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Wheat bran xylooligosaccharides improve blood lipid metabolism and antioxidant status in rats fed a high-fat diet

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ABSTRACT

Functional oligosaccharides have been used extensively both as food ingredients and pharmacological supplements. The objective of the present study was to evaluate effects of wheat bran xylooligosaccharides substituted with arabinose as novel functional oligosaccharides on blood lipid metabolism and antioxidant status in rats fed a high-fat diet for 6 weeks. The high-fat diet resulted in hyperlipidemia and an increase in oxidative stress, indicated by a significant rise in body weight, blood glucose and lipids levels, and malondialdehyde level of serum, liver and heart, and a significant decrease in the ratio of reduced glutathione (GSH) to oxidized glutathione (GSSG) and antioxidant enzymes activity in them, however, supplementation of 5% wheat bran xylooligosaccharides had a beneficial effect on controlling body weight, improving blood glucose and lipid levels and increasing antioxidant status. These results demonstrated that wheat bran xylooligosaccharides might be effective in protecting humans against high-fat diet-induced oxidative stress.

1. Introduction

High-fat diets lead to excessive energy intake and are reported to increase oxidative stress in a variety of tissues of rodents (Sreekumar et al., 2002). Oxidative stress has been defined as a disturbance to the equilibrium status of prooxidant and antioxidant systems in favor of prooxidation due to excess formation of free radicals and decreased activity of antioxidant defense systems (Kennedy et al., 2005). Excess reactive oxygen species (ROS) are toxic as they can attack and damage various biomolecules such as DNA, proteins, carbohydrate and membrane lipids leading to cell death and tissue injure. Oxidative stress and ROS have been associated with a variety of chronic health problems, such as cardiovascular disease, certain cancers, malaria, rheumatoid arthritis, diabetes, Alzheimer's disease, Parkinson's disease, other neurological disorders and also aging process (Aruoma, 1998).

Recently, there are a few reports which demonstrate that some oligosaccharides can reduce effectively oxidative stress in high-fat diet-treated rats or in streptozotocin-induced diabetic rats. Yamatoya, Shirakawa, Kuwano, Suzuki, and Mitamura (1996) examined the effect of hydrolyzed xyloglucan from the tamarind plant using edno-1,4-β-glucanase on lipid metabolism rats fed a high-fat diet and demonstrated that the plasma and hepatic lipid levels were significantly decreased by the hydrolyzed xyloglucan. Chen, Liu, Zhu, Xu, and Li, (2010) reported that soybean oligosaccharides significantly reduced abnormal blood glucose, lipid levels and oxidative stress in rats fed a high-fat diet. Haw pectic oligosaccharides showed anti-hyperlipidemic ability and protected against oxidative stress in experimental hyperlipidemia mice induced by high-fat diet (Li et al., 2010). Imaizum, Nakatsu, Sato, Sedarnawati, and Sugano (1991) reported that the dietary XOS improved growth retardation, hyperphagia, polydipsia, elevation of serum glucose, triglyceride and cholesterol, reduction of liver triglyceride, and fatty acid composition of liver phosphatidylcholine in diabetic rats. The dietary supplementation with xylo-oligosaccharides (XOS) was effective in improving the blood sugar and serum lipids in patient with type 2 diabetes mellitus (Sheu, Lee, Chen, & Chan 2008). Gobinath, Madhu, Prashant, Srinivasan, and Prapulla (2010) discussed the beneficial effect of XOS and fructo-oligosaccharides in streptozotocin-induced diabetic rats. Both the oligosaccharides could be used as an adjunct to dietary therapy to derive antidiabetic benefits, and to delay secondary complications.

XOS as non-digestible oligosaccharides can be attained by chemical and/or enzymatic methods from a variety of xylan-containing raw materials. Wheat bran, an important by-product of the cereal industry, is produced worldwide in large quantities and recognized as a good source of dietary fiber. It consists mainly of cell wall polysaccharides, among which xylans represent 40% of dry matter (Benamrouche, Crônier, Debeire, & Chabbert, 2002). Xylans consist of a linear backbone of β-(1→4) linked D-

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xylopyranosyl residues containing individual α-L-arabinofuranosyl residues attached through O-2 and/or O-3 (Izydorczyk & Biliaderis, 1993). The xylan backbone can be hydrolyzed randomly by endoxylanases (endo-1,4-β-D-xylan xylanohydrolase, EC 3.2.1.8). Endoxylanases attack the xylan main chain internally in a random manner to release a mixture of various XOs. In our previous study, wheat bran insoluble dietary fiber was hydrolyzed by commercial xylanase preparation Sunzymes to release XOS with a degree of polymerization (DP) of 2–7 and the ratio of arabinose to xylose 0.27 (Wang, Sun, Cao, Tian, & Wang, 2009).

XOS can be used as low-calorie sweeteners and soluble dietary fiber since they are not metabolized by the human digestive system. Further, they exhibit many excellent physiological properties, including improvement in bowel function, calcium absorption, lipid metabolism, and reduction of the risk of colon cancer by forming short-chain fatty acids (SCFA) in the large intestine during fermentation and a prebiotic effect promoting the growth of beneficial intestinal bacteria, such as *Bifidobacterium* and *Lactobacillus* (Grootaert et al., 2007; Kabel, Kortenoeven, Schols, & Voragen, 2002; Mussatto & Mancilha, 2007). In addition, XOS have acceptable organoleptic properties and do not exhibit toxicity or negative effects on human health (Montané, Nabarlatz, Martorell, Torné-Fernández, & Fierro, 2006). However, to the best of our knowledge, XOS has not been studied in any depth regarding its effect on oxidative stress in rats fed a high-fat diet. The main objective of this study was to investigate the effect of XOS substituted with arabinose as novel oligosaccharides from wheat bran insoluble dietary fiber on blood lipid metabolism and antioxidant status in high-fat diet treated rats.

2. Materials and methods

2.1. Materials

Wheat bran was obtained from Chengde Rehe Flour Factory, Hebei, PR China. The bran was milled and passed through a 0.5 mm sieve. Sunzymes, which contains the glycoside hydrolase 10 family endo-1,4-β-xylanase from *Bacillus subtilis*, was obtained from Sunhy Biology Co., Ltd, Wuhan, PR China. Heat-stable α-amylase Termamyl 120 L (EC 3.2.1.1 from *Bacillus licheniformis*, 120 KNU/g), protease Alcalase 2.4 L (EC 3.4.21.62, from *Bacillus licheniformis*, 2.4 AU/g), and amyloglucosidase AMG 300 L (EC 3.2.1.3, from *Aspergillus niger*, 300 AGU/g) were from Novo Nordisk (Bagsvaerd, Denmark). Amberlite XAD-2 was obtained from Rohm and Haas Company (Philadelphia, USA). All other chemicals and solvents in this study used were of analytical grade.

2.2. Preparation of XOS

The preparation of XOS from wheat bran insoluble dietary fiber was based on a procedure described previously (Wang et al., 2009). Briefly, wheat bran (100 g) was autoclaved to destroy the activities of endogenous cell wall-degrading enzymes (*e.g.* endogenous arabinoxylanase) and subsequently swollen in a 2 L beaker with 1 L water at 60 °C for 6 h. After that, 7.5 mL α-amylase was added to the suspension, and then was heated in a boiling water bath for 40 min with continuous stirring. After treatment with α-amylase, the pH of the suspension was adjusted to 7.5 with 275 mM NaOH, and 3.0 mL protease was then added to the samples. After incubation at 60 °C for 30 min, the pH of the suspension was acidified with 325 mM HCl to 4.5. Then, 3.5 mL amyloglucosidase was added and the mixture was incubated at 60 °C for 30 min with continuous agitation. The suspension was centrifuged at 10,000 × *g* for 10 min, and the residue was washed twice with hot water and cold water respectively until no cloudiness was evident. The washed residue was dried at 40 °C overnight in a vacuum oven to obtain wheat bran insoluble dietary fiber (WBIDF). Ten grams of WBIDF were incubated in 200 mL of 0.5% (w/v) Sunzymes (in 50 mM acetate buffer at pH 5.0) at 50 °C in the dark for 16 h with constant stirring. After heat inactivation of the enzyme at 100 °C for10 min, the hydrolysate was centrifuged at10,000 × *g* for 20 min, and the supernatant solution was passed through a 0.45 μm filter. The filtrate was concentrated to 100 mL by rotary evaporation. The concentrated solution was applied to an open column (80 cm × 2.5 cm i.d.) packed with Amberlite XAD-2 (previously washed with 95% (v/v) ethanol and then water). Elution was successively carried out with 4 column volumes of distilled water. The eluted fraction was concentrated and lyophilized with a freeze dry system (AlPHA1-4, Christ, Germany) to get XOS.

Table 1
Composition of experiment diets (g/100 g diet).

Ingredient	Group			
	Normal	Normal + XOS	High-fat diet	High-fat diet+XOS
XOS	–	5	–	5
Corn starch	55	50	55	50
Casein	20	20	20	20
Sucrose	10	10	10	10
Soybean oil	5	5	5	5
Lard	–	–	25	25
Cellulose	5	5	5	5
Mineral mix. (AIN-93)	3.5	3.5	3.5	3.5
Vitamin mix. (AIN-93)	1	1	1	1
L-Cystine	0.3	0.3	0.3	0.3
Choline bitatrate	0.2	0.2	0.2	0.2

2.3. Animals, diet and experimental design

Forty pathogen-free Wistar rats (20 females, 20 males) weighing between 100 ± 12 g were obtained from Beijing Vitalriver Laboratory Animal Co., Ltd. (Beijing, PR China). The animals were housed in stainless steel cages in a room with 12 h light/12 h dark cycle at a temperature of 22–24 °C and a relative humidity of 70 ± 5%. Following one week of acclimatization with basal feed and tap water *ad libitum* for 1 week, the rats were randomly divided into four groups ($n = 10$) for a period of 6 weeks (normal diet, N group; normal diet +5% XOS, NS group; high-fat diet, HF group; high-fat diet +5% XOS, HFS group). The food consumption and body weight were measured every other day and every week, respectively. The experimental diets of four groups are described in Table 1.

At the end of the experimental period, all rats were fasted 24 h before operation. After sacrifice, blood samples were drawn from the ether-anesthetized rats by cardiac puncture using a syringe containing heparin, and serum was obtained by sediment for 30 min, centrifugation at 1740 × *g* for 10 min. the liver and heart were rapid removed, rinsed with physiological saline and cleared of adhering fat. All samples were stored at −80 °C until analysis.

2.4. Biochemical analysis

2.4.1. Analysis of blood biochemical values

Blood glucose was analyzed by the enzymatic reaction method using a commercially available kit by DiaSys Diagnostic Sysmtem (Holzheim, Germany). Serum total cholesterol (TC), triacylglycerols (TG), low-density lipoprotein cholesterol (LDL-c), and high-density lipoprotein cholesterol (HDL-c) levels were measured by enzymatic and colorimetric methods, using assay kits from BioSino Biotech. Sci, Inc. (Bejing, PR China). Activity of serum glutamic oxaloacetic transminase (GOT) and glutamic pyruvic transaminase (GPT) was

Table 2
Effect of dietary supplementation of XOS on body weight, food intake and food efficiency ratio (FER) of rats for 6 weeks (values are mean ± SD, $n = 10$).[a]

Group	Initial body weight (g)	Final body weight (g)	Daily weight gain (g)	Daily food intake (g/day)	FER[b]
N	111.36 ± 8.50^{A}	372.02 ± 18.34^{A}	6.18 ± 0.32^{A}	18.7 ± 1.3^{A}	0.33 ± 0.01^{A}
NS	110.53 ± 8.76^{A}	350.53 ± 17.87^{B}	5.43 ± 0.21^{B}	19.1 ± 1.2^{A}	0.30 ± 0.01^{A}
HF	109.67 ± 9.02^{A}	408.65 ± 20.46^{c}	7.04 ± 0.47^{c}	15.9 ± 1.7^{B}	0.44 ± 0.02^{B}
HFS	108.98 ± 9.27^{A}	381.46 ± 19.21^{A}	6.47 ± 0.34^{A}	16.3 ± 1.5^{B}	0.40 ± 0.02^{B}

[a] Values with the same superscript letter within column are not significantly different at $P < 0.05$.
[b] FER, body weight gain (g)/food intake (g).

analyzed using an automatic analyzer (Symchron CX-7 systems, Beckman, USA) according to the methods by Karmen, Wróblewski, and LaDue (1955).

2.4.2. Assessment of thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH), oxidized glutathione (GSSG) and antioxidant enzymes activity

The protein content in each sample was measured by the Lowry, Rosenbrough, Farr, and Randall's method (1951) with Bovine Serum Albumin (BSA) as the standard. The values of TBARS, GSH, GSSG and the activities of SOD, GSH-Px and CAT were measured using commercially available assay kits according to the manufacturers' protocols. The values of TBARS, an index of lipid peroxidation were determined with a malondialdehyde (MDA) Assay Kit A003 (Nanjing Jianchen Bioengineering Institute, Jiangsu, PR China); The contents of GSH and GSSG were determined using a GSH and GSSG Assay Kit A0061 (Nanjing Jianchen Bioengineering Institute, Jiangsu, PR China); catalase (CAT) activity was determined with CAT Assay Kit A007 (Nanjing Jianchen Bioengineering Institute, Jiangsu, PR China); superoxide dismutase (SOD) activity was determined with SOD Assay Kit A001 (Nanjing Jianchen Bioengineering Institute, Jiangsu, PR China); glutathione peroxidase (GSH-Px) activity was determined with a GSH-Px Assay Kit A005 (Nanjing Jianchen Bioengineering Institute, Jiangsu, PR China).

2.5. Statistical treatment of data

All the data were reported as the mean ± standard deviation (SD) of triplicate determinations. Duncan's multiple range tests were used to estimate significant differences among the mean at the 5% probability level. Results were processed by Statistica (Version 6.0).

3. Results and discussion

3.1. Effects of wheat bran XOS on the growth of rats fed a high-fat diet

High-fat diets have been shown to produce more rapid weight gain in rodents (Maeda, Hosokawa, Sashima, Murakami-Funayama, & Miyashtta, 2009). In the present study, the effects of wheat bran XOS on the growth performance of rats fed a high-fat diet were evaluated. As shown in Table 2, the final body weight and daily weight gain of rats fed a high-fat diet (HF group) was significantly higher than that of the other groups. There were significant differences in final body weight and daily weight gain between with and without XOS in rats fed with either normal or high-fat diet, and both the final body weight and daily weight gain were lower in group fed either normal or high-fat diet with XOS compared with the group without XOS, and the final body weight of group fed high-fat diet with XOS was similar to that the group fed normal diet, indicating that XOS had beneficial effect on controlling body weight. In contrast, Yamatoya et al. (1996) reported that the hydrolyzed xyloglucan supplementation did not affect body weight gain, food intake and food efficiency in rats fed a high-fat diet. The soy bean oligosaccharides supplementation did not significantly decrease the final boy weight in rats fed a high-fat diet (Chen et al., 2010). During the experimental period, compared with the N and NS groups, the food intake of HF and HFS groups was significantly lower, and their food efficiency ratios were also significantly higher. However, there were no significant differences in food intake and food efficiency ratio between with and without XOS in rats fed either normal or high-fat diet.

3.2. Effects of wheat bran XOS on blood biochemical values in rats fed a high-fat diet

Adequate dietary fat intake serves a number of essential bodily functions such as energy metabolism and body mass control, however, high-fat diets often lead excessive energy intake and are positively associated with impairments in blood glucose regulation and lipid metabolic disturbance (Li, Li, Sigmon, McCort, & Ren, 2005; Li et al., 2010; Liu, Zhu, Xu, & Li, 2010). Recently, various physiological effects of dietary fiber, such as its ability to control blood glucose and reduce cholesterol, have received considerable attention (Knopp et al., 1999; Yamatoya et al., 1996). In the present study, the effects of wheat bran XOS on blood glucose, the activity of GOT and GPT and lipid metabolism in rat fed a high-fat diet were evaluated.

3.2.1. Effects of wheat bran XOS on blood glucose in rats fed a high-fat diet

As shown in Table 3, the high-fat diet induced a significant elevation of the serum glucose level, which was increased by 77.7% compared with that of the rats fed a normal diet. A long-term (45 consecutive days) of high-fat diet administration increased blood glucose level 2.5 folds in rats (Chen et al., 2010). However, the addition of XOS to the high-fat diet reduced significant the blood glucose level. The level of blood glucose was not significant difference between rats fed the normal diet with and without XOS (NS and N groups). It has been reported that XOS supplementation for 8 weeks could reduce the blood glucose in type 2 diabetes mellitus patients

Table 3
Blood biochemical values in rats supplemented with XOS for 6 weeks (values are mean ± SD, $n = 10$).[a]

Group	Blood glucose (mmol/1)	GOT (U/l)	GPT (U/l)	TC (mmol/1)	TG (mmol/1)	HDL-c (mmol/1)	LDL-c (mmol/1)
N	5.34 ± 0.69^{A}	118.69 ± 9.16^{A}	38.51 ± 2.14^{A}	1.76 ± 0.13^{A}	0.71 ± 0.10^{A}	1.14 ± 0.11^{A}	0.49 ± 0.08^{A}
NS	4.75 ± 0.33^{A}	109.45 ± 8.45^{A}	34.72 ± 2.36^{A}	1.53 ± 0.11^{A}	0.56 ± 0.08^{A}	1.02 ± 0.16^{A}	0.45 ± 0.07^{A}
HF	9.49 ± 0.84^{B}	149.04 ± 11.98^{B}	65.14 ± 3.79^{B}	3.52 ± 0.40^{B}	1.65 ± 0.15^{B}	0.71 ± 0.07^{B}	0.96 ± 0.23^{B}
HFS	6.83 ± 0.47^{c}	112.76 ± 10.23^{A}	41.26 ± 2.73^{A}	2.15 ± 0.34^{c}	0.83 ± 0.12^{c}	0.83 ± 0.07^{c}	0.71 ± 0.21^{c}

[a] Values with the same superscript letter within column are not significantly different at $P < 0.05$.

Table 4
Levels of oxidative stress biomarkers and antioxidant enzymes in serum from rats fed with XOS for 6 weeks (values are mean ± SD, $n = 10$).[a]

Group	MDA (nmol/mL)	GSH (nmol/mL)	GSSG (nmol/mL)	GSH/GSSG	SOD (U/mL)	CAT (U/mL)	GSH-Px (U/mL)
N	9.40 ± 0.78^{A}	4.95 ± 0.58^{A}	1.84 ± 0.21^{A}	2.69 ± 0.36^{A}	198.47 ± 12.32^{A}	13.67 ± 1.02^{A}	319.54 ± 16.28^{A}
NS	6.79 ± 0.51^{B}	6.01 ± 0.49^{B}	1.48 ± 0.19^{B}	4.14 ± 0.32^{B}	237.06 ± 13.14^{B}	19.13 ± 1.68^{B}	387.42 ± 25.19^{B}
HF	14.93 ± 1.15^{C}	3.35 ± 0.41^{c}	2.37 ± 0.28^{c}	1.42 ± 0.19^{c}	120.29 ± 10.95^{c}	7.94 ± 0.83^{c}	175.56 ± 10.34^{c}
HFS	8.95 ± 0.66^{A}	4.87 ± 0.36^{A}	1.82 ± 0.22^{A}	2.65 ± 0.29^{A}	192.85 ± 11.43^{A}	14.05 ± 1.37^{A}	328.06 ± 19.45^{A}

[a] Values with the same superscript letter within column are not significantly different at $P < 0.05$.

(Sheu et al., 2008). However, Chung, Hsu, Ko, and Chan (2007) found that 4 g/d XOS supplementation for 8 weeks showed no influence on the fasting sugar in normal glycemia elderly subjects.

3.2.2. Effects of wheat bran XOS on the activity of GOT and GPT in serum of rats fed a high-fat diet

The GOT and GPT activities in HF group were significantly higher than that of the other groups as shown in Table 3. Compared with the rats fed a normal diet, the GOT and GPT levels were increased about 25.6% and 69.2% respectively in rats fed a high-fat diet, however, there were no significant differences among N, NS and HFS groups. The GOT and GPT activities were decreased by supplementation of a high-fat diet with 5% XOS about 24.3% and 36.7%, respectively. Yamatoya et al. (1996) reported that plasma GOT activity in rats fed a high-fat diet was reduced by 33.5% by hydrolyzed xyloglucan. In contrast, Sheu et al. (2008) demonstrated that the XOS supplementation did not change the GOT and GPT activities in patient with type 2 diabetes mellitus. GOT and GPT exist primarily in hepatocytes and occur in trace amounts in serum, which are released into the bloodstream upon damage to the liver cell. They are the most sensitive markers employed in the diagnosis of liver damage and diseases (Pari & Ashod, 2002). Therefore, the obtained results suggest that XOS may prevent hepatic damage in rats fed a high-fat diet.

3.2.3. Effects of wheat bran XOS on lipid metabolism in rats fed a high-fat diet

Abnormal blood lipid levels were also observed in all rats fed the high-fat diet (HF group) as shown in Table 3. Serum TC, TG, and LDL-c Levels in the HF group were significantly higher, whereas HDL-c was significantly lower than those in the normal group (N group). In general, soluble food fibers have the ability to lower the levels of TC and TG in the blood (Knopp et al., 1999). The presented study confirmed that XOS supplementation with a high-fat diet resulted in improved significantly lipid levels, which were indicated by the significantly decreased TC, TG, LDL-c levels and increased HDL-c level. Sheu et al. (2008) reported that XOS supplementation for 8 weeks could lower lipid levels of type 2 diabetes mellitus subjects. Associated with the results of body weight mentioned above, these results demonstrated that XOS could greatly block the accumulation of tissue fat in rats fed the high-fat diet.

The improvement of blood glucose and lipid levels in rats fed a high-fat diet by the supplementation XOS may be related to their prebiotic effect. It has been reported that the gut bifidobacterial content of high-fat diet-induced diabetes in mice could be increased through the use of the prebiotic oligosaccharides (Cani et al., 2007). XOS are fermented by several kinds of bacteria in the large intestine to stimulate selectively the growth of bifidobaceria and increase the SCFA production such as acetic, propionic and butyric acids (Manisseri & Gudipati, 2010; Mussatto & Mancilha, 2007). The propionic acid supplementation decreased the fasting serum glucose and insulin sensitivity and influenced lipid metabolism in healthy volunteers (Venters, Vorster, & Cummings, 1990). The increase in the amounts of SCFA during XOS supplementation leads to inhibition of cholesterol synthesis or modification in the bile acid metabolism, and thereby improving blood glucose and lipid metabolism in rats fed a high-fat diet.

3.3. Effects of wheat bran XOS on antioxidant defense system in rats fed a high-fat diet

Consumption of a high-fat diet promotes excessive ROS production by nicotinamide adenine dinucleotide phosphate–oxidase activation (Folmer, Soares, Gabriel, & Rocha, 2003). ROS oxidize biological molecules such as lipids, proteins, DNA, and sugars to induce their modification. During the course of evolution, the living organisms have developed a fine defense network system against oxidative stress, in which various enzymes, protein, and small molecules with different functions play an important protective role against oxidative stress *in vivo*. In the present study, the effect of wheat bran XOS on the antioxidant defense system in rats fed a high-fat diet was assessed by measuring the formation of MDA and the levels of such key antioxidants as GSH, SOD, CAT and GSH-PX. Tables 4–6 show the MDA level, ratio of GSH to GSSG, and CAT, SOD, GSH-Px activities in serum, liver and heart of rats fed a normal or high-fat diet supplemented with 5% XOS.

3.3.1. Effects of wheat bran XOS on the formation of MDA in rats fed a high-fat diet

Among the biological targets of oxidative stress, lipids are the most involved class of biomolecules. Lipid oxidation gives rise to a number of secondary products. MDA is the principal and most studied product of polyunsaturated fatty acid peroxidation. This aldehyde is a highly toxic molecule and should be considered as more than just a marker of lipid peroxidation. Its interaction with DNA and proteins has often been referred to as potentially mutagenic and atherogenic. MDA is widely used as a biomarker of oxidative stress. The level of MDA was significantly higher in serum, liver and heart of rats fed the high-fat diet (HF group) than that of the other groups as shown in Tables 4–6. Compared with the rats fed the normal diet (N group), the levels of MDA in serum, liver

Table 5
Levels of oxidative stress biomarkers and antioxidant enzymes in liver from rats fed with XOS for 6 weeks (values are mean ± SD, $n = 10$).[a]

Group	MDA (nmol/mg protein)	GSH (nmol/mg protein)	GSSG (nmol/mg protein)	GSH/GSSG	SOD (U/mg protein)	CAT (U/mg protein)	GSH-Px (U/mg protein)
N	1.92 ± 0.13^{A}	10.46 ± 1.23^{A}	1.98 ± 0.21^{A}	5.28 ± 0.57^{A}	46.89 ± 3.42^{A}	18.48 ± 1.79^{A}	10.41 ± 1.43^{A}
NS	1.87 ± 0.14^{A}	11.03 ± 1.08^{A}	1.96 ± 0.19^{A}	5.61 ± 0.64^{A}	49.23 ± 3.51^{A}	20.31 ± 2.05^{A}	13.02 ± 1.56^{A}
HF	4.63 ± 0.48^{B}	8.59 ± 0.94^{B}	2.31 ± 0.35^{B}	3.72 ± 0.43^{B}	28.54 ± 2.13^{B}	11.62 ± 1.34^{B}	6.05 ± 0.57^{B}
HFS	2.14 ± 0.17^{A}	9.27 ± 0.83^{A}	1.79 ± 0.18^{A}	5.19 ± 0.46^{A}	43.27 ± 3.65^{A}	19.94 ± 1.98^{A}	12.73 ± 1.44^{A}

[a] Values with the same superscript letter within column are not significantly different at $P < 0.05$.

Table 6
Levels of oxidative stress biomarkers and antioxidant enzymes in heart from rats fed with XOS for 6 weeks (values are mean ± SD, $n = 10$).[a]

Group	MDA (nmol/mg protein)	GSH (nmol/mg protein)	GSSG (nmol/mg protein)	GSH/GSSG	SOD (U/mg protein)	CAT (U/mg protein)	GSH-Px (U/mg protein)
N	1.87 ± 0.15^{A}	13.26 ± 0.14^{A}	1.73 ± 0.19^{A}	7.82 ± 0.83^{A}	43.58 ± 4.16^{A}	51.24 ± 4.37^{A}	13.48 ± 1.23^{A}
NS	1.79 ± 0.13^{A}	13.41 ± 1.38^{A}	1.69 ± 0.17^{A}	7.90 ± 0.64^{A}	59.36 ± 6.04^{B}	56.48 ± 5.03^{A}	17.43 ± 1.52^{B}
HF	4.39 ± 0.28^{B}	9.75 ± 0.99^{B}	2.84 ± 0.21^{B}	3.43 ± 0.26^{B}	25.14 ± 2.37^{C}	37.15 ± 3.42^{B}	6.35 ± 0.59^{c}
HFS	1.98 ± 0.14^{A}	12.93 ± 1.16^{A}	1.74 ± 0.18^{A}	7.43 ± 0.65^{A}	48.25 ± 4.73^{A}	50.69 ± 4.83^{A}	14.71 ± 1.25^{A}

[a] Values with the same superscript letter within column are not significantly different at $P < 0.05$.

and heart of rats fed the high-fat diet (HF group) were elevated by 58.8%, 141.2% and 134.8%, respectively. However, the levels of MDA in serum, liver and heart of rats fed the high-fat diet supplemented with 5% XOS (HFS groups) were near those of the rats fed the normal diet (N groups). The levels of MDA in liver and heart of rats fed the normal diet did not change between with and without XOS. Interestingly, the content of MDA in serum of rat fed the normal diet supplemented with 5% XOS (NS group) were significantly lower than that of rats fed the normal diet (N group). In contrast, XOS supplementation did not influence the level of TBARS in erythrocyte in type 2 diabetes mellitus subjects but it did significant reduce CAT activity (Sheu et al., 2008).

3.3.2. *Effects of wheat bran XOS on non-enzymatic antioxidant in rats fed a high-fat diet*

Intracellularly, GSH is a major antioxidant that helps eliminate peroxides and other oxidants. It reacts with superoxide radical, peroxy radical and singlet oxygen to form GSSG and other disulfides (Ashfaq et al., 2006). The GSSG generated is later converted back to GSH by glutathione reductase in which reduced nicotinamide adenine dinucleotide phosphate is the hydrogen donor. When cellular GSSG levels increase during oxidative stress, GSSG is exported into extracellular fluid to avoid thiol toxicity. The level of GSSG was significantly increased, whereas the content of GSH, ratio of GSH to GSSG were significantly decreased in serum, liver and heart of rats fed the high-fat diet (HF group) as shown in Tables 4–6. Compared with the rats fed the normal diet (N group), the levels of GSSG in serum, liver and heart of rats fed the high-fat diet (HF group) were depleted by 28.8%, 16.8% and 64.2%, respectively, and the content of GSH in them were decreased by 32.3%, 17.9%, and 26.5%, respectively, and the ratio of GSH to GSSG were decreased by 47.2%, 29.6%, and 56.1%, respectively. However, the levels of GSH and GSSG in serum, liver and heart of rats fed the high-fat diet supplemented with 5% XOS were near those of the rats fed the normal diet. The levels of the determined oxidative stress biomarkers in liver and heart of rats fed the normal diet did not change between with and without XOS. Interestingly, the content of GSSG in serum of rat fed the normal diet supplemented with 5% XOS (NS group) were significantly lower than that of rats fed the normal diet (N group), and the levels of GSH, GSH/GSSG and the activities of SOD, CAT and GSH-Px were significantly higher.

3.3.3. *Effects of wheat bran XOS on enzymatic antioxidant in rats fed a high-fat diet*

The primary antioxidant enzymes such as SOD, CAT and GSH-Px form part of this defense system and are capable of eliminating ROS and removing cytotoxic peroxides in mammalian systems. SOD catalyzes the dismutation of the highly reactive superoxide to the less reactive hydrogen peroxide, CAT efficiently catalyzes the conversion of H_2O_2 to water (preventing the generation of hydroxyl radicals) and molecular oxygen, and GSH-Px reduces lipid hydroperoxides to their corresponding alcohols and free hydrogen peroxide to water. Depletion in the activities of theses antioxidant enzymes can be owed to an enhanced radical production. The activities of SOD, CAT and GSH-Px were significantly lower in serum, liver and heart of rats fed the high-fat diet (HF group) than that of the other groups as shown in Tables 4–6. Compared with the rats fed the normal diet (N group), the levels of SOD in serum, liver and heart of rats fed the high-fat diet (HF group) were decreased by 39.4%, 39.1% and 42.3%, respectively, and the activities of CAT in them were decreased by 41.9%, 37.1%, and 27.5%, respectively, and the GSH-Px activity was decreased by 45.1%, 41.9%, and 52.9%, respectively. However, the activities of these antioxidant enzymes in serum, liver and heart of rats fed the high-fat diet supplemented with 5% XOS were near those of the rats fed the normal diet. Interestingly, the activities of SOD, CAT and GSH-Px in serum of rat fed the normal diet supplemented with 5% XOS (NS group) were significantly higher than that of rats fed the normal diet (N group). In addition, the activity of SOD in heart of rats fed the normal diet supplemented with 5% XOS (NS group) were significantly higher than that of rats fed the normal diet (N group). However, except for SOD level in heart, the levels of antioxidant enzymes in liver and heart of rats fed the normal diet did not change between with and without XOS. Gobinath et al. (2010) reported that XOS supplementation significantly increased the activity of CAT and GSH-Px in the blood of diabetic rats. In contrast, Sheu et al. (2008) reported that XOS supplementation did not influence the activities of SOD and GSH-Px in erythrocyte in type 2 diabetes mellitus subjects but it did significant reduce CAT activity.

4. Conclusion

The present study demonstrated that a high-fat diet resulted in hyperglycemia, hyperlipidemia and an increase of oxidative stress. XOS supplementation with a high-fat diet was found to effective in improving blood glucose and lipid levels, together with reducing MDA and GSSG levels and increasing the content of GSH and the ratio of GSH to GSSG and the activity of SOD, CAT and GSH-Px in serum, liver and heart of rats. These results suggested that dietary supplementation of XOS might attenuate the damage of oxidative stress induced by high-fat diet through modulating lipid metabolism and antioxidant defense system and have a beneficial effect for human health. The precise mechanisms of these beneficial effects exerted by XOS will be further investigated.

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