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Metabolic effects of β-glucans (Saccharomyces cerevisiae) per os administration in rats with streptozotocin-induced diabetes

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Abstract

Introduction: beta-glucans (BG) derived from plant tissues are reported to show metabolic effects. In contrast, those fibers isolated from yeast seem to be more related to immune response modulation. Since diabetic individuals are more susceptible to exacerbation of inflammatory signs, the ingestion of fibers that could conjugate both metabolic and immune effects would be of great importance.

Objective: we investigated the effect of BG - Saccharomyces cerevisiae - ingestion on glycemic and lipoprotein profile of diabetic rats.

Design: twenty-four adult Wistar rats were used, distributed into 4 groups in a design of entirely casualized delineation with a 2 x 2 factorial model (with and without diabetes; with and without BG). Diabetes Mellitus was induced by an intraperitoneal injection of 80mg/kg of streptozoticin. Thus, animals with fasting glycemia of over 250 mg/dl were considered diabetic. Forty-eight hours after induction, the rats received daily doses of 30 mg/kg of BG or saline solution by gavage during 28 days.

Results and discussion: the Groups with DM presented a higher glycemic index and lower C peptide levels than the control groups, in addition to lower weight gain and urinary volume. Total cholesterol levels (CT), LDL-C + VLDL-C, plasma triacylglycerides (TAG) and alanine aminotransferase (ALT) were also higher in the diabetic animals (p < 0.05), and there were no alterations in the HDL-C levels. The ingestion of BG reduced blood glucose concentrations (30%), TAG (32%) and ALT (41%) (p < 0.05). No histopathological hepatic alterations were observed in any of the groups. Furthermore, the diabetic animals present increase in villous:crypt ratio (V:C) in the duodenum, without interference of BG. No alterations in the carcass were observed between the groups.

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EFEKTOS METABÓLICOS DE LOS β-GLUCANOS (SACCHAROMYCES CEREVISIAE) A TRAVÉS DE LA ADMINISTRACIÓN ORAL EN RATAS CON DIABETES INDUCIDA POR ESTREPTOZOTOCINA

Resumen

Introducción: los beta-glucanos (BG) derivados de tejidos vegetales se ha informado que muestran efectos metabólicos. Por el contrario, esas fibras aisladas de levadura parecen estar más relacionadas con la modulación de la respuesta inmune. Dado que los individuos con diabetes son más susceptibles a la exacerbación de los signos inflamatorios, la ingestión de fibras sí podría conjugar ambos efectos metabólicos e inmunológicos, lo cual sería de gran importancia.

Objetivo: el objetivo de este estudio fue investigar los efectos de la ingestión de los BG —Saccharomyces cerevisiae— en el perfil glucémico y la lipoproteína de ratas diabéticas.

Métodos: en el diseño de delineación, totalmente precario, fueron utilizadas 24 ratas Wistar macho adultas distribuidas en cuatro grupos, con un modelo factorial 2 x 2 (con y sin diabetes, con y sin BG). La diabetes mellitus fue inducida por la inyección intraperitoneal de un 80 mg/kg de estreptozotocina. Por lo tanto, los animales con glucemia en ayunas de más de 250 mg/dl fueron considerados diabéticos. Cuarenta y ocho horas después de la inducción, las ratas recibieron dosis diarias de 30 mg/kg de BG o solución salina mediante alimentación forzada durante 28 días.

Resultados y discusión: los grupos con DM presentaron el mayor índice glucémico y menores niveles de péptido C que los grupos de control, además de reducir el aumento de peso y un mayor consumo de la ración, la ingestión de agua y el volumen urinario. Los niveles de colesterol total (CT), LDL-C + VLDL-C, triacilglicéridos plasmáticos (TAG) y alanina aminotransferasa (ALT) también fueron más altos en los animales diabéticos (p < 0,05), y había alteraciones en los niveles de HDL-C. La ingestión de BG redujo las concentraciones de glucosa en sangre (30%), TAG (32%) y ALT (41%) (p < 0,05). No se observaron alteraciones hepáticas en ninguno de los grupos. Además, los animales diabéticos presentaron un aumento de la relación criptas:vellosidades (V:C) en el duodeno, sin interferencia de BG. No se observaron alteraciones en la carcaza entre los grupos.
Conclusions: it was concluded that the use of BG significantly reduced the glycemic, TAG and ALT levels, showing its therapeutic potential.

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Key words: Metabolic disease. Diabetes mellitus. Polysaccharide. Glucans.

Introduction

Diabetes mellitus (DM) is a disease characterized by the increase in glycaemia due to inadequate regulation of carbohydrate, protein and lipid metabolism by the pancreatic hormone, insulin. It is one of the main world public health problems that has rapidly worsened, especially in developing countries. Moreover, the majority of the chronic complications of the disease are highly incapacitating for the performance of daily and productive activities, compromising the quality of life of affected individuals.

The prevalence of diabetes patients estimated in the year 2010 was 285 million persons, representing 6.6% of the adult population in the world, with a predicted increase to 435 million by 2030. The forms of treatment for DM consist of nutritional therapy, regular physical activity, insulin therapy and/or use of oral hypoglycemic drugs, depending on the type (I or II) and stage of the disease. However, the medications used in the treatment may present adverse effects, such as feeling unwell, weakness, diarrhea and hypoglycemia, in addition to some inconvenience, such as daily insulin applications. Furthermore, the treatment is extremely costly to the health system. Therefore, the investigation of new alternatives for treatment of the disease is highly relevant.

Among the alternatives used in the control of glycaemia, the beta-glucans (BG) are outstanding. These polymers are found in the cell wall of different plants and yeasts, which have been shown to be effective in the reduction of blood glucose concentrations, possibly because of their capacity to increase the viscosity of the intestinal content, forming a protective barrier that delays the absorption of carbohydrates and lipids.

Furthermore, researches have suggested that BG may increase insulin sensitivity and reduce the cholesterol levels, thereby diminishing the risks of cardiovascular diseases. Moreover, in addition to the beneficial effects of this polymer, its immune-modulating capacity against infection by both bacteria and protozoa, with potent effects on innate and adaptive immunity, which also contribute to its anticancerogeneous properties.

The effectiveness of consumption of this polysaccharide by patients with DM is attributed to and depends on the molecular mass of this substance, chemical conformation, solubility, viscosity and the positioning of their ramifications. In addition, it is important to point out that the dose and time of consumption of this substance are factors that may determine its efficiency. Polysaccharides showing \(\beta-1,3/1,4\) conformation, characteristic of plants, tend to diminish blood cholesterol, glycaemia and insulin secretion levels, while those showing \(\beta-1,3/1,6\) conformation, characteristics of fungi, tend to present antitumor, antiviral, antimicrobial and immunomodulating characteristics. Nevertheless, both chemical structures perform both metabolic and immunological activity.

Therefore, these polymers are used as adjuvant substances in the treatment of diverse syndromes, and are also important sources of raw material for the pharmaceutical industry. However, a large portion of studies have researched the hypoglycemic and hypolipidemic effects of the consumption of BC coming from plants, and there is a scarcity of studies that investigate metabolic and physiologic parameters related to BG coming from yeasts.

Thus, considering DM a multifactorial pathology with a chronic characteristic, the aim of the present study was to evaluate the effects of BG from the fungus \textit{Saccharomyces cerevisae} on metabolic parameters in diabetic rats.

Material and methods

Animals

The present study was approved by the ethics commission on the use of animals - CEUA (Ethics Committee on Animal Use of Federal University of Lavras) of the Federal University of Lavras, under Protocol 083/11 in accordance with the National Legislation in force of the National Council on Control of Animal Experimentation - CONCEA.

A total of 24 adult male rats (\textit{Rattus norvegicus albinus}, \textit{Wistar}), in a healthy state, with initial weight of 187 ± 25 g were initially selected. The animals were obtained from the Central Bioterium of the Federal University of Lavras - UFLA. After this, the rats were submitted to a period of nine days of acclimatization to the environment and team conducting the experiment. The room was acclimatized at a temperature of 20-25°C and with 12/12 hour light-dark cycles. Commercial rations and water were provided \textit{ad libitum} during the entire experimental period.


Metabolic effects of \(\beta\)-glucans \((\textit{Saccharomyces cerevisae})\) per os administration in rats with...
After a week of acclimatization, the animals were randomly divided into the experimental groups (Table I). The design used was an entirely casualized delineation with a 2x2 factorial scheme (diabetic or not, treated or not treated with BG), with six repetitions of one animal each. The animals were kept in individual metabolic cages for measurement of daily urinary excretion volume, ration and water consumption. These measures were obtained directly by weighing and volume measurements.

**Experimental Protocol**

For the induction of DM, 18 animals were induced to a fast of four hours, and received an intraperitoneal injection of 80 mg/kg of streptozotocin. After 48 hours, blood samples were collected by amputating the tip of the tail for measurement of glycemia (with prior fasting for eight hours), which was performed with an Accu-Chek® glucosemeter, Roche brand (Roche Brasil, São Paulo, SP, Brazil). After cardiac puncture, the animals were submitted to ample opening of the abdominal cavity until the internal organs were exposed. Then the pancreas, liver and fragments of each intestinal segment (duodenum, jejunum and ileum) were collected, which were fixed in 10% buffered formaldehyde, and routinely processed for preparing histological slides that were Hema-toxylin-Eosin stained and analyzed by optical microscopy.

In the histopathological evaluation of the pancreas, the aspects of integrity of the Langerhans islet cells were observed, in which the slides of each animal were evaluated and classified in accordance with the following score: (-) normal (normal number of islet cells), (+) slight lesion (number of islet cells slightly reduced, few inflammatory cells, cytoplasm slightly increased), (+++) severe lesion (number of islet cells moderately reduced, moderate number of inflammatory cells, cytoplasm moderately increased) and (++++) severe lesion (number of islet cells severely reduced, elevated number of inflammatory cells, cytoplasm severely increased). In addition, histopathological analysis of the liver was performed to identify possible microscopic lesions in this organ. All the histomorphometric analyses were obtained, using an image capture and analysis system, consisting of a binocular Olympus CX31 (Olympus Optical do Brasil Ltda, São Paulo, SP, Brazil) microscope with camera coupled to it (SC30 CMOS Color Camera for Light Microscopy, Olympus Optical do Brasil Ltda, São Paulo, SP, Brazil). The measurements were made using the software program Image-Pro® Express (Targetware Informática do Brasil Ltda, Água Branca, SP, Brazil).

At the end of the experimental period the animals were induced to a fast of eight hours and were euthanized by cardiac puncture. For this procedure, the rats were anesthetized with 50 mg/kg of sodium thiopental via intraperitoneal injection. The blood samples were collected with a heparinized syringe and the plasma was used to determine the concentrations of total cholesterol (TC), high density lipoprotein (HDL-C), low density lipoprotein + very-low density lipoprotein (LDL-C + VLDL-C), triacylglycerol (TAG), glucose, C peptide and alanine aminotransferase (ALT), using colorimetric enzymatic Kits. Figure 1 presents the stages developed during the course of the experimental period.

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<th>Table I Distribution of experimental groups</th>
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<td><strong>Experimental Groups</strong></td>
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*Beta-glucan Solution at dose of 30 mg/Kg of body weight.

β-glucan (BG) Administration

The BG used was a commercial product and presented the following composition: β-glucans- Mín. 60.0 %; Crude Protein- Max. 8.0%; pH (solution 2%) 4.0 - 7.0; ash - Max.10.0 g/100g. Particle size distribution: mean - 41 μm - <20 μm 19%; 20 - 50 μm 43%; 50 - 100 μm 28%; 100 - 200 μm 10%; >200 μm 0%. Fluidity (sec) - 70.2; Angle of repose (degrees) 31.2; Compressibility 37%; Water retention ability (average) 7.4, Water solubility index 7.9. The BG used was from cell wall of Saccharomyces cerevisiae, and was provided to the animals in daily doses of 30 mg/kg dissolved in distilled water. The solution containing BG was administered via oral for 28 days, whereas the control group animals received saline solution for an equal period, administered by gavage in equal volumes of 0.3 mL.

At the end of the experimental period the animals were induced to a fast of eight hours and were euthanized by cardiac puncture. For this procedure, the rats were anesthetized with 50 mg/kg of sodium thiopental via intraperitoneal injection. The blood samples were collected with a heparinized syringe and the plasma was used to determine the concentrations of total cholesterol (TC), high density lipoprotein (HDL-C), low density lipoprotein + very-low density lipoprotein (LDL-C + VLDL-C), triacylglycerol (TAG), glucose, C peptide and alanine aminotransferase (ALT), using colorimetric enzymatic Kits. Figure 1 presents the stages developed during the course of the experimental period.

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Thus, in the small intestine slides, the ratio between the crypt depths/villous heights was evaluated. The villous height was defined as the vertical distance (μm) between the top of the villous and the villous-crypt junction. The crypt depth was obtained by measuring the vertical distance from the villous-crypt junction up to the final inferior limit of the crypt. In each section, ten distances were measured for the crypt depth and for the villous height, and the values obtained for each segment (duodenum, jejunum or ileum) of each animal were represented by the mean value of the three histological sections.

For evaluation of carcass, the skin was dissected and the viscera were removed. These carcasses of the animals were weighed and dried for analysis of the ether extract and crude protein. The percentage of fat on the carcass was obtained by the Soxhlet method and protein by the Kjeldahl method.

Statistical Analysis

Statistical analyses were performed by the Analysis of Variance (ANOVA) for comparison of means between the studied groups in a 2x2 factorial model (diabetic or not, treated and not treated with BG). When the F values indicated significant difference in the interactions, these were split between the factors. The analyses were performed using the SAS statistical software program. The level of significance was fixed with p<0.05.

Results

As regards the mean daily ration consumption, it was verified that the diabetic animals presented a higher food consumption in comparison with the control animals (p < 0.05), and treatment offered showed no influence. Whereas in the body weight evaluation, the results showed that the diabetic animals lost weight in comparison with the non-diabetic animals (p < 0.05), and the consumption of BG showed no influence (Fig. 2).

When water ingestion and urinary production were evaluated, we observed a higher water consumption and increase in urinary volume of the diabetic animals in comparison with the non-diabetic animals (p < 0.05). The use of BG did not interfere in these parameters (Fig. 2).

The diabetic animals presented an increase in glycemic level and lower values of C peptide in comparison with the non-diabetic animals (p < 0.05). The use of BG led to a reduction in glycemia (30%) only in the diabetic animals, and there was no interference in the blood glucose levels in the control animals (Fig. 3). In the other biochemical parameters of blood, in increase was observed in the CT and LDL-c + VLDL-c levels of the diabetic animals (p < 0.05) in comparison with the healthy animals, without significant improvement with the use of BG. No alteration was observed in the HDL-c levels (Fig. 3). As regards the serum TAG levels, the rats with induced DM presented an increase in this parameter with comparison to the non-induced rats. The use of BG caused a reduction in TAG (32%) in the diabetic animals (p < 0.05) (Fig. 3). Whereas the blood Alanine aminotransferase (ALT) levels were higher in the diabetic animals when compared with the healthy rats, and BG was also efficient in reducing these levels (41%) in the animals with induced DM (Fig. 3).

With regard to the histopathological evaluation, it was observed that the healthy treated animals maintained the histological characteristics of the pancreas and liver unchanged, without indication of lesion from the treatment. However, according to the evaluation criteria used in the present study, the rats induced to DM by STZ demonstrated a significantly reduction in the quantity of Langerhans islets, when compared with the normal animals (Fig. 4 - Table II). The histopathological analysis of the animals’ liver showed no visible microscopic alterations in any of the groups.
In the histomorphometric evaluations of the intestinal measurements, no difference was observed in the V:C ratio of the jejunum and ileum. The V:C ratio of the duodenum was higher in the diabetics animals, without influence of the treatment with BG (Fig. 5).

In the chemical analysis of the carcass, no differences were observed in the percentage of protein, fat and water in the animals with DM when compared with the healthy animals, nor as a result of consuming BG (Fig. 6).

Discussion

The induction of DM by means of STZ is a well described experimental model that has commonly been used to investigate the mechanisms of the disease. In the present study, the induction of DM type 1 with dose of 80 mg/kg of STZ via intraperitoneal administration was successfully achieved, with the induced animals presenting an increase in glycemia in comparison with the rodents not induced by the drug. According to Tay et al., the intravenous or intraperitoneal pathways are more effective and the present greater reproducibility and permanence of the experimental disease. In addition, the above-mentioned authors have suggested that the beta cells of the Langerhans islets of these rodents are more sensitive to the toxic effects of STZ, so that doses between 40 and 80 mg/kg of this substance promote the desired effects.

The administration of BG had a beneficial effect on the glycemic profile of the diabetic animals, with a reduction being observed in the blood glucose levels of these animals. These results corroborate those found by Miranda-Nantes et al., in which it was observed that the BG coming from the fungus Botryosphaeria rhodina (MAMB) administered to diabetic rats induced with a dose of 50 mg/kg of STZ, reduced the glycemia of the diabetic animals by 52% and 17%, at the doses of 12 and 6 mg/Kg respectively. Therefore, the reduction in glycemia with the use of BG may be related to its capacity to act directly on the gastro-intestinal tract, forming a protector layer, thus producing a reduction in the absorption of carbohydrates and lipids.
The dose of 30 mg/kg/day of BG coming from *Saccharomyces cerevisiae* generally promote modulation of the immune response as described in other studies. However, according to the results of the present study, the properties of the BG from yeast go beyond the immunomodulating effects, since there was an improvement of 30% in the glycemia of diabetic animals treated with this polymer. Thus, it was observed that the BG from yeasts has immunomodulating and metabolic properties, and may become a great ally to diabetic patients, seeing that these individuals present not only hyperglycemia, but immune system limitations as well.

The use of BG in this study did not promote improvements in the clinical manifestations arising from DM, which include symptoms such as polyphagia, polyuria, polydipsia and slimming. However, in spite of the 30% reduction in blood glucose caused by BG, the diabetic animals still presented high levels of glycemia (on an average 366 mg/dL), which probably explains the non-remission of all the clinical signs related.

With respect to the biochemical blood parameters, it was observed that there was an increase in TC, LDL-C + VLDL-C and TAG levels, without alteration in HDL-C, of the diabetic animals in comparison with the normal animals. The use of BG was shown to be efficient in reducing only the blood concentrations of TAG (32%) of the diabetic rats. These results corroborate the reports of Miranda-Nantes et al. in which the hypolipidemic action of BG coming from the fungus *Botryosphaeria rhodina* (MAMB) was observed, at
the dose of 2.4 mg/kg, in Wistar rats fed on a diet rich in fats, without alteration in the HDL-C.

These results differ from those found by Gao et al.\textsuperscript{17} in which the BG coming from barley, administered at the dose of 200 mg/Kg reduced CT, TAG, LDL-c levels, which were shown to be elevated in diabetic animals when compared with the normal animals. In this study the BG also promoted an increase in HDL-C in the animals with induced DM. This fact may be related to the greater capacity of BG of vegetable origin in diminishing the absorption and reabsorption of cholesterol and biliary acids in the intestine, in addition to inhibiting the lipogenic enzymes responsible for cholesterol synthesis\textsuperscript{44}. Furthermore, it has been proved \textit{in vitro} that the BGs are capable of inhibiting the uptake of long chain fatty acids in the intestine\textsuperscript{10,18}. Therefore, it is suggested that the dose of BG provided (30 mg/Kg) in the present study may have been limited for promoting more pronounced hypolipidemic effects.

As regards the hepatic enzyme ALT, there was an increase in the blood levels of this transaminase in the animals with induced DM, since the BG was efficient in the reduction of this parameter (41% reduction), thus demonstration a hepatic protective effect. In the same way, Kim et al.\textsuperscript{45} observed and increase in aspartate aminotransferase (AST) and ALT in rats with DM induced by STZ, in which the BG at the dose of 200 mg/kg, coming from oats was efficient in the reduction of these parameters. Therefore, it is suggested that the use of BG could be an ally as a hepatic protector, since it has been observed that severe hyperglycemia may contribute to liver dysfunction\textsuperscript{42}.

The results found in the present study with regard to the histological analyses of the pancreas confirm the diabetogenic action of STZ, since the number of islet cells was severely reduced. As regards the histology of the liver, no type whatever of visible alteration was verified in the different groups. Thus, no sign of toxicity was detected as a result of using this polysaccharide. Different studies have suggested that when this substance is provided via oral administration, for a short period (up to four weeks), it is harmless to the body, even when administered in large quantities\textsuperscript{46-50.}

With respect to the intestinal evaluation, an increase was observed in the villous: crypt ratio (V:C) in the duodenum of the diabetic animals. The BG used in the present study was not effective in reducing this parameter in these animals. Furthermore, no difference was observed in the V:C ratio of the jejunum or ileum in any of the groups. The V:C ratio is considered an indicators of the absorptive capacity in the small intestine, and is directly proportional to the efficiency of nutrient absorption\textsuperscript{11}. Previous studies have also demonstrated a similar increase in the height of villous and depth of crypts in the small intestines of diabetic animals. Therefore, it is suggested that the increase in the V:C ratio in the animals with induced DM is an compensatory adaptive change of the body in response to polyphagia, in an attempt to maintain sufficient quantities of nutrients\textsuperscript{52,53}. However, the dose of BG used in the present study was not capable of promoting complete remission of the DM condition (the animals, in spite of the significant reduction in glycemia, continued to be diabetic), so that these intestinal adaptations persisted even due to the decrease in glycemia.

The results observed with regard to the chemical analysis parameters of the carcass corroborate those found by Lo et al.\textsuperscript{54} in which the content of protein, fat and water in the animals with induced DM presented no differences when compared with the control animals. However, it was expected that the quantity of protein and body fat of the diabetic animals would be reduced, since the glucose deficiency in the body promotes an increase in lipolysis and proteolysis, causing the lipid reserves of the adipocytes, and amino acids of the muscles to be consumed to generate energy\textsuperscript{5}. From this aspect, it is suggested that the period of 28 days of experimentation may not have been sufficient to promote alterations in the carcass.

Conclusions

The daily consumption of BG coming from the cell wall of yeast, at the dose of 30 mg/Kg in diabetic rats, reduced glycemia (30%), triacylglycerides (32%) and ALT (41%) levels. Therefore, the use of this BG may be considered an auxiliary tool in the metabolic control of diabetic individuals, and must not be indicated as an isolated therapy.

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