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Protection against Oxidative Stress in Diabetic Rats by Wheat Bran Feruloyl Oligosaccharides

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Diabetes is one of the most costly of the chronic diseases and is increasing in epidemic proportions in developing countries. It has been found that some antioxidants play a role in protection against oxidative stress, which is associated with diabetes. In this study, enzyme-released feruloyl oligosaccharides from wheat bran were given intragastrically (ig) to test their effect on antioxidant capacity, body weight restoring capacity, and serum glucose level in alloxan-induced diabetic Sprague−Dawley (SD) rats, using sodium ferulate and vitamin C as positive control groups. The levels of blood glucose, total antioxidant capacity (TAOC), and malondiadehyde (MDA) and the activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and xanthine oxidase (XOD) were determined in rat serum, liver, and testes. Feruloyl oligosaccharides significantly increased TAOC level, GSH-Px, and SOD activities, but decreased blood glucose and MDA levels and XOD activity in serum, liver, and testes of diabetic rats compared to diabetic controls. Feruloyl oligosaccharides were, overall, more efficient in mitigating oxidative damage in diabetic rats than sodium ferulate and vitamin C. In this feruloyl oligosaccharide feeding study, the antioxidant restoring capacity varied across the tissues observed, and also the activity change of the various antioxidant enzymes varied within a single tissue. Feruloyl oligosaccharides showed greater antioxidant capacity in vivo than in vitro when compared with vitamin C.

KEYWORDS: Feruloyl oligosaccharides; wheat bran; diabetes; oxidative stress, total antioxidant activity (TOAC); alloxan

INTRODUCTION

Free radicals have been associated with the pathogenesis of various disorders such as cancer, diabetes, cardiovascular diseases, autoimmune diseases, and neurodegenerative disorders and are also implicated in aging. Antioxidants are emerging as prophylactic and therapeutic agents, which scavenge free radicals and prevent the damage caused by them (*1*). Wheat bran is a major source of dietary fiber and phenolic compounds such as hydroxycinnamic acids and hydroxybenzoic acids, which are known for their antioxidant activity (*2*−*4*). Among wheat bran phenolic compounds, ferulic acid has been the most extensively investigated because of its physiological roles in antioxidant, antimicrobial, anti-inflammatory, antithrombotic, and anticancer activities and in the prevention of coronary disease, lowering cholesterol in serum and liver, and increasing sperm viability (*4*). There are many reports on the release of ferulic acid from wheat bran and other cereal brans to produce ferulic acid and its oligosaccharide esters (*5*−*11*). It was reported that ferulic acid bound to arabinoxylans from wheat bran is more bioavailable in the rat than the free compound by increasing the plasma elimination half-life period in the organism and decreasing the level of urinary excretion in 24 h (*12*). In vitro studies also indicated that feruloyl oligosaccharides showed a higher antioxidant activity than free ferulic acid (*13*−*15*). Whether this higher antioxidant activity is found in vivo is still to be elucidated. In this study, rats with diabetes mellitus induced by alloxan injection were used to test the antioxidant activity of feruloyl oligosaccharides prepared from wheat bran.

Currently, 6.2% of the U.S. population is estimated to have diabetes, with 35% of these cases undiagnosed (*16*). Slightly lower percentages suffer from diabetes in newly industrializing countries such as China, but these percentages are increasing. Diabetes is associated with a risk of atherosclerosis, which involves endothelial dysfunction. Platelet activation in the narrowed arteries is the most proximate event in the culmination of acute myocardial infarction and stroke (*17*). Hyperglycemia is associated with atherosclerogenesis. The effect of diabetes (hyperglycemia) is mediated, in large part, by a state of enhanced oxidative stress, which is associated with disruption and uncoupling of several key oxidative reactions that result in excessive production of reactive oxygen species at the mitochondrial and cellular levels (*17*, *18*).

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Many antioxidants or antioxidant-containing foods have been investigated as protection against diabetic oxidative stress, and it has been shown that some antioxidants play a large role in the mitigation of oxidative stress in diabetes mellitus (*1*, *19*−*22*). Recently, much attention has been paid to replacing synthetic antioxidants with natural alternatives, primarily plant phenolics (*23*). Feruloyl oligosaccharides, the ferulic acid ester of oligosaccharides, produced either by microorganisms in the colon or from cereal bran fermentation (*4*), are natural phenolic antioxidants. As purified natural extracts, they have the potential for industrial scale production as nutraceutical products. This investigation of the antioxidant activity of feruloyl oligosaccharides in diabetic rats demonstrates the potential of a natural antioxidant for ameliorating the symptoms of diabetes.

MATERIALS AND METHODS

Materials. Wheat bran was provided by Nanfang Flour Co. in Guangzhou.

Viscozyme L (25−55 units/mL), heat-stable α-amylase Termamyl 120 L (EC 3.2.1.1, from *Bacillus licheniformis*, 120000 units/g), Alcalase 2.4 L protease (EC 3.4.21.62, from *B. licheniformis*, 2.4 units/g), and AMG 300 L amyloglucosidase (EC 3.2.1.3, from *Aspergillus niger*, 300 units/g) were purchased from Novo Nordisk (Bagsvaerd, Denmark).

Anion macroporous resin D201, which is a strong-alkali anion macroporous resin, was purchased from the Chemical Company of Nankai University (Nanjing, Jiangsu Province, China).

Male Sprague−Dawley (SD) rats and their feed were purchased from Guangdong Medical Experimental Animal Center (Guangzhou, Guangdong Province, China).

Alloxan, ferulic acid, and vitamin C were purchased from Sigma-Aldrich (St. Louis, MO). All other chemicals and solvents used were of analytical grade.

OneTouch Brand serum glucose test strips were purchased from Lifescan Inc. (Milpitas, CA). Test kits for total antioxidant capacity (TAOC), the activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and xanthine oxidase (XOD), and the level of malondiadehyde (MDA) were purchased from Nanjing Jiancheng Bioengineering Co., Nanjing, Jiangsu Province, China (www.njjcbio-.com).

Pretreatment of Wheat Bran. Fresh wheat bran was oven-dried at 110 °C for 6 h and then ground to pass an 80-mesh sieve. Dried wheat bran (2000 g) was destarched and deproteinized in a 30-L biological reactor (Zhenjiang East Bioengineering Co., Zhenjiang, Jiangsu Province, China) by heat-stable α-amylase, amyloglucosidase, and protease sequentially, and the water-insoluble dietary fiber was separated as described by Yuan et al. (*13*).

Enzymatic Hydrolysis of Destarched and Deproteinized Wheat Bran. One thousand grams of pretreated wheat bran was added to 15 L of Viscozyme L at a concentration of 0.2% (v/v) and reacted in a 30-L biological reactor (Zhenjiang East Bioengineering Co.) at pH 4.5 and 40 °C for 6 h. Reaction was stopped by heating at 95 °C for 30 min and cooled by cycling cold water, and the supernatant was collected by a basket centrifuge and filtered at 5 kDa using an NUF model ultrafiltrator from Wuxi Ultrafiltration Equipment Co. (Jiangsu, China); the filtrate was collected for further purification of feruloyl oligosaccahrides.

Preparation of Feruloyl Oligosaccharides. Free ferulic acid was removed from the ultrafiltration filtrates by anion exchange chromatography as described previously (*24*). Ferulic acid was separated on anion macroporous resin D201. The combined eluents, containing 3.42 mmol/L bound ferulic acid and 5.6% oligosaccharides based on xylose, were vacuum-concentrated to 5 L, bottled in separate 500-mL containers, sterilized at 85 °C for 30 min, cooled, and refrigerated.

Rats, Diets, and Experimental Design. Male SD rats weighing between 180 and 200 g were maintained at 25 ± 2 °C and 75% relative humidity on an alternating 12-h light/12-h dark cycle. Diabetes was induced in rats by intraperitoneal injection of freshly prepared alloxan solution in normal saline on three successive days: day 1, 100 mg/kg of body weight; day 2, 130 mg/kg of body weight; day 3, 100 mg/kg of body weight. Rats with serum glucose at 72 h (blood collection from tail) of ≥11.1 mmol/L were regarded as diabetic.

A total of 50 rats were randomly divided into 5 groups (10 per group): group 1, normal control rats; group 2, diabetic rats; group 3, diabetic rats fed with feruloyl oligosaccharides intragastrically (ig) at a dose that contained 50 μmol of bound ferulic acid/kg of body weight (bw); groups 4 and 5, diabetic rats fed, respectively, with sodium ferulate and vitamin C ig at a dose of 50 μmol/kg of bw.

Feed and water were provided. At the end of the 42-day feeding period, the rats were deprived of food, body weight was determined, and blood was collected from tails into tubes containing 10% EDTA. Serum was separated for estimating blood glucose, enzymatic antioxidants, total antioxidant activity, and lipid peroxides. The rats were killed by cervical dislocation, and their liver and testes were collected and homogenized. The homogenate was centrifuged at 10000*g* for 20 min at 2 °C and the supernatant used for the determination of antioxidant content and enzymatic activity.

Analytical Methods. Protein content was estimated according to the method of Lowry et al. (*25*) using bovine serum albumin as standard at 640 nm. Serum glucose levels were estimated with OneTouch Brand serum glucose test strips from Lifescan Inc..

Total antioxidant capacity (TAOC), MDA (malondialdehyde), and activities of GSH-Px, XOD, and SOD were determined using commercially available kits from Nanjing Jiancheng Bioengineering Co. TAOC was determined spectrophotometrically at 593 nm by ferric reducing/antioxidant power assay with kit A015; MDA was determined by thiobarbituric acid (TBA) reaction according to the method of Nagababu and Lakshmaiah (*26*) using kit A003; GSH-Px was assayed on the basis of the method of Rotruck et al. (*27*) using kit A005; SOD and XOD were assayed according to the method of Zhu et al. (*28*) using kit A001 and kit A002, respectively. The activities of the antioxidant enzymes were defined as follows: 1 unit/mL for GSH-Px was defined as 1 μmol/L of glutathione consumed by 1 mL of enzyme per minute at 37 °C; 1 unit/mL for SOD was defined as 1 mL of enzyme required to inhibit 50% of nitrite formation in 1 mL of reaction solution in 1 min at 37 °C; 1 unit for XOD was defined as 1 L of enzyme required to transform 1 μmol of hypoxanthine to xanthine in 1 min at 37 °C.

Ferulic acid in feruloyl oligosaccharides was determined spectrophotometrically at 320 nm using a UV-2101 PC UV−vis scanning spectrophotometer (Shimadzu) (*29*); oligosaccharides were acid-hydrolyzed and determined according to the method of Yuan et al. (*14*).

Statistical Analysis. Data on body weight and blood measurements were analyzed using the analysis of variance procedure of the SPSS 13.0 for Windows.

RESULTS

Data for change in rat body weight and blood measurements are presented in **Table 1**. A significant increase in blood glucose and a decrease in body weight were observed in the alloxan-induced rats. Feeding feruloyl oligosaccharides for 42 days reversed the diabetic weight loss by 27.6%, reaching 89.1% of the normal level (calculated from **Table 1**). Feeding feruloyl oligosaccharides proved to be more effective in reversing diabetic weight loss than feeding sodium ferulate and vitamin C. Feeding feruloyl oligosaccharides decreased the serum glucose level by >70% but did not reach the normal level and showed no significant difference from feeding sodium ferulate and vitamin C (**Table 1**).

Diabetic rats fed feruloyl oligosaccharides significantly increased serum antioxidant capacity. In comparison to diabetic controls, SOD and GSH-Px activities increased 27.9 and 30.6%, respectively; MDA content and XOD activity (an enzyme increasing the oxidative stress level in the body) decreased 29.2 and 9.7%, respectively.

Table 1. Body Weight and Serum Glucose of Rats in Different Groups[a]

	body weight (g)		serum glucose (mmol/L)	
group	prior to alloxan	end of feeding trial	prior to alloxan	end of feeding trial
normal	250.3 ± 21.5 a	378.5 ± 23.0 d	4.41 ± 0.49 a	4.09 ± 0.25 a
diabetic	248.9 ± 19.8 a	264.3 ± 32.2 a	13.89 ± 2.82 b	17.63 ± 3.67 c
diabetic fed SF	249.9 ± 29.3 a	302.8 ± 34.3 b	14.86 ± 3.15 b	10.29 ± 2.81 b
diabetic fed Vc	243.7 ± 26.3 a	318.5 ± 33.9 b	14.31 ± 3.67 b	10.76 ± 2.56 b
diabetic fed FO	250.8 ± 22.9 a	337.4 ± 36.6 c	14.00 ± 2.42 b	10.04 ± 2.71 b

[a] Values (means ± SD, $n = 10$) with different letters within a column are significantly different at the 5% level. SF, sodium ferulate; Vc, vitamin C; FO, feruloyl oligosaccharides.

Table 2. TAOC, Activities of SOD, GSH-Px, and XOD, and Content of MDA in Serum of Rats[a]

group	TAOC (units/mL)	SOD (units/mL)	GSH-Px (units/mL)	XOD (units/L)	MDA (nmol/mL)
normal	8.0 ± 1.8 b	137.1 ± 11.3 bc	419.9 ± 22.8 b	5.7 ± 1.6 a	5.5 ± 1.2 ab
diabetic	6.2 ± 1.2 a	114.5 ± 13.9 a	339.2 ± 27.6 a	7.2 ± 1.2 c	8.9 ± 1.6 c
diabetic fed SF	7.2 ± 1.2 a	133.0 ± 14.5 ab	439.6 ± 23.7 c	5.8 ± 1.4 a	4.9 ± 0.8 a
diabetic fed Vc	6.9 ± 1.6 a	123.4 ± 12.3 a	428.7 ± 25.3 b	6.7 ± 2.0 b	6.0 ± 0.8 b
diabetic fed FO	7.1 ± 1.5 a	146.5 ± 15.0 d	443.1 ± 19.7 c	6.5 ± 1.1 b	6.3 ± 1.6 b

[a] Values (means ± SD, $n = 10$) with different letters within a column are significantly different at the 5% level. SF, sodium ferulate; Vc, vitamin C; FO, feruloyl oligosaccharides.

Table 3. TAOC, Activities of SOD, GSH-Px, and XOD, and Content of MDA in the Liver of Rats[a]

group	TAOC (units/mL)	SOD (units/mL)	GSH-Px (units/mL)	XOD (units/L)	MDA (nmol/mL)
normal	1.4 ± 0.3 c	218.1 ± 23.7 d	63.4 ± 12.2 b	7.8 ± 1.5 b	1.2 ± 0.2 b
diabetic	0.3 ± 0.1 a	156.8 ± 21.6 a	48.8 ± 9.2 a	9.6 ± 1.1 c	1.9 ± 0.4 c
diabetic fed SF	0.9 ± 0.2 b	197.8 ± 20.6 b	70.1 ± 13.4 c	7.8 ± 1.1 b	0.9 ± 0.2 a
diabetic fed Vc	0.4 ± 0.1 a	219.2 ± 18.4 d	58.7 ± 10.3 b	9.4 ± 1.3 c	0.9 ± 0.3 a
diabetic fed FO	0.7 ± 0.2 b	206.9 ± 19.1 c	63.1 ± 13.5 b	6.6 ± 1.8 a	0.8 ± 0.2 a

[a] Values (means ± SD, $n = 10$) with different letters within a column are significantly different at the 5% level. SF, sodium ferulate; Vc, vitamin C; FO, feruloyl oligosaccharides.

Table 4. TAOC, Activities of SOD, GSH-Px, and XOD, and Content of MDA in the Testes of Rats[a]

group	TAOC (units/mL)	SOD (units/mL)	GSH-Px (units/mL)	XOD (units/mL)	MDA (nmol/mL)
normal	1.1 ± 0.3 bc	113.3 ± 22.7 b	9.1 ± 1.6 c	6.7 ± 1.8 a	0.7 ± 0.1 b
diabetic	0.7 ± 0.2 a	82.3 ± 13.4 a	7.2 ± 2.0 a	11.3 ± 1.2 c	0.9 ± 0.2 c
diabetic fed SF	1.0 ± 0.2 b	128.7 ± 17.1 c	8.4 ± 2.1 b	6.7 ± 1.4 a	0.7 ± 0.1 b
diabetic fed Vc	0.9 ± 0.2 b	119.1 ± 14.6 b	8.0 ± 2.7 b	8.2 ± 1.9 b	0.7 ± 0.2 b
diabetic fed FO	1.3 ± 0.2 c	118.8 ± 12.2 b	8.1 ± 2.4 b	8.4 ± 1.9 b	0.5 ± 0.1 a

[a] Values (means ± SD, $n = 10$) with different letters within a column are significantly different at the 5% level. SF, sodium ferulate; Vc, vitamin C; FO, feruloyl oligosaccharides.

Feeding feruloyl oligosaccharides increased the level of antioxidant capacity about 1 unit/mL in serum, but this increase was not statistically significant at the 5% level for $N = 10$ (**Table 2**). Feeding feruloyl oligosaccharides, compared to feeding sodium ferulate and vitamin C, significantly increased SOD activity and GSH in the serum of diabetic rats, but showed no significant difference for XOD activity or MDA content (**Table 2**).

Compared to diabetic controls, the tested indices in liver and testes of rats fed feruloyl oligosaccharides were all significantly improved (**Tables 3** and **4**). In the liver, feeding feruloyl oligosaccharides increased TAOC, SOD activity, and GSH-Px activity by 133.3, 32.0, and 29.3%, respectively, and decreased XOD activity and MDA content by 31.3 and 57.9%, respectively. In the testes, feeding feruloyl oligosaccharides increased TAOC, SOD activity, and GSH-Px activity by 85.7, 44.3, and 12.5%, respectively, and decreased XOD activity and MDA content by 25.7 and 44.4%, respectively. These findings strongly indicate that feruloyl oligosaccharides could protect against oxidative stress in diabetic rats. Also, compared with feeding sodium ferulate and vitamin C, feeding feruloyl oligosaccharides showed a higher capacity for the prevention of oxidative stress in liver than in testes.

DISCUSSION

Oxidative free radical induced degeneration of pancreatic β-islet cells has been implicated in the etiopathogenesis of clinical diabetes mellitus. In keeping with this postulate, experimental diabetogenic agents such as alloxan have been designed. Alloxan is converted to dialuric acid by a two-electron reduction, and dialuric acid is unstable and converted back to alloxan, a reaction accompanied by the reduction of oxygen to the oxidative free radical, O_2, and H_2O_2. The latter, through the Fenton reaction in the presence of Fe^{2+} ions, generates the highly toxic hydroxyl radical. Use of radical scavengers protects animals against alloxan-induced diabetes mellitus (*21*, *30*).

Enzyme-released feruloyl oligosaccharides from wheat bran exhibited restoring capacity for serum glucose level in diabetic rats induced by alloxan injection. Being a potential antioxidant,

as the extract of some plants are, feruloyl oligosaccharides may bring about an anti-hyperglycemic effect through insulin secretion from the remnant β-cells and from regenerated β-cells and exert hypoglycemic activity through insulin release stimulatory effects (*31*, *32*), thus mitigating the syndrome of diabetes and restoring lost body weight.

Feruloyl oligosaccharides also restored antioxidant capacity by increasing the activities of SOD and GSH-Px and decreasing XOD activity and MDA content. Many studies have proved the reduction in the antioxidant enzyme activities of SOD, CAT, and GPx in diabetic condition, which is possibly due to increased oxygen metabolites causing a decrease in the activity of the antioxidant defense system and due to nonenzymatic glycosylation of the enzymes (*33*). Feruloyl oligosaccharides a play role in the inhibition of lipid peroxidation and the scavenging of free radicals by their antioxidant nature and protected against damage of pancreatic tissue and restored antioxidant enzyme activities. However, the reasons why feruloyl oligosaccharides have shown less antioxidant capacity in vitro than in vivo (*15*) and have different restoring capacities in serum, liver, and testes must be further investigated. Also, their effects on human subjects need to be studied.

Moreover, the diabetic rats fed feruloyl oligosaccharides showed a higher protection effect against oxidative stress than the diabetic rats fed the same concentration of sodium ferulate and vitamin C. Test results indicated that feruloyl oligosaccharides ranked differently according to antioxidant capacity in the serum, liver, and testes in diabetic rats and also ranked differently according to the activity of antioxidant enzymes in the same tissue. The precise mechanism for these effects requires further investigation.

In summary, feeding feruloyl oligosaccharides significantly increased the antioxidant capacity of alloxan-induced diabetic rats and restored it to almost normal level or even higher, thus greatly mitigating the diabetes syndrome. Moreover, the rats fed feruloyl oligosaccharides showed less serious diabetes syndrome than rats fed sodium ferulate and vitamin C, leading to the conclusion that feruloyl oligosaccharides are a suitable antioxidant for protection against oxidative damage in diabetes. Furthermore, feruloyl oligosaccharides have other benefits. First, they are a nonionic chemical species that may pass through cell membranes with a high density of inner negative charges more easily than the negative antioxidants vitamin C and free phenolic compounds. Second, they can form resonance-stabilized free radicals that would not actively attack other substances after free radical formation, and, third, they contain oligosaccharides that are beneficial for gastrointestinal function and immunology (*34*).

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