

β -glucan-rich Extract of Oat Bran Ameliorates Exercise-induced Fatigue and Its Antioxidative Effects *in Vitro* and *in Vivo*

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Abstract β -glucan-rich extract of oat bran (oat β -glucan), a good source of soluble dietary fiber with well-recognized health benefits, was investigated to identify correlation between its antioxidative ability and anti-fatigue effects using exercise model. The antioxidant activities of oat β -glucan were determined both *in vitro* and *in vivo*. *In vitro* assays showed its free radical scavenging capacities at respective levels of 33.07, 25.53, and 52.95 $\mu\text{mol TE/g}$ against DPPH $\cdot$, peroxy, and hydroxyl radicals. On the other hand, *in vivo* studies showed significant alleviation of oxidative stress in exercise rats, with significant increase of the superoxide (SOD) activity and reduction of the malondialdehyde (MDA) in skeletal muscle and serum, along with increasing catalase (CAT) activity and decreasing OH $\cdot$ levels. Therefore, the anti-fatigue mechanism of oat β -glucan could be associated with its antioxidant effects. Thus, oat β -glucan is a promising and novel dietary antioxidant and anti-fatigue ingredient, which may contribute to enhancement of exercise endurance.

Keywords: oat β -glucan, anti-fatigue, antioxidant activities, *in vitro*, *in vivo*

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1. Introduction

Oat β -glucan, a plant cell wall component that contains mainly β -(1,3-1,4)-D-glucan, is a good source of soluble dietary fiber with a number of well-recognized health benefits [1,2]. Kirwan et al. [3] reported that consumption of an oat meal with a high dietary fiber content and moderate glycemic index 45 min before prolonged moderately intense exercise significantly enhances exercise capacity. According to the ancient record of traditional Chinese medicine, oats have been regarded as an anti-fatigue grain in northern part of China [4], and it has been considered as a good feed for racing horse till now. In a previous study, we also found that oat β -glucan could alleviate exercise-induced fatigue in a mice model [5]. However, there are scarce evidences elucidating the correlations between oat β -glucan and exercise-induced fatigue.

The role of free radical in exercise-induced fatigue has been identified [6,7]. Free radicals are intermediate metabolites of many vital biochemical events in the body, and they are in a dynamic balance between their production and clearance. Intense exercise can produce an imbalance between the body's oxidation system and its

anti-oxidation system. The accumulation of reactive free radicals will put the body in a state of oxidative stress and bring injury to the body by attacking large molecules and cell organs [8,9,10]. Decreased concentration of antioxidative enzymes such as superoxide dismutase (SOD), and increased level of peroxidation products such as malondialdehyde (MDA) and ROS were detected in exercised-induced fatigue [11,12]. For this reason, oxidative stress has been considered to be a common factor in the development of fatigue and its complications [13]. And oxidative stress during exercise can be used as a manifestation of fatigue [5]. Some reports showed that exogenous dietary antioxidants can also decrease the contribution of exercise-induced oxidative stress and improve the animal's physiological condition [14,15,16]. Such a phenomenon could be attributed to the fact that exogenous antioxidants can promote or interact with endogenous antioxidants to form a cooperative network of cellular antioxidants [17]. Therefore, it is reasonable to postulate that by supplying the body with an effective antioxidant, the level of fatigue could be reduced.

It is noteworthy that some controversies arise from contradicting results reported in the literature on the oxidative/anti-oxidative activity of β -glucan. While some studies showed that glucans stimulate cytokine release,

causing generation of reactive oxygen metabolites and releasing arachidonic acid metabolites [18,19], the antioxidant effects of oat β -glucan in animal models have also been reported [20,21]. Other studies further demonstrated that β -glucan could effectively reduce oxidative injury in rats [22,23], additional to its *in vitro* antioxidative activities [24]. Nevertheless, the antioxidant activities of oat β -glucan still need to be extensively investigated, especially on the antioxidative effect of oat β -glucan on exercise-induced fatigue using animal models.

In the present study, comprehensive assessments of antioxidative effects of β -glucan-rich extract of oat bran (oat β -glucan) in correlation with its anti-fatigue potential were conducted combining *in-vitro* and *in-vivo* approaches. The *in vitro* antioxidant activities of β -glucan were determined by evaluating the radical scavenging capacities, such as DPPH, peroxyl, and hydroxyl radicals, whereas *in vivo* investigations were focused on biochemical approaches using exercise animal models. A variety of biochemical indicators, such as malondialdehyde (MDA) and superoxide dismutase (SOD) activities in liver, skeletal muscle and serum, hydroxyl radical levels, as well as catalase (CAT) were measured.

2. Materials and Methods

2.1. Oat β -Glucan Purification

Naked oats (*Avena nuda*) were provided by researchers from Dingxi Dryland Agriculture Center in 2022, Gansu Province, China. After removing impurities, the oat seeds were steamed for 20 minutes to inactivate lipoxidase and then put into a hot air dryer at 38°C for 24 h to reduce the moisture content to 12%. After that, oat seeds were peeled to get oat bran powder with particle diameter < 0.8 mm using Satake mill (TM-05C, Jiangsu, China). The milled oat bran samples were stored at 4°C.

2.2. Isolation and Purification of Oat β -Glucan

β -glucan was extracted from oat bran according to the method of Wood et al. [26]. The oat bran powder was mixed with water (1:15, w/w) at 50 °C for 1h, and the residues were removed using centrifugation (3000×g). The protein was precipitated and removed at its pI (pH 4.3), whereas the starch that remained in the supernatant was hydrolyzed with alpha amylase. The suspended gum containing β -glucan in the supernatant was separated by pouring the supernatant into absolute ethyl alcohol (1:1, v/v), followed by centrifugation at 4000×g for 10 min. The precipitant was freeze-dried and stored at 4°C until use.

2.3. Determination of β -Glucan Antioxidant Activities *in Vitro*

2.3.1. Determination of DPPH Radical Scavenging Activity

Trolox was used as the antioxidant standard. The reaction mixture contained 100 μ L of 0.2 mM working DPPH• solution and 100 μ L β -glucan solution. The

absorbance was measured at 515 nm every minute for 40 min by using a Victor³ multilabel plate reader (PerkinElmer, Turku, Finland). The DPPH radical scavenging capacity was calculated from the area under the curve and expressed as μ mol TE/g β -glucan.

2.3.2. Determination of Peroxyl Radical Scavenging Capacity

The peroxyl radical absorbance capacity was conducted based on the report of Moore et al. [27] with some modifications. Trolox, a water soluble vitamin E derivative, was used as the standard. The reaction mixture consisted of 30 μ L of blank, standard, or sample, 225 μ L of 8.16×10^{-8} M FL and 25 μ L of 0.36 M AAPH. The mixture was recorded every 2 min at 37°C for 2 h. Trolox equivalents were calculated for samples based on area-under-the-curve calculation. Results were expressed as μ mol TE/g β -glucan.

2.3.3. Determination of Hydroxyl Radical Scavenging Activity

The hydroxyl radical scavenging activity of β -glucan was determined according to the reports [28]. Fluorescein (FL) was used as the fluorescent probe and Trolox was used as standard. The final mixture contained 30 μ L of sample, 40 μ L of freshly prepared 0.1990 M H₂O₂, 60 μ L FeCl₃ and 170 μ L of 9.28×10^{-8} M FL. The fluorescence was recorded every 4 min for 7 h. Results were expressed as μ mol TE/g β -glucan.

2.4. Determination of β -Glucan Antioxidant Activities *in Vivo*

2.4.1. Animals

Thirty-two male SD rats (Fourth Military Medical University, Xi'An, Shaanxi, China; License NO. SCXK 2010-007), weighting 152.3-163.8 g, were used. They were kept under the conditions of a constant temperature (22.0 $\pm$ 2 °C) and humidity (55%), and were allowed to freely access food and water with 12-h light and dark cycles during the experiment.

2.4.2. Animal Design and Experimental Design

After adapting to their environment for 1 week, the rats were randomly divided into four groups (eight for each) as described in Table 1: control group, oat β -glucan group, control exercise group, and β -glucan + exercise group. Rats in β -glucan treatment groups were administered with oat β -glucan (312.5 mg/kg body weight, according to the FDA recommended dosage [29]) dissolved in 2 mL distilled water every morning. Meanwhile, rats in other two groups were administered with 2 mL physiological saline. In the current study, β -glucans were administered to rats by gastric gavage rather than adding to their food source. Accurate dose of β -glucan was fed to rats by gastric gavage, thus individual appetite difference can be avoided.

Rats in the training groups were habituated to running on a treadmill. For the first 5 weeks, namely adaptation training, rats run at the speed of 15, 22, 27, 31, 35 m/min for 20 min per day. In the following 2 weeks of intensive training, rats were running at a speed of 35 m/min for 20 min. They were continued daily for consecutive 5 days in

both running treatments. After 7 weeks training, all the rats in these four groups were running at 35 m/min for 30 min, and then they were sacrificed for further test.

Table 1. Experiment groups and treatments

Group	Group name	Diets and training
1	Control group	Basal diet ^a + physiological saline
2	β -Glucan group	Basal diet + β -glucan (312.5 mg/kg.d) ^b
3	Control exercise group	Basal diet + physiological saline + running
4	β -Glucan + exercise group	Basal diet + β -glucan (312.5 mg/kg.d) + running

^a Basal diet was made in laboratory; ^b312.5 mg/(kg.d) means 312.5 mg oat β -glucan supplementation per kg body weight daily.

2.4.3. Bioassay of Blood, Liver and Skeletal Muscle

Rats were anesthetized with 10% chloral hydrate. Blood from the celiac artery was collected into dry test tube using syringes and allowed to coagulate at ambient temperature for 30 min. The serum was collected for further analysis after centrifugation at 200 $\times g$ for 10 min. Liver and gastrocnemius muscles were quickly excised and washed in ice-cold saline solution to remove the blood, and then frozen in liquid nitrogen. All these samples were stored at -80 °C in a deep freezer for further biochemical test. Malondialdehyde (MDA) and OH $\cdot$ levels, as well as catalase (CAT), and superoxide dismutase (SOD), were determined by using commercial reagent kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the instruction manuals.

2.5. Statistics

The data was reported as mean $\pm$ SD ($n = 8$). Differences among means were assessed by one-way ANOVA (SAS Institute, Cary, NC). Statistical significance was declared at $P < 0.05$ as determined by Duncan's new multiple range test. Bar and line charts were drawn using Graph Pad Prism 5 software (Graphpad Software, San Diego, CA).

3. Results

3.1. Purification of Oat β -Glucan and Its Physicochemical Properties

In this study, the chemical components of purified sample were 91.3% β -glucan, 1.4% protein, and 2.6% ash on the dry weight bases. The molecular weight was measured by HPLC with a size exclusion column. Based on a calibration curve obtained from the elution retention times of dextran with various molecular weight cutoffs, the average molecular weight of β -glucan was estimated to be 1.92×10^5 Da (data not shown).

3.2. Antioxidant Activities of β -Glucan *in Vitro*

The DPPH radical scavenging capacity of β -glucan was determined in the present study (Table 2). DPPH radical is

a stable radical and can accept an electron or hydrogen radical to become a stable diamagnetic molecule. It can be scavenged by antioxidants due to its hydrogen-donating ability [30], and thus it has been widely used as an indicator for free-radical scavenging capacities of antioxidants [31]. As shown in Table 2, the DPPH radical scavenging capacity was 33.07 μ mol TE/g.

Peroxyl radical scavenging capacity is also a very important index to estimate antioxidant activities. While peroxyl itself is not very reactive, yet it may generate OH $\cdot$ which is toxic to cells. Thus, the ability to remove peroxyl in a biological system is important to keep away possible toxic damage. As shown in Table 2, the peroxyl radical scavenging capacity of oat β -glucan was determined to be 25.53 μ mol TE/g.

Among the oxygen radicals, the hydroxyl radical is the most reactive and can severely damage the adjacent biomolecules. The hydroxyle radical is thought to be deteriorative and can lead to cell damages [32]. The hydroxyl radical scavenging capacity of β -glucan was determined to be 52.95 μ mol TE/g in this present study (Table 2).

Table 2. Antioxidant activities of β -glucan *in vitro*^a

Sample	DPPH $\cdot$ scavenging capacity (μ mol TE/g)	Peroxyl radical scavenging capacity (μ mol TE/g)	OH $\cdot$ scavenging capacity (μ mol TE/g)
β -glucan	33.07 $\pm$ 3.34	25.53 $\pm$ 2.37	52.95 $\pm$ 5.27

^aData are mean $\pm$ SD($n=8$)values.

3.3. Antioxidant Activities of β -Glucan *in Vivo*

3.3.1. Superoxide Dismutase (SOD) Activity

Superoxide dismutase (SOD) activities in liver, muscle, and serum were determined (Figure 1). The liver SOD activities in liver were significantly higher in rats in the β -glucan + Exercise group than those in other three groups ($P < 0.01$) (Figure 1a). Also, rats in β -glucan + Exercise group had the highest SOD activity in muscle tissue compared to the control group ($P < 0.01$) (Figure 1b). In addition, rats fed with β -glucan in group 2 had significantly higher SOD activity in muscle (64.32 ± 6.05 U/mL) compared to either control or exercise group ($P < 0.05$). In serum, the trend of SOD activity in rats was similar to that observed in liver (Figure 1c).

3.3.2. Catalase (CAT) Activity in Serum

Compared to normal control group, rats in the β -glucan + Exercise group had the highest CAT activities (6.91 U/mL) ($P < 0.01$), followed by the β -glucan group (5.59 U/mL) ($P < 0.05$) (Figure 2). But exercise showed no significant difference compared to the control group. In addition, rats in the β -glucan + Exercise group had significant higher CAT activities when compared to the exercise group ($P < 0.01$).

3.3.3. Determination of Lipid Peroxidation

MDA, the main product of lipid peroxidation, is an indicator of lipid peroxidation. In our study, the levels of MDA were determined in liver, muscle, and serum. Rats in

the β -glucan + Exercise group showed significantly lower MDA levels in liver (18.21 ± 3.88 nmol/mL) when compared to either the normal control or the exercise group ($P < 0.05$) (Figure 3a). In muscle, rats fed with β -glucans in group 2 showed the lowest MDA level (13.33 nmol/mL) compared to either the normal control or the exercise group ($P < 0.05$). Also, MDA levels observed in the β -glucan +

Exercise group were significantly lower (13.63 ± 3.67 nmol/mL) when compared to those in the control group (Figure 3b). Furthermore, MDA level in serum in the β -glucan + Exercise group was 12.45 ± 1.34 nmol/mL, (Figure 3c), which had a similar trend to that in liver. However, there was no significant difference observed between the control and exercise groups ($P > 0.05$).

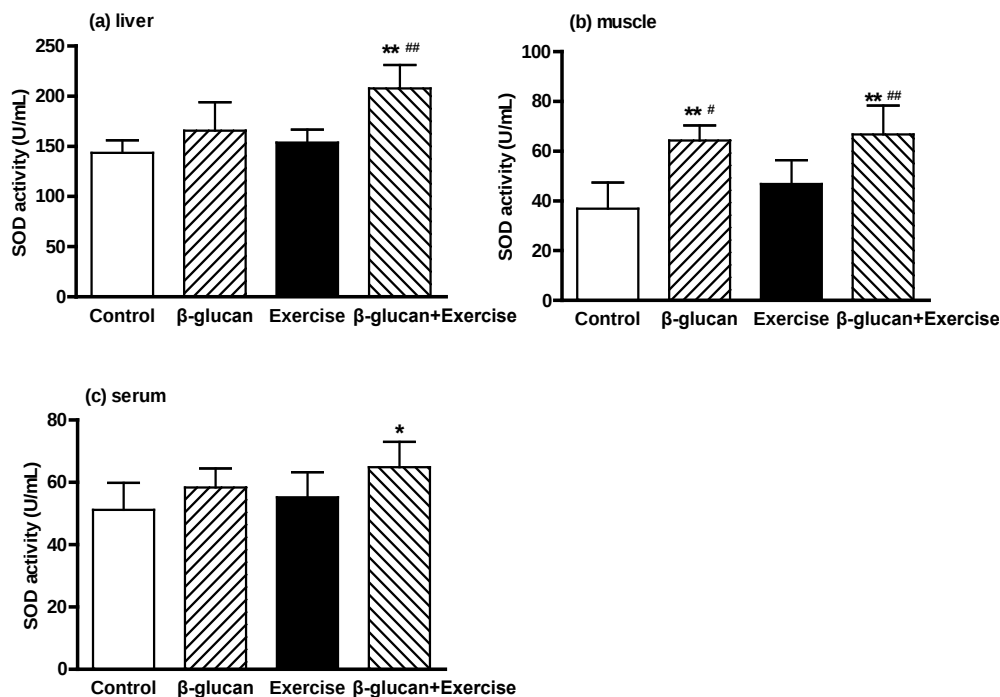


Figure 1. Figure 1. Superoxide dismutase (SOD) activities in liver (a), muscle (b), and serum (c) *: $P < 0.05$; **: $P < 0.01$ compared to control; #: $P < 0.05$, ##: $P < 0.01$ compared to Exercise group

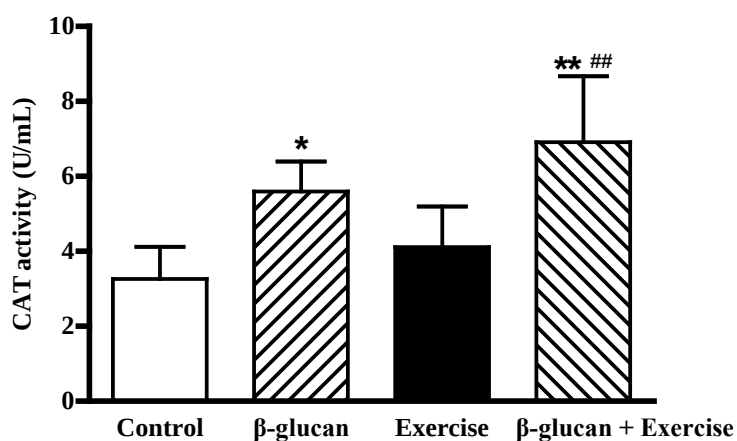


Figure 2. Catalase (CAT) activity in serum. *: $P < 0.05$; **: $P < 0.01$ compared to control; ##: $P < 0.01$ compared to Exercise group

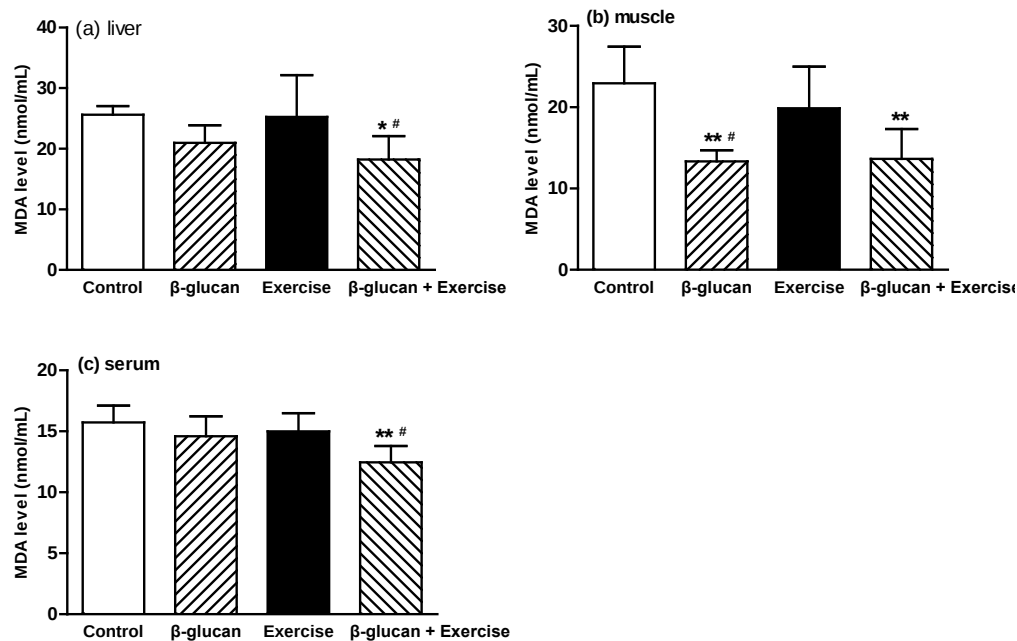


Figure 3. MDA levels in liver (a), muscle (b), and serum (c). *: $P < 0.05$; **: $P < 0.01$ compared to control; #: $P < 0.05$, compared to Exercise group

3.3.4. Serum Hydroxyl Radical

Rats in the β-glucan + Exercise group had the lowest value (629.44 U/mL) of hydroxyl radical in serum among the four groups ($P < 0.01$) (Figure 4). In addition, compared to control group, hydroxyl radical levels in the β-glucan and Exercise group were also significantly lower ($P < 0.05$), 704.00 and 712.09 U/mL, respectively.

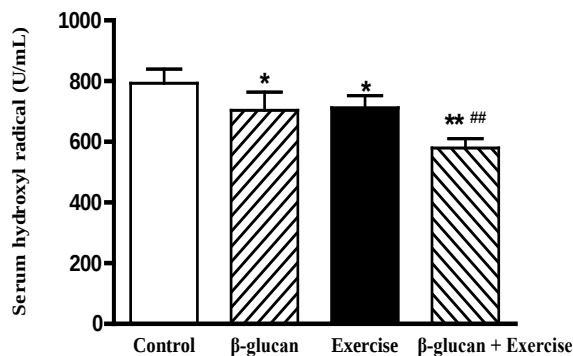


Figure 4. Hydroxyl radical levels in serum. *: $P < 0.05$; **: $P < 0.01$ compared to control; #: $P < 0.01$ compared to Exercise group

4. Discussion

Most previously studies used radical remaining or quenched percentage to report the radical scavenging capacities of polysaccharides [33], which is highly dependent on the reaction time and the initial concentration of the radicals and the antioxidants in the samples. But the approaches of radical scavenging capacities used in our study take into account the thermodynamic and kinetic measurements of the reactions and expressed as trolox equivalents in μmol on a per

sample weight basis. While it might be difficult to quantitatively compare the results with others, our findings were in line with those of KAI Z and coworkers [24] who reported that the hydroxyl scavenging capacity of β-glucan was significantly high. Thus, it is evident that oat β-glucan does possess effective antioxidative activity *in vitro*.

Oat β-glucan could enhance the endurance capacity of rats while facilitating their recovery from fatigue. Evaluation on anti-fatigue effect of oat β-glucan suggested that: Compared with control group (NC), oat β-glucan group (OG) could increase the exhaustive running time, and reduce serum urea nitrogen and serum creatine kinase of rats significantly ($P < 0.01$). Also, it could increase the liver glycogen contents, serum free fatty acid content and lactate dehydrogenase enzyme activity, and reduce serum urea nitrogen levels significantly ($P < 0.05$). Results showed that oat β-glucan has significant anti-fatigue properties, by combined with oat β-glucan and moderate intensity training, it could improve the body's endurance, delay fatigue [5]. However, whether the intake of Oat beta-glucan improves oxidative stress damage in exercise rats is unclear

SOD and CAT are two major antioxidant enzymes, which can scavenge reactive oxygen species (ROS) *in vivo* during oxidative stress [34,35]. SOD is the only enzyme that disrupts $\text{O}_2^\bullet$ radicals and exists in high amounts in erythrocytes. CAT can destroy H_2O_2 generated by free radicals or by SOD reaction. Thus, it can prevent the formation of $\text{OH}^\bullet$ radicals [36]. The balance between these enzymes is crucial for determining the steady-state level of ROS. To date, reports on the SOD and CAT activities after exercise training remain controversial. Some studies demonstrated that SOD and CAT activities in skeletal muscle increased significantly after training [37,38,39,40], whereas others failed to detect SOD training adaption [41] and any change in muscle CAT with training [42,43]. The

results of the present study demonstrated that oat β -glucans can increase the muscle SOD and serum CAT activities, although no significant increase was detected in liver and serum after exercise. However, the results clearly showed a positive correlation with synergistic effects of β -glucan and exercise on elevating the SOD and CAT activities not only in the serum but also in liver and muscle tissues ($P < 0.05$).

Lipid peroxidation, mediated by oxygen free radicals, was reported to be a crucial cause of destruction and damage to cell membranes, since the polyunsaturated fatty acids in the cellular membranes were degraded and disrupted by this process. A lower MDA level indicates that there was weaker oxidant stress and less lipid peroxidation [39,44]. As evidenced by detectable changes in the MDA levels in muscle, it clearly demonstrated that the oxidative injury could be ameliorated by β -glucan treatment. Our results were in agreement with the observation reported by Benlier N et al. [25]. Furthermore, β -glucan combined with exercise significantly reduced the MDA values in liver, muscle, and serum, suggesting that β -glucan and moderate-intensity exercise could produce synergistic antioxidant effects. Similarly [45,46], also verified that β -glucan could reduce MDA levels and preserve cellular integrity, which supports the observation that β -glucan could prevent methotrexate-induced lipid peroxidation [46,47].

It has been well recognized that the lower the hydroxyl radical concentration in serum, the stronger the hydroxyl radical scavenging capacity. Based on the results acquired in the present study, it becomes apparent that the hydroxyl radical scavenging capacity of oat β -glucans could be amplified after exercise training of rats. It is suggested that oat β -glucan has the effect of scavenging hydroxyl radicals *in vivo*, which may be related to the same effect *in vitro*.

5. Conclusion

β -glucan-rich extract of oat bran (oat β -glucan) was found to not only exhibit *in vitro* antioxidant activities, but also *in vivo* protection against exercise-induced oxidative stress in rats. Acted as the scavenger for DPPH, peroxy, and hydroxyl radicals, oat β -glucan can also significantly alleviate oxidative stress in exercise rats by increasing SOD and CAT activities and decreasing the MDA and $\text{OH}^\bullet$ levels. Therefore, it is evident that a positive correlation exists between the anti-fatigue mechanism of oat β -glucan and its anti-oxidative activities. These results provide an important basis for developing oat β -glucan as a novel dietary antioxidant and anti-fatigue ingredient. Further research needs to be carried out to evaluate its anti-fatigue activity on humans as well as its antioxidant mechanism(s) at the cellular and molecular levels.

Author Contributions

Conceptualization, Y.Z. and C.X.; methodology, Y.Z.; software, Y.W.; validation, Y.Z., X.X. and C.X.; formal analysis, Y.Y.; investigation, K.C.; resources, Z.A.; data curation, X.X.; writing-original draft preparation, Y.Z.; writing-review and editing, Y.W.; visualization, X.X.;

supervision, B.S.; project administration, C.X.; funding acquisition, C.X. All authors have read and agreed to the published version of the manuscript."

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