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Astaxanthin protects lipopolysaccharide-induced inflammatory response in *Channa argus* through inhibiting NF-κB and MAPKs signaling pathways

Mu-Yang Li[a], Li Sun[a,b], Xiao-Tian Niu[a], Xiu-Mei Chen[a], Jia-Xin Tian[a], Yi-Di Kong[a], Gui-Qin Wang[a,*]

[a] College of Animal Science and Technology, Jilin Agriculture University, 2888 Xincheng Road, Changchun, Jilin, China
[b] Changchun Testing Center of Quality and Safety in Aquatic Product, 777 CaiYu Road, Changchun, Jilin, China

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ABSTRACT

The present study was conducted to evaluate the protective effects of astaxanthin against lipopolysaccharide (LPS)-induced inflammatory responses in *Channa argus* in vivo and ex vivo. Primary hepatocytes were exposed to different concentrations of LPS for 24 h to induce an inflammatory response, and the protective effects of astaxanthin against LPS-induced inflammation were studied ex vivo and in vivo. Hepatocytes exposed to LPS (5–20 μg mL^{-1}) alone for 24 h resulted in a significant increase in lactate dehydrogenase release (LDH), Nitric oxide (NO) production and Malondialdehyde (MDA) content, 10 μg mL^{-1} LPS could induced inflammatory response in hepatocytes. Gene expression of TLR4, NFkBp65, MAPKp38, TNF-α, IL-6 and IL-1β mRNA expression were also enhanced ex vivo ($p < 0.05$). In vivo test demonstrated that pretreatment with astaxanthin prevented the LPS-induced upregulation of pro-inflammatory cytokines TNF-α, IL-6 and IL-1β. Besides, astaxanthin blocked the expression of Toll-like receptor 4 (TLR4) and then suppressed the phosphorylation of nuclear transcription factor-kappa B (NF-κB) p65 and degradation inhibitor of NF-κBα (IκBα). Further study showed that astaxanthin could suppress the phosphorylation of p38, extracellular signal-regulated kinase (ERK) and c-jun NH2-terminal kinase (JNK) in mitogen-activated protein kinase (MAPK) signal pathway. In conclusion, our results suggest that astaxanthin played an anti-inflammatory role by regulating TLR4 and the NF-κB and MAPK signaling pathways in *C. argus*.

1. Introduction

Lipopolysaccharide (LPS), a component of the cell envelope of gram-negative bacteria, is often associated with disease processes, it is also one of the most potent initiators of inflammation. As we know, pro-inflammatory response is initiated as a defense mechanism following stimulation from environmental stimuli, including pathogens, damage, or other foreign products [1]. However an excessive or inappropriate production of inflammatory mediators can result in numerous chronic diseases [2].

LPS is recognized by Toll-like receptor (TLR) 4 on the surface of the host cell and initiates an inflammatory signaling cascade. TLR4-activated macrophages signal via inflammatory pathways to up-regulate the expression and release of various inflammatory factors. Activation of TLR4 by LPS eventually results in the activation of NF-kB, which is believed to play a central role in the regulation of genes encoding for the inflammatory cytokines, adhesion molecules, chemokines, and other growth factors involved in mammary inflammation [3]. This occurs primarily via the translocation of nuclear factors, such as nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and signal transducer and activator of transcription 3 (STAT3) [4]. While inflammation is usually required as a natural defense mechanism, excessive and prolonged immune activation can result in the destruction of healthy tissues. Therefore, for many inflammatory-related diseases, pharmaceutical intervention is required to restrain the excessive release of such inflammatory mediators.

NF-κB and mitogen-activated protein kinases (MAPKs) signaling pathways have long been considered the classical pathways to modulate the inflammatory response and process. Activation of these pathways leads to the release of inflammatory factors, regulates oxidative stress response and accelerates the inflammation progress [5]. Once activated, nuclear factor κB translocates into the nucleus to regulate the target genes [6]. Moreover, MAPK pathway plays an essential role in regulating cellular processes, especially inflammation, to involve the production of inflammatory mediators [7].

Astaxanthin is a naturally occurring carotenoid pigment that

* Corresponding author.
E-mail address: wangguiqing131@163.com (G.-Q. Wang).

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belongs to a phytochemical class known as terpenes and has significant antioxidant capacity [8,9]. It is found in many kinds of marine organisms, such as microalgae, yeast, salmon, trout, krill, and shrimp [10]. Astaxanthin has been reported to possess a wide variety of biological functions, including antioxidant and anti-tumor activities [11,12]. Recently astaxanthin are used gainfully in aquaculture as feed additive and dietary supplement [13–15]. As a dietary supplement, it has been reported as having anti-aging, anti-inflammatory, and sun proofing properties, positively influence growth, reproductive performance, and boost immune system in fish and shellfish [16–18]. However, the protective effects of astaxanthin against LPS-induced inflammatory responses is little known, the present study was conducted to evaluate the anti-inflammatory effect of astaxanthin in *Channa argus*, the mechanisms and signaling pathways underlying this property was also determined.

2. Materials and methods

2.1. Reagent

Astaxanthin, ≥98% (HPLC) purchased from Shanghai aladdin Bio-Technology, LPS were prepared in our lab and it was isolated from *Aeromonas veronii*-JS-1 in *Parabramis pekinensis* The following antibodies were used in this study: Rabbit anti-phospho-p65, rabbit anti-phospho-ERK, rabbit anti-phospho-p38, rabbit anti-p65, rabbit anti-ERK, rabbit anti-p38 and TLR4 were purchased from Hangzhou HuaAn Bio-Technology, Zhejiang, China. LDH, NO and MDA commercial kits were purchased from Nanjing Jiancheng Bioengineering Institute, China.

2.2. Ex vivo study

2.2.1. Culture of primary C. argus hepatocyte

Primary cultured hepatocyte was prepared as previously described by Giri et al. [19]. Cells were cultured in DMEM containing 10% fetal bovine serum (FBS), 100 IU streptomycin sulfate mL^{-1} and 100 IU penicillin mL^{-1} at a concentration of 1×10^6 cells mL^{-1}. Cultures were maintained in an incubator at 26 ± 0.5 °C in 5% CO_2. Cells were allowed to attach to 24-well plates for 72 h.

2.2.2. LPS induced inflammatory response in C. argus hepatocyte

To determine a suitable concentration of LPS, primary culture hepatocytes were treated with a serious of concentrations of LPS (5–20 μg mL^{-1}). The cells were incubated for 24 h in fresh medium containing 0, 5, 10, 15 and 20 μg mL^{-1} LPS. The levels of lactate dehydrogenase (LDH) and Nitric oxide (NO) production and Malondialdehyde (MDA) content were measured by commercial kits according to the manufacturer's instructions. All the test were replicated three times.

2.2.3. Ex vivo effect of astaxanthin against LPS-induced inflammatory in C. argus hepatocyte

C. argus hepatocyte were seeded in 6-well plates and divided into 5 groups: Group I (control group) treated with no astaxanthin, Group II treated with no astaxanthin but 10 μg mL^{-1} LPS, Group III, Group IV and Group V pretreated with 50, 100 and 200 μg mL^{-1}astaxanthin for 72 h prior to treatment with 10 μg mL^{-1} LPS in a 27 °C incubator, correspondingly. After 72 h incubation, each group were exposed to 10 mg L^{-1} LPS (based on 2.1.2, this concentration could induce inflammatory response) for 24 h, respectively.

Cell lysates were collected to detect TLR4, NFkBp65, MAPKp38, TNF-α, IL-6 and IL-1β mRNA expression. Total RNA was extracted by TRIzol Reagent (simgen). cDNA was then synthesized using the Reverse Transcriptase M-MLV Kit (TaKaRa) following the instructions. The real-time quantitative PCR was performed using THUNDERBIRD SYBR qPCR Mix Kit (TOYOBO), and carried out in astratagene MxProSystem (stratagene mx3005p, USA) in 96-well reaction plates. The β-actin gene was used as a house keeping gene. The reaction mixture included 10 μl of THUNDERBIRD SYBR qPCR Mix, 1 μl of forward and reverse primer (10 mM) and 1 μl of cDNA, and was then filled up with ultra-pure water to a final total volume of 20 mL. All PCRs were performed at least three times. Additional dissociation-curve analysis was performed and showed a single melting curve in all cases. Data were analyzed by the stratagene MxPro software (stratagene mx3005p, USA).

2.3. In vivo

2.3.1. Feed and experimental design

The experimental design was completely randomized with five treatment diets, each of which was replicated three times. For each treatment replicate, 30 *C. argus* (58.8 ± 6.5 g) were randomly chosen and placed in 300 L cycling-filtered plastic tanks containing continuously circulating aerated water. The five treatment diets were as follows: Group I (control group) were fed with control (basic) diet throughout the feeding trial; Group II, III, IV and V were fed 0, 50, 100 and 200 mg astaxanthin kg^{-1} body weight, respectively. Fish were fed twice a day (9:00 and 15:00) at a rate of 3% of the bodyweight and kept in glass aquaria at 23 ± 1 °C with laboratory conditions as mentioned above for 8 weeks. Based on the increases in body weight, the total amount of the diet was adjusted every ten days, and the amount consumed every time was recorded by calculating the differences in the weight of the diet before and after feeding. Tank bottom debris was removed by siphon daily and about one third of the water was replaced daily. All the experimental animals used in the study were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee of Jilin Agricultural College.

2.3.2. Cytokine assay

After 8 weeks administration, 10 fish were collected in each tank of every treatment for cytokine assays. Tissue samples (liver) were collected from the individual fish after the blood samples. The gene expression of TNF-α, IL-6 and IL-1β mRNA were determined according to the method mentioned in 2.1.3.

2.3.3. Western blotting

After 8 weeks administration, liver from ten fish of each groups were collected and the levels of TLR4, NF-kBp65 and IκBα, p38, ERK, and JNK were determined by immuno-blotting Opal et al. [20]. Protein concentrations were measured by BCA assay and were then separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto a polyvinylidene difluoride (PVDF) membrane. The membrane was incubated with a specific antibody at 4 °C overnight and was blocked in 5% skim milk for 2 h on a rotary shaker. Subsequently, the membrane was washed with TBST followed by incubation with the anti-rabbit secondary antibodies at room temperature for 1 h. The membrane was again washed with TBST and developed with the ECL Plus Western blotting Detection System.

2.4. Data analysis

The data in this study were analyzed by Statistical Product and Service Solutions (SPSS 16.0) and expressed as the arithmetic mean ± standard deviation. The homogeneity of the replicates of the samples was checked by the Mann–Whitney *U* test. Differences between the three groups were measured and considered statistically different at $P < 0.05$ or $P < 0.01$.

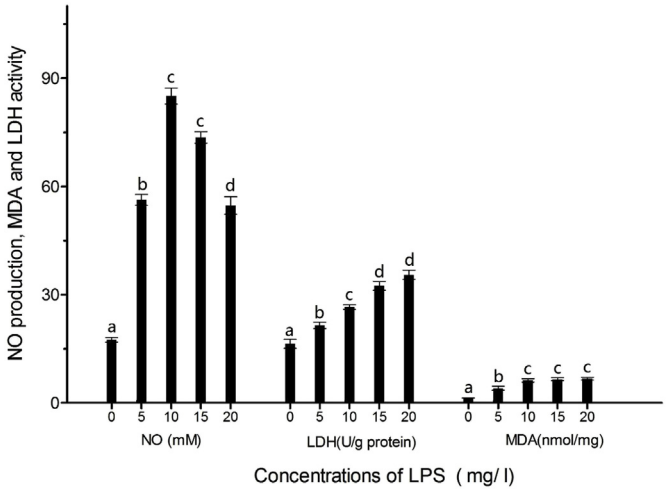

Fig. 1. Effect of different concentrations of LPS on NO production, MDA and LDH activity in *Channa argus* hepatocytes. Note: Data are expressed as mean ± SD with different letters as significantly difference ($P < 0.05$).

3. Results

3.1. Ex vivo study

3.1.1. LPS-induced inflammatory response in C. argus hepatocyte

The results of LPS-induced inflammatory response in *C. argus* hepatocyte were listed in Fig. 1. As showed in Fig. 1, LPS markedly increased the LDH activity in a dose-dependent manner which indicated the injury was induced by LPS. The NO production in LPS treated group were higher than that of control group, it reached the highest when the LPS concentration was 10 μg mL^{-1}. As for MDA, all the LPS-treated group resulted in a statistically significant increase as compared to the control group ($P < 0.05$).

3.1.2. Protective effect of astaxanthin against LPS-induced inflammatory response in C. argus hepatocyte ex vivo

The transcriptional changes of TLR4, NFkBp65, MAPKp38, TNF-α, IL-6 and IL-1β genes in hepatocyte were shown in Fig. 2. All the test genes transcription in LPS treated only group were markedly increased as compared with control group. Compared with Group II (LPS treated only), transcriptional level of TLR4, NFkBp65, MAPKp38, TNF-α, IL-6 and IL-1β in astaxanthin treated group were decreased. As for TLR4, NFkBp65 and MAPKp38, the mRNA expression levels were down-regulated after treated with astaxanthin, however, only group V and group III showed the statistical difference ($P < 0.05$). As for IL-1β, slight decrease in TNF-αcontent were detected, but only Group V showed the statistical difference ($P < 0.05$). With regard to the expression of IL-1β and IL-6, all the astaxanthin-treated group led to a statistically significant decrease as compared to the LPS-treated group ($P < 0.05$).

3.2. In vivo study

3.2.1. Effects of astaxanthin on cytokine production

The effects of astaxanthin on TNF-α, IL-1β and IL-6 mRNA expression in the liver of fish following LPS exposure are presented in Fig. 3. As showed in Fig. 3. Expression of TNF-α, IL-1β and IL-6 were significantly increased in group II (LPS treated but with no astaxanthin pre-treated) as compared with control group. However, pre-treatment with astaxanthin prior to LPS administration led to a significant lower of TNF-α, IL-1β and IL-6 mRNA expression ($P < 0.05$).

3.2.2. Astaxanthin inhibits the activation of NF-KB and MAPKs signal pathways

The levels of TLR4 were shown in Fig. 4. The results showed that infusion of LPS marked increased the expression of TLR4. Astaxanthin (50, 100 and 200 mg kg^{-1}) significantly inhibited the activation of the expression of TLR4 compared with the LPS-treated group.

The results of immuno-blotting assays were presented in Figs. 4–5. As shown in Fig. 4, the levels of NF-KBp65 and IκBα in the NF-KB signal pathway, and levels of p38, ERK, JNK in the MAPKs signal pathway in group II (LPS treated but with no astaxanthin pre-treated) were significantly increases as compared with the control group. However, administration of astaxanthin inhibited the up-regulation of phosphorylation of NF-kBp65 and IκBα, p38, ERK, and JNK as compared with the group II (Fig. 5).

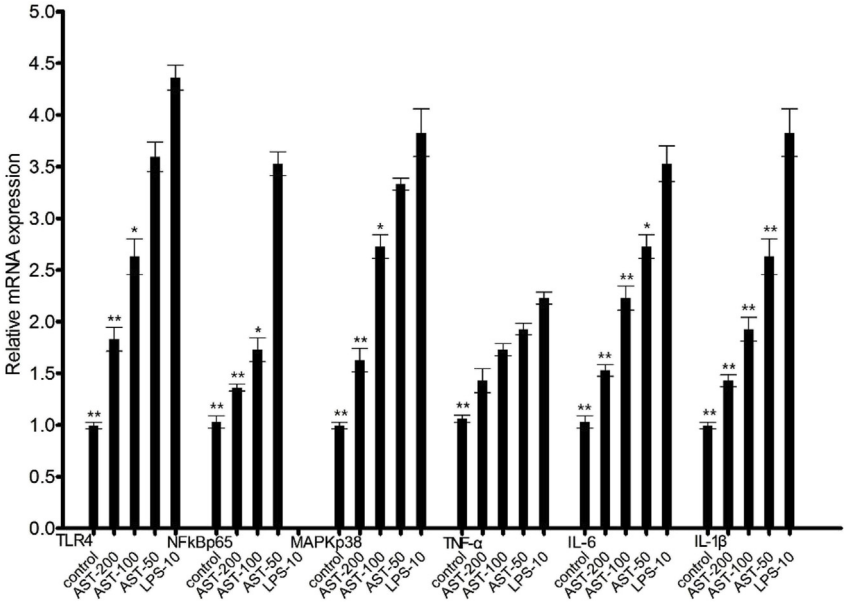

Fig. 2. Effect of various concentrations of astaxanthin on TLR4, NFkBp65, MAPKp38, TNF-α, IL-6 and IL-1β mRNA expression in *Channa argus* hepatocytes after treated with LPS. The values presented are mean ± SEM. (n = 10 in each group). P* < 0.05, P** < 0.01vs. LPS group (only).

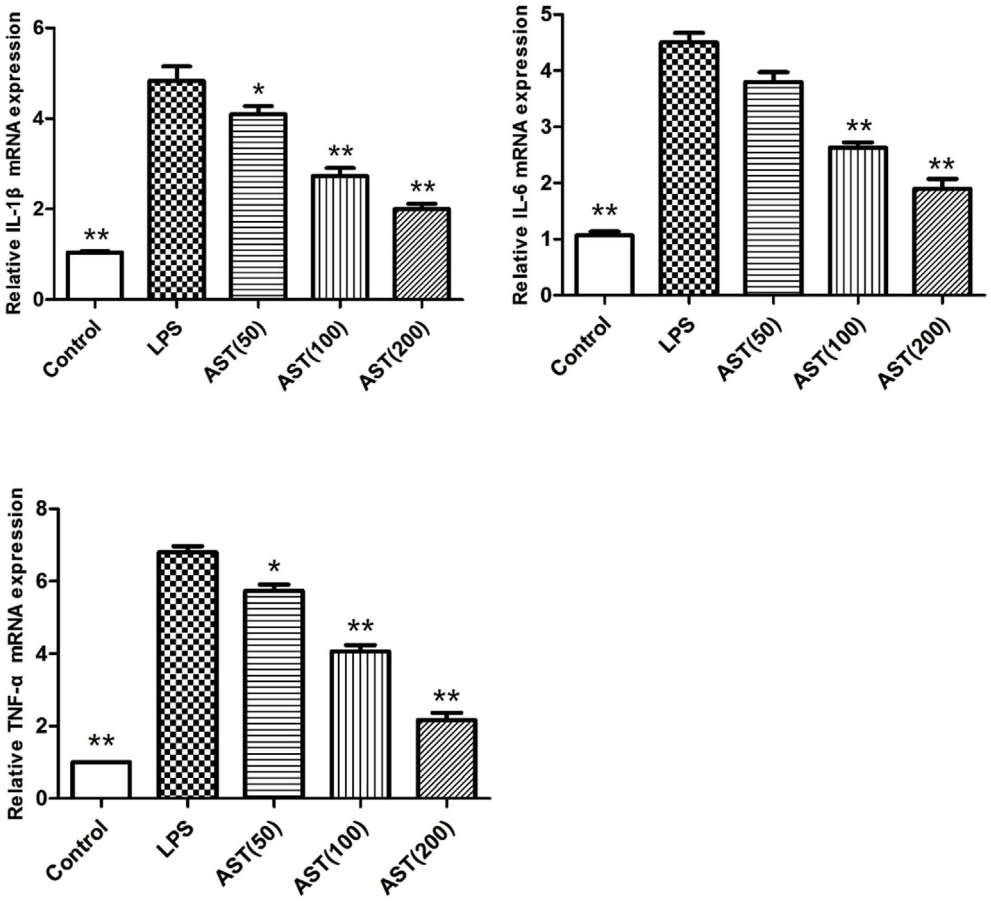

Fig. 3. Effect of different concentrations of AST on TNF-a, IL-1b, and IL-6 mRNA expression in LPS induced *Channa argus*. The values presented are mean ± SEM. (n = 10 in each group). P* < 0.05, P** < 0.01vs. LPS group (only).

4. Discussion

Inflammation play an important role in host defense reaction in response to the invasion of pathogens, tissue damage, or other environmental assaults. The body must achieve a balance of injury and restoration during an inflammatory response to resolve a threat while incurring a minimal level of damage. If the balance is broken as a result of an excessive or unregulated pro-inflammatory response, chronic tissue damage ensues [2]. Recently, the molecular mechanisms of the initiation and functionality of the cellular processes involved in a pro-inflammatory response have shed light on potential targets for novel anti-inflammatory therapeutics [21]. In the present study, we evaluated lipopolysaccharide (LPS)-induced inflammatory responses in *Channa argus,* and found that 10 μg mL^{-1} LPS could induced inflammatory response in hepatocytes. Our result is similar with Solem et al. (1995) [22] whose results showed that 10 μg mL^{-1} LPS result in respiratory burst and phagocytic activity in Atlantic salmon. However, Luo et al. (2019) [23] found that when the concentration of LPS reached 100 μg mL^{-1} LPS inflammatory responses could be induced in *Epinephelus coioides*, this may because LPS of different bacterial species and serotypes, even of the same origin but from different production batches have widely differing potency to induce inflammation and mortality in zebrafish [24].

Nitric oxide is considered as an indicator for inflammation and it plays a key role in various forms of inflammation and carcinogenesis [25]. Excessive NO production can lead to cytotoxicity, inflammation, carcinogenicity, and autoimmune disorders [25]. Therefore, NO production may provide a measure to assess the effects of chemopreventive agents on the inflammatory process. So, in the present study, we use NO production as an indicator to determine the dose of LPS to induce inflammatory response, and the ex vivo results showed that, 10 μg mL^{-1} LPS was the optimum concentration. LDH levels is another a critical marker of cell toxicity [26,27]. Inflammatory cytokines trigger the generation of ROS and antioxidant defense [28]. MDA is an important oxidant enzyme and index of tissue damage [29]. The present results demonstrated that exposure to LPS (10 μg mL^{-1}) alone significantly increased LDH levels and MDA content in the medium, indicating severe enterocyte damage. Therefore, to test the protective effects of astaxanthin, 10 μg $mL^{-1}$of LPS was used for inducing inflammation in *C. argus* hepatocytes (ex vivo).

Persistent pro-inflammatory signals can lead to tissue damage and even various immune-related diseases. Thus, there are a significant number of regulatory factors that counteract the release of pro-inflammatory cytokines and restrict the inflammatory response [30]. TNF-α, IL-1β and IL-6 are the most important cytokines. They are the signal of various kinds of interaction between cells, play important roles in the process of host defense and infection and inflammation pathological development [31]. TNF-α generated by macrophages or mammary epithelial cells can trigger the inflammatory cascade and induce other cells to produce other cellular factors [32]. TNF-α can elicit infiltration and activation of neutrophils, impair vascular endothelial cells, enhance cellular adhesion molecules. IL-1β is a pro-inflammatory cytokine and has been confirmed to have the ability to induce the production of several secondary cytokines. IL-6 is a proinflammatory cytokine and is typically one of the first to be produced following immune activation. Moreover, it is involved in the formation of C-reactive protein in the plasma of the patients with acute or chronic inflammation [33]. Additionally, IL-6 acts as a proinflammatory cytokine by activating the JAK2/STAT3 signaling pathway. In the current study, the concentrations of TNF-α, IL-1β and IL-6 both in LPS-induced *C. argus*

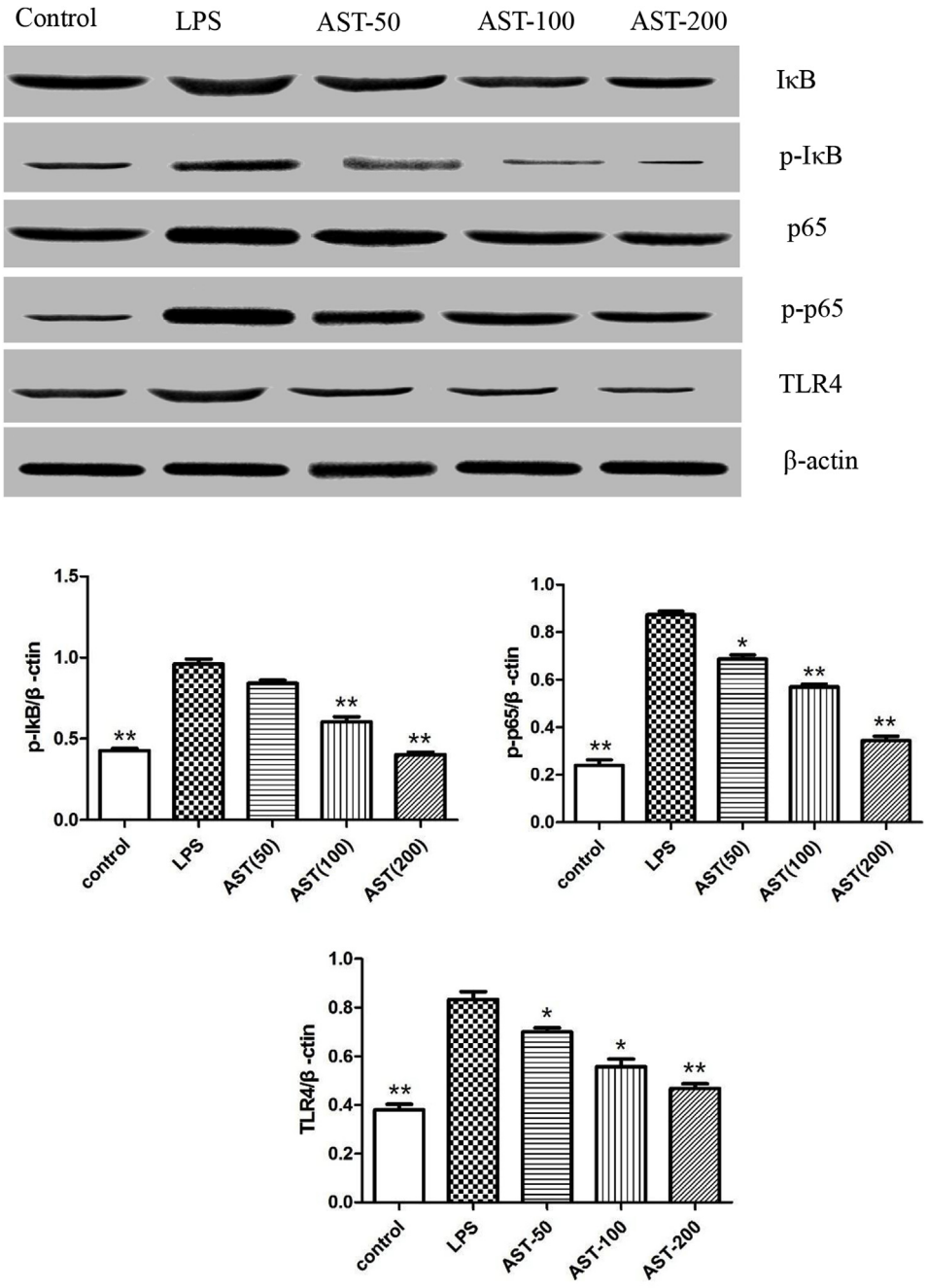

Fig. 4. Effect of AST on the TLR4 and NF-κB pathway in LPS induced *Channa argus*. Western blot analysis was employed to the expression of P-65 and IκB. β-actin was used as a control. The values presented are mean ± SEM. (n = 10 in each group). P* < 0.05, P** < 0.01vs. LPS group (only).

and LPS-stimulated primary hepatocyte were attenuated after pre-treatment with astaxanthin as compared with the control group. These results indicated that astaxanthin reduced inflammatory responses may be attributed to the inhibition of inflammatory cytokines TNF-α, IL-1β and IL-6.

TLR4, one of the best characterized TLRs located on the cell plasma membrane, is a pattern recognition receptor for LPS from Gram-negative bacteria [34]. In mammals, the lipid A portion of the LPS acts as a toxin by overstimulating the TLR-4 innate immune signaling, which induces pathogenic inflammatory responses [35]. Activation of TLR4 by LPS eventually results in the activation of NF-kB, which is believed to play a central role in the regulation of genes encoding for the inflammatory cytokines, adhesion molecules, chemokines, and other growth factors involved in mammary inflammation [36]. Thus, the inhibition of TLR4/NF-κB signaling pathways may have a therapeutic effect on inflammation. The results of the present study showed that astaxanthin inhibited the expression of TLR4 in LPS stimulated inflammatory responses.

The key signaling pathway mediating the inflammatory response, from MAPKs to NF-kB activation has been well established in various inflammatory diseases and cancers [37]. NF-κB plays an important role in regulating inflammatory and immune responses to extracellular stimulus [38]. It is known as an important target for anti-inflammatory molecules. NF-κB is normally sequestered in the cytoplasm by a family of inhibitory proteins known as inhibitors of NF-κB (IκBs). Therefore, this signaling pathway is a noble target for anti-inflammatory drug development. To further explore the potential molecular mechanism of astaxanthin suppressing the production of proinflammatory cytokines,

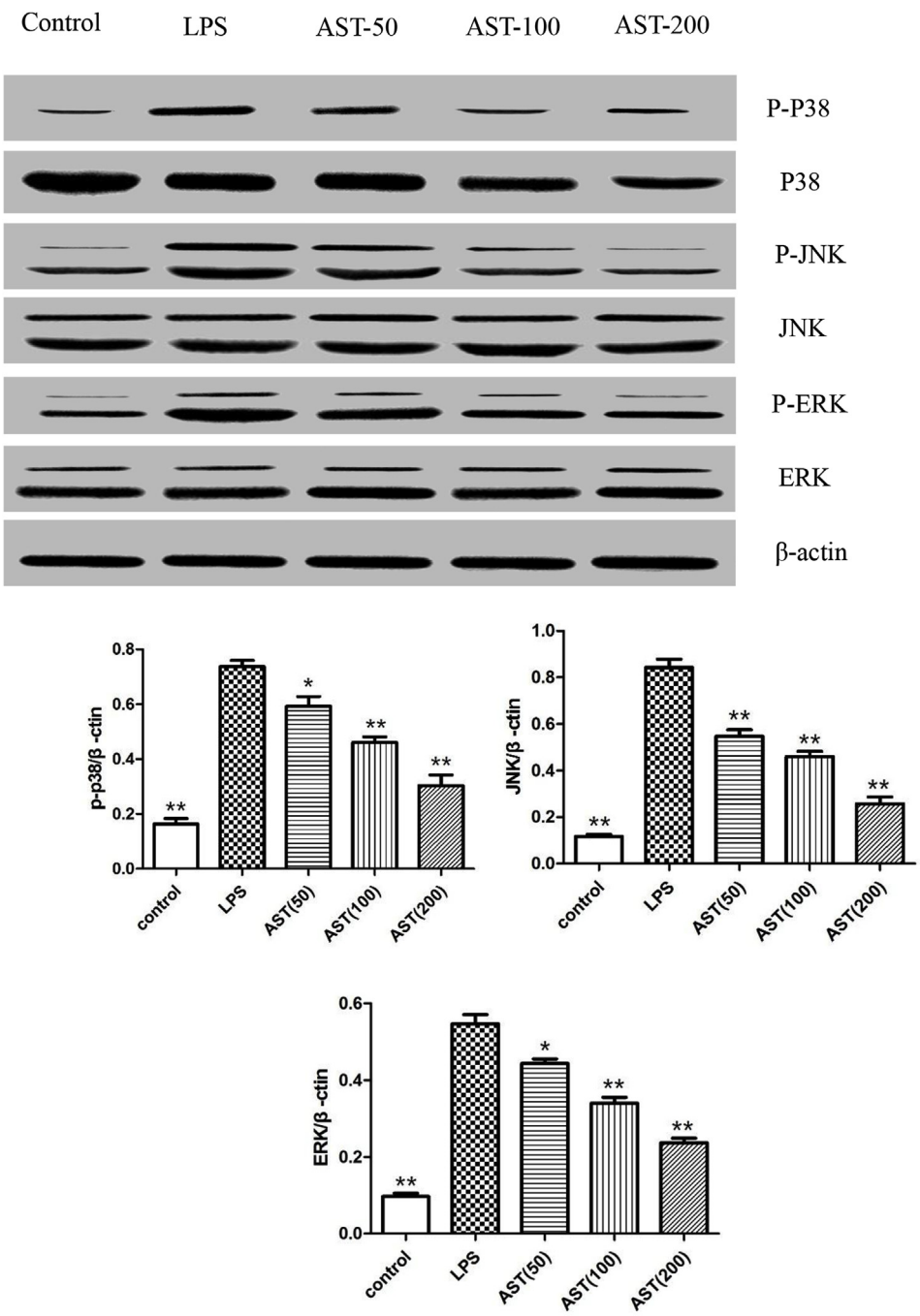

Fig. 5. Effect of AST on the MAPK pathway in LPS induced *Channa argus*. Western blot analysis was employed to the expression of JNK, P-38 and ERK. β-actin was used as a control. The values presented are mean ± SEM. (n = 10 in each group). P* < 0.05, P** < 0.01vs. LPS group (only).

we examined the activation of NF-κB p65 subunit and IκBα in the NF-κB signaling pathway. Our data showed that the phosphorylation of IκBα and NF-κB protein were increased by LPS, but astaxanthin inhibited NF-κB activation and IκBα degradation. Therefore, the results indicated that anti-inflammatory effect of astaxanthin correlated with the inhibition of NF-κB activation.

In addition, MAPKs have been implicated in LPS-induced inflammation pathways, it also play an important role during inflammatory responses [39]. To further characterize the inhibitory mechanism of astaxanthin on cytokine production, we also tested its' effect on the activation of MAPK. Our results demonstrated that phosphorylation of p38, ERK and JNK were accentuated after LPS induction. However, phosphorylation of p38, ERK and JNK in astaxanthin treated groups were significantly inhibited ($p < 0.05$). From the above, our results suggested that astaxanthin suppressed pro-inflammatory cytokine production by inhibiting the activation of NF-κB and MAPKs signaling pathway.

In conclusion, our results demonstrated that pretreatment with astaxanthin develops a potent protective effect on LPS-induced inflammatory response which may be associated with its inhibition of NF-κB and MAPKs signaling pathways activation.

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References

[1] M.E. Kotas, R. Medzhitov, Homeostasis, inflammation, and disease susceptibility, Cell 160 (2015) 5816–5827.

[2] Y.A. Ivanenkov, K.V. Balakin, Y. Lavrovsky, Small molecule inhibitors of NF-κB and JAK/STAT signal transduction pathways as promising anti-inflammatory therapeutics, Mini Rev. Med. Chem. 11 (2011) 55–78.

[3] K. Asehnoune, D. Strassheim, S. Mitra, J.Y. Kim, E. Abraham, Involvement of PKCalpha/beta in TLR4 and TLR2 dependent activation of NF-kappaB, Cell. Signal. 17 (2005) 385–394.

[4] T. Hanada, A. Yoshimura, Regulation of cytokine signaling and inflammation, Cytokine Growth Factor Rev. 13 (2002) 413–421.

[5] S. Rivas-Arancibia, L.F. Zimbrón, E. Rodríguez-Martínez, P.D. Maldonado, G. Borgonio Pérez, M. Sepúlveda-Parada, Oxidative stress-dependent changes in immune responses and cell death in the substantia nigra after ozone exposure in rat, Front. Aging Neurosci. 7 (2015) 65.

[6] A. Mediero, M. Perez-Aso, B.N. Cronstein, Activation of EPAC1/2 is essential for osteoclast formation by modulating NFκB nuclear translocation and actin cytoskeleton rearrangements, Faseb. J. 28 (2014) 4901–4913.

[7] Y. Yang, S.C. Kim, T. Yu, Y.S. Yi, M.H. Rhee, G.H. Sung, B.C. Yoo, J.Y. Cho, Functional roles of p38 mitogen-activated protein kinase in macrophage-mediated inflammatory responses, Mediat. Inflamm. (2014) 352–371.

[8] W. Miki, K. Yamaguchi, S. Konosu, Comparison of carotenoids in the ovaries of marine fish and shellfish, Comp. Biochem. Physiol. B 71 (1982) 7–11.

[9] Y.M. Naguib, Antioxidant activities of astaxanthin and related carotenoids, J. Agric. Food Chem. 48 (2000) 1150–1154.

[10] T. Tanaka, H. Makita, M. Ohnishi, H. Mori, K. Satoh, A. Hara, Chemoprevention of rat oral carcinogenesis y naturally occurring xanthophylls, astaxanthin and canthaxarathin, Cancer Res. 55 (1995) 4059–4064.

[11] F.J. Pashkow, D.G. Watumull, C.L. Campbell, Astaxanthin: A novel potential treatment for oxidative stress and inflammation in cardiovascular disease, Am. J. Cardiol. 101 (2008) 58–68.

[12] G. Hussein, H. Goto, S. Oda, U. Sankawa, K. Matsumoto, H. Watanabe, Antihypertensive potential and mechanism of action of astaxanthin III. Antioxidant and histopathological effects spontaneously hypertensive rats, Biol. Pharm. Bull. 29 (2006) 684–688.

[13] S.R. Garner, B.D. Neff, M.A. Bernards, Dietary carotenoid levels affect carotenoid and retinoid allocation in female Chinook salmon *Oncorhynchus tshawytscha*, J. Fish. Biol. 76 (2010) 1474–1490.

[14] J.I.P. Angeles, Y.H. Chien, M.M. Tayamen, Effects of different dosages of astaxanthin on giant freshwater prawn *Macrobrachium rosenbergii* (De Man) challenged with *Lactococcus garvieae*, Aquacult. Res. 41 (2009) 70–77.

[15] Y.H. Chien, W.C. Shiau, The effects of dietary supplementation of algae and synthetic astaxanthin on body astaxanthin, survival, growth, and low dissolved oxygen stress resistance of kuruma prawn, *Marsupenaeus japonicus* Bate, J. Exp. Mar. Biol. Ecol. 318 (2005) 201–211.

[16] I. Higuera-Ciapara, L. Felix-Valenzuela, F. Goycoolea, Astaxanthin: a review of its chemistry and applications, Crit. Rev. Food Sci. Nutr. 46 (2006) 185–196.

[17] R. Lorenz, G. Cysewski, Commercial potential for Haematococcus microalgae as a natural source of astaxanthin, Trends Biotechnol. 18 (2000) 160–167.

[18] M. Guerin, M. Huntley, M. Olaizola, Haematococcus astaxanthin: applications for human health and nutrition, Trends Biotechnol. 21 (2003) 210–216.

[19] S.S. Giri, S.S. Sen, J.W. Jun, V. Sukumaran, S.C. Park, Protective effects of leucine against lipopolysaccharide-induced inflammatory response in *Labeo rohita* fingerlings, Fish Shellfish Immunol. 52 (2016) 239–247.

[20] S.M. Opal, T. Gluck, Endotoxin as drug target, Crit. Care Med. 31 (Suppl) (2003) 57–64.

[21] H.Y. Chung, M. Cesari, S. Anton, E. Marzetti, S. Giovannini, A.Y. Seo, C. Carter, B.P. Yu, C. Leeuwenburgh, Molecular inflammation: underpinnings of aging and age-related diseases, Ageing Res. Rev. 8 (2009) 18–30.

[22] S.T. Solem, J.B. Jørgensen, B. Roberrtsen, Stimulation of respiratory burst and phagocytic activity in Atlantic salmon (*S. salar L*) macrophages by lipopolysaccharide, Fish Shellfish Immunol. 5 (1995) 475–491.

[23] S.W. Luo, H. Kang, R.C. Xie, W. Wei, Q.J. Liang, Y. Liu, W.N. Wang, N-terminal domain of EcC1INH in *Epinephelus coioides* can antagonize the LPS-stimulated inflammatory response, Fish Shellfish Immunol. 84 (2019) 8–19.

[24] H. Mottaz, R. Schonenberger, S. Fischer, R.L. Eggen, K. Schirmer, K.J. Groh, Dose-dependent effects of morphine on lipopolysaccharide (LPS)-induced inflammation, and involvement, Environ. Pollut. 221 (2017) 105–115.

[25] S.Y. Choi, J.H. Hwang, S.Y. Park, Y.J. Jin, H.C. Ko, S.W. Moon, S.J. Kim, Fermented guava leaf extract inhibits LPS induced COX-2 and i-NOS expression in mouse macrophage cells by inhibition of transcription factor NF-kB, Phytother. Res. 22 (2008) 1030–1034.

[26] K. Gerloff, C. Albrecht, A.W. Boots, I. Forster, R.P. Schins, Cytotoxicity and oxidative DNA damage by nanoparticles in human intestinal Caco-2 cells, Nanotoxicology 3 (2009) 355–364.

[27] J.Y. Koh, D.W. Choi, Quantitative determination of glutamate mediated cortical neuronal injury in cell culture by lactate dehydrogenase efflux assay, J. Neurosci. Methods 20 (1987) 83–90.

[28] I.A. Giebelen, D.J. Westerloo, G.J. LaRosa, D.A.F. Vos, T.V.D. Poll, Local stimulation of alpha 7 cholinergic receptors inhibits LPS-induced TNFalpha release in the mouse lung, Shock 28 (2007) 700–703.

[29] P. Palsamy, S. Sivakumar, S. Subramanian, Resveratrol attenuates hyperglycemia mediated oxidative stress, proinflammatory cytokines and protects hepatocytes ultrastructure in streptozotocin-nicotinamide-induced experimental diabetic rats, Chem. Biol. Interact. 186 (2010) 200–210.

[30] C.N. Serhan, J. Savill, Resolution of inflammation: the beginning programs the end, Nat. Immunol. 6 (2005) 1191–1197.

[31] N. Boudjellab, H.S. Chan-Tang, X. Zhao, Bovine interleukin-1 expression by cultured mammary epithelial cells (MAC-T) and its involvement in the release of MAC-T derived interleukin-8, Comp. Biochem. Physiol. A Mol. Integr. Physiol. 127 (2000) 191–199.

[32] U.K. De, R. Mukherjee, Expression of cytokines and respiratory burst activity of milk cells in response to *Azadirachta indica* during bovine mastitis, Trop. Anim. Health Prod. 41 (2009) 189–197.

[33] F.N. Markus, F. Susetta, IL-6 signaling in autoimmunity, chronic inflammation and inflammation-associated cancer, Cytokine Growth Factor Rev. 22 (2011) 83–89.

[34] K. Miyake, Y. Nagai, S. Akashi, M. Nagafuku, M. Ogata, Y. Iwakura, Essential role of MD-2 in LPS responsiveness and TLR4 distribution, Nat. Immunol. 3 (2002) 667–672.

[35] J. Santander, J. Kilbourne, J.-Y. Park, T. Martin, A. Loh, I. Diaz, R. Rojas, C. Segovia, D. DeNardo, R. Curtiss, Inflammatory effects of *Edwardsiella ictaluri* lipopolysaccharide modifications in catfish gut, Infect. Immun. 82 (2014) 3394–3404.

[36] K. Asehnoune, D. Strassheim, S. Mitra, J.Y. Kim, E. Abraham, Involvement of PKCalpha/beta in TLR4 and TLR2 dependent activation of NF-kappaB, Cell. Signal. 17 (2005) 385–394.

[37] J. Liu, A. Lin, Wiring the cell signaling circuitry by the NF-kB and JNK1 crosstalk and its applications in human diseases, Oncogene 26 (2007) 3267–3278.

[38] B.B. Gu, J.F. Miao, Y.M. Zhu, Y. Deng, S.X. Zou, Protective effect of retinoid against endotoxin-induced mastitis in rats, Inflamm. Res. 58 (2009) 81–88.

[39] S.D. Kim, Y.J. Lee, J.S. Baik, J.Y. Han, C.G. Lee, K. Heo, Baicalein inhibits agonist and tumor cell-induced platelet aggregation while suppressing pulmonary tumor metastasis via cAMP-mediated VASP phosphorylation along with impaired MAPKs and PI3K-Akt activation, Biochem. Pharmacol. 92 (2014) 251–265.