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ORIGINAL ARTICLE

Yeast culture improve CCl_4-induced liver damage, inflammatory response via inhibition of TLR2/NF-kB signaling pathway expression in *Pseudobagrus ussuriensis*

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Abstract

The aim of this study was to investigate the underlying hepatoprotective effects of yeast culture (YC) against carbon tetrachloride (CCl_4)-induced hepatic damage in *Pseudobagrus ussuriensis*. The fish were randomly divided into three experimental groups (Control, CCl_4 and YC+CCl_4) with three replicates of 30 fish in each replicate. Firstly, the Control and CCl_4 groups were fed basal diet without yeast culture, and the YC+CCl_4 group was fed diet with 20 g/kg YC for 8 weeks. After the end of feeding experiment, Control group was intraperitoneally injected olive oil with 0.05 ml/15 g fish body weight, while CCl_4 and YC+CCl_4 groups were intraperitoneally injected CCl_4 olive oil solution (CCl_4: olive oil = 3:7) with 0.05 ml/15 g fish body weight for 48 h. The results indicated that fish fed with 20 g/kg yeast culture not only ameliorated injured hepatic cell, as evidenced by well-preserved liver architecture, but also significantly decreased plasma AST activity in the CCl_4-induced hepatic injury model. Next, we found that dietary 20 g/kg YC supplementation could improve hepatic antioxidant activity and inhibit lipid peroxidation induced by CCl_4. Fish fed 20 g/kg YC could suppress the decrease of plasma IgM and plasma ACH50 content caused by CCl_4 ($p < .05$). In addition, we also found that fish treated with CCl_4 up-regulated the expression of immune-related genes (TLR2, MyD88 and NF-κBp65), proinflammatory cytokines (IL-1β and IL-8) and Hsp70 mRNA expression in liver compared with the Control group; meanwhile, fish fed with 20 g/kg YC down-regulated the above-mentioned genes expressions in liver compared with CCl_4 group. In general, the results mentioned above suggested that the dietary yeast culture could relieve the oxidative stress, immune damage and liver injury induced by CCl_4 and could also suppress CCl_4-induced inflammation through inhibiting the TLR2/NF-κB signalling pathway.

KEYWORDS
antioxidant capacity, carbon tetrachloride, NF-κB signalling pathway, *Pseudobagrus ussuriensis*, TLR2, yeast culture

1 | INTRODUCTION

Liver is an important organ that may regulate various physiochemical functions including synthesis, secretion and metabolism of xenobiotics. Damage to the liver may lead to these physiochemical function disorder. Many risk factors in aquaculture may induce such damage, including environmental pollution and the abuse of antibiotics and pesticides. At present, there are few breakthroughs in the preventive and control of fish liver damage (Yin et al., 2011). Therefore, research identifying improve hepatic damage agents is urgently necessary. And the hepatic injury models induced by chemical substances were often used to investigate its pathogenesis and the underlying protective effect of drugs. These chemical substances mainly include carbon tetrachloride (CCl_4), alcohol, acetaminophen (Jaeschke et al., 2011) and thioacetamide (Ming et al., 2006). Presently, carbon tetrachloride (CCl_4) is widely used as a well-established hepatic injury model chemical with the merits of convenient accessibility, easy model making and little damage to other organs. Previous reports demonstrated that the metabolites of CCl_4 such as toxic trichloromethyl free radical (•CCl3) and trichloromethyl peroxyl (•OOCCl3) free radicals may lead to severe damage to the function and structure of liver cells in the grouper and jian carp (*Cyprinus carpio var.* Jian) (Al-Harbi et al., 2014; Cao et al., 2015; Singh et al., 2008; Sun et al., 2019). Therefore, researchers always used carbon tetrachloride (CCl_4) as a classic liver hepatotoxicant to gain an insight into the pathophysiological processes in aquaculture (Zou et al., 2018).

In recent years, it is gaining increasing attention that probiotics was used as dietary supplements for the improvement of liver damage and diseases in animals. (Ayiku et al., 2020; Banerjee & Ray, 2017; Bu et al., 2019; Kong et al., 2020). Yeast culture (YC) a member of probiotic family possesses many immunomodulatory constituents (β-glucan, nucleotide and mannose oligosaccharide) and antioxidant substances (B-vitamin, glutathione) (Jensen et al., 2008; Liu et al., 2004; Xiong et al., 2017), respectively. Previous studies have demonstrated that yeast culture or yeast hydrolysate as dietary supplements could improve growth performance, immune response, intestinal health, antioxidant capacity and disease resistant in aquatic animals (Ayiku et al., 2020; Bu et al., 2019; Cheng et al., 2019; Jin et al., 2018; Zhang et al., 2018). The above-mentioned positive effect was probably attributed to the β-glucan and mannose oligosaccharide in YC, which could inhibit the colonization of pathogens in the intestine, improve the structure of intestinal flora. In addition, β-glucan as immune regulatory ligand could co-act with immune-receptors such as Toll-like receptors (TLRs) to elicit a series of immune cells containing dendritic cells, macrophages, monocytes, natural killer cell and neutrophils resulting in various immune responses (Dalmo & Bøgwald, 2008; Goodridge et al., 2009). TLRs identified as a mediator of inflammatory response may associate with their corresponding adaptor molecules myeloid differentiation factor 88 (MyD88) to active downstream signal, eventually stimulate the transcription of nuclear factor κB (NF-κB), thereby inducing the expression of proinflammatory cytokines such as interleukin-1β (IL-1β) and interleukin-8 (IL-8) (Sun et al., 2017; Thompson & Locarnini, 2007; Zhang et al., 2017). TLR2 plays a crucial role in innate initiating immune responses and influences subsequent adaptive immune responses (Fan et al., 2015). Growing number of researches have proved that the liver damage induced by CCl_4 may be correlated with the up-regulation of TLR2/NF-κB signalling pathway (Gan et al., 2018). And the inhibition of this pathway by dietary yeast culture supplementation could alleviate liver injury and inflammatory response induced by gossypol in *Pseudobagrus ussuriensis* (Bu et al., 2019). Nevertheless, it is still lack of enough information about the effectiveness and the associated mechanisms of YC as dietary additives to relieve hepatic damage.

Pseudobagrus ussuriensis is an important indigenous species in China and East Asia because of its great economic value and better productive performance. However, this fish is facing the problem of immune suppression and liver damage with the development of intensive farming. To date, some experiments about dietary nutrition requirement of this fish have been conducted and the effect of different vegetable protein (soybean meal, vegetable meal, cotton meal and corn protein) and animal protein (meat and bone meal, mussel meal) replacing fish meal had been studied in our laboratory (Bu et al., 2017; Luo, 2019; Wang et al., 2020). Moreover, a preliminary study about the health function of YC on *Pseudobagrus ussuriensis* has been explored (Bu et al., 2019). But little information is available on YC against liver damage of *Pseudobagrus ussuriensis*. Thus, the objectives of this study were to investigate the effects of dietary yeast culture against CCl_4-induced liver damage in *Pseudobagrus ussuriensis*.

2 | MATERIAL AND METHODS

2.1 | Reagents

Yeast culture was purchased from Beijing Enhalor International Tech Co., Ltd. (Beijing, China). CCl_4 (analytical grade) was purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China) and dissolved in olive oil to generate CCl_4 solution (CCl_4: olive oil = 3: 7).

2.2 | Diets and feeding management

To investigate the underlying protective mechanism of yeast culture against CCl_4 challenge, growth experiment was conducted ahead of the CCl_4 challenge trial. The basal diet and experimental diet with 20 g/kg yeast culture were formulated (Table 1), and referred as control and YC, respectively. The diet was produced according to the method described by Bu et al. (2019) and stored at −20°C until used. The diets ingredients were determined based on AOAC procedures. And the dry matter of diet was measured by desiccation at 105°C until constant weight. Crude protein was measured by Auto Kjeldahl System (2300-Auto-analyzer; FOSS, USA) according to the Kjeldahl method. Crude lipid was measured using Soxhlet extraction. Ash was measured by placing the sample in a muffle furnace at 550°C for 24 h. Gross energy was measured by the adiabatic bomb calorimeter (Parr 6300, USA).

TABLE 1 Formulation and chemical composition of the test diet (g kg^{-1} dry matter)

Ingredients	Diet Control	Diet YC
Fish meal[a]	280	260
Soybean meal[b]	300	300
Cottonseed meal[c]	110	110
Corn gluten meal[d]	70	70
Wheat meal	164	164
Soybean lecithin	10	10
Soybean oil	33	33
Vitamin premix[e]	5	5
Mineral premix[f]	5	5
Choline	3	3
$Ca(H_2PO_4)_2$	20	20
Yeast cultured[g]	0	20
Proximate composition (g kg^{-1} dry matter)		
Dry matter	932.7	928.0
Crude protein	458.3	455.5
Crude lipid	77.7	76.4
Gross energy (kJ g^{-1})	184.9	186.0
Ash	121.3	115.3

[a]Fish meal: crude protein: 645.0 g kg^{-1}, crude lipid: 85.0 g kg^{-1}.

[b]Soybean meal: crude protein: 467.9 g kg^{-1}, crude lipid: 31.4 g kg^{-1}.

[c]Cottonseed meal: crude protein: 482.0 g kg^{-1}, crude lipid: 14.7 g kg^{-1}.

[d]Corn gluten meal: crude protein: 602.0 g kg^{-1}, crude lipid: 14.7 g kg^{-1}. These ingredients were supplied by Huada feed Co., Ltd. (Harbin, China).

[e]Vitamin premix (IU or mg kg^{-1} dry diet): retinol (VA) 3000 IU; cholecalciferol (VD) 1500 IU; tocopherol (VE) 40 mg; menadione (VK) 4.5 mg; thiamin (VB1) 8 mg; riboflavin (VB2) 8.5 mg; pyridoxine (VB6) 6.5 mg; cyanocobalamin (VB12) 0.02 mg; nicotinic acid 45 mg; nicotinamide 45 mg; D-Ca pantothenate 17 mg; inositol 40 mg; biotin 0.15 mg; folic acid 1.3 mg; antiscorbic acid 110 mg.

[f]Trace mineral mixture use providing the following concentration (mg kg^{-1} dry diet): copper 6.5 mg; iron 45 mg; selenium 0.35 mg; zinc 70 mg; manganese 8.5 mg; magnesium 100 mg; cobalt 1 mg; iodine 1.2 mg.

[g]Yeast culture: crude protein: 487.0 g kg^{-1}, crude lipid: 42.1 g kg^{-1}, supplied by Beijing Enhalor International Tech Co., Ltd. (Beijing, China).

Fish were obtained from Fisheries Research Institute of Harbin Academy of Agricultural Sciences (Harbin, China), and were fed the control diet for 2 weeks to acclimate to the experimental diets before starting the feeding trial. After fasting for 24 h, the fish randomly allotted to three groups (Control, CCl_4 and YC+CCl_4) in triplicate (1.0 × 0.5 × 0.8 m, water depth 50–60 cm) with 30 fish to each aquarium. The Control and CCl_4 groups were fed control diet, and YC+CCl_4 group were fed YC diet. All fish were hand-fed two times (08:30 and 16:30) a day to apparent satiation for 8 weeks. During the experimental period, the flow rate of water in each tank was maintained at 2.4 L min^{-1} and the water conditions were dissolved oxygen >6 mg L^{-1}, temperature 24 ± 1°C pH7.0 ± 0.5, respectively. The experimental photoperiod was set at 12-h light and 12-h dark.

2.3 | CCl_4 challenge and Sample collection

After the 8-week feeding trial, the fish in each tank were weighed and collected for CCl_4 challenge. Fish in the Control group were intraperitoneally injected with olive oil (0.05 ml/15 g bodyweight) for 48 h. Fish in CCl_4 and YC+CCl_4 groups were intraperitoneally injected with CCl_4 solution (0.05 ml/15 g bodyweight) for 48 h. At the end of challenge trial, three fish from each tank were anaesthetized with eugenol (1:12,000) (Shanghai Reagent Corporation, Shanghai, China) and used for the collection of blood and liver samples. The blood samples were collected via caudal vein into tubes containing 32 g/kg sodium citrate and then centrifugated at 4000 *g* for 10 min and stored at −80°C until used. The liver was immediately frozen in liquid nitrogen and stored frozen at −80°C until analysed.

2.4 | Liver histology

One portion of liver tissue per fish was obtained and stained according to the method of Chunhua and Hongyan (2017). The slides were stained with the conventional haematoxylin–eosin (HE) protocol, mounted with neutral resin and examined with optical microscopy.

2.5 | Plasma biochemical index

The alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (AKP), albumin (ALB) and lysozyme (LZM) were determined by commercial reagent kits (Nanjing Jiancheng Bioengineering Institute, China). The plasma immunoglobulin M (IgM) and alternative complement pathway activity (ACH50) level were measured by the kit of enzyme-linked immunosorbent assay as described by Yu et al. (2014).

2.6 | Hepatic antioxidant status analysis

Liver tissues were homogenized and centrifuged according to the method of Bu et al. (2019). Superoxide dismutase (SOD) and glutathione peroxidase (GPX) activities, and hepatic malondialdehyde MDA content were determined using commercial kits according to the instructions of the kit (Nanjing Jiancheng Bioengineering Institute, China).

2.7 | Real-time polymerase chain reaction (PCR) analysis

The total RNA from each liver was extracted with *TransZol* Up Plus Kit (TransGen Biotech, China) according to the manufacturer's instructions. The quantity and concentration of RNA were measured on an ultra-micro spectrophotometer (Implen, Germany), respectively. The cDNA was synthesized using *TransScript*® One-Step gDNA Removal and cDNA Synthesis SuperMix (TransGen Biotech, China) according

to the manufacturer's instructions. According to the reference (Bu et al., 2019), the primers were designed and showed in Table 2. β-actin was used as the internal control gene. Real-time PCR assays were performed on Applied Biosystems® 7500 (USA) in a final volume of 20 μl with 2 × *TransStart*® Top Green qPCR SuperMix (TransGen Biotech, China) and ROX Reference Dye II following the manufacturer's instruction. The real-time PCR condition uses the following temperature profile: 30 s at 94°C, 5 s at 94°C for 40 cycles, 30 s at 60°C. The gene expression level was determined by $2^{-\Delta\Delta CT}$ method (Livak & Schmittgen, 2001).

2.8 | Statistical analysis

Data in this paper were statistical analysed by SPSS 25, and the results are presented as means ± standard error of means (SEM) with superscript letters indicating differences between groups. All data were checked for homogeneity of variance using Levene's equal variance test. One-way analysis of variance (ANOVA) followed by Duncan's multiple comparison test was chosen to determine the significant differences among the means when the data met the homogeneity of variance. In addition, the Kruskal–Wallis test was used to determine the significant differences among the means when the data did not pass homogeneity of variance test. The value of statistical significance was set at $p < .05$.

3 | RESULTS

3.1 | Growth

The growth performance parameters in different groups are shown in Table 3. As expected, there was no difference in the initial body weight among three groups. After feeding trial, final body weight, weight gain (WG), feed intake (FI) and specific growth rate (SGR) in YC+CCl_4 group were significantly higher than Control and CCl_4 groups ($p < .05$). And the survival rate (SR) and feed conversion ratio (FCR) were not significantly different among three groups ($p > .05$).

3.2 | Histological observation of liver

Regular morphological structure of liver tissue was observed in the control group (Figure 1A). The administration of CCl_4 caused histopathological changes in the liver such as hepatocyte swelling, vacuolar degeneration, necrosis and nuclei shifting to the cellular periphery. (Figure 1B). Meanwhile, the structure of cell membrane was incomplete in CCl_4 group. However, fish fed yeast culture showed a lower occurrence rate of above histopathological symptoms compared with those fish in CCl_4 group (Figure 1C).

3.3 | ALT and AST activities

Plasma ALT and AST activities are shown in Figure 2. After treated with CCl_4 for 48 h, the plasma activities of AST ($p > .05$) and ALT ($p < .05$) in CCl_4 group were higher than that in Control group, and ALT activity was significantly different between the above-mentioned two groups. Compared with CCl_4 group, the AST and ALT activities in YC+CCl_4 group were reduced, but AST activity in YC+CCl_4 group was significantly lower than that in CCl_4 group ($p < .05$).

3.4 | Antioxidant capacity

As shown in Table 4, the MDA content in CCl_4 group was significantly increased than the Control group after treated with CCl_4 ($p < .05$).

TABLE 2 Primer utilized for gene expression analysis (qPCR)

Primer names	Sequence (5′-3′)	Product (bp)	Tm (°C)	Reference or accession number
TLR2-F TLR2-R	TTGTACAGCTGGATGAGTTG TGTCGTCAGTGAAATGTCTC	206	54	Bu et al. (2019)
MyD88-F MyD88-R	TCAGACAGCTGGAGCAGACA CGCTGGTGATGGTCCAAACA	93	59	Bu et al. (2019)
NF-κB p65-F NF-κB p65-R	AAGAACCAGCCATACAAGCCACAC TCAGGCAGGTCCGCTTCGTAG	83	60	Bu et al. (2019)
IL-1β-F IL-1β-R	CCTGAACACCTTCGAGTCGG AGGTGGCTGGTTTGCTGATT	102	58	Bu et al. (2019)
IL-8-F IL-8-R	ATCGAAGGAAAAGCAGAGCG CTTTGCACAGGAGCCACTTG	111	57	Bu et al. (2019)
Hsp70-F Hsp70-R	GACTGTCCTGATCAAACGCAAC TGGCTCTTTCACCCTCATACACG	116	59	XM027173973
β-actin-F β-actin-R	CCTCCGTCTGGATTTGGCTG TCAAGGGCGACGTAGCAGAG	141	60	Bu et al. (2019)

Abbreviations: Hsp70, heat shock protein 70; IL-1β, interleukin-1β; IL-8, interleukin-8; MyD88, myeloid differentiation factor 88; NF-κB p65, nuclear factor kappa-B p65; TLR2, toll-like receptor 2.

MDA content in YC+CCl_4 group was reduced than that in CCl_4 group, but there was no significant difference ($p > .05$). The determination of liver SOD activity showed that the activity in CCl_4 group was significantly reduced compared with that in Control group ($p < .05$). But the activity of SOD in YC+CCl_4 group was significantly higher than that in CCl_4 group ($p < .05$). Simultaneously, there was no significant difference in the GPX activity among three groups ($p > .05$), but there was an improvement for that of fish in YC+CCl_4 group compared with that of fish in CCl_4 group. Those results showed that dietary yeast culture supplementation could alleviate *Pseudobagrus ussuriensis* liver damage induced by CCl_4 to a certain extent, which may be related to its functions of anti-stress ability and anti-lipid peroxidation.

TABLE 3 Growth performance of *Pseudobagras ussuriensis* in different groups

Index	Control	CCl_4	YC+CCl_4
IBW (g)[a]	180.15 ± 0.55	180.7 ± 0.00	180.46 ± 0.38
FBW (g)[b]	409.43 ± 5.13[b]	405.30 ± 1.00[b]	448.10 ± 3.77[a]
WG (%)[c]	127.28 ± 3.54[b]	124.29 ± 0.55[b]	148.29 ± 1.56[a]
SGR (%)[d]	1.47 ± 0.03[b]	1.44 ± 0.04[b]	1.62 ± 0.01[a]
SR (%)[e]	95.00 ± 1.67	98.33 ± 1.67	94.44 ± 2.93
FI (%)[f]	1.47 ± 0.01[b]	1.50 ± 0.04[b]	1.57 ± 0.01[a]
FCR [g]	1.22 ± 0.02	1.27 ± 0.03	1.19 ± 0.01

Note: Data represent as mean ± SEM. Mean with different superscripts in the same row is significantly different ($p < .05$).

[a]IBW, initial body weight.

[b]FBW, final body weight.

[c]WG, weight gain (%) = 100 × (final body weight - initial body weight) / (initial body weight).

[d]SGR, Specific growth rate (%) = 100 × (Ln (final body weight) – Ln (initial body weight))/days.

[e]SR, Survival Rate (%) = 100 × final fish number / initial fish number.

[f]FI, Feed intake (% per day) = 100× dry feed intake / [(initial body weight + final body weight) / 2 × t].

[g]FCR: Feed conversion ratio = feed consumed / weight gain.

3.5 | Immune ability

Compared with control group, the lysozyme activity in CCl_4 group was significantly increased ($p < .05$), while the activity in YC+CCl_4 group was significantly decreased compared with that in CCl_4 group ($p < .05$) (Figure 3). The IgM ($p < .05$) and ACH50 ($p > .05$) content in CCl_4 group was decreased compared with the control group. But compared with CCl_4 group, the plasma ACH50 in YC+CCl_4 group was significantly increased and IgM content also increased with no significant difference. There were no significant differences in AKP activity among three groups ($p > .05$).

3.6 | Gene expression

After treated with CCl_4, the expression levels of hepatic TLR2, MyD88 and NF-κBp65 gene in CCl_4 group were significantly increased compared with Control group ($p < .05$) (Figure 4). The

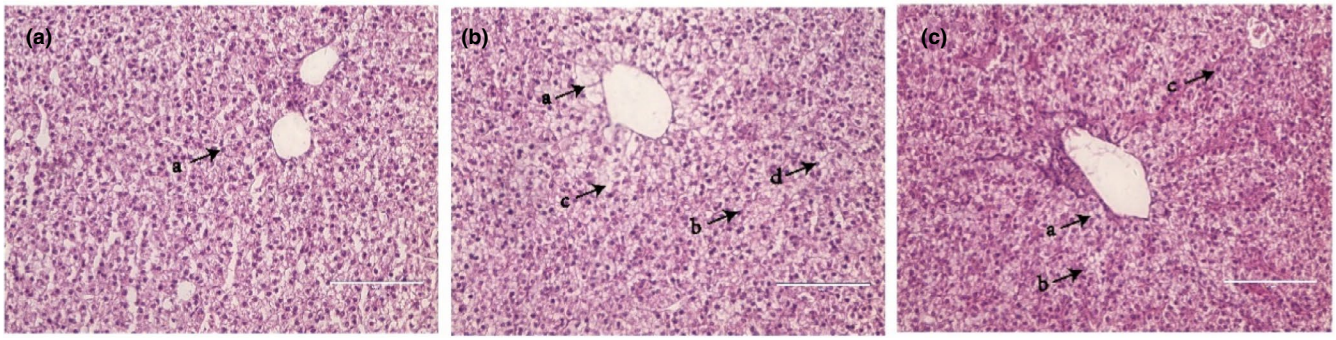

FIGURE 1 (A) Liver structure of fish in control group: (a) hepatocyte (H & E). (B) Liver structure of fish in CCl_4 group: (a) cell membrane lysis, (b) karyolysis, (c) hydropic degenerations, (d) nuclei shifting to the cellular periphery. (C) Liver structure of fish in YC+CCl_4 group: (a) hydropic degenerations, (b) nuclei shifting to the cellular periphery, (c) karyolysis. Scale bar = 100 μm

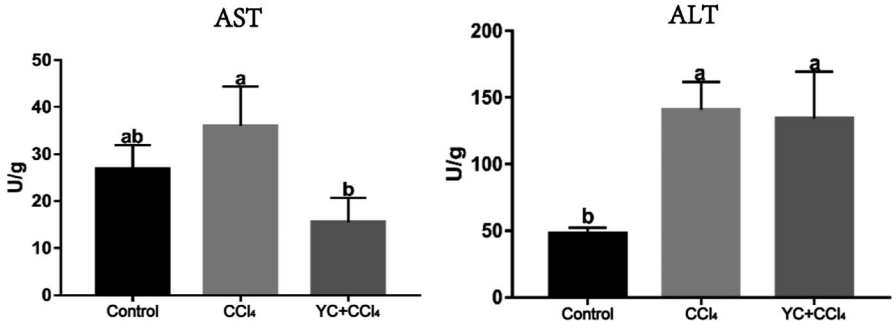

FIGURE 2 The effect of dietary YC on ALT and AST activities in plasma of CCl_4-treated *Pseudobagrus ussuriensis*. Vertical bars represented the means ± SEM of three replicates. Different letters in each figure represented significant difference among dietary treatments ($p < .05$). ALT, alanine transaminase; AST, aspartate transaminase

TABLE 4 The effect of YC on antioxidant capacity in liver of CCl_4-treated *Pseudobagrus ussuriensis*

Index	Control	CCl_4	YC+CCl_4
SOD (U mg^{-1})	23.39 ± 0.69[a]	20.46 ± 0.86[b]	23.32 ± 0.80[a]
MDA (nmol mg^{-1})	0.24 ± 0.02[b]	0.37 ± 0.02[a]	0.32 ± 0.01[a]
GPX (U mg^{-1})	20.56 ± 0.90	17.62 ± 0.61	19.46 ± 2.43

Note: Data represent as mean ± SEM. Mean with different superscripts in the same row is significantly different ($p < .05$).

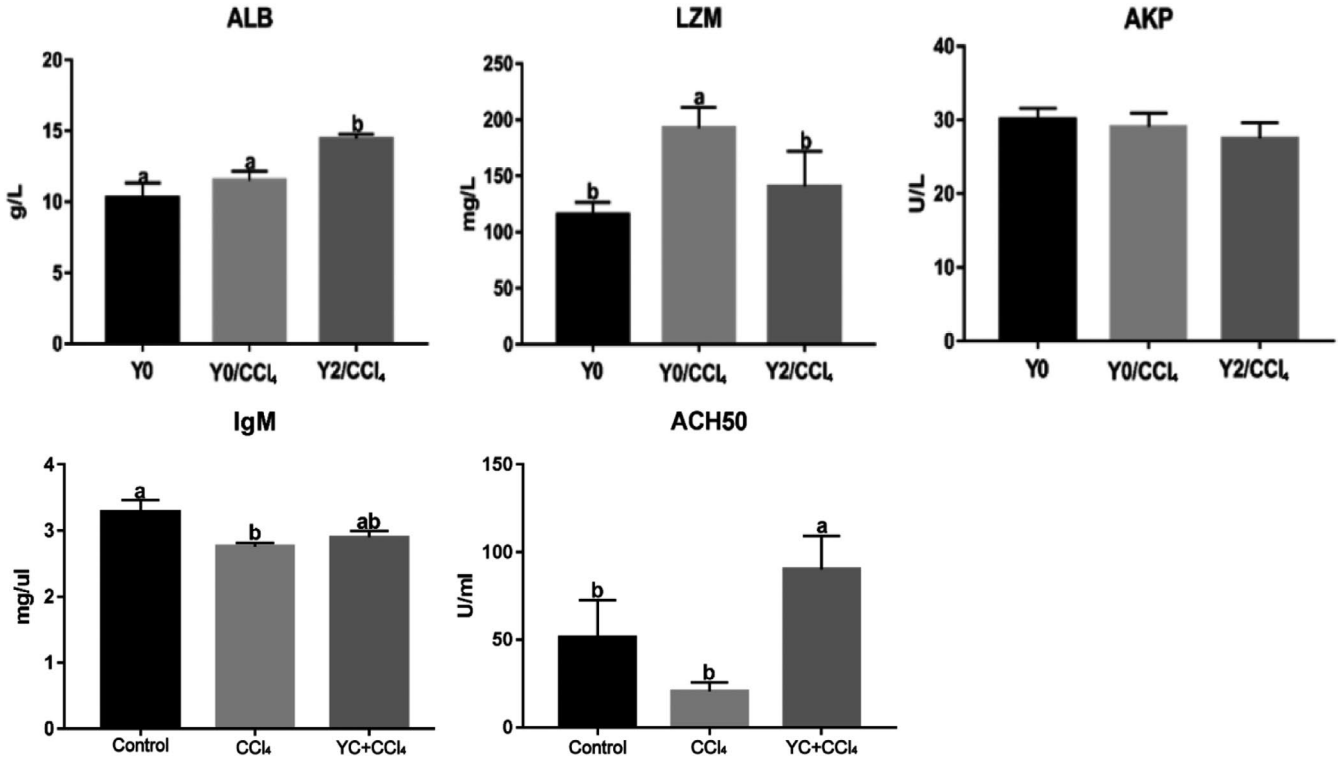

FIGURE 3 The effect of YC on immune ability of hepatic injury induced by CCl_4 in *Pseudobagrus ussuriensis*. Vertical bars represented the means ± SEM of three replicates. Different letters in each figure represented significant difference among dietary treatments ($p < .05$). ACH50, alternative complement pathway activity; ALB, albumin; AKP, alkaline phosphatase; IgM, immunoglobulin M; LZM, lysozyme

decrease of TLR2, MyD88 and NF-κBp65 was found in YC+CCl_4 group compared with CCl_4 group, but hepatic NF-κBp65 gene expression level in YC+CCl_4 group was significantly reduced ($p < .05$). The expression levels of hepatic IL-8 and Hsp70 gene in CCl_4 group was significantly increased compared with the Control group ($p < .05$). But compared with CCl_4 group, the hepatic IL-8 and Hsp70 gene in YC+CCl_4 group were significantly decreased ($p < .05$). The hepatic 1L-1β gene expression level was not significantly different among three groups ($p > .05$).

4 | DISCUSSION

The aim of this study was to investigate the hypothesis that yeast culture as an additive may have a protective effect against CCl_4-induced liver damage in *Pseudobagrus ussuriensis*. CCl_4 is one of the most commonly used hepatotoxins in the study of acute liver injury. Metabolism of CCl_4 produces the trichloromethyl free radical ($CCl3•$) and trichloromethyl peroxy radical ($CCl3OO•$) (Dong et al., 2013). And $CCl3OO•$ attacks and destroys polyunsaturated fatty acids, which initiates the chain reaction of lipid peroxidation resulting in the exudation of soluble cytoplasmic enzymes and increased serum AST and ALT (Al-Harbi et al., 2014). Serum enzymes such as AST and ALT are employed in the evaluation of hepatic disorders, and the increase of these enzyme activities in serum reflects acute liver damage and inflammatory hepatocellular disorder. The present results showed that CCl_4 administration caused severe acute liver damage in *Pseudobagrus ussuriensis* as evidenced by elevation of serum ALT ($p < .05$) and AST ($p > .05$) activities and classical histopathological changes, which is similar with previous reports that CCl_4 solution (3 ml/kg b.w., 1:1 in groundnut oil) can increase the activities of serum ALT and serum AST and change the hepatic histopathology in rat (Singh et al., 2008). However, when YC was supplemented in groups exposed to CCl_4, it was seen that serum AST activities dropped to a level similar to that of control in this study. Similar results were also reported by Bu et al. (2020), in which Ussuri

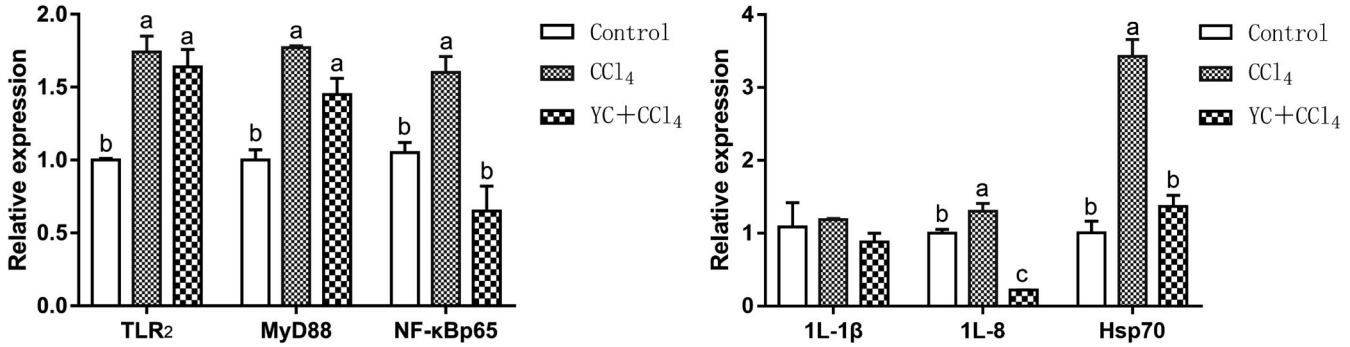

FIGURE 4 The effect of YC on hepatic inflammation related genes expression in CCl_4-treated *Pseudobagrus ussuriensis*. Vertical bars represented the means ± SEM of three replicates. Different letters in each figure represented significant difference among dietary treatments ($p < .05$). IL-1β, interleukin-1β; IL-8, interleukin-8; MyD88, myeloid differentiation factor 88; NF-κB p65, nuclear transcription factor κB p65; TLR2, toll-like receptor 2

catfish (*Pseudobagrus ussuriensis*) prefed diet with 10 g/kg yeast culture significantly decreased serum AST activities and alleviate the gossypol-induced liver damage.

The first line of defence against free radical consists of various endogenous antioxidants such as SOD, GPX, which maintains a complex immune response in animals. Their activities could reflect the antioxidant capacity for removing superoxide radicals and assisting the recovery of normal physiological functions of the cells (Yousefi et al., 2020). CCl_4 metabolite $CCl_3O_2\bullet$ could suppress SOD, GPX activities, resulting in the accumulation of large amounts of radicals and the lipid peroxidation product MDA, which lead liver cell necrosis, further aggravating liver damage. Previous study reports that the fish (*Cyprinus carpio var.* Jian) treated with CCl_4 can significantly increase hepatic MDA level and reduce SOD activity (Cao et al., 2015). In this study, CCl_4 had a decreasing effect on the activities of hepatic SOD and GPX; nevertheless, incorporation of yeast culture into diets restored the activities of antioxidant enzymes and resulted in a decline in the MDA content. Some studies have indicated that oligosaccharides, glutathione, vitamins and β-glucan could increase the antioxidant activity and regulate immune response (Liu et al., 2020; Lu et al., 2019; Takavar & Mandieh, 2019). Thus, it can be presumed that the protective effect of YC against CCl_4-induced liver damage in *Pseudobagrus ussuriensi* may be related to the mannose oligosaccharides (MOS), vitamins, β-glucan, glutathione and other antioxidants in yeast culture. The antioxidative effect of those substances (oligosaccharides, vitamins, β-glucan and glutathione) has been reported in crabs (*Eriocheir sinensis*), gibel carp (*Carassius auratus gibelio* CAS) and yellow drum (*Nibea albiflora*), and these studies have demonstrated that the above-mentioned substances can improve antioxidant ability by increasing SOD and GPX activities and reducing MDA production (Liu et al., 2020; Lu et al., 2019; Wang et al., 2019; Zhang et al., 2018).

The innate immune system of fish is an essential defence mechanism providing the first line of defence to against pathogens for the host (Ke & Zhang, 2019). Recently, in order to maintain fish health and improve disease resistance, immunostimulants, probiotics and nucleotides have been used as dietary additives to improve the immunity and resist the diseases (Vallejos-Vidal et al., 2016; Zhao et al., 2020). In this paper, indicators of innate immunity including plasma IgM, ACH50, LZM, AKP and ALB were evaluated to explore the adverse influence of CCl_4 on immunity of *Pseudobagrus ussuriensi* and the effects of yeast culture against immune damage induced by CCl_4.

IgM level and ACH50 activity as good biomarkers for evaluating the immune status may be adversely affected by toxicants in aquatic animals (Sharifian et al., 2015; Wang et al., 2014). In the current study, a reduction in IgM and ACH50 was found in the group exposed to CCl_4 alone, which may be considered as a damage to immune system. These results provide further support for the fact that CCl_4 may lead to the disorder of immune system in *Pseudobagrus ussuriensi*. Furthermore, fish in YC+CCl_4 group had higher IgM levels ($p > .05$) and ACH50 activities ($p < .05$) than fish in CCl_4 group, suggesting that fish prefed yeast culture could relieve the immune damage response induced by CCl_4 for *Pseudobagrus ussuriensi*. These results probably were demonstrated by the positive effect from the dietary addition of β-glucan as the main component of yeast culture, which could stimulate the complement activities. Some studies showed that the fish (*Oreochromis niloticus*) fed β-glucan had significantly higher ACH50 and IgM contents than the fish in control group (Amphan et al., 2018; El-Murr et al., 2019).

Albumin, the most abundant plasma protein, is primarily synthesized in liver. In this paper, fish prefed 20 g/kg yeast culture had a higher ALB level than fish in Control and CCl_4 groups. Similar results were also reported by Hardy et al. (2020), in which juvenile Nile tilapia (*Oreochromis niloticus*) fed diet with 10 or 30 g/kg yeast hydrolysate had markedly higher ALB than those of fish fed diets without yeast hydrolysate. Albumin also acts as a leading carrier for some cations, nutrients, hormones and metabolites and plays a role in tissue repairment. The body's physiological function would be affected by the concentration of ALB in plasma with the transportation of nutrients and hormones to the tissues and organs according to the requirement. Thus, we assume that the elevation of ALB was also responsible for the alleviation of liver damage, where it would accelerate the transportation of nutrients and hormones to meet the requirements of metabolic activities.

Moreover, lysozyme as a bactericidal enzyme is an important component of the immune system that can degrade pathogenic

bacteria by breaking down their structure of cell wall (Hauge et al., 2002; Lushchak et al., 2001). In this study, the fish in CCl_4 group had higher LZM activity than fish in Control group. Similar results were also reported by Demers and Bayne (1997), in which the plasma lysozyme activity was significantly increased briefly in Rainbow trout (*Oncorhynchus mykiss*) treated with handling stressor, and the reason might be attributed to that the acute stress may help humoral components of innate defences where it is most needed.

Several researches have indicated that TLRs interact with myeloid differentiation factor 88 (MyD88), an adaptor molecule shared by all the members of TLRs except for TLR3, 21, 22 to activate NF-κB and induce several types of cytokines, such as interleukin, tumour necrosis factor and chemokines (Chou et al., 2019; Montero et al., 2008; Seki et al., 2005). Previous study indicated that TLR2 and its downstream signalling are stimulated by CCl_4-induced oxidative stress, as determined by the remarkable up-regulation of TLR2, MyD88 and NF-κB mRNA expression in rat (Gan et al., 2018). In present study, we found that the expression of TLR2, MyD88 and NF-κBp65 mRNA was significantly enhanced in CCl_4 group compared with Control group, and YC supplementation reversed the CCl_4-induced up-regulations of TLR2, MyD88 and NF-κBp65. Those data indicated that dietary yeast culture supplementation may relieve the hepatic damage of Ussuri catfish through inhibiting TLR2/NF-κB signalling pathway. NF-κB a transcription factor could up-regulate the release of inflammatory cytokines including IL-8 and IL-1β, which may further perpetuate the inflammatory cascades resulting in liver damage (Jung et al., 1995). In this paper, fish in CCl_4 group had higher hepatic IL-1β ($p > .05$) and IL-8 ($p < .05$) mRNA expression than fish in Control group; however, hepatic IL-1β ($p > .05$) and IL-8 ($p < .05$) mRNA expressions of fish in YC+CCl_4 group were lower than fish in CCl_4 group, suggesting that CCl_4 may have a stimulatory action upon proinflammatory processes and dietary yeast culture supplementation, to some extent, inhibited the inflammatory response.

Heat shock proteins 70 (HSP70), a member of heat shock proteins (HSPs) family, are engaged in overall proteome maintenance while under conditions of environmental alterations (Song et al., 2007). At an early stage after CCl_4 administration, transient increases in the gene expression of the stress-inducible HSP70 are preceded (Schiaffonati & Tiberio, 1997). Furthermore, Song et al. (2007) reported that HSP70 knock out mice injected with CCl_4 showed higher alanine transaminase level and a more severe degree of neutrophilic infiltration and necrosis than those of wild-type mice injected with CCl_4. In this study, the significant increase was observed in case of hepatic HSP70 mRNA in CCl_4 group ($p < .05$), and hepatic HSP70 mRNA expression of fish in YC+CCl_4 group was significantly lower than fish in CCl_4 group ($p < .05$). These data suggested that yeast culture could have protective role against the hepatic damage induced by CCl_4.

5 | CONCLUSIONS

In conclusion, fish injected CCl_4 olive oil solution could induce inflammatory response, reduce antioxidant capacity and destroy hepatocyte structure. Dietary yeast culture supplementation could relieve the oxidative stress, immune damage and liver injury induced by CCl_4 and could also suppress CCl_4-induced inflammation through inhibiting the TLR2/NF-κB signalling pathway.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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