good cholesterol” was found in the healthy group not treated with oligosaccharides. The highest HDL concentrations were detected in the diabetic groups (non-significant).

The usefulness of both of the oligosaccharides for treatment of lipid abnormalities is controversial because OS1 and OS2 increase TAG levels in the diabetic (DOS1) and non-diabetic (NDOS2) groups, respectively. Also, OS2 has opposite effects on the serum TAGs and TC levels: in the diabetic rats it significantly increases the concentration of TAGs but slightly lowers the TC levels compared to the non-diabetic rats fed a standard diet. This indicates that the tested galacto-oligosaccharides and xylo-oligosaccharides do not exert a significant effect on the lipid profile in both the control and diabetic rats.

Diabetes mellitus is characterized by a low-grade systemic chronic inflammation [42]. A study has shown a direct link between hyperglycemia, the inflammatory process, and oxidative stress which contribute to the development of chronic diseases [43] but another has reported elevated serum levels of inflammatory mediators such as cytokines and CRP in patients with diabetes [42]. Elevated CRP and IL-6 levels have also been observed in rats with STZ-induced diabetes [44, 45].

In the present study, we demonstrate that OS2 has a stronger significant effect on hs-CRP levels than OS1 in the diabetic rats. Nevertheless, both oligosaccharides have no beneficial effect on the serum C-reactive protein in the diabetic rats because lower amount of hs-CRP is observed in the control groups. On the other hand, OS1 tends to lower IL-6 levels in the diabetic rats compared to the non-diabetic rats fed a standard diet. Further research is needed to study in detail the effect of the two oligosaccharides on inflammation.

5 Conclusions

The streamlined design of our experiment in which rats of approximately equal weight were divided into groups (some with successfully induced diabetes and others left as healthy controls) and fed a standard diet with a free access to water, allowed us to test the effects of two commercially available oligosaccharides, OS1 and OS2, on the antioxidant, lipid, and inflammatory profiles.

We report that xylo-oligosaccharides (OS1) and galacto-oligosaccharides (OS2) exert the most beneficial effect on the antioxidant protection in diabetic rats but have just a minor effect on the various fractions of serum lipids and pro-inflammatory cytokines.

Further research is warranted for a full examination of the capacity of action of OS1 and OS2 for them to be used in
the prevention of diabetes mellitus. The antioxidant activity we have established to-date turns them into potential therapeutic agents (to be confirmed by future research) in conditions associated with increased oxidative stress.

**Abbreviations**

| Abbreviation | Description |
|--------------|-------------|
| OS1          | xylo-oligosaccharides |
| OS2          | galacto-oligosaccharides |
| MDA          | malondialdehyde |
| 8-OHdG       | 8-hydroxy-2′-deoxyguanosine |
| FRAP         | ferric reducing ability of plasma |
| TAGs         | triacylglycerols |
| TC           | total cholesterol |
| HDL          | high-density lipoproteins |
| CRP          | C-reactive protein |
| IL-6         | interleukin-6 |
| DIO1         | diabetic rats, treated with xylo-oligosaccharides |
| DIO2         | diabetic rats, treated with galacto-oligosaccharides |
| DUT          | diabetic rats, untreated (fed a standard diet) |
| NDIO1        | non-diabetic rats, treated with xylo-oligosaccharides |
| NDIO2        | non-diabetic rats, treated with galacto-oligosaccharides |
| NDDUT        | non-diabetic rats, untreated (fed a standard diet) |
| STZ          | streptozotocin |
| DP/dp        | degrees of polymerization |
| ROS          | reactive oxygen species |
| DNA          | deoxyribonucleic acid |
| LDL          | low-density lipoproteins |

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