Effects of Wheat Bran Feruloyl Oligosaccharides on Plasma and Tissue Antioxidant Capacity in Diquat-Induced Oxidative Stress Rats: Postprint

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Abstract

This experiment utilized Saccharomyces cerevisiae and Bacillus subtilis as mixed cultures to ferment wheat bran, and prepared wheat bran feruloyl oligosaccharides (FOs) through separation and purification by Amberlite XAD-2 column, to investigate whether wheat bran FOs have a mitigating effect on diquat-induced oxidative stress in rats. The experiment selected 48 weaned male rats with similar body weight, which were randomly divided into a non-challenged group, a challenged group, a challenged + 100 mg/kg BW wheat bran FOs group, a challenged + 200 mg/kg BW wheat bran FOs group, a challenged + 300 mg/kg BW wheat bran FOs group, and a challenged + 100 mg/kg BW vitamin C group, with 8 replicates per group and 1 rat per replicate. All groups were fed the same commercial diet. Wheat bran FOs and vitamin C were prepared as aqueous solutions and administered by gavage; the non-challenged and challenged groups received normal saline instead, with a gavage volume of 0.2 mL for 15 consecutive days. On the day of gavage completion, rats in the non-challenged group were injected with 0.3 mL of normal saline, while the other five groups received an intraperitoneal injection of 0.3 mL diquat at a dose of 0.1 mmol/kg BW. Samples were collected 12 h after diquat challenge, and total antioxidant capacity (T-AOC), catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) activities, as well as glutathione (GSH) and 8-hydroxydeoxyguanosine (8-OHdG) contents in plasma, liver, kidney, and ileum of rats in each group were analyzed. The results showed: 1) Wheat bran FOs were prepared through mixed-culture fermentation of wheat bran and purified using an Amberlite XAD-2 column, obtaining a wheat bran FOs concentration of 0.059 mmol/g. 2) Intraperitoneal injection of diquat significantly decreased SOD activity and GSH content in rat plasma ($P<0.05$), significantly decreased T-AOC, CAT and GSH-Px activities and GSH content in rat liver ($P<0.05$), significantly decreased T-AOC and CAT, SOD activities in rat kidney ($P<0.05$),

significantly decreased T-AOC, CAT, GSH-Px activities and GSH content in rat ileum ($P<0.05$), and significantly increased 8-OHdG content in rat plasma and all tissues ($P<0.05$). 3) Under oxidative stress conditions induced by diquat, gavage of certain doses of wheat bran FOs could significantly increase SOD (400 mg/kg BW), GSH-Px activities (100 and 200 mg/kg BW) and GSH content (100 and 200 mg/kg BW) in rat plasma, significantly increase T-AOC (100, 200 and 400 mg/kg BW), CAT (200 and 400 mg/kg BW), SOD (100, 200 and 400 mg/kg BW) and GSH-Px activities (100, 200 and 400 mg/kg BW) and GSH content (100, 200 and 400 mg/kg BW) in rat liver, significantly increase T-AOC (400 mg/kg BW), CAT (200 mg/kg BW) and GSH-Px activities (200 and 400 mg/kg BW) and GSH content (400 mg/kg BW) in rat kidney, significantly increase T-AOC (200 mg/kg BW), SOD (400 mg/kg BW) and GSH-Px activities (100, 200 and 400 mg/kg BW) and GSH content (100, 200 and 400 mg/kg BW) in rat ileum, and significantly decrease 8-OHdG content in plasma and all tissues (plasma, kidney, ileum: 100, 200 and 400 mg/kg BW; liver: 100 mg/kg BW) ($P<0.05$); moreover, after gavage with 200 and 400 mg/kg BW wheat bran FOs, some antioxidant-related indices in rat plasma and tissues could be restored to normal physiological levels. In conclusion, the wheat bran FOs prepared in this experiment could effectively alleviate oxidative stress induced by diquat by effectively increasing antioxidant enzyme activities and GSH content in rat plasma and tissues, and decreasing the content of DNA oxidative stress metabolite 8-OHdG.

Full Text

Effects of Wheat Bran Feruloyl Oligosaccharides on Plasma and Tissue Antioxidant Capacities of Diquat-Induced Oxidative Stress Rats

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Abstract

This study investigated the protective effects of wheat bran feruloyl oligosaccharides (FOs) against diquat-induced oxidative stress in rats. Wheat bran FOs were prepared through mixed fermentation of wheat bran using *Saccharomyces cerevisiae* and *Bacillus subtilis*, followed by purification via Amberlite XAD-2 column chromatography. Forty-eight weaned male rats with similar body weights were randomly assigned to six groups: a non-challenged group, a challenged group, a challenged + 100 mg/kg BW wheat bran FOs group, a challenged + 200 mg/kg BW wheat bran FOs group, a challenged + 300 mg/kg BW wheat bran FOs group, and a challenged + 100 mg/kg BW vitamin C group. Each

group contained eight replicates with one rat per replicate. All rats were fed identical commercial diets throughout the experiment. Wheat bran FOs and vitamin C were administered as aqueous solutions via gavage at a volume of 0.2 mL for 15 consecutive days, while the non-challenged and challenged groups received physiological saline instead. On the final day of gavage, rats in the non-challenged group were injected with 0.3 mL physiological saline, whereas the other five groups received an intraperitoneal injection of 0.3 mL diquat at a dose of 0.1 mmol/kg BW. Samples were collected 12 hours post-challenge to analyze total antioxidant capacity (T-AOC), catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) activities, as well as glutathione (GSH) and 8-hydroxydeoxyguanosine (8-OHdG) contents in plasma, liver, kidney, and ileum.

The results demonstrated: (1) Wheat bran FOs prepared through mixed bacterial fermentation and purified by Amberlite XAD-2 column chromatography reached a concentration of 0.059 mmol/g. (2) Intraperitoneal diquat injection significantly decreased plasma SOD activity and GSH content ($P<0.05$), hepatic T-AOC and CAT, GSH-Px activities and GSH content ($P<0.05$), renal T-AOC and CAT, SOD activities ($P<0.05$), and ileal T-AOC, CAT, GSH-Px activities and GSH content ($P<0.05$), while significantly increasing 8-OHdG content in plasma and all tissues ($P<0.05$). (3) Under diquat-induced oxidative stress, oral administration of wheat bran FOs significantly increased plasma SOD activity (400 mg/kg BW), GSH-Px activity (100 and 200 mg/kg BW), and GSH content (100 and 200 mg/kg BW). In liver, wheat bran FOs significantly elevated T-AOC (100, 200, and 400 mg/kg BW), CAT activity (200 and 400 mg/kg BW), SOD activity (100, 200, and 400 mg/kg BW), GSH-Px activity (100, 200, and 400 mg/kg BW), and GSH content (100, 200, and 400 mg/kg BW). In kidney, wheat bran FOs significantly improved T-AOC (400 mg/kg BW), CAT activity (200 mg/kg BW), GSH-Px activity (200 and 400 mg/kg BW), and GSH content (400 mg/kg BW). In ileum, wheat bran FOs significantly increased T-AOC (200 mg/kg BW), SOD activity (400 mg/kg BW), GSH-Px activity (100, 200, and 400 mg/kg BW), and GSH content (100, 200, and 400 mg/kg BW). Additionally, wheat bran FOs significantly reduced 8-OHdG content in plasma and tissues (plasma, kidney, ileum: 100, 200, and 400 mg/kg BW; liver: 100 mg/kg BW) ($P<0.05$). Notably, pretreatment with 200 and 400 mg/kg BW wheat bran FOs restored several antioxidant indices in plasma and tissues to normal physiological levels. In conclusion, wheat bran FOs effectively alleviated diquat-induced oxidative stress by enhancing antioxidant enzyme activities and GSH content while reducing the DNA oxidative stress metabolite 8-OHdG in rat plasma and tissues.

Keywords: wheat bran feruloyl oligosaccharides; weaned rats; oxidative stress; antioxidant capacity

Introduction

Ferulic acid (FA) is a derivative of cinnamic acid and a phenolic acid widely distributed in gramineous plants, primarily cross-linked with cell wall polysaccharides and lignin as an integral component of the cell wall structure [1]. Feruloyl oligosaccharides (FOs) are important compounds formed through ester bonds connecting the carboxyl group of FA with the hydroxyl group of oligosaccharides. Due to variations in the oligosaccharides linked via ester bonds, numerous FOs exist, including feruloylated arabinoxyloses, feruloylated arabinoses, feruloylated galactoses, feruloylated xylobioses, feruloylated xylotrioses, and feruloylated xylotetraoses [2]. The unique ester bond structure in FOs enhances certain physiological activities. Research has demonstrated that FOs exhibit potent antioxidant, antibacterial, and anti-inflammatory properties. In vitro studies by Ge Lihua [3] and Zeng Fengcai [4] revealed that FOs possess strong scavenging capacities against 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2' -azinobis-3-ethylbenzthiazoline-6-sulphonate (ABTS), and hydroxyl radicals ($\cdot$ OH). Yao et al. [5] found that FOs supplementation in hydrogen peroxide (H_2O_2)-treated cell culture media promoted cell growth, increased adherent cell numbers, reduced rounded cells, restored cellular synapses, and enhanced intracellular SOD activity. Zhang et al. [6] reported that FOs derived from enzymatic wheat bran hydrolysis alleviated oxidative stress induced by 2,2' -azobis[2-methylpropionamidine] dihydrochloride (AAPH) in rats, increasing CAT, SOD, GSH-Px activities and GSH content in oxidative stress rat tissues. Further investigations by Xie Chunyan [7] and Chen Zhou [8] demonstrated that FOs not only inhibited *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella* but also promoted the proliferation of several lactobacilli and streptococci.

Current preparation methods for FOs include acid hydrolysis, enzymatic hydrolysis, and microbial fermentation, with the latter predominantly employing edible fungi and molds [3,9]. Reports on non-mycelial strains as fermentation microorganisms remain scarce. This study utilized *Saccharomyces cerevisiae* CGMCC 2.119 and *Bacillus subtilis* CGMCC 1.0892 as mixed strains to ferment wheat bran, with subsequent purification via Amberlite XAD-2 column chromatography to prepare wheat bran FOs. Numerous studies have established that intraperitoneal diquat injection induces oxidative stress in rats [10], weaned piglets [11], and broilers [12]. Vitamin C, a recognized antioxidant, effectively mitigates diquat-induced oxidative stress [13]. Therefore, this experiment employed intraperitoneal diquat injection to establish a rat oxidative stress model, using vitamin C as a positive control to investigate the protective effects of prepared wheat bran FOs against oxidative stress, thereby providing theoretical support for the application of wheat bran FOs in improving animal health and promoting growth in production settings.

Materials and Methods

Preparation of Wheat Bran FOs Wheat bran FOs were prepared through mixed fermentation of wheat bran using *Saccharomyces cerevisiae* CGMCC 2.119 and *Bacillus subtilis* CGMCC 1.0892. Fermented samples were dried in an oven (45 °C, 24 h), ground, and extracted at a solid-to-water ratio of 1:20 (mass ratio) in a water bath at 80 °C. Following centrifugation (5,000 r/min, 10 min), the supernatant was collected. Purification was performed via Amberlite XAD-2 column chromatography according to the method described by Yao Huiyuan et al. [14], sequentially eluting with 2 column volumes of distilled water, 3 column volumes of 50% aqueous methanol, and 2 column volumes of absolute methanol. The 50% methanol eluate was collected, concentrated, and lyophilized to obtain wheat bran FOs for animal experiments.

UV Spectral Analysis of Wheat Bran FOs An appropriate amount of the 50% methanol eluate was subjected to UV spectral scanning across a wavelength range of 200–400 nm.

Determination of Wheat Bran FOs Concentration in Purified Samples The concentration of wheat bran FOs in purified samples was determined using the dual-wavelength method according to literature [15]. Briefly, 0.1 mL of wheat bran FOs aqueous solution was mixed with 0.9 mL of borax-glycine buffer (0.1 mol/L, pH=10). Absorbance (OD) values were measured at 345 nm and 375 nm. Based on the molar extinction coefficients of ferulic acid (' $_{345}$=19,662, ' $_{375}$=7,630) and FOs ($_{345}$=23,064, $_{375}$=31,430), the wheat bran FOs concentration was calculated using the formula:

$$C = \frac{(\varepsilon'_{345} \times A_{375} - \varepsilon'_{375} \times A_{345}) \times b}{(\varepsilon'_{345} \times \varepsilon_{375} - \varepsilon_{345} \times \varepsilon'_{375})} \div C_0$$

where:
- C = wheat bran FOs concentration (mmol/g)
- b = cuvette thickness (cm)
- A_{345} = OD value at 345 nm
- A_{375} = OD value at 375 nm
- C_0 = concentration of wheat bran FOs aqueous solution (mg/mL)

Experimental Animals and Design Forty-eight healthy weaned male Wistar rats (purchased from the Animal Experiment Center of Inner Mongolia University) were randomly allocated to six groups based on similar body weight using a completely randomized design. The groups were: non-challenged group, challenged group, challenged + 100 mg/kg BW wheat bran FOs group, challenged + 200 mg/kg BW wheat bran FOs group, challenged + 300 mg/kg BW wheat bran FOs group, and challenged + 100 mg/kg BW vitamin C group. Each group comprised eight replicates with one rat per replicate. Wheat bran FOs

and vitamin C were prepared as aqueous solutions and administered via timed gavage at 0.2 mL volume for 15 consecutive days, while the non-challenged and challenged groups received physiological saline at the same volume. All rats were fed identical basal diets. On the final gavage day, non-challenged rats received an intraperitoneal injection of 0.3 mL physiological saline, while the other five groups received 0.3 mL diquat at 0.1 mmol/kg BW to establish the oxidative stress model. The basal diet was purchased from Jiangsu Xietong Pharmaceutical Bioengineering Co., Ltd., with guaranteed nutrient levels (per kg diet): moisture $ $100 g, crude protein $ $180 g, crude fat $ $40 g, crude fiber $ $50 g, crude ash $ $80 g, calcium 10–18 g, total phosphorus 6–12 g, methionine + cysteine $ $5.3 g. Diquat was purchased from Chongqing Shurong Chemical Co., Ltd. (effective concentration 200 g/L). The experiment was conducted at the College of Animal Science, Inner Mongolia Agricultural University, with conventional management and ad libitum access to feed and water.

Sample Collection and Preparation Twelve hours post-diquat challenge, rats were subjected to cardiac blood collection. Blood was placed in heparin sodium anticoagulant tubes, stored at 4 °C for 30 min, and centrifuged at 3,000 r/min for 10 min to prepare plasma, which was stored at -80 °C until analysis. Following blood collection, liver, ileum, and kidney tissues were rapidly excised, rinsed with ice-cold physiological saline, snap-frozen in liquid nitrogen, and stored at -80 °C.

Determination of Antioxidant Indices Appropriate amounts of tissue were homogenized in phosphate-buffered saline (PBS, 0.01 mol/L, pH 7.4) to prepare 10% (mass) tissue homogenates. After centrifugation at 3,000 r/min for 10 min, the supernatant was collected for antioxidant index determination. Total antioxidant capacity (T-AOC) and SOD and CAT activity assay kits were purchased from Nanjing Jiancheng Bioengineering Institute, while GSH-Px activity and GSH and 8-hydroxydeoxyguanosine (8-OHdG) content assay kits were from Wuhan GeneMay Biotechnology Co., Ltd. All procedures were performed according to manufacturer instructions.

Statistical Analysis Experimental data were analyzed using the General Linear Model (GLM) in SAS 9.2, with Duncan' s multiple range test for intergroup comparisons. Each rat served as the statistical unit. Results are expressed as "mean ± standard deviation," with $P<0.05$ considered statistically significant.

Results

UV Spectral Analysis and Concentration of Wheat Bran FOs Wheat bran was fermented with mixed *S. cerevisiae* and *B. subtilis* cultures, and FOs were purified via Amberlite XAD-2 column chromatography. The UV spectral scan [Figure 1: see original paper] revealed characteristic absorption peaks near

283.6 nm and 325.0 nm, indicating the presence of esterified and free ferulic acid. The dual-wavelength method determined the FOs concentration in the purified sample to be 0.059 mmol/g.

Effects of Different Wheat Bran FOs Doses on Plasma Antioxidant Indices in Diquat-Induced Oxidative Stress Rats The effects of various wheat bran FOs doses on plasma antioxidant indices are presented in Table 1 . Intraperitoneal diquat injection significantly decreased plasma SOD activity and GSH content ($P<0.05$) while significantly increasing 8-OHdG content ($P<0.05$). Compared with the challenged group, 100 mg/kg BW wheat bran FOs significantly reduced plasma CAT activity ($P<0.05$); 400 mg/kg BW wheat bran FOs significantly increased plasma SOD activity ($P<0.05$); 100 and 200 mg/kg BW wheat bran FOs significantly elevated plasma GSH-Px activity and GSH content ($P<0.05$); and 100, 200, and 400 mg/kg BW wheat bran FOs significantly decreased plasma 8-OHdG content ($P<0.05$). Vitamin C at 100 mg/kg BW showed no significant effects on plasma antioxidant indices except for 8-OHdG content ($P>0.05$).

Effects of Different Wheat Bran FOs Doses on Hepatic Antioxidant Indices in Diquat-Induced Oxidative Stress Rats Table 2 summarizes the effects on hepatic antioxidant indices. Diquat challenge significantly decreased hepatic T-AOC, CAT and GSH-Px activities, and GSH content ($P<0.05$) while significantly increasing 8-OHdG content ($P<0.05$). Compared with the challenged group, 100, 200, and 400 mg/kg BW wheat bran FOs significantly increased hepatic T-AOC, SOD and GSH-Px activities, and GSH content ($P<0.05$). Additionally, 200 and 400 mg/kg BW wheat bran FOs significantly enhanced CAT activity ($P<0.05$), and 100 mg/kg BW wheat bran FOs significantly reduced 8-OHdG content ($P<0.05$). Vitamin C at 100 mg/kg BW significantly increased hepatic T-AOC, CAT activity, and GSH content ($P<0.05$) while decreasing 8-OHdG content ($P<0.05$).

Effects of Different Wheat Bran FOs Doses on Renal Antioxidant Indices in Diquat-Induced Oxidative Stress Rats The effects on renal antioxidant indices are shown in Table 3 . Diquat injection significantly decreased renal T-AOC, CAT, and SOD activities ($P<0.05$) and significantly increased 8-OHdG content ($P<0.05$). Compared with the challenged group, 400 mg/kg BW wheat bran FOs significantly increased renal T-AOC, GSH-Px activity, and GSH content ($P<0.05$); 200 mg/kg BW wheat bran FOs significantly enhanced CAT and GSH-Px activities ($P<0.05$); and 100, 200, and 400 mg/kg BW wheat bran FOs significantly reduced renal 8-OHdG content ($P<0.05$). Vitamin C at 100 mg/kg BW significantly increased renal T-AOC and GSH-Px activity ($P<0.05$) while decreasing 8-OHdG content ($P<0.05$).

Effects of Different Wheat Bran FOs Doses on Ileal Antioxidant Indices in Diquat-Induced Oxidative Stress Rats Table 4 presents the

effects on ileal antioxidant indices. Diquat challenge significantly decreased ileal T-AOC, SOD and GSH-Px activities, and GSH content ($P<0.05$) while significantly increasing 8-OHdG content ($P<0.05$). Compared with the challenged group, 200 mg/kg BW wheat bran FOs significantly increased ileal T-AOC ($P<0.05$); 400 mg/kg BW wheat bran FOs significantly enhanced SOD activity ($P<0.05$); 100, 200, and 400 mg/kg BW wheat bran FOs significantly elevated GSH-Px activity and GSH content ($P<0.05$); and all three doses significantly reduced ileal 8-OHdG content ($P<0.05$). Vitamin C at 100 mg/kg BW significantly increased ileal T-AOC activity and GSH content ($P<0.05$) while decreasing 8-OHdG content ($P<0.05$).

Discussion

Wheat bran is rich in ferulic acid, but its utilization by animals is limited due to ester linkages with lignin and cellulose side chains. Numerous studies have shown that moderate acid hydrolysis, enzymatic hydrolysis, and microbial fermentation can produce FOs. Ge Lihua [3] prepared FOs at a concentration of 1.45$×10^{-5}$ mol/g through oxalic acid hydrolysis of wheat bran. Chen Zhou [8] and Yuan Xiaoping [16] obtained FOs at concentrations of 1.326 and 1.546 mmol/L, respectively, through xylanase hydrolysis from different sources. Xie Chunyan [7] produced FOs at 35.4 mol/L through fermentation of wheat bran by the medicinal fungus *Agrocybe aegerita*. While acid hydrolysis is economically cost-effective, issues such as extraction reagent residue and low extraction rates limit its application. Enzymatic hydrolysis, though milder, generates substantial wastewater and incurs high production costs. Microbial fermentation integrates enzyme production and hydrolysis processes, eliminating the need for enzyme purification and reducing production costs. This study employed mixed fermentation with *S. cerevisiae* and *B. subtilis* followed by Amberlite XAD-2 purification, achieving a relatively high FOs concentration of 0.059 mmol/g.

During metabolism, organisms generate substantial reactive oxygen species (ROS), including superoxide anion (O_2^-), $\cdot OH$, and H_2O_2. Under normal conditions, ROS production and elimination maintain equilibrium; however, various harmful stimuli disrupt this redox balance, causing oxidative stress [17]. Defense systems comprising enzymatic and non-enzymatic components protect against oxidative damage. The enzymatic system primarily includes antioxidant enzymes such as CAT, SOD, and GSH-Px, while the non-enzymatic system consists mainly of GSH and vitamins [18]. SOD catalyzes O_2^- conversion to H_2O_2 and oxygen (O_2). H_2O_2 is converted by CAT into non-toxic H_2O and O_2, or transformed into H_2O or alcohol during GSH-Px-catalyzed GSH oxidation [19]. Concurrently, GSH is oxidized to glutathione disulfide (GSSG) by GSH-Px, reducing strongly oxidative peroxides to harmless hydroxyl compounds, thereby protecting cellular structure and functional integrity [20]. 8-OHdG, produced by ROS oxidation of the guanine 8-carbon atom in DNA strands, is exclusively generated through DNA oxidative damage, remains stable in vivo,

and is minimally metabolized, serving as an internationally recognized sensitive biomarker for DNA oxidative damage and oxidative stress status [21].

Diquat generates O_2^- and H_2O_2 using molecular oxygen, subsequently producing other free radicals that induce cellular peroxidation [22] and has been widely applied in oxidative stress model establishment [10-12]. This study selected diquat as the oxidative stress inducer at 0.1 mmol/kg BW, with antioxidant indices measured 12 hours post-challenge. The results showed that intraperitoneal diquat injection dramatically reduced T-AOC, CAT, SOD, and GSH-Px activities and GSH content in plasma, liver, kidney, and ileum, while significantly increasing 8-OHdG content, confirming successful oxidative stress model establishment.

Previous studies have demonstrated that FOs possess strong scavenging capacities against ferrous ions (Fe^{2+}), H_2O_2, and $\cdot OH$ [23-25]. The present findings indicate that oral administration of wheat bran FOs significantly increased T-AOC, SOD and GSH-Px activities, and GSH content in plasma, liver, kidney, and ileum of diquat-challenged rats, while significantly decreasing the DNA oxidative damage product 8-OHdG, demonstrating that wheat bran FOs effectively alleviate oxidative stress by enhancing antioxidant enzyme activities and antioxidant substance content. Wang et al. [26] reported that dietary supplementation with 160 mg/kg FOs for 4 weeks increased serum SOD, CAT, and GSH-Px activities in rats by 56.7%, 24.4%, and 23.0%, respectively, compared with controls. Yu Xiaohong et al. [27] demonstrated that FOs significantly inhibited erythrocyte auto-oxidative hemolysis in vitro in a dose-dependent manner and significantly increased SOD and GSH-Px activities while decreasing malondialdehyde (MDA) content in tumor-bearing mice. Rondini et al. [28] showed that FOs administration significantly increased plasma SOD, CAT, and GSH-Px activities in AAPH-induced oxidative stress rats. These collective findings indicate that FOs protect against oxidative stress damage by scavenging free radicals and enhancing non-enzymatic components and antioxidant enzyme expression.

FOs are compounds composed of ferulic acid and oligosaccharides linked by ester bonds. Following ingestion, FOs are partially metabolized in the digestive tract into free ferulic acid and oligosaccharides [29], possessing both phenolic activity and oligosaccharide characteristics [16]. The synergistic interaction between hydrophobic ferulic acid and hydrophilic oligosaccharides renders FOs' antioxidant function superior to ferulic acid alone. The phenolic hydroxyl and phenoxy groups in ferulic acid' s unsaturated side chain can terminate free radical chain reactions, preventing membrane damage and maintaining cellular integrity [30]. Gerin et al. [31] demonstrated that ferulic acid (50 mg/kg) alleviated formaldehyde-induced hepatic injury in rats, significantly increasing hepatic T-AOC, GSH-Px, and SOD activities. Liao Changxiu et al. [32] and Ji Yiping [33] further confirmed that sodium ferulate significantly increased SOD activity in rat tissues. Additionally, oligosaccharides exhibit strong antioxidant properties by promoting SOD and GSH-Px expression to eliminate O_2^- [34].

Studies have also shown that feruloylated xylooligosaccharides can be obtained from wheat bran through xylanase hydrolysis and *Aureobasidium pullulans* fermentation [14,35]. Xylooligosaccharides display potent antioxidant functions, significantly increasing CAT and GSH-Px activities in mouse blood and liver [36], and dietary supplementation with 0.02% xylooligosaccharides enhanced serum GSH-Px activity and antioxidant capacity in growing pigs [37]. The present results indicate that 200 and 400 mg/kg BW wheat bran FOs restored several antioxidant indices in plasma, liver, kidney, and ileum to normal physiological levels, suggesting that wheat bran FOs effectively alleviate antioxidant capacity reduction caused by oxidative stress. Interestingly, hepatic T-AOC in rats receiving 100 mg/kg BW wheat bran FOs exceeded those receiving 200 and 400 mg/kg BW, warranting further investigation. Moreover, hepatic antioxidant enzyme activities and GSH content responded more prominently to wheat bran FOs administration than other tissues, possibly because the liver is the primary target organ for diquat toxicity [22], which may explain the superior protective effect of wheat bran FOs against hepatic oxidative damage.

Vitamin C, a recognized antioxidant, plays a crucial regulatory role in redox metabolic reactions [38]. This study found that 100 mg/kg BW vitamin C had no significant effects on hepatic CAT, SOD, and GSH-Px activities or GSH content in oxidative stress rats, but significantly increased renal T-AOC and GSH-Px activity, ileal T-AOC activity and GSH content, and decreased 8-OHdG content in liver, kidney, and ileum, indicating partial alleviation of diquat-induced oxidative stress. However, its efficacy in restoring antioxidant capacity was inferior to 200 and 400 mg/kg BW wheat bran FOs. This discrepancy with Zhang et al. [6] may be attributed to the relatively low vitamin C dosage (100 mg/kg BW) employed in this study.

Conclusions

1. Mixed bacterial fermentation of wheat bran followed by Amberlite XAD-2 purification yielded wheat bran FOs at a high concentration of 0.059 mmol/g.
2. Intraperitoneal diquat injection suppressed antioxidant enzyme secretion and caused oxidative damage in rats.
3. Under diquat-induced stress, oral administration of wheat bran FOs effectively enhanced antioxidant enzyme activities and GSH content while reducing the DNA oxidative stress metabolite 8-OHdG, thereby improving the antioxidant capacity of rat plasma and tissues.

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Note: Figure translations are in progress. See original paper for figures.