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Hypoglycemic and Hypocholesterolemic Effects of Botryosphaeran from *Botryosphaeria rhodina* MAMB-05 in Diabetes-Induced and Hyperlipidemia Conditions in Rats

Carolina C. B. O. Miranda-Nantes[1], Eveline A. I. Fonseca[1†], Cassia T. B. V. Zaia[2], Robert F. H. Dekker[3], Neelam Khaper[4], Inar A. Castro[5] and Aneli M. Barbosa[1*]

[1]*Department of Biochemistry and Biotecnology, CCE, State University of Londrina, P. O. Box 6001, Londrina 86051-990, PR, Brazil*
[2]*Department of Physiological Sciences, CCB, State University of Londrina, P. O. Box 6001, Londrina 86051-990, PR, Brazil*
[3]*Biorefining Research Initiative, Lakehead University, Thunder Bay, ON P7B 5E1, Canada*
[4]*Medical Sciences Division, Northern Ontario School of Medicine, Lakehead University, Thunder Bay, ON P7B 5E1, Canada*
[5]*Department of Food and Experimental Nutrition, Faculty of Pharmaceutical Sciences , University of Sao Paulo, Sao Paulo, SP 05508-900, Brazil*
[†]***Present Address:*** *Department of Pharmacology, Institute of Biomedical Science, University of Sao Paulo, Sao Paulo, SP 05508-900, Brazil*

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Botryosphaeran, a water-soluble exopolysaccharide of the β-(1 → 3;1 → 6)-D-glucan type that has been isolated from the culture medium of *Botryosphaeria rhodina* MAMB-05 grown in submerged fermentation using glucose as the sole carbon source, was previously demonstrated to be non-genotoxic in peripheral blood and bone marrow, and exhibited strong anticlastogenic activity. In the present study, the effects of botryosphaeran were investigated in streptozotocin-induced diabetic rats as well as in high-fat diet-fed hyperlipidemic Wistar rats. The plasma glucose level was reduced by 52% in the diabetic group of rats after administration of 12 mg botryosphaeran/kg body weight of the rats (b.w.)/day by gavage over 15 days. A reduction in the median ration intake was accompanied by an increase in the median body weight gain, as well as the efficiency of food conversion. These results demonstrate that botryosphaeran has protective effects by reducing the symptoms of cachexia in *Diabetes mellitus*. Botryosphaeran administered by gavage at a concentration of 12 mg botryosphaeran/kg b.w./day over 15 days also reduced the plasma levels of total cholesterol and low density lipoprotein-cholesterol by 18% and 27%, respectively, in hyperlipidemic rats. Based on these findings, we conclude that botryosphaeran possesses hypoglycemic and hypocholesterolemic properties in conditions of diabetes mellitus and hyperlipidemia, respectively, and may be used as an oral anti-diabetic agent.

KEYWORDS : β-(1 → 3;1 → 6)-Glucan, *Botryosphaeria rhodina*, Hypocholesterolemia, Hypoglycemia, Streptozotocin-induced diabetes

The incidence of diabetes worldwide is increasing at an alarming rate. The control of diabetes-associated mortality and decreased quality and expectancy of life still remains unsatisfactory [1]. Despite the current options available for treating diabetes, there is an increasing demand for oral hypoglycemic agents that are non-toxic with little or no side-effects, such as those experienced with insulin use and present oral hypoglycemic agents [2]. Fungal β-(1 → 3;1 → 6)-D-glucans have been demonstrated to possess medicinal properties effective in treating human disease conditions such as cancer, microbial infections, Alzheimer's disease, acquired immunodeficiency syndrome (AIDS), multiple sclerosis, hypercholesterolemia, and diabetes [3]. In this respect, β-glucans may serve as an alternative treatment based on their protective roles in treating several diseases, including diabetes and associated cardiovascular complications [1, 3].

Some fungal exopolysaccharides have been reported to have the ability to lower cholesterol levels in blood (hypocholesterolemia) and these have the potential to decrease high lipid blood content caused by diet [4]. The hypocholesterolemic effect of β-glucans has been reported in some previous studies involving animals and humans [2], but the mechanism behind this effect still remains unclear [5]. According to Wang *et al*. [6], the hypocholesterolemic effect of β-glucans appeared to be associated with the enhanced viscosity promoted by these biopolymers in the small intestine. This resulted in decreased absorption of

*Corresponding author <E-mail : anelibarbosa@gmail.com>

cholesterol and triacylglycerol, a reduction in digestive enzyme activities, and a higher cholesterol excretion in the faeces.

The fungus *Botryosphaeria rhodina* (isolate MAMB-05) produces a water-soluble exopolysaccharide (EPS) of the β-D-glucan type, which has been structurally characterized as a β-(1 → 3;1 → 6)-D-glucan, and named botryosphaeran [7]. This EPS consists of a main chain of (1 → 3)-linked β-glucose units branched at carbon-6 with glucose and gentiobiose residues, with a degree of branching of ~22%. Botryosphaeran exists in a triple helix conformation [8], which has been associated with the biological response activities reported for β-(1 → 3)-D-glucans [3, 9]. When the fungus was grown on different carbohydrate carbon sources, *B. rhodina* MAMB-05 produced a family of botryosphaerans that varied in the degree and frequency of branching [10].

It is still largely unknown which structural features of β-(1 → 3)-glucans best manifest biological activities [1]. Structurally-different β-(1 → 3)-glucans are known to exhibit different efficacies of action and clinical applications [3]. The anti-mutagenic and anti-clastogenic effect of botryosphaeran was previously demonstrated. The protective effect was concentration-dependent with strong antimutagenic activity observed at low doses of botryosphaeran. Furthermore, there was no weight gain in mice treated with botryosphaeran [11].

Most of the literature on the hypoglycemic and hypocholesterolemic effects of β-(1 → 3)-glucans are based on the β-glucans obtained through extraction of mushroom fruiting bodies [2, 12], or naturally present in oats and barley [13]. However, only a few studies using polysaccharides secreted by microorganisms (EPS) have been reported [14, 15].

No studies on the hypoglycemic and hypocholesterolemic activities of botryosphaeran from *B. rhodina* MAMB-05 exist in the scientific literature. Therefore, in the present study, the effects of botryosphaeran on both of these activities in streptozotocin (STZ)-induced diabetic rats, as well as in high-fat diet-induced hyperlipidemic rats were evaluated.

Materials and Methods

Microorganism and growth conditions. *Botryosphaeria rhodina* MAMB-05 was cultivated on glucose as previously described [7], and left to grow for 72 hr at 28°C under shaking conditions of 180 rpm.

Production and determination of botryosphaeran. After 72 hr of cultivation, the fungal cells were harvested by centrifugation (1,250 ×g/15 min) and the supernatant collected and dialyzed (48 hr) against several changes of de-ionized water. The dialyzate was then treated with 3 volumes of absolute ethanol and left at 4°C for 24 hr. The precipitated material (botryosphaeran) was filtered, re-dissolved in water, and dialyzed against de-ionized water (48 hr). The dialyzate was lyophilized, and the dried material (EPS) stored at −20°C.

Preparation of botryosphaeran solutions. Stock solutions of botryosphaeran (EPS) were prepared in an isotonic saline solution at concentrations of 1.5 and 3.0 g/L, autoclaved at 121°C for 20 min, and used in the biological assay.

Experimental designs. Adult male Wistar rats were obtained from the rat breeding colony at Universidade Estadual de Londrina (UEL), housed individually in plastic cages, and preconditioned for 3 days before beginning the treatment protocol. The rats received a rodents ration of food (Nuvilab CR1; Nuvital®, Apucarana, PR, Brazil) and water ad-libitum, and were kept in a room where the temperature was maintained at 22 ± 2°C, relative humidity at 55 ± 10%, and a light-dark cycle of 12 hr under fluorescent lighting. Animals with body weights ranging from 170~210 g were randomly divided into 4 groups, with 10 rats in each group, to evaluate the hypoglycemic effect of botryosphaeran. Animals with body weights ranging from 170~200 g were randomly divided into 3 groups, with 10 rats per group, to evaluate the hypocholesterolemic effects of botryosphaeran. Median body weight gains as well as median ration intake (g of ration consumed/100 g body weight) were recorded daily during the treatment protocol.

The study on rats was conducted in accordance with ethical procedures and policies approved by the Animal Care and Use Committee at Universidade Estadual de Londrina.

Experimental design for hypoglycemic effects. All animals, except for the control group, received intramuscular injection of STZ (Sigma, St. Louis, MO, USA) freshly dissolved in 0.01 M sodium citrate buffer solution (pH 4.5) at a dose of 50 mg/kg body weight of the rats (b.w.) [16]. Two days after the injections of STZ, urinary glucose was monitored using a glucose oxidase diagnostic kit (Diastix®; Bayer, Sao Paulo, SP, Brazil), and the rats presenting urinary glucose levels above 500 mg/dL were considered diabetic and used in the diabetic groups.

The animals were divided into four groups: control, STZ (diabetic) and 2 groups of diabetic animals treated with botryosphaeran. The control group received a placebo of physiological saline solution by gavage (0.4 mL) twice a day for 15 days, with 12 hr intervals between treatments. Botryosphaeran (1.5 and 3.0 g/L solutions) was administered twice a day by gavage (0.4 mL containing 0.6 and 1.2 mg EPS, respectively) for 15 days during the same interval between treatments. The botryosphaeran doses used

were based on a previous study where the maximum antimutagenic activity was observed [11]. These botryosphaeran concentrations (1.5 and 3.0 g/L) resulted in doses of 1.2 and 2.4 mg/day, respectively, per rat, or 6 and 12 mg botryosphaeran/kg b.w./day based upon the mean b.w. of 200 g/rat. At the end of the treatment period, and following a 16 hr fast, the rats were euthanized by decapitation. Blood was collected in heparinized tubes (5,000 IU, Liquemine®; Roche, Sao Paulo, SP, Brazil) and centrifuged (1,250 ×g/30 min) to recover the plasma. Immediately thereafter, the plasma was assayed for glucose using biochemical analysis kits (Glucose Bioliquid Biodiagnóstica®; Laborclin, Pinhais, PR, Brazil). Ration intake and mean body weight gain was also monitored and the ration conversion efficiency was calculated.

Experimental design for hypocholesterolemic effect. After the initial three-day adaptation period, the hypocholesterolemic activity of botryosphaeran was evaluated in two phases, each lasting 15 days. During the first phase, from day 1 to 15, the animals were fed two diets. The control group received a diet prepared according to the AIN-93M formulation [17] where cellulose (C6288; Sigma) was added. Cellulose, which is an insoluble fiber, has no apparent hypolipidemic action, and has been used as a "control" in diet-related animal studies [18]. The two other groups, (the high-fat diet, HFD groups) were rendered hypercholesterolemic by receiving a diet containing cholesterol, saturated fats and colic acid (referred as HYPER diet, see Table 1 [17, 19]). In the second phase (days 16 to 30), the control and one of the HFD groups of animals continued receiving the same diet as shown in Table 1. The other HFD (HFD + EPS-12 mg) group received 3.0 g/L botryosphaeran (0.4 mL solution containing 1.2 mg EPS) twice a day by gavage at each application, which corresponded to 2.4 mg/day per animal treated or 12 mg botryosphaeran/kg b.w./day. The animals in the other two groups (control and HFD) were given a placebo of sterilized physiological saline solution by gavage (0.4 mL) at the intervals mentioned above in addition to their diet. After the last treatment with botryosphaeran or physiological saline, all animals were fasted for 16 hr and then euthanized by decapitation. Blood was collected in heparinized tubes (Liquemine®) and the plasma recovered. The lipid and free fatty acid content in the plasma was determined as described below.

Table 1. Composition of the diets (control and hyperlipidaemic) used to feed rats

Ingredients[a]	Control[b] (g/100 g b.w.)	HYPER[c] (g/100 g b.w.)
Casein	14.00	14.00
DL-methionine	0.18	0.18
Corn starch[d]	46.57	29.06
Corn dextrin[d]	15.50	15.50
Vitamin mixture[e]	1.00	1.00
Mineral mixture[e]	3.50	3.50
Choline	0.25	0.25
Sucrose	10.00	10.00
Cellulose	5.00	-----
BHT (mg)	0.80	5.20
Soybean oil	4.00	----
Coconut oil	----	25.00
Cholic acid	----	0.50
Cholesterol	----	1.00

b.w., body weight of the rats; BHT, butylhydroxytoluene, an antioxidant.
[a]Sigma chemicals (St. Louis, MO, USA).
[b]AIN-93M control [17].
[c]HYPER: hyperlipidaemic diet modified from Zulet *et al.* [19].
[d]Corn products Brazil (Diadema®, Diadema, SP, Brazil).
[e]Mixtures according to Reeves *et al.* [17].

Chemical analysis. Total cholesterol, high density lipoprotein (HDL)-cholesterol, triacylglycerol and plasma glucose concentrations were analyzed by enzymatic methods using kits (Colesterol Bioliquid, Biodiagnóstica®; Laborclin). The free fatty acid content was determined spectrophotometrically according to the method described by Falholt *et al.* [20]. Low density lipoprotein (LDL)-cholesterol was calculated using the Friedewald formula [21]:

LDL cholesterol = total cholesterol
– HDL cholesterol – (triacylglycerol/5).

The atherogenic index was measured using the following equation: total cholesterol – HDL-cholesterol/HDL-cholesterol.

Statistical analysis. All data were expressed as mean ± SD and analyzed by ANOVA. Values of $p < 0.05$ were considered statistically significant. The animal groups that presented $p < 0.05$ after comparison with the respective control groups were further analyzed by the Tukey test using STATISTICA ver. 6 (Statsoft Inc., Tulsa, OK, USA).

Results

Analysis of the hypoglycemic effect of botryosphaeran. The results of the median ration intake, the median body weight gain, and the ration conversion efficiency (body weight gain/ration intake) are presented in Table 2. The median body weight gain and the median ration intake were in proportion to 100 g of body weight of each animal during the 15-day experiment. The ration intake of the diabetic group increased by 48.14% ($p < 0.01$) relative to the control group, and for the STZ-EPS groups (6 and 12 mg/kg b.w./day), the ration intake increased from 48.05 to 11.50%, respectively. The median body weight gains of the rats in the STZ group were reduced by 76.32%

Table 2. Effect of botryosphaeran on ration consumption, median body weight gain, and ration conversion efficiency in diabetic rats

Treatment group	Median ration intake (g/100 g b.w./day)	Median body weight gain (g/day)	Ration conversion efficiency[a]
Control (saline)	10.78 ± 0.15 a	5.49 a	0.51 a
STZ	15.97 ± 0.29 b**	1.30 b**	0.08 b**
STZ + EPS (6 mg)[b]	15.96 ± 0.33 b**	0.89 b**	0.06 b**
STZ + EPS (12 mg)[b]	12.02 ± 0.47 c*	2.25 c*	0.18 c*

a, b, and c are statistically different; $^{*}p < 0.05$; $^{**}p < 0.01$.
b.w., body weight of the rats; STZ, streptozotocin; EPS, exopolysaccharide.
[a]Ration conversion efficiency = body weight gain/ration intake.
[b]Botryosphaeran dose/kg b.w./day based upon a mean b.w. of 200 g/rat.

($p < 0.01$), and for the animals treated with botryosphaeran (6 and 12 mg/kg b.w./day) these values ranged from 83.78 to 59.01% respectively.

The rate of food conversion efficiency for the STZ group and EPS-6 mg/kg b.w./day group was lower than the control group and the EPS-12 mg/kg b.w./day group (Table 2). The botryosphaeran administered at the latter concentration resulted in an increased rate of conversion of food compared to the STZ group.

Treatment with 12 mg botryosphaeran/kg b.w./day did not fully attenuate the disease symptoms in relation to the STZ group. However, botryosphaeran at the highest dose reduced the median ration intake by 24.7% in the diabetic animals, and increased the median body weight gain by 73.1%. Under these conditions, the ration conversion efficiency increased 125% when compared to the STZ group.

Table 3. Plasma glucose concentration and percentage reduction of glucose in normal and diabetic rats after treatment with botryosphaeran

Treatment group	Plasma glucose	
	Concentration (mg/dL)	Reduction (%)
Control (saline)	126.81 ± 9.77 a	-
STZ	532.18 ± 30.68 b	0
STZ + EPS (6 mg)[a]	443.83 ± 57.81 c*	17
STZ + EPS (12 mg)[a]	254.66 ± 45.49 d**	52

a, b, c, and d are statistically different.
STZ, streptozotocin; EPS, exopolysaccharide; b.w., body weight of the rats.
[a]Botryosphaeran dose/kg b.w./day based upon a mean b.w. of 200 g/rat; $^{*}p < 0.05$ in relation to the diabetic control group (STZ); $^{**}p < 0.01$ in relation to the diabetic control group (STZ).

The effect of botryosphaeran on plasma levels of glucose is presented in Table 3. STZ effectively induced diabetes in the STZ group as confirmed by the increased plasma glucose levels (532.2 mg/dL), which indicates the reproducibility of this model. Botryosphaeran administered at 12 mg/kg b.w./day reduced the plasma glucose levels by 52% ($p < 0.01$) relative to the diabetic group. At the lower concentration (6 mg/kg b.w./day), botryosphaeran reduced the plasma glucose concentration by 17% ($p < 0.05$).

Analysis of the hypocholesterolemic effect of botryosphaeran. Data on total plasma cholesterol concentration, LDL-cholesterol, HDL-cholesterol and very low density lipoprotein (VLDL)-cholesterol are presented in Table 4. There were statistically significant differences ($p < 0.05$) in the total cholesterol and LDL-cholesterol ($p < 0.01$) concentrations between the high-fat diet HFD group and the control group. The total cholesterol and LDL-cholesterol levels decreased by 18.6% and 27.0%, respectively, in the botryosphaeran-treated group when compared to the HFD group. The HDL-cholesterol and VLDL-cholesterol levels did not differ significantly in any

Table 4. Effect of botryosphaeran on cholesterol levels in hyperlipidaemic rats

Treatment group	Cholesterol (mg/dL)			
	Total	LDL	HDL	VLDL[a]
Control	69.15 a ± 5.3	13.19 a ± 2.7	31.43 ± 7.5	24.53 ± 1.7
HFD[b]	137.51 b* ± 6.7	77.91 b** ± 3.5	37.27 ± 5.9	22.34 ± 1.9
HFD + EPS (12 mg)[c]	111.95 b* ± 7.4 (18.6%)[d]	56.85 c* ± 4.0 (27.0%)	35.14 ± 4.3 (5.9%)	19.96 ± 1.8 (10.6%)

a, b, and c are statistically different; $^{*}p < 0.05$; $^{**}p < 0.01$.
LDL, low-density lipoprotein; HDL, high-density lipoprotein; VLDL, very low density lipoprotein; EPS, exopolysaccharide; b.w., body weight of the rats.
[a]VLDL = triacylglycerol/5.
[b]High-fat diet (HYPER diet containing added cholesterol (1%), saturated fats (25%) and colic acid (0.1%) (see Table 1).
[c]Botryosphaeran dose/kg b.w./day based upon a mean b.w. of 200 g/rat.
[d]Values in parenthesis represent % reduction over HYPER-fed animals.

Table 5. Effect of botryosphaeran on the atherogenic index, ratio of HDL-cholesterol to total cholesterol and level of free fatty acids in the plasma of hyperlipidaemic rats

Treatment group	Plasma atherogenic index[a]	Cholesterol ratio (HDL/total)	Free fatty acid (μmol/dL)
Control	1.20 a ± 0.21	0.45 ± 0.02	265.76 a ± 19.4
HFD[b]	2.68 b* ± 0.13	0.27 ± 0.05	314.09 b* ± 20.1
HFD + EPS (12 mg)[c]	2.18 b* ± 0.17	0.31 ± 0.07	313.86 b* ± 22.2

a and b are statistically different; $^{*}p < 0.05$.

HDL, high-density lipoprotein; EPS, exopolysaccharide; b.w., body weight of the rats.

[a]Total cholesterol - HDL-cholesterol/HDL-cholesterol.

[b]High-fat diet (HYPER) containing added cholesterol (1%), saturated fats (25%) and colic acid (0.1%).

[c]Botryosphaeran dose/kg b.w./day based upon a mean b.w. of 200 g/rat.

of the groups, indicating that the ration didn't alter these components in the blood circulation.

Table 5 shows the effect of botryosphaeran on the atherogenic index and on the HDL-cholesterol-to-total cholesterol ratio, which are reliable indexes of development of coronary artery disease [22]. The plasma free fatty acid concentrations are presented in Table 5. The plasma atherogenic index was higher in the HFD group relative to the control. Treatment with botryosphaeran did not attenuate this effect. The HDL-cholesterol/total cholesterol ratio did not change in any of the groups.

Discussion

Different types of oral hypoglycemic agents exist and act in association with insulin for the treatment of diabetes mellitus. Recently, there has been increasing interest in using natural products that possess anti-diabetic activity and minimal side effects and can be administered orally. There is a paucity of information on the hypoglycemic activity of fungal EPS's.

The water-soluble EPS botryosphaeran, which is a β-(1 → 3;1 → 6)-D-glucan secreted in the liquid culture medium when *B. rhodina* MAMB-05 is grown in submerged fermentation. This EPS belongs to the gel-forming group of β-(1 → 3)-glucans and its hypoglycemic properties in the STZ-induced diabetic rat model, as well as its hypocholesterolemic effect in rats on a high-fat (HYPER) diet were examined.

STZ is a selective genotoxicant employed to induce diabetes mellitus in experimental animals through its toxic effects on pancreatic β-cells [23]. Selective destruction of pancreatic β-cells by STZ results in decreased plasma insulin levels, which, in turn, lead to the condition of hyperglycemia. The STZ-induced diabetes in rats was confirmed by the increased plasma glucose concentration in the diabetic group when compared to the control group. Lower concentrations of botryosphaeran (6 mg/kg b.w./day) reduced plasma glucose level by 17% relative to the STZ group, whereas treatment with 12 mg botryosphaeran/kg b.w./day reduced plasma glucose level by 52%. The role of β-glucans in decreasing the blood glucose concentration has also been previously demonstrated by others [1, 2, 15]. Most previous studies that evaluated the hypoglycemic effects of EPS used the extracts of mushroom fruiting bodies, fruiting body powders and extracts of fungal mycelia; however, few studies have examined the effects of crude fungal EPSs [14]. A crude EPS produced by *Phellinus baumii* showed a 52.3% reduction in the plasma glucose level in STZ-induced diabetic rats [15], and this value was similar to that observed with botryosphaeran, although the dose of EPS used in that study was 200 mg/kg b.w./day, which was 17-fold higher than the botryosphaeran concentration used in this study. The structural moiety responsible for this effect and the receptor involved in binding the β-(1 → 3)-glucan is still unknown.

Orally administered β-(1 → 3)-glucans are considered to be resistant to digestion by gastric juices, and are entrapped by macrophage receptors present in the intestinal wall. Water-soluble polysaccharides from *Tremella mesenterica* fruiting bodies demonstrated significant hypoglycemic activity in STZ-induced and genetic models of diabetes. The anti-diabetic activity correlated with an increase in insulin secretion that accelerated glucose metabolism in the liver [12, 24]. Furthermore, an increase in insulin sensitivity was also suggested to play a role in the anti-diabetic activity of β-glucan. The hypoglycemic activities of an EPS isolated from submerged cultures of *Lentinus edodes*, *Cordyceps militaris* and *C. sinensis* were also reported [4, 25, 26]. Grifolan, which is a β-(1 → 3)-glucan produced by the fungus *Grifola frondosa*, was effective in activating macrophages as well as increasing insulin production and contributing to its beneficial effect in diabetes. Grifolan consists of two β-(1 → 6)-linked branched glucose residues to every five glucose residues along the β-(1 → 3)-glucan chain with a degree of branching ranging from 31 to 36% [9, 27], whereas botryosphaeran contains one β-(1 → 6) branched glucose or gentiobiose residues for every five β-(1 → 3) glucose residues, with 22% branching at C-6 [7]. According to Bohn and BeMiller [9], the β-(1 → 3)-D-glucan backbone is essential to the biological response activities, and the most active β-glucans have degrees of branching ranging between 20 to 33%.

Diabetes mellitus is associated with an increase in ration

consumption and decrease in the efficiency of food conversion as a result of defects in carbohydrate metabolism by pancreatic β-cells [28]. Botryosphaeran at a concentration of 12 mg/kg b.w./day increased the median body weight gain when compared to that of 6 mg/kg b.w./day, which suggests that botryosphaeran has an anti-cachectic property (Table 2). The ration conversion efficiency was significantly decreased in the STZ group relative to the control group. The higher concentration (12 mg/kg b.w./day) of botryosphaeran was effective in improving the efficiency of ration conversion when compared to the lower concentration (6 mg/kg b.w./day). These data are in agreement with studies that evaluated the effects of EPS on median body weight gain [24, 25]. The concentration of botryosphaeran used in the present study (12 mg/kg b.w./day) was relatively low compared to other reported studies (100 mg/kg b.w./day) [4, 26] indicating the efficiency of botryosphaeran in providing beneficial effects. Future studies will involve evaluating the use botryosphaeran as a coadjuvant of insulin for the treatment of diabetes.

Although fungal β-(1 → 3)-glucans are known to possess hypocholesterolemic activities [3, 14], only a few studies have examined the hypocholesterolemic effects with β-(1 → 3)-glucans produced through submerged fermentation [15]. In the present study, we also analyzed the hypocholesterolemic effect of botryosphaeran in HFD rats. The Hyper diet resulted in a significant increase in total cholesterol and LDL-cholesterol in the HFD group when compared to the control group (Table 4). Treatment with botryosphaeran at a concentration of 12 mg/kg b.w./day resulted in a modest decrease in total cholesterol level (18.6%) relative to the HFD group (Table 4). Another study reported no significant decrease in plasma cholesterol and triacylglycerol levels in hyperlipidemic rats treated with polysaccharides [29]. In contrast, Yang *et al*. [4] reported that an EPS from *Auricularia polytricha* administered at high concentrations (50~100 mg/kg b.w.) to hyperlipidemic rats decreased the plasma LDL-cholesterol concentration by 70%, which was associated with the inhibition of the micellar formation in the small intestine, thereby altering the physical characteristics in the intestinal mucosa of the rats. In the present study, however, the concentration of botryosphaeran was relatively low (12 mg/kg b.w./day) compared to that used by Yang *et al*. [4], which may have been the reason for the modestly low reduction in cholesterol levels in our study.

Although the mechanism of the hypocholesterolemic activity of botryosphaeran is presently unclear, it has been proposed that the increased viscosity of these biopolymers may decrease the absorption of cholesterol and triacylglycerols, or may decrease the activity of digestive enzymes thereby reducing the lipid levels [6]. Other studies have suggested that the decrease of the total cholesterol and LDL-cholesterol levels by β-(1 → 3)-glucans may be related with the up-regulation of the activity of cholesterol 7-α-hydroxylase, which is involved in the conversion of cholesterol into bile acids [30] resulting in lowering cholesterol levels.

In conclusion, botryosphaeran at a concentration of 12 mg/kg b.w./day exhibited significant hypoglycemic activity (blood glucose reduction of 52%) in diabetic rats, but at the same concentration did not exhibit significant hypocholesterolemic activity, which suggests that higher concentrations of this EPS should be further investigated. Future studies will be directed towards the analysis of the rheological properties of botryosphaeran as well as the development of derivatised botryosphaeran aimed at decreasing the viscosity of this EPS in order to allow an increased dose to be used in the diet.

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