Polish Journal of Veterinary Sciences Vol. 28, No. 2 (2025), 311–320

DOI 10.24425/pjvs.2025.154952

Original article

Protective effect of mannose oligosaccharides on cadmium-induced hepatic oxidative damage in rats

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Abstract

The objective of this study was to investigate the effect of mannose oligosaccharides (MOS) against cadmium (Cd)-induced hepatic oxidative damage and analyze its underlying antioxidant mechanism. Thirty male Sprague-Dawley (SD) rats were randomly divided into five groups: control group and four others treated with cadmium chloride ($CdCl_2$)(2 mg/kg body weight (b.w.)) and different MOS levels at 0, 100, 300, 500 mg/kg b.w.. The results demonstrated that administration of MOS at a dose of 500 mg/kg significantly reduced Cd-induced oxidative damage in rat livers. This was evidenced by an increase in body weight gain (BWG) and thymus index. Additionally, liver superoxide dismutase (SOD), catalase (CAT) and total antioxidant capacity (T-AOC) activities were significantly improved compared to the group exposed to Cd alone. Conversely, MOS resulted in significant reductions in the liver index, liver malondialdehyde (MDA), hydrogen peroxide (H_2O_2), glutathione (GSH), and serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels. Morphological analysis showed that MOS ameliorated Cd-induced histopathology of the rat liver. Notably, Nrf2 gene expression levels increased, while heme oxygenase 1 (HO-1) and quinone oxidoreductase 1 (NQO1) mRNA levels decreased in the MOS group. In conclusion, MOS effectively attenuate Cd-induced oxidative damage in rat liver and the Nrf2 signaling pathway is involved in this process. This study provides valuable insights for the implementation of MOS applications in livestock and poultry production.

Keywords: mannan oligosaccharides, Cadmium, rat liver, oxidative damage, antioxidant

Introduction

When animals are exposed to various harmful stimuli, the oxidative and antioxidant systems in the body become imbalanced, and the body produces excessive amounts of highly reactive molecules such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), which lead to oxidative damage in cells and tissues (Ullah et al. 2023). In modern animal husbandry, the feeding environment, feeding mode, and diet composition can lead to oxidative stress in livestock and poultry. Oxidative damage caused by oxidative stress can directly lead to low immunity, chronic inflammation, reduced production performance and impaired quality of animal products (Cram et al. 2015). The liver is an essential metabolic organ, and there are thousands of mitochondria in liver cells, which are highly susceptible to oxidative conditions. Cadmium (Cd) is a toxic heavy metal that is widely distributed in the environment and can cause toxicity in almost all organs of humans and animals. Acute or chronic exposure to Cd can lead to liver and kidney damage, bone necrosis, testicular atrophy, etc., with the liver being the main accumulation site and target organ of Cd (Theocharis et al. 1991, Souza-Arroyo et al. 2022, Bautista et al. 2024). Cd exposure induces oxidative stress through multiple mechanisms, including the oxidation of cellular lipids and proteins, reduction of hematocrit and hemoglobin concentration, and generation of ROS and malondialdehyde (MDA) (Yan and Allen 2021). Furthermore, Cd impairs the antioxidant defence system by suppressing the activity of crucial antioxidant enzymes, such as superoxide dismutase (SOD) and catalase (CAT), thereby compromising their ability to scavenge free radicals and leading to subsequent oxidative damage (Nemmiche et al. 2007). Additionally, Cd exposure induces significant morphological changes and DNA strand breaks in both liver and heart tissues (Bhattacharjee et al. 2019). Cd can cause liver damage; our previous study also successfully established a rat liver oxidative damage model by continuous intraperitoneal injection of 2 mg/kg body weight (b.w.) cadmium chloride ($CdCl_2$) for 7 days, which laid the foundation for this experiment (Gao et al. 2021).

The ability of animals to counteract oxidative stress is closely related to the body's immunity and production performance. As a prebiotic, mannan oligosaccharides (MOS) regulate gut microbiota balance, enhance immune system function, and combat oxidative stress caused by harmful free radicals (Ahmad et al. 2023). MOS is not only widely used in livestock and poultry production, but is also used as prebiotics in aquaculture to improve the resistance of animals. Studies have shown that MOS had significant free radical scavenging capacity and can improve the antioxidant defence system in animals by reducing MDA levels and upregulating the activity of key antioxidant enzymes such as SOD, glutathione peroxidase (GSH-Px) and total antioxidant capacity (T-AOC) (Cheng et al. 2019, Lu et al. 2019). MOS can prevent cell membrane lipid peroxidation, inhibit the release of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymes into plasma, and maintain hepatic cell integrity (Xue et al. 2022). MOS promotes the levels of total protein, albumin and globulin, thereby improving immune function. And it optimizes the structure of the intestinal flora, improves digestive efficiency and enhances nutrient absorption, ultimately leading to improved growth performance (Torrecillas et al. 2013).

The nuclear factor erythroid 2-related factor 2 (Nrf2) is the key transcription factor regulating the antioxidant response. The antioxidant defense system in animals is regulated by the Nrf2 signaling pathway (Vemula et al. 2024). This pathway mainly regulates the expression of antioxidant enzymes such as SOD, GSH-Px, glutamate cysteine ligase (GCL), CAT, haem oxygenase (HO-1) and phase II detoxification enzymes such as UDP-glucuronosyltransferase 1A6 (UGT1A6) and quinine oxidoreductase (NQO1), to scavenge free radicals and mitigate oxidative damage (Ahmed et al. 2017, Dai et al. 2018, Widjiati et al. 2024). Therefore, this experiment aimed to study the antioxidant capacity of MOS based on the Cd-induced oxidative damage model in the rat liver, which may provide a reference for revealing the mechanism of oxidative stress and developing new antioxidant feed additives.

Materials and Methods

Animals and feeding

Male Sprague-Dawley (SD) rats, specific-pathogen-free (SPF), aged 6-8 weeks, and weighing 160-180 g, were used as animal models. The rats were housed in an environmentally controlled room (20-25°C, 40% - 60% relative humidity, 12 hours of light per day) with ad libitum access to feed and water. All animal procedures were approved by the animal care review committee of Henan University of Technology with the approval number HAUTETHI-2017-0485.

Experimental design

Thirty male SD rats were adapted to the new laboratory environment for 7 days and then randomly allocated into 5 groups, there were 6 replicates per treatment and 1 rat per replicate in a single cage. $CdCl_2$ (Comeo Chemical Reagent Co., Ltd, Tianjin, China)

Table 1. Experimental design and treatment of rats.

Groups	1st week	2nd week	3rd week
Control	Saline	Saline	Saline
$CdCl_2$	Saline	Saline	$CdCl_2$ (2)
100 mg/kg MOS	MOS (100)	MOS (100)	MOS (100)+$CdCl_2$ (2)
300 mg/kg MOS	MOS (300)	MOS (300)	MOS (300)+$CdCl_2$ (2)
500 mg/kg MOS	MOS (500)	MOS (500)	MOS (500)+$CdCl_2$ (2)

Note: The units of $CdCl_2$ and mannose oligosaccharides (MOS) are mg/kg b.w..

was administered via injection following the method previously described by Gao et al. (2021), MOS (Angel Yeast Co., Ltd, Hubei, China) was administered by oral gavage. The experimental period was 3 weeks. The detailed experimental design and treatments are presented in Table 1.

Determination of body weight gain (BWG) and relative organ weight

The initial and final body weights of the rats were recorded accordingly. Twenty-four hours after the last injection, the rats were euthanised by cervical dislocation to obtain liver, spleen, and thymus samples, which were then washed thoroughly with ice-cold physiological saline, dried with filter paper, and subsequently weighed. The relative organ weight was calculated using the following formula according to previous studies (Khatoon et al. 2024).

Body weight gain (g) = final weight (g) – initial weight (g)

Relative organ weight (%) = (organ weight / body weight) × 100%

Determination of AST and ALT activity

Blood samples were obtained by removing the eyeballs of the rats, and serum was separated following standard protocols as previously described (Dik et al. 2023). AST and ALT were detected strictly according to the manufacturer's instructions provided with the kits (Cat.No. C010, C009, Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Determination of oxidative damage and antioxidant indexes in the liver

After weighing, the left lobes of liver tissue were immediately snap-frozen in liquid nitrogen and stored at -80°C for subsequent analysis. MDA, hydrogen peroxide (H_2O_2), L-glutathione (GSH), SOD, CAT and T-AOC levels were measured using commercial kits (Cat.No. A003, A064, A006, A001, A007, A015, Nanjing Jiancheng Bioengineering Institute). Briefly, 1 g of liver tissue was weighed and mixed with 9 times its volume of saline. The mixture was then homogenized in an ice-water bath and centrifuged at 3500 rpm for 15 minutes. The supernatant was analyzed according to the manufacturer's instructions.

Histopathological analysis

Right lobes of rat liver tissues were collected and stained with hematoxylin-eosin (HE) as previously described (Mohammad et al. 2023), and histopathological changes were observed under a fluorescence microscope (Discover Echo, San Diego, CA, USA).

Relative quantitative RT-PCR

The mRNA levels of Nrf2, HO-1 and NQO1 were determined via relative quantitative RT-PCR with GAPDH as the internal control. Total RNA was extracted from the liver tissue following the instructions provided by Tsingke's RNAprep FastPure Tissue & cell kit (Beijing, China). cDNA was synthesized using a Goldenstar^{TM} RT6 cDNA Synthesis Kit Ver.2 (Tsingke). Relative quantitative RT-PCR was performed by the Master qPCR Mix (SYBR Green I) system (Tsingke) according to the manufacturer's instructions. Relative gene expression levels were calculated using the $2^{-\Delta\Delta CT}$ method. The primer sequences are listed in Table 2.

Statistical analysis

All data were tested for homogeneity and normal distribution using Levene's test and Shapiro-Wilk test, respectively, before statistical analysis. Data are presented as mean ± SD. One-way ANOVA was performed using SPSS 23.0 statistical software (IBM, Armonk, NY, USA), and differences between samples were considered significant at $p \leq 0.05$. Correlation analysis was performed using Pearson's correlation test. Data visualization was performed using GraphPad 8.0 (GraphPad Software, Inc., San Diego, CA, USA) and R (v4.0.2), and Hiplot online tools according to the method described in Lu et al. (2023).

Table 2. The sequences of primers used for relative quantitative RT-PCR.

Genes	Forward primer (5′→ 3′)	Reverse primer (5′→ 3′)
Nrf2	TGGAGACGGCCATGACTGATT	CACTGTAACTCGGGAATGGAAA
NQO1	GCGGTGAGAAGAGCCCTGAT	GCTCCCCTGTGATGTCGTTTC
HO-1	CAGGTCCTGAAGAAGATTGCG	GGCCTCTGGCGAAGAAACTC
GAPDH	CTGGAGAAACCTGCCAAGTATG	GGTGGAAGAATGGGAGTTGCT

Table 3. Determination of body weight gain (BWG) and relative organ weights in rats.

Groups	BWG, g	Liver index, %	thymus index, %	spleen index, %
Control	95.42±11.58^{a}	4.22±0.44^{c}	0.27±0.05^{a}	0.34±0.05^{a}
$CdCl_2$	45.22±10.24^{c}	5.2±0.31^{a}	0.17±0.02^{b}	0.29±0.02^{a}
100 mg/kg MOS	42.63±10.24^{c}	5.07±0.52^{ab}	0.17±0.03^{b}	0.28±0.03^{a}
300 mg/kg MOS	48.72±7.1b^{c}	4.71±0.5^{abc}	0.22±0.04^{ab}	0.3±0.04^{a}
500 mg/kg MOS	66.46±11.86^{b}	4.36±0.3^{bc}	0.24±0.04^{a}	0.32±0.04^{a}

Note: Different letters indicate significant differences at p≤0.05.

Results

Effects of MOS on BWG and relative organ weights in Cd-induced rats

Previous studies have confirmed that intraperitoneal injection of $CdCl_2$ at a dose of 2mg/kg b.w./day for one week can establish an oxidative damage model in rat liver. Therefore, in this experiment we used a dose of 2 mg/kg b.w. $CdCl_2$ to establish an oxidative damage model in rat liver. As shown in Table 3, compared to the control group, the BWG and thymus index in the $CdCl_2$ group were decreased by 52.61% and 37.04%, respectively (p≤0.05), and the liver index significantly increased by 23.22% (p≤0.05), while the spleen index decreased by 14.71%, but there was no significant difference (p>0.05). Compared to the $CdCl_2$ group, MOS increased the BWG, thymus and spleen indices and decreased the liver index. Specifically, the 500 mg/kg MOS group showed a significant increase of 46.97% (p≤0.05) in body weight gain and 41.17% (p≤0.05) in thymus index compared to the $CdCl_2$ group, along with a decrease of 16.15% (p≤0.05) in liver index and an increase of 9.46% in spleen index; however, the latter did not reach the statistical significance level (p>0.05). Furthermore, the liver and thymus indices of the 300 mg/kg and 500 mg/kg MOS groups were not significantly different from those of the control group (p>0.05).

MOS reduced the content of AST and ALT in Cd-induced rat serum

AST and ALT are crucial factors in liver function because the activities of AST and ALT in the serum are positively correlated with liver cell damage. To detect the effect of MOS on liver damage, serum AST and ALT activities were assessed. As shown in Table 4, serum AST and ALT activities increased by 93.72% (p≤0.05) and 200.24% (p≤0.05), respectively, in the $CdCl_2$ group compared to the control group. By contrast, MOS decreased the activities of AST and ALT in a dose-dependent manner compared to the $CdCl_2$ group. In the 300 mg/kg MOS group, AST and ALT activities decreased by 27.54% (p≤0.05) and 12.24% (p≤0.05), respectively. In the 500 mg/kg MOS group, AST and ALT activities decreased by 32.39% (p≤0.05) and 32.37% (p≤0.05), respectively. Additionally, ALT activity was significantly lower in the 500 mg/kg MOS group than in the 300 mg/kg MOS group (p≤0.05) (Table 4).

Effects of MOS on hepatic antioxidant capacity in rats

As shown in Table 5, compared to the control group, the levels of MDA, H_2O_2 and GSH increased by 200.18%, 92.41% and 12.35%, respectively, while the levels of SOD, CAT and T-AOC decreased by 21.60%, 40.69% and 25.00%, respectively, in $CdCl_2$ group (p≤0.05). In contrast, MOS decreased the levels of MDA, H_2O_2 and GSH, and increased the levels of SOD, CAT and T-AOC in a dose-dependent manner compared to the $CdCl_2$ group. The results are as follows: the levels of MDA, H_2O_2 and GSH in the 500 mg/kg MOS group decreased by 40.64% (p≤0.05), 32.11% (p≤0.05) and 14.82% (p≤0.05), respectively; the activities of SOD, CAT and T-AOC increased by 27.81% (p≤0.05), 32.26% (p≤0.05) and 20.00% (p≤0.05), respectively, while only MDA and GSH were significantly lower and

Table 4. Determination of AST and ALT activity in the rat serum.

Groups	AST, U/L	ALT, U/L
Control	22.15±3.37^{c}	14.06±1.69^{d}
$CdCl_2$	42.91±3.69^{a}	45.51±3.25^{a}
100 mg/kg MOS	39.11±2.75^{a}	43.69±2.56^{ab}
300 mg/kg MOS	31.09±2.15^{b}	38.66±3.15^{b}
500 mg/kg MOS	29.01±2.39^{b}	30.78±3.88^{c}

Note: Different letters indicate significant differences ($p \leq 0.05$).
AST – aspartate aminotransferase, ALT – alanine aminotransferase.

Table 5. Determination of oxidative damage and antioxidant indexes in the rat liver.

Indexes	Control	$CdCl_2$	100 mg/kg MOS	300 mg/kg MOS	500 mg/kg MOS
MDA	0.80±0.13^{c}	2.51±0.49^{a}	2.15±0.11^{a}	1.68±0.13^{b}	1.49±0.09^{b}
H_2O_2	7.77±1.11^{c}	14.95±1.22^{a}	13.79±0.99^{a}	13.32±0.93^{a}	10.15±0.8^{b}
GSH	42.65±4.33^{c}	55.00±4.07^{a}	50.37±2.52^{ab}	47.88±5.86^{bc}	46.85±2.62^{bc}
SOD	111.11±8.17^{a}	87.02±6.68^{b}	88.37±4.78^{b}	95.25±6.23^{b}	111.22±6.06^{a}
CAT	55.26±4.00^{a}	32.77±3.97^{b}	32.37±2.18^{b}	35.04±2.10^{b}	43.34±4.62^{c}
T-AOC	0.60±0.01^{a}	0.45±0.03^{d}	0.44±0.02^{d}	0.49±0.01^{c}	0.54±0.02^{b}

Note: Different letters indicate significant differences ($p \leq 0.05$).
MDA – malondialdehyde (nmol/mg protein), H_2O_2 – hydrogen peroxide (mmol/g protein), GSH – L-glutathione (μmol/g protein), SOD – superoxide dismutase (U/mg protein), CAT – catalase (U/mg protein), T-AOC – total antioxidant capacity (mmol/g protein).

T-AOC significantly higher in the 300 mg/kg MOS group than in the $CdCl_2$ group. Furthermore, SOD activity and GSH content in the 500 mg/kg MOS group did not significantly differ from the control ($p>0.05$) (Table 5).

Effects of MOS on histopathology in the rat liver

To evaluate the effect of MOS on Cd-induced hepatic toxicity, histopathological analysis was performed to observe the morphological characteristics of the liver tissue. Results from the control group revealed complete morphology and structure of liver tissue with neatly arranged cords of liver cells without any hepatocyte necrosis (Fig. 1A). Compared to the control, Cd-induced abnormal morphology was characterized by hepatocyte necrosis and inflammatory cell infiltration (Fig. 1B). And there was no significant improvement was observed in the liver of the 100 and 300 mg/kg MOS groups where there were still instances of hepatocyte necrosis or nuclear condensation or karyolysis (Fig. 1C-D). However, in the 500 mg/kg MOS group, there was no apparent damage to the morphology or structure of the liver tissue, with stable nuclear morphology (Fig. 1E).

Effects of MOS on Nrf2 signaling pathway in rat liver exposed to Cd

Nrf2 is an essential transcription factor in the animal antioxidant defense system. To evaluate the effect of MOS on Cd-induced liver oxidative damage, the gene expression of Nrf2 and its downstream factors HO-1 and NQO1 were detected by RT-PCR. As indicated in Fig. 2A, compared to the control group, Nrf2 mRNA levels were significantly decreased by 42.66% ($p \leq 0.05$) in the $CdCl_2$ group. However, administration of 500 mg/kg MOS notably increased Nrf2 mRNA levels by 48.78% ($p \leq 0.05$) compared with the $CdCl_2$ group. Furthermore, there were no significant differences in Nrf2 mRNA levels between the 500 mg/kg MOS group and the control group ($p>0.05$). Additionally, as shown in Fig. 2B-C, exposure to Cd resulted in elevated HO-1 and NQO1 mRNA levels in contrast to the control group; however, treatment with MOS led to a decrease in both HO-1 and NQO1 mRNA levels compared with the $CdCl_2$ group ($p \leq 0.05$).

Correlation analysis

To investigate the correlation between the expression of biomarkers related to hepatic oxidative damage, cor-

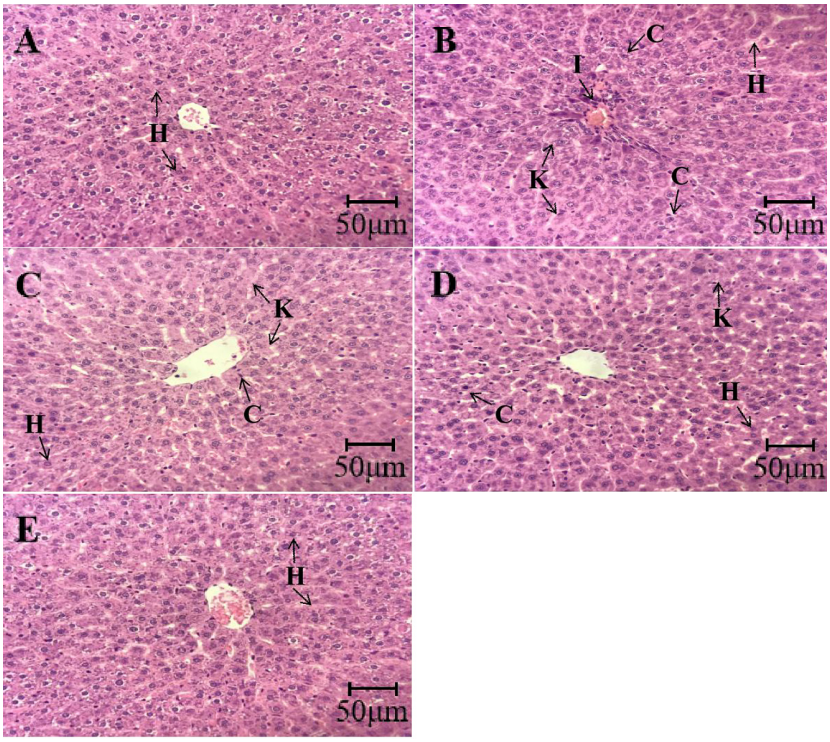

Fig. 1. The histopathology of the rat liver (hematoxylin and eosin stain; x200). A. Control group, B. $CdCl_2$ group, C. 100 mg/kg MOS group, D. 300 mg/kg MOS group, E. 500 mg/kg MOS group. H, Hepatocyte; C, Nuclear condensation; K, Nuclear karyolysis; I, Inflammatory cells.

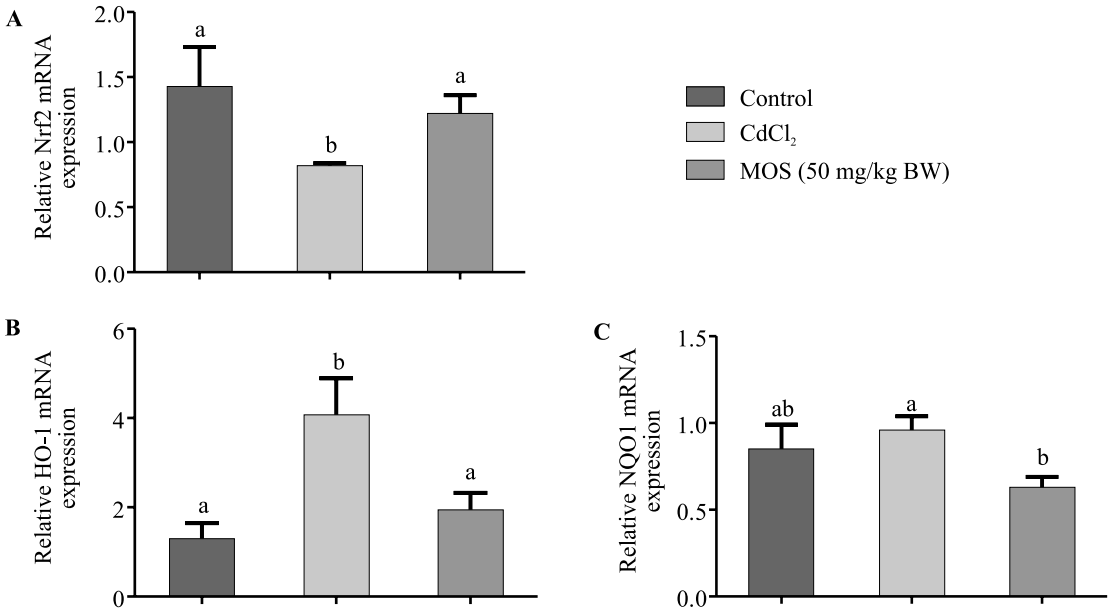

Fig. 2. Effects of mannose oligosaccharides (MOS) on antioxidant status in the rat liver. Relative quantitative RT-PCR analysis of Nrf2 (A), heme oxygenase-1 (HO-1) (B) and quinone oxidoreductase 1 (NQO1) (C) mRNA levels in the rat liver; Data represent the mean ± SD of six rats in each group. Different letters indicate significant differences ($p \leq 0.05$).

relation analysis of related indices was performed in the rats of the 500 mg/kg MOS group. Fig. 3 provides the diagram of the correlation analysis. The data showed that the activity of serum AST was positively correlated with the levels of ALT, T-AOC and CAT in the rat liver, and negatively correlated with the contents of MDA and SOD. Another hepatocyte injury factor, serum ALT, was positively correlated with the levels of T-AOC, H_2O_2 and GSH in the liver. Besides, MDA levels were positively correlated with SOD, and negatively correlated with T-AOC and CAT in the liver. H_2O_2 was positively correlated with GSH and ALT. In addition, the level of the antioxidant enzyme CAT presented a positive correlation with T-AOC, and a negative correlation with GSH and

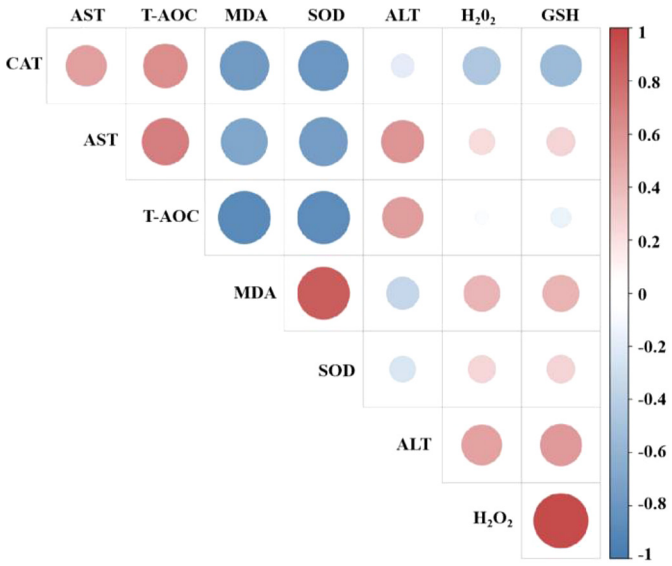

Fig. 3. Correlation analysis of parameters related to hepatic oxidative damage in the 500 mg/kg MOS rat group.

SOD. The activity of T-AOC was negatively correlated with SOD.

Discussion

Cd can bind to carboxyl, amino or sulfhydryl groups in proteins, peptides and fatty acids (Sabbioni and Marafante 1975, Rani et al. 2014). Cd binds to functional sulfhydryl groups which are located in the active centers of digestive enzymes, thereby inhibiting the activity of digestive enzymes and impairing the digestive and absorptive function of the gastrointestinal tract in animals, ultimately impeding their growth. MOS is a functional oligosaccharide that can maintain the balance of intestinal flora, promote digestion, absorption, and utilization of nutrients, and improve growth performance (Leone and Ferrante 2023, Yuan et al. 2023). In this study, the BWG of rats in the MOS groups was increased in a dose-dependent manner, and the 500 mg/kg MOS group was significantly increased compared to the $CdCl_2$ group. Oral exposure to $CdCl_2$ has been shown to induce acute intestinal inflammation and villus damage in mice (Zhao et al. 2006). Our previous study found that MOS can enhance intestinal health, and activate the intestinal immune system in rats through regulating the proinflammatory cytokines, lysozyme and secretory immunoglobulin A (sIgA) (Qiao et al. 2024). Based on these findings, MOS supplementation may alleviate Cd-induced intestinal inflammation, improve nutrient absorption efficiency and consequently enhance growth performance in this study.

The liver is the primary metabolic as well as detoxification organ (Karabekir et al. 2024), while the thymus and spleen are considered as important immune organs of the body (Al-Suwailem et al. 2024). An increase in the organ index indicates congestion and enlargement, while a decrease indicates atrophy and necrosis. The study demonstrated that supplementation with 500 mg/kg MOS significantly ameliorated $CdCl_2$-induced liver enlargement and reduction in thymus index, indicating that MOS improves the immune function and metabolic activity of the $CdCl_2$-damaged rat organism.

AST and ALT are direct indicators of liver disease. When the liver is damaged, AST and ALT are released into the bloodstream, thus the activity of AST and ALT in the serum reflects the degree of damage to the hepatocytes. Our study found that the activities of AST and ALT were significantly higher in the $CdCl_2$ group compared to the control group. However, the MOS groups showed a decreasing trend in the activities of AST and ALT compared to the $CdCl_2$ group, and this effect is particularly pronounced in the 300 mg/kg and 500 mg/kg MOS groups. These data imply that MOS alleviated $CdCl_2$-induced liver damage, which was further confirmed by histopathology of the rat liver. Histopathological examinations revealed disordered arrangements of hepatocytes in the $CdCl_2$ group, with a large number of condensed or lysed nuclei and infiltration of inflammatory cells, whereas the morphology and structure of hepatocytes in the 500 mg/kg MOS group were basically normal, and there was no obvious nuclear condensation or lysis. All these results confirmed that MOS has a protective effect on the liver against $CdCl_2$ – induced damage, and 500 mg/kg MOS could reverse the liver damage caused by $CdCl_2$.

H_2O_2 is one of the most common ROS, and MDA is an index of oxidative injury and lipid peroxidation

(Janero 1990, Ito et al. 2019). The results showed that $CdCl_2$ could increase the contents of MDA and H_2O_2, and decrease the activities of SOD and CAT and the level of T-AOC in rat liver tissues, while supplementation with MOS could reverse the above phenomena to varying degrees. Among the dose gradients evaluated in the experiment, 500 mg/kg MOS had the most obvious effect, which could significantly reduce the contents of MDA and H_2O_2 and significantly increase the activities of SOD and CAT and the level of T-AOC in liver tissue, and there was no significant difference in SOD activity between the 500 mg/kg MOS group and the control group. The 300 mg/kg MOS group also significantly reduced the content of MDA and increased the level of T-AOC, but the activity of SOD in liver tissue was not significantly increased in this group. Collectively, these data indicate that MOS can contribute to resistance to oxidative stress by improving the activity of antioxidant enzymes to maintain the homeostasis of redox balance, and the antioxidant effect of MOS is dose-dependent, which is consistent with previous report (Attia et al. 2017).

In this experiment, $CdCl_2$ induced a significant increase in the levels of GSH, a key non-enzymatic antioxidant, while MOS supplementation attenuated the Cd-induced GSH elevation. This observation is inconsistent with previous reports demonstrating Cd-induced GSH depletion (Olayan et al. 2020), and MOS-mediated enhancement of GSH activity (Cheng et al. 2019, Lu et al. 2019). Correlation analysis revealed a significant positive relationship between GSH levels and H_2O_2 concentration, and previous studies have reported that GSH is protective against H_2O_2-induced oxidative damage (Scott 2006). Based on these results, we hypothesize that the increased GSH levels may facilitate H_2O_2 detoxification. Notably, although the 500 mg/kg MOS group showed higher GSH levels compared to the control group, the difference was not statistically significant, suggesting that MOS may help maintain redox homeostasis in Cd-induced rats.

The transcription factor Nrf2 plays a pivotal role in the antioxidant stress response of animals, functioning as a central regulator of the oxidative stress response. Analysis revealed that the mRNA and protein expression levels of Nrf2 in the $CdCl_2$ group were significantly lower than those in the control group, whereas the mRNA and protein levels of Nrf2 in the 500 mg/kg MOS group exhibited a notable elevation compared to the $CdCl_2$ group, and there was no significant difference compared to the control group. However, the mRNA expression levels of HO-1 and NQO1 exhibited an inverse correlation with Nrf2. Administration of $CdCl_2$ resulted in overexpression of HO-1 and NQO1 mRNA, whereas MOS was able to inhibit this overexpression and exhibited no significant difference compared to the control group. This may be due to the excessive production of ROS induced by $CdCl_2$, which consumes a significant amount of Nrf2 to activate gene transcription of the antioxidant enzymes HO-1 and NQO1 for subsequent protein synthesis. In contrast, MOS restored the expression of Nrf2, HO-1 and NQO1 to levels similar to those observed in the control group. The results demonstrated that MOS could alleviate $CdCl_2$-induced oxidative damage in rat liver by regulating the Nrf2 signaling pathway. These findings are consistent with the concept that the Nrf2 signaling pathway is involved in regulating the expression of antioxidant genes (Levonen et al. 2007, Saha et al. 2020).

In conclusion, our results demonstrate that MOS supplementation at 500 mg/kg b.w. effectively alleviates Cd-induced hepatic oxidative damage by modulating the Nrf2 signaling pathway, and maintaining redox homeostasis. Additionally, MOS supplementation regulates intestinal immune function and improves growth performance. These findings provide an intergrated view of MOS as a feed additive in livestock and poultry production.

Acknowledgements

This work was supported in part by the Cultivation Programme for Young Backbone Teachers in Henan University of Technology (21421208); the key Technologies R&D Programme of Henan Province (242102111012, 242102110006); the Natural Science Fund of Henan (242300420142), and the Cultivation Plan Project of Henan University of Technology (31490143).

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