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Astragaloside IV Ameliorates Inflammatory Damage in Mice with Acute Liver Failure

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Abstract

Acute liver failure is a life-threatening clinical syndrome with a high mortality rate. Currently, the research on Astragaloside IV in liver diseases primarily focuses on liver cancer, and there is limited understanding of its mechanism in acute liver failure's innate immunity. Therefore, this study aims to investigate the potential protective effect of Astragaloside IV on acute liver failure and its impact on innate immune cells. The study employed D-GalN/LPS-induced acute liver failure mouse models and employed various techniques such as a range of molecular and analytical techniques. The experimental results demonstrated that treatment with Astragaloside IV significantly reduced the inflammatory response, alleviated liver injury, and improved the survival rate of mice with acute liver failure induced by D-GalN/LPS. Further investigations revealed that AS-IV played a beneficial role by regulating the proportion of $CD11b^{+}Ly6C^{hi}$ monocytes and the secretion of inflammatory cytokines and anti-inflammatory metabolites. These findings suggest that the pharmacological mechanism of AS-IV may involve targeted regulation of $CD11b^{+}Ly6C^{hi}$ monocytes in both peripheral blood and liver. The implications of this study's results are twofold. Firstly, they provide a basis for the clinical application of AS-IV in treating liver failure, offering potential therapeutic benefits. Secondly, they serve as a reference for further development of safer and more effective modified compounds.

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Keywords

Astragaloside IV, Acute Liver Failure, Inflammation, Monocyte, Autophagy

1. Introduction

Liver failure is a life-threatening clinical syndrome characterized by coagulation disorders, hepatic encephalopathy, ascites, and other complications. It exhibits rapid progression and high mortality with rates, reaching up to 80% [1]. Currently, liver transplantation [2] and artificial liver supporting system (ALSS) [3] are considered the most viable treatments for this condition with a high-mortality rate. However, liver transplantation is limited to a select few due to the scarcity of liver donors and the associated high costs. Furthermore, liver regenerative capacity is often impaired after transplantation or in the presence of liver failure [4] [5] [6]. As a result, there has been an increased focus on exploring new therapeutic strategies to prevent liver failure. Patients with liver failure experience extensive immune activation, inflammatory responses, and sepsis. Dysregulation of the inflammatory response compromises the liver's defensive function, resulting in widespread hepatocyte necrosis, acute liver injury, and eventual liver failure [7]. Innate immunity, particularly the excessive activation of monocytes/ macrophages, plays a crucial role in the onset and progression of the disease. Thus, restoring immune balance by modulating liver immune inflammation is considered a potential strategy for liver failure treatment.

Astragalus membranaceus has a long history of use in treating various diseases. Astragaloside IV (AS-IV), a natural saponin extracted from Astragalus membranaceus, has been extensively studied and shown to have a wide range of beneficial effects in experimental models. These effects include cardiovascular diseases, neurological diseases, lung diseases, diabetes, renal diseases, and gynecological diseases. AS-IV is well-established to possess immunomodulatory, anti-inflammatory, and antioxidant properties [8] [9]. Furthermore, research indicates that AS-IV can inhibit TGF-beta to exert anti-fibrotic effects [10]. Clinical studies have demonstrated that Astragali glycoside sodium chloride injection can improve electrocardiogram performance [11] and alleviate heart failure by activating PPARα to shift from glycolysis to fatty acid β-oxidation [12]. AS-IV also inhibits the progression of liver cancer by modulating macro-phage polarization through the TLR4/NF-κB/STAT3 signaling pathway [13]. Additionally, it has been found to attenuate the migration and invasion of cancer cells and enhance the chemosensitivity of chemotherapy drugs [14]. Currently, research on the mechanism of Astragaloside IV in liver diseases primarily focuses on liver cancer, with relatively few studies investigating its effects on inflammatory cells in acute liver failure. Therefore, the aim of this study is to investigate the therapeutic effect of AS-IV on liver failure and its regulatory mechanism on innate immune cells through in vitro and in vivo experiments.

2. Materials and Methods

2.1. Materials

2.1.1. Animals

Male C57Bl/6 mice, aged 6 - 8 weeks, were obtained from GemPharmatech Co. Ltd. (Jiangsu, China).

2.1.2. Regeants

1) Chemical compounds

Astragaloside IV (AS-IV, #E-0146) was obtained from TAUTO Biotech Co., Ltd. in Shanghai, China. D-galactosamine (D-GalN, #G0500) and lipopolysaccharide (LPS, #L4391) were sourced from Sigma-Aldrich (St. Louis, MO, USA).

2) Autophagy related reagents

The Autophagy PCR Array-mice (#WC-MRNA0268-M, Wcgene® biotech) was purchased from Nuo Yang Sheng Wu (Shanghai, China). Cyto-ID (#ENZ-KIT175-0050) was purchased from Zneo (New York, USA).

3) Immunological reagent

Anti-mouse antibodies CD45 (APC-Cy7, #557659), CD11b (PE-Cy7, #101216), Ly6C (BV605, #563011), and F4/80 (BV786, #744340) were acquired from BD Biosciences in NJ, USA. 7-AAD (#420404) was obtained from Biolegend (CA, USA). The Anti-Myeloperoxidase antibody (#ab208670) was sourced from Abcam (Cambridge, MA, USA). The Quantibody® Human Th1/Th2/Th17 Array Q1 (#QAH-TH17-1-1) was purchased from Raybiotech (RayBiotech Inc., USA).

4) Metabolic reagents

For the metabolomics study, the Dansyl-labeling Kit for Amine & Phenol Metabolomics (#NMT-4101-KT) and HP-CIL Metabolomics Analysis Reagent: RT Calibrants (#NMT-2134-L) were obtained from Xiamen Meliomics Technology Co., Ltd. (Xiamen, China).

2.2. Methods

2.2.1. Animal Model

In the model group, acute liver failure was induced in C57BL/6 mice through intraperitoneal injection of a mixture containing LPS and D-GalN (5 μg/kg and 500 mg/kg, respectively). In the drug treatment group, mice were intraperitoneally administered AS-IV (400 mg/kg) 30 minutes prior to the injection of the D-GalN/LPS mixture. The control group consisted of mice that received either 400 mg/kg AS-IV or normal saline. Throughout the studies, the animals were housed in an environmentally controlled room with constant temperature and humidity. The protocol for mouse usage was approved by the Research Ethics Committee of the First Affiliated Hospital, College of Medicine, Zhejiang University. Researchers were provided with appropriate training in the care and handling of animals by the hospital's Animal Laboratory Center, and all experiments adhered to the guidelines outlined in the Guide for the Care and Use of Experimental Animals.

2.2.2. Survival Analyses

In this study, four groups of mice were established, each consisting of ten mice. These groups included a normal saline control group, an AS-IV drug control group, a D-GalN/LPS model group, and an AS-IV treatment group. Following the injection of the D-GalN/LPS mixture, the mice were monitored for mortality every 6 hours.

2.2.3. Biochemical Analysis and Histological Staining

Mouse liver tissue and serum samples were collected 6 hours after injection of the D-GalN/LPS mixture for further analysis. The levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the serum samples were determined using a biochemical analyzer (Beckman AU-5421; Beckman-Coulter, Brea, CA, USA). A portion of the liver tissues was fixed in a 4% paraformaldehyde solution for 2 days and subsequently embedded in paraffin wax. Tissue slices with a thickness of 5 μm were prepared for histopathological examination (HE) and MPO histochemical examination. All images were captured using an optical microscope (Olympus, Tokyo, Japan).

2.2.4. High-Throughput Quantitative PCR

mRNA was extracted from liver tissues and subsequently reverse transcribed into cDNA. The resulting cDNA was utilized in the Autophagy PCR Array—mouse to detect the expression of autophagy-related genes using Real-time fluorescence quantitative PCR (QuantStudio).

2.2.5. Flow Cytometry

Immune cells were extracted from mouse peripheral blood and liver tissue for flow cytometry analysis. To exclude dead cells, 7-AAD was utilized. Monocytes were double-labeled with CD11b and Ly6C, macrophages were double-labeled with CD11b and F4/80, and neutrophils were double-labeled with CD11b and Ly6G. Cyto-ID was employed to detect autophagy levels in the immune cells. Flow cytometry (BD™ LSRFortessa, BD Biosciences) was performed to determine the proportion of immune cells and measure autophagy levels. FlowJo v10.0 (BD Biosciences) was used for data analysis of each sample.

2.2.6. Cell Culture

THP-1 cells were selected for in vitro cell studies. In the cell experiments, four groups were established: a normal saline control group, an AS-IV drug control group, an LPS experimental group, and an AS-IV drug treatment group. The concentrations of AS-IV and LPS used were 10 μg/mL and 1 μg/mL, respectively. After 24 h of LPS stimulation, the cell fusion degree reached over 90%. The supernatant was collected and transferred to a 15 mL centrifuge tube. Following centrifugation (2000 rpm, 4˚C, 10 min), the supernatant was collected and divided into 1.5 mL EP tubes, then stored at −80˚C.

2.2.7. Protein Microarray

Following the instructions, the supernatant of THP-1 cells cultured in each

group was subjected to protein microarray analysis (Quantibody® Human Th1/Th2/Th17 Array Q1). This array could detect 20 inflammatory mediators, including GM-CSF, IFN-γ, IL-1β, IL-10, IL-12 p70, IL-13, IL-17A, IL-17F, IL-2, IL-21, IL-22, IL-23, IL-28A (IFN-λ2), IL-4, IL-5, IL-6, MIP-3 alpha (CCL20), TGF beta1, TNF-α, and TNF-β (TNFSF1B). Subsequently, the R language was utilized for conducting Gene Ontology Biological Process (GO_BP) (biological process), Gene Ontology Cellular Component (GO_CC) (cellular component), and Gene Ontology Molecular Function (molecular function) analyses, as well as KEGG enrichment analysis.

2.2.8. Metabolomics Test

Metabolomic analysis was conducted on the THP-1 cells and the previously collected supernatant using Amine & Phenol Metabolomics. The experimental procedures included sample collection and preparation, dansylation labeling, sample-wise normalization, LC-MS, and data processing and analysis (refer to the supplementary material for detailed information). The obtained metabolomic data underwent SIMCA and MetaboAnalyst pathway analysis. Additionally, volcano maps and Venn diagrams were generated. These detection results enable a systematic analysis of the regulatory effect of AS-IV on metabolites.

2.2.9. Statistical Analysis

Each experiment was conducted in triplicate. Statistical analyses were performed using GraphPad Prism 7.0 software. The data are presented as means ± standard errors of the means (SEM). A value of $p < 0.05$ (two-tailed) was considered statistically significant. Significance levels were denoted as follows: *$p < 0.05$, **$p < 0.01$. The symbol "NS" indicates that the observed value is not statistically significant.

3. Results

3.1. AS-IV Ameliorates Liver Injury in Mice with Acute Liver Failure

This study utilized a D-GalN/LPS-induced acute liver failure model, which closely mimics the pathogenesis of acute liver failure in clinical settings [15]. **Figure 1(A)** presents the results of the initial analysis, demonstrating the beneficial effects of AS-IV in mitigating liver injury. It was observed that AS-IV treatment significantly increased the survival rate of mice compared to the model group (60% vs 20%). Further examination of liver function revealed that AS-IV notably reduced the levels of ALT and AST in the serum, which are indicative of hepatocyte damage (**Figure 1(B)**, $p < 0.05$; **Figure 1(C)**, $p < 0.01$). Moreover, in the AS-IV treatment group, there were no apparent signs of diffuse hyperemia and edema (**Figure 1(D)**). Additionally, the evaluation of Hematoxylin and eosin (HE)-stained liver sections demonstrated infiltration of inflammatory cells, necrosis of liver cells, and blurred hepatic sinuses in the D-GalN/LPS model group (**Figure 1(E)**). Notably, **Figure 1(F)** illustrates a significant decrease in the proportion of MPO-positive cells in the Astragaloside IV-treatment group

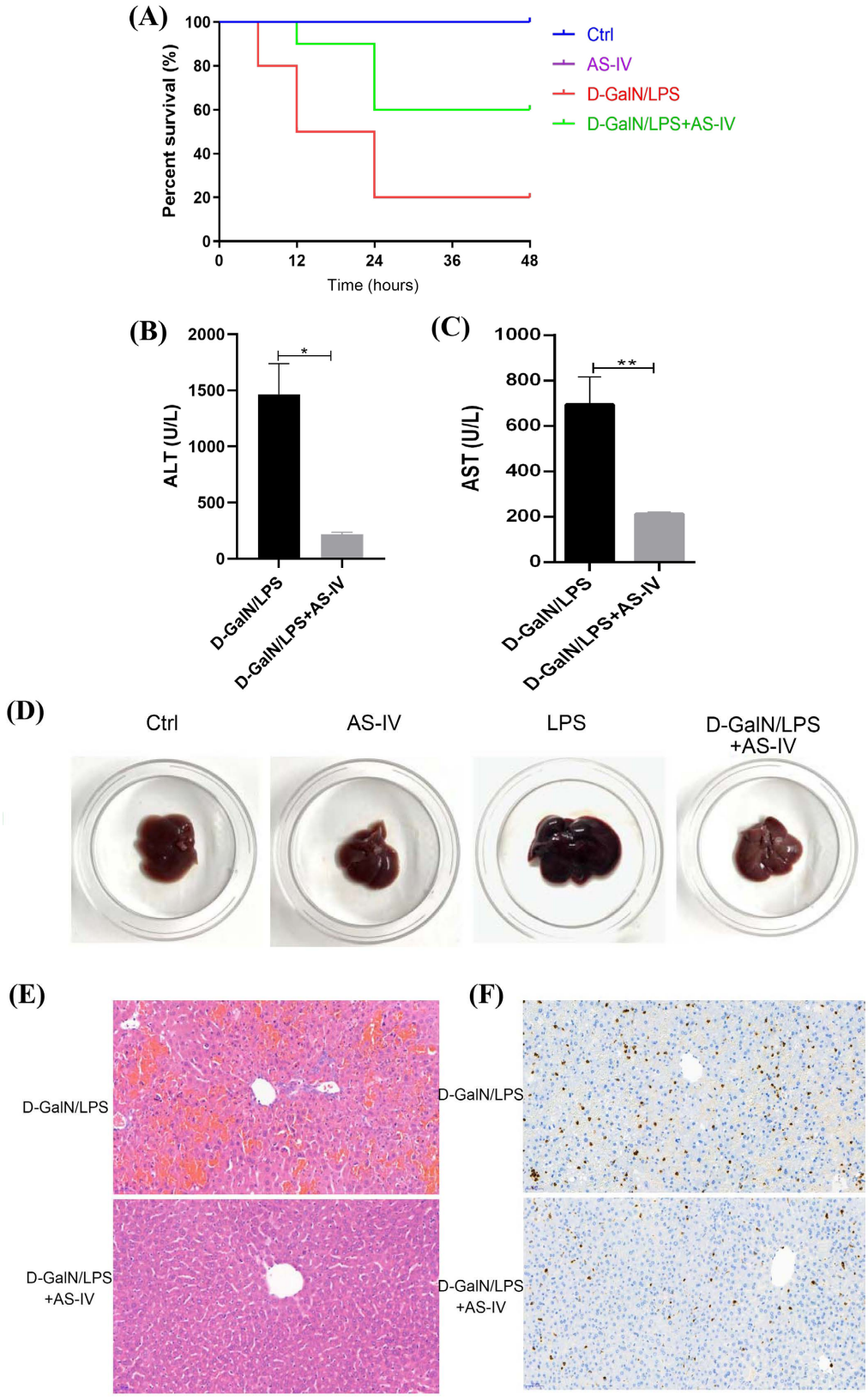


Figure 1. AS-IV can improve survival rate and reduce liver injury. (A). Survival curves of mice in each group. (B-C). Detection of serum biochemical AST, ALT. (D). Pictures of mouse livers. (E). HE staining of the liver section. (F). Anti-myeloperoxidase (MPO)—stained liver sections. *p < 0.05, **p < 0.01.

compared to the model group (92 dots vs 150 dots). These findings collectively indicate that AS-IV effectively ameliorates liver injury and improves the survival rate in mice with D-GalN/LPS-induced acute liver failure.

3.2. AS-IV Down-Regulates the Proportion of Monocytes

In this study, peripheral blood samples were collected from mice in each group through eyeball blood extraction. Flow cytometry was then performed to detect the proportion of monocytes, macrophages, and neutrophils in the experimental mice's peripheral blood. This involved steps such as red blood cell lysis and immune cell staining. The results revealed a significant decrease in the proportion of $CD11b^{+}Ly6C^{hi}$ monocytes and $CD11b^{+}F4/80^{hi}$ macrophages in the peripheral blood of AS-IV-treated mice (**Figure 2(A)**, **Figure 2(B)**). However, the proportion of $CD11b^{+}F4/80^{lo}$ macrophages and $CD11b^{+}Ly6G^{+}$ neutrophils remained unaffected by AS-IV treatment (**Figure 2(B)**, **Figure 2(C)**). Furthermore, analysis of liver tissue indicated that AS-IV only reduced the proportion of $CD11b^{+}Ly6C^{hi}$ monocytes and had no impact on the proportion of macrophages and neutrophils (**Figure 2(D)**).

3.3. AS-IV Increases the Level of Liver Autophagy in Acute Liver Failure Mice

In this study, flow cytometry was used to detect the proportion of inflammatory cells in the peripheral blood of mice, along with measuring the mean fluorescence intensity of the autophagy signal in cells. The results revealed that AS-IV significantly increased the level of autophagy in monocytes and macrophages (**Figure 2(A)**, **Figure 2(B)**). The proportion of $CD11b^{+}F4/80^{lo}$ macrophages in the peripheral blood of AS-IV-treated mice with acute liver failure remained unchanged, but the level of autophagy was increased. AS-IV had no effect on the proportion of $CD11b^{+}Ly6G^{+}$ neutrophils and their level of autophagy (**Figure 2(C)**). Furthermore, mRNA was extracted from the liver tissue of experimental mice to extensively analyze the expression levels of autophagy-related genes. The results showed that 90 genes exhibited significant changes (**Figure 3**). Among these genes, 35 were up-regulated by more than 1.2-fold and were associated with autophagy functions. These functions contain 4 categories, 1) Autophagic Vacuole Formation; 2) autophagy and apoptosis such as Co-Regulators of Autophagy and Apoptosis, Co-Regulators of Autophagy and the Cell Cycle; 3) autophagy response or induction such as Autophagy in Response to Other Intracellular Signals, Autophagy Induction by Intracellular Pathogens; 4) multiple aspects of protein such as Protease Activity Responsible for Protein Targeting to Membrane/Vacuole, Protein Transport, and Protein Ubiquitination. On the other hand, 24 genes were significantly downregulated (≤0.83-fold) and were linked to autophagy functions. These functions contain 4 categories, such as 1) autophagy and apoptosis such as Co-Regulators of Autophagy and Apoptosis, Co-Regulators of Autophagy and the Cell Cycle, 2) autophagy process such as

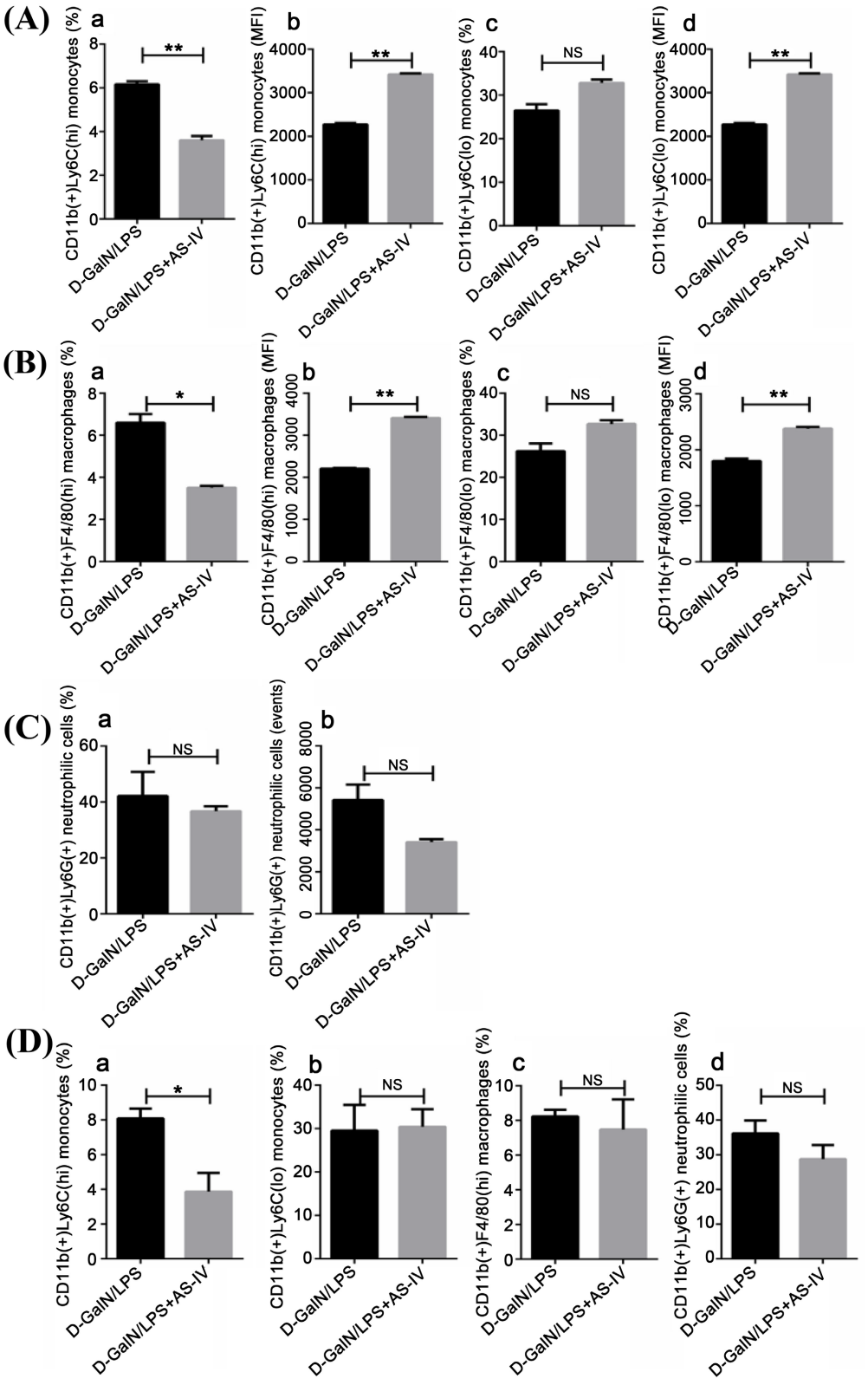


Figure 2. Proportion of cell and autophagy level. (A). Proportion of monocytes and autophagy level in blood. (B). Proportion of macrophages and autophagy level in blood. (C). Proportion of neutrophils and autophagy level in blood. (D). Proportion of monocytes, macrophages and neutrophils in liver. NS: no significant difference, *$p < 0.05$, **$p < 0.01$.

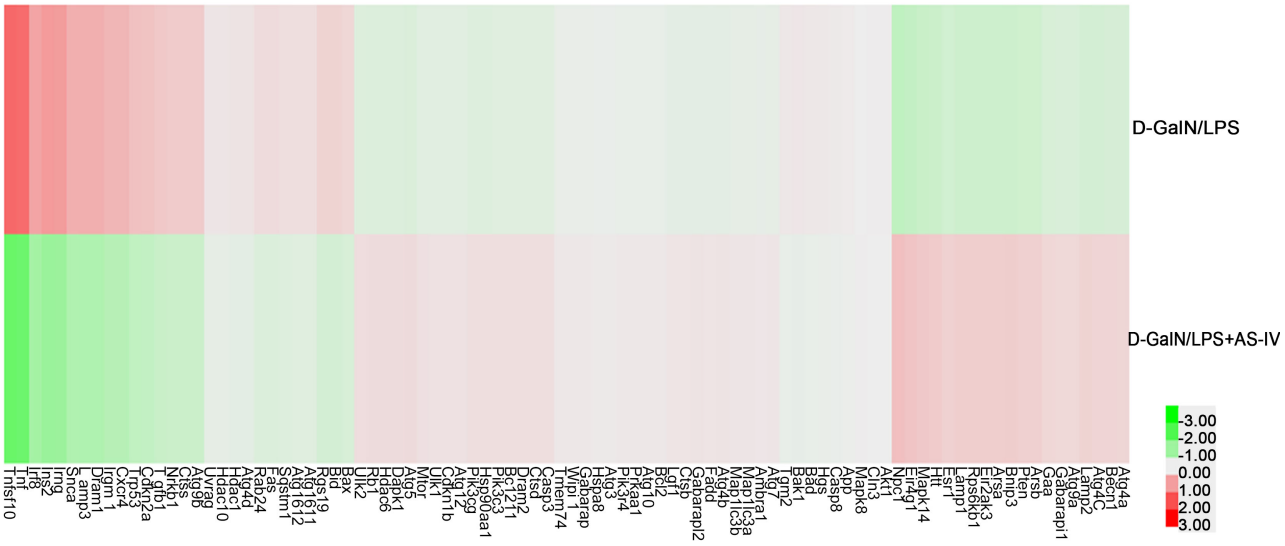


Figure 3. Autophagy-related gene expression measured by high-throughput quantitative PCR.

Autophagic Vacuole Formation, and Linking Autophagosome to Lysosome, 3) Protein Transport.

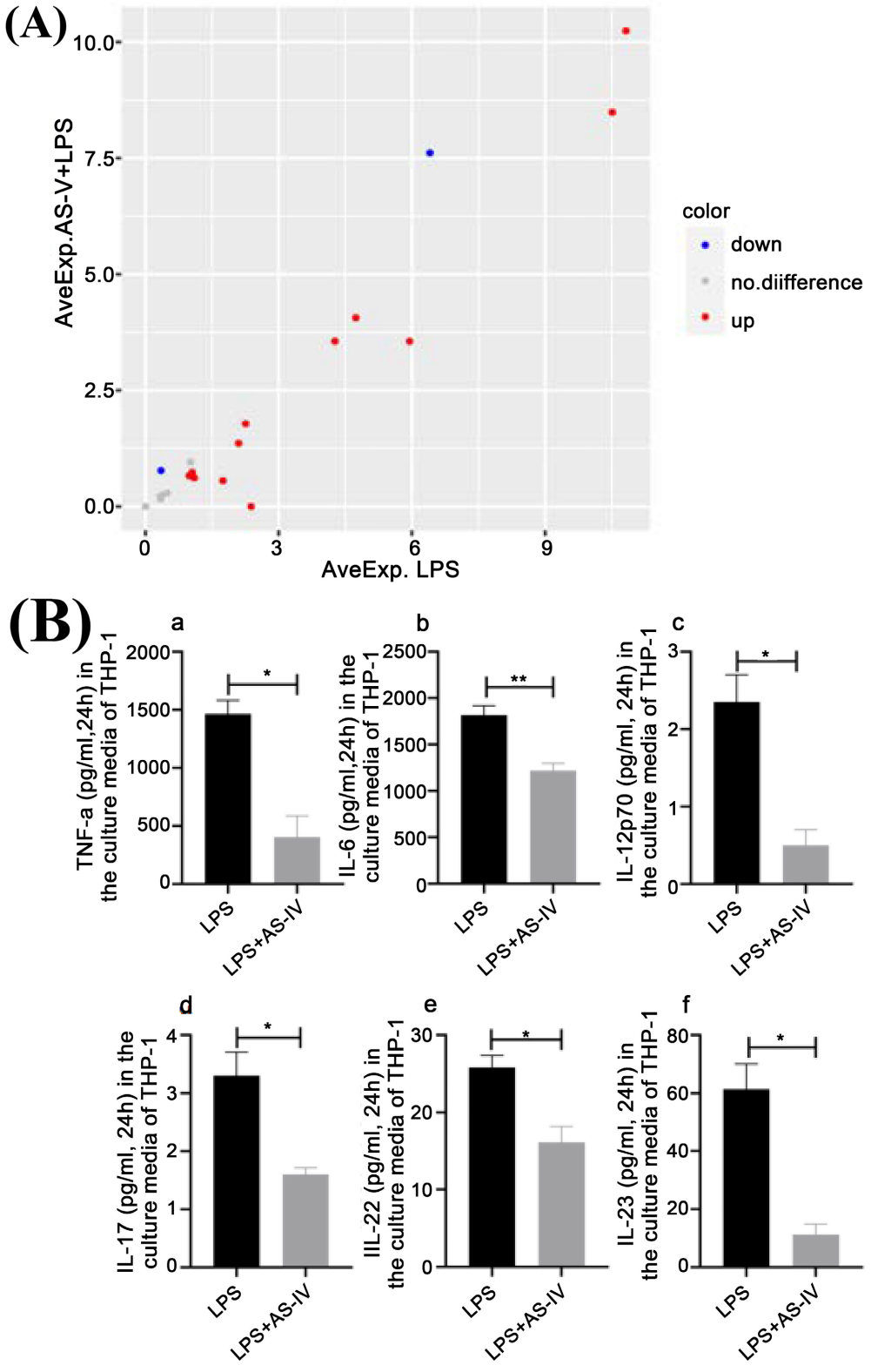
3.4. AS-IV Reduces Inflammatory Cytokine Secretion of D-GalN/LPS-Induced Monocyte

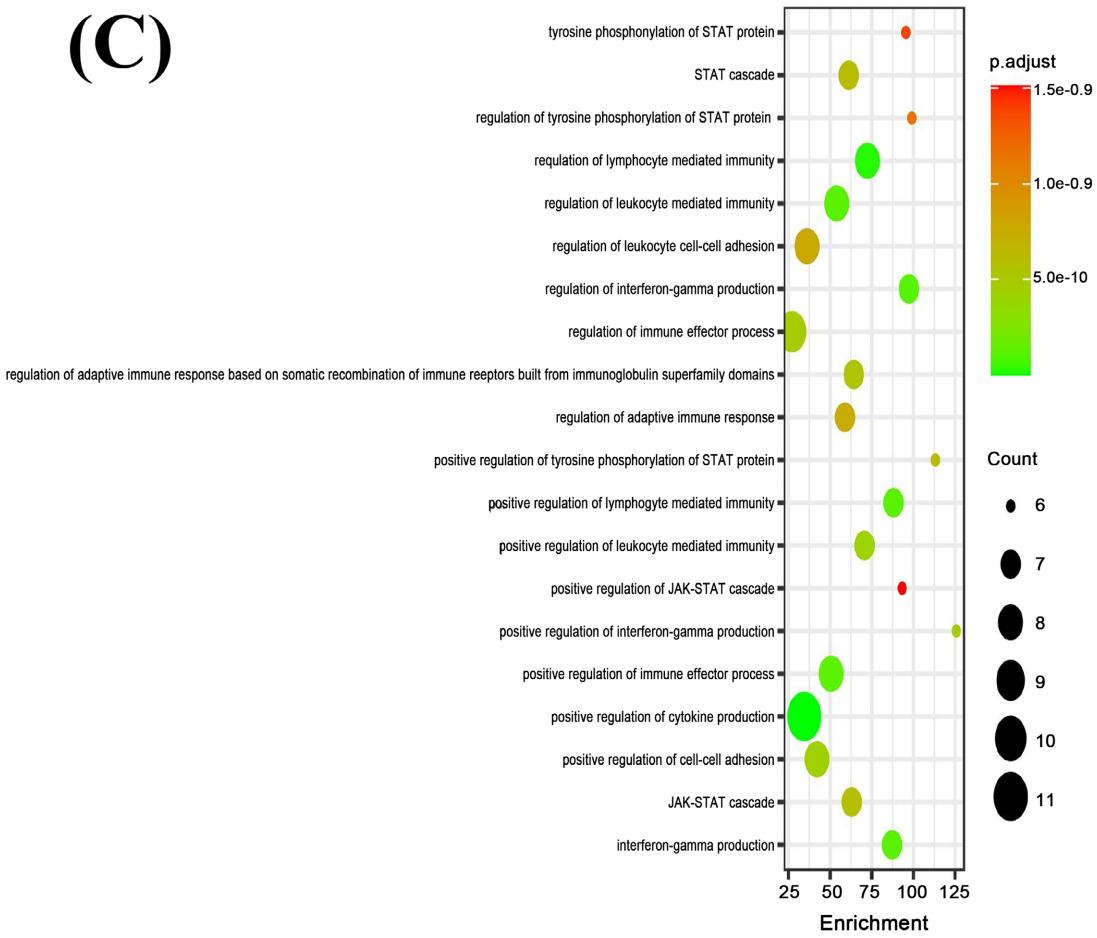
The study involved the collection of culture media from in vitro cell experiments for protein microarray analysis. A comparison between the AS-IV treatment group and the group without AS-IV treatment revealed significant alterations in 14 inflammatory mediators (**Figure 4(A)**). Notably, TNF-*α*, IL-6, IL-12p70, IL-17, IL-22, and IL-23 (**Figure 4(B)**) exhibited significant down-regulation in the AS-IV treatment group. Additionally, GO_BP, GO_CC, and GO_MF analyses were conducted, revealing that the biological processes were associated with immune cell responses and cytokine expression (**Figure 4(C)**, **Figure 4(E)**). The localized cellular components were identified as late endosome lumen and endoplasmic reticulum lumen, while the molecular functions were predominantly related to cytokine activity, receptor ligand activity, and cytokine receptor binding. Furthermore, KEGG enrichment analysis demonstrated that these inflammatory mediators were associated with inflammation-related diseases, immune-related diseases, and infectious diseases (**Figure 4(F)**).

3.5. AS-IV Regulates Metabolite Secretion of D-GalN/LPS-Induced Monocyte

In this study, the effect of AS-IV on monocyte metabolism stimulated by D-GalN/LPS was investigated. The THP-1 monocyte cell line and cell supernatant from both AS-IV intervention and non-intervention groups in the in vitro cell experiment were collected for Amine & Phenol Metabolomics analysis. The results, shown in **Figure 5(A)**, **Figure 5(B)**, demonstrated significant changes in the ex-

pression levels of metabolites. Comparing the AS-IV treatment group with the LPS group, Figures 5(A)(a-c) revealed up-regulated expression of S-Sulfo-L-c, L-gamma-Glutamyl-(3R)-L-beta-ethynylserine, and L-Methionine after AS-IV




(C)
tyrosine phosphonylation of STAT protein
STAT cascade
regulation of tyrosine phosphorylation of STAT protein
requlation of lymphocyte mediated immunity
regulation of leukocyte mediated immunity
regulation of leukocyte cell-cell adhesion
regulation of interferon-gamma production
regulation of immune effector process
regulation of adaptive immune response based on somatic recombination of immune reeptors built from immunoglobulin superfamily domains
regulation of adaptive immune response
positive regulation of tyrosine phosphorylation of STAT protein
positive regulation of lymphogyte mediated immunity
positive regulation of leukocyte mediated immunity
positive regulation of JAK-STAT cascade
positive regulation of interferon-gamma production
positive regulation of immune effector process
positive regulation of cytokine production
positive regulation of cell-cell adhesion
JAK-STAT cascade
interferon-gamma production
25 50 75 100 125
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1.0e-0.9
5.0e-10
Count
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8
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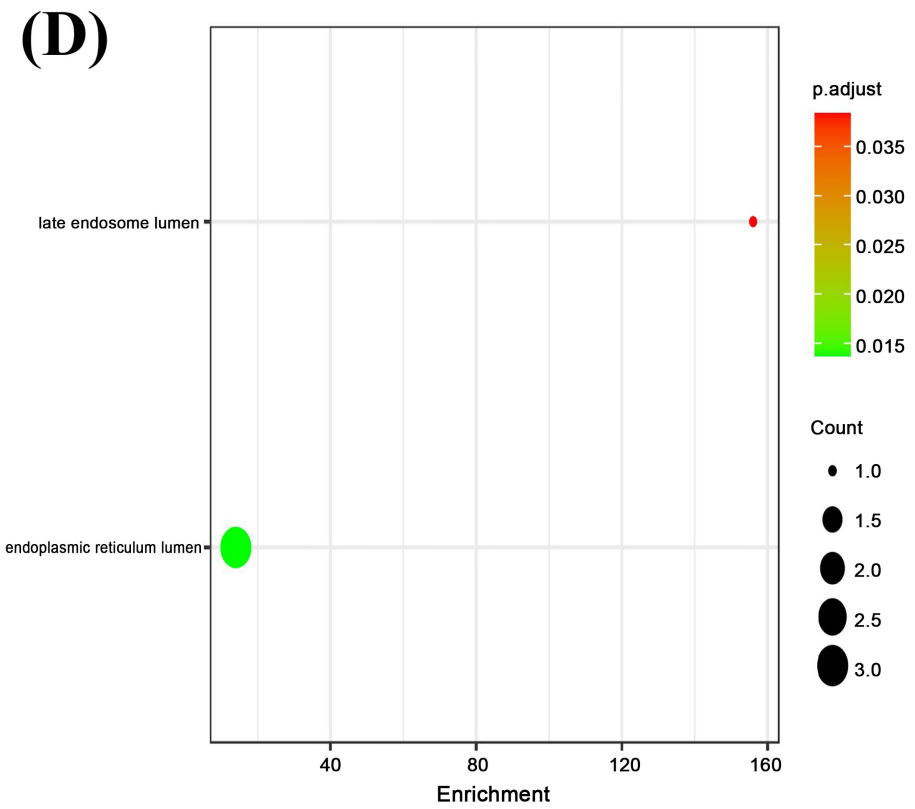
10
11


(D)
late endosome lumen
endoplasmic reticulum lumen
40 80 120 160
Enrichment
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2.0
2.5
3.0

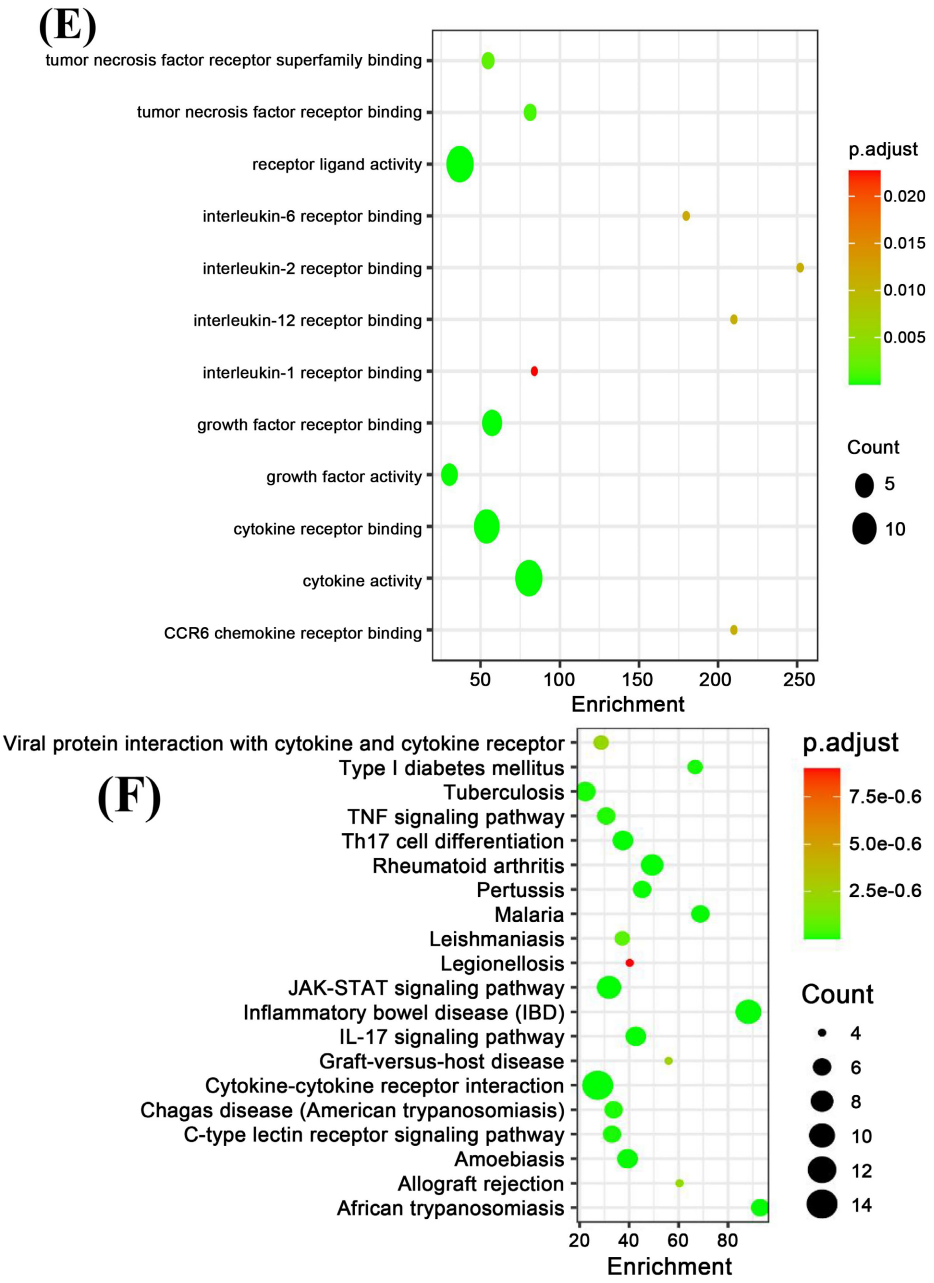
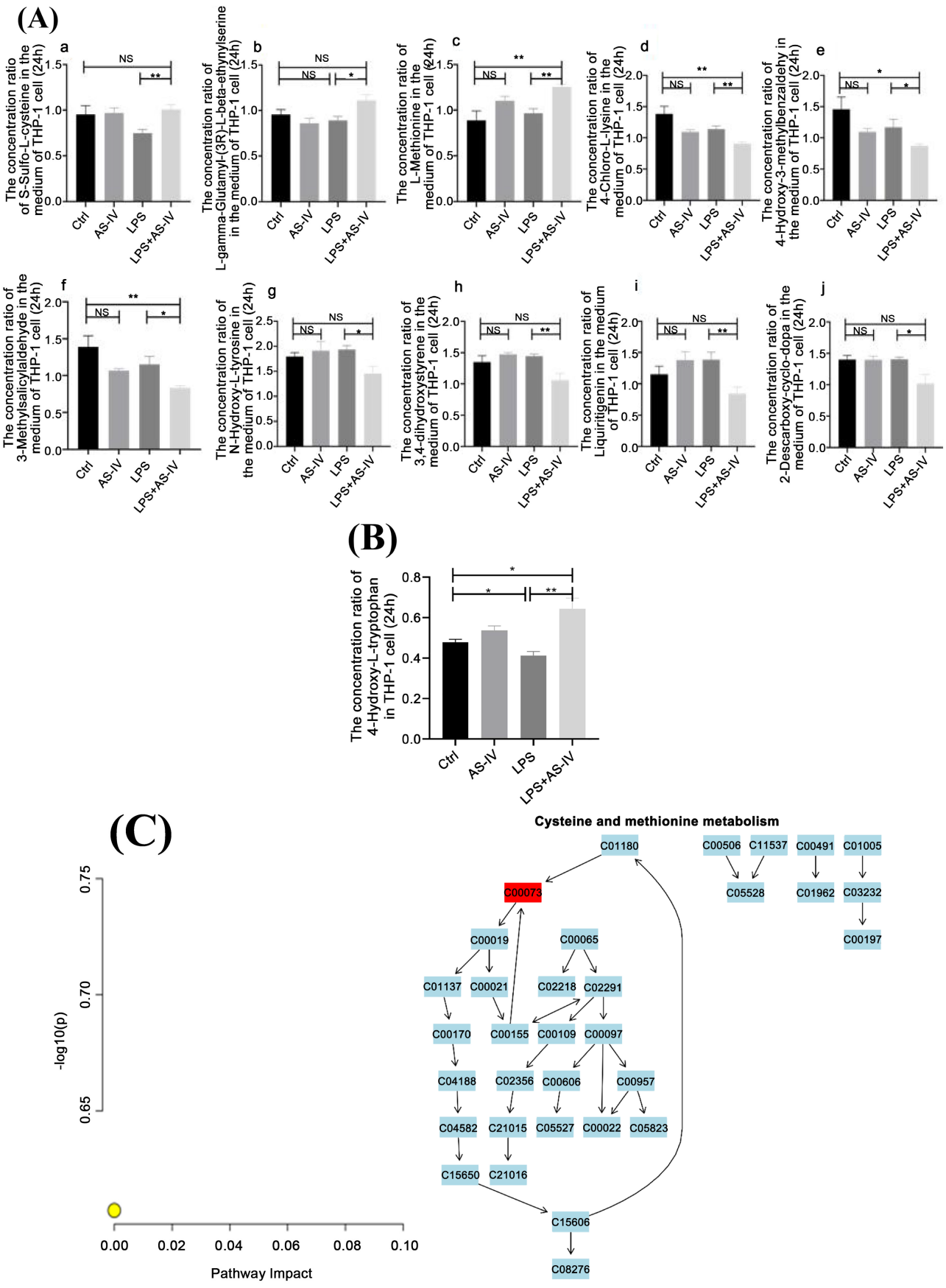


Figure 4. Protein microarray cytokines concentration of the supernatant in the culture was measured. (A-B). Significantly altered cytokines. (C). GO_BP analyses. (D). GO_CC analyses. (E). GO_MF analyses. (F).KEGG systematically analyzed gene function of significant changes in cytokines.

treatment. On the other hand, Figures 5(A)(d-j) displayed down-regulated expression of 4-Chloro-L-lysine, 4-Hydroxy-3-methylbenzaldehyde, 3-Methylsalicylaldehyde, N-Hydroxy-L-tyrosine, 3,4-dihydroxystyrene, Liquiritigenin, and 2-Descarboxy-cyclo-dopa in the AS-IV plus LPS group compared to the LPS group. Furthermore, Metaboanalyst analysis identified the pathways involved in L-Methionine, including Cysteine and methionine metabolism, and Aminoacyl-tRNA biosynthesis (Figure 5(C)).


(A)
a
The concentration ratio of S-Sulfo-L-cysteine in the medium of THP-1 cell (24h)
b
The concentration ratio of L-gamma-Glutamyl-(3R)-L-beta-ethynylserine in the medium of THP-1 cell (24h)
c
The concentration ratio of L-Methionine in the medium of THP-1 cell (24h)
d
The concentration ratio of 4-Chloro-L-lysine in the medium of THP-1 cell (24h)
e
The concentration ratio of 4-Hydroxy-3-methylbenzaldehy in the medium of THP-1 cell (24h)
f
The concentration ratio of 3-Methylsalicylaldehyde in the medium of THP-1 cell (24h)
g
The concentration ratio of N-Hydroxy-L-tyrosine in the medium of THP-1 cell (24h)
h
The concentration ratio of 3,4-dihydroxystyrene in the medium of THP-1 cell (24h)
i
The concentration ratio of Liquiritigenin in the medium of THP-1 cell (24h)
j
The concentration ratio of 2-Descarboxy-cyclo-dopa in the medium of THP-1 cell (24h)
Ctrl
AS-IV
LPS
LPS+AS-IV
NS
(B)
The concentration ratio of 4-Hydroxy-L-tryptophan in THP-1 cell (24h)
(C)
Cysteine and methionine metabolism
C01180
C00506
C11537
C00491
C01005
C00073
C05528
C01962
C03232
C00019
C00065
C00197
C01137
C00021
C02218
C02291
C00170
C00155
C00109
C00097
C04188
C02356
C00606
C00957
C04582
C21015
C05527
C00022
C05823
C15650
C21016
C15606
C08276
-log10(p)
0.75
0.70
0.65
0.00
0.02
0.04
0.06
0.08
0.10
Pathway Impact

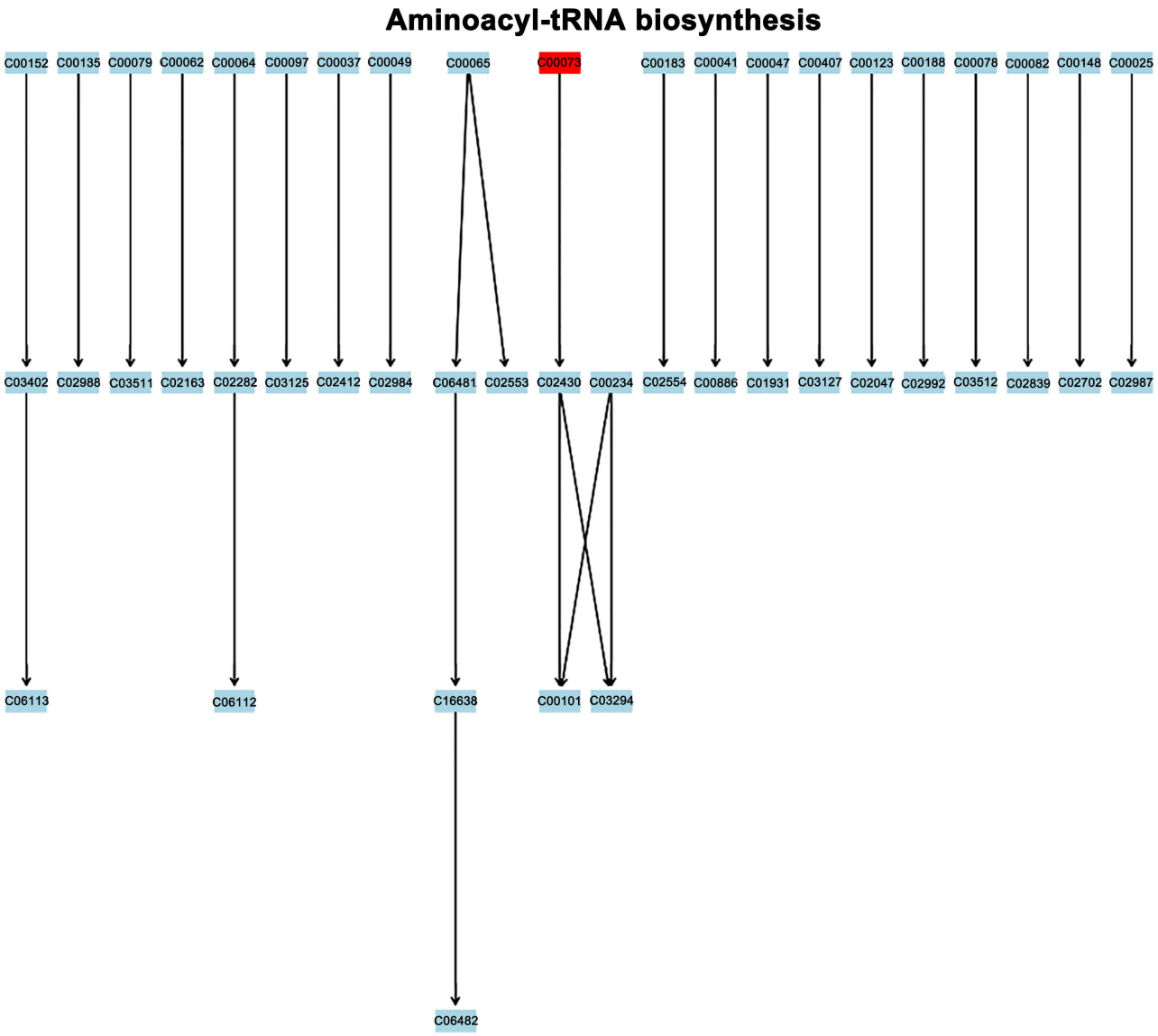


Figure 5. Metabolomics test. Amine & Phenol Metabolomics were tested on the THP-1 cells and supernatant collected above. (A). Histogram of metabolite changes in cell culture medium. (B). Significant changes of 4-Hydroxy-L-tryptophan were observed in the cells. (C). Metaboanalyst pathway analysis.

4. Discussion

AS-IV exhibits significant potential in reducing the inflammatory response, alleviating liver damage, and improving the survival rate in mice with acute liver failure. Further investigations have indicated that AS-IV achieves these beneficial effects primarily through the regulation of $CD11b^{+}Ly6C^{hi}$ monocyte proportions, the secretion of inflammatory cytokines, the autophagy levels in the liver and the mononuclear macrophage system, and the modulation of anti-inflammatory metabolites.

Acute liver failure poses a high mortality risk in patients. Current clinical treatments for liver failure include artificial liver supports, stem cell transplantation, liver transplantation, and medical drugs. This study employed LPS com-

bined with GalN to establish a mouse model of acute liver failure [16]. This model [7] [17] exhibits a dramatic activation of the mononuclear macrophage system in the liver, leading to the production of numerous pro-inflammatory mediators [18]. Extensive hepatocyte apoptosis and massive hepatocyte necrosis initiated by end-effector cells contribute to the inflammatory cascade. In this study, AS-IV improved 24-hour survival rates. Histological analysis also demonstrate the beneficial effects of AS-IV on acute liver failure.

Hepatic monocytes/macrophages play a crucial role in the progression of acute liver damage and the maintenance of liver homeostasis [19]. Upon activation of Kupffer cells (KC cells), pro-inflammatory cytokines such as monocyte chemotactic protein 1 (MCP-1), Interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and Interleukin-8 (IL-8) are produced and released. This activation leads to a feedback loop amplifying inflammation and promoting neutrophil infiltration in the hepatic microcirculation [20]. AS-IV has been shown to modulate macrophage phenotype and mitigate inflammation through remodeling the STAT signaling pathway [21]. Wang's research has demonstrated that AS-IV inhibits the production of pro-inflammatory cytokines from peritoneal macrophages in vitro [22]. Therefore, this study initially examined the proportion of mononuclear macrophages and neutrophils in the peripheral blood of the model mice. The results indicated a significant reduction in the proportion of monocytes and macrophages, particularly $CD11b^{+}Ly6C^{hi}$ monocytes and $CD11b^{+}F4/80hi$ macrophages, in the peripheral blood following AS-IV treatment. Further analysis of the proportion of mononuclear macrophages and neutrophils in the liver revealed a significant decrease only in $CD11b^{+}Ly6C^{hi}$ monocytes, while the proportions of macrophages and neutrophils remained unaffected. Monocytes expressing high levels of Ly6c are known to possess pro-inflammatory and antibacterial activity, accumulating at sites of inflammation. Conversely, monocytes with low Ly6c expression are considered patrolling monocytes that surveil blood vessels and participate in early inflammatory and tissue repair responses [23]. In the context of chronic neuroinflammation, classical monocytes with high Ly6C expression accumulate in the inflammatory sites of the central nervous system and contribute to the formation of inflammatory macrophages through terminal differentiation [24]. Based on this understanding, we hypothesize that AS-IV may primarily modulate inflammation by targeting $CD11b^{+}Ly6C^{hi}$ monocytes.

To further elucidate the mechanism of action of AS-IV, we conducted in vitro cell experiments using THP-1 cells. Protein chip analysis of the cell supernatant revealed significant down-regulation of IL-6, IL-12p70, IL-17, IL-22, IL-23, and TNF-α. These findings suggest that AS-IV exerts an anti-inflammatory effect by suppressing the expression of these inflammatory mediators. Furthermore, analysis of GO_BP, GO_CC, and GO_MF demonstrated that the expression changes of the 14 inflammatory mediators, which were either increased by more than 1.2-fold or decreased by less than 0.83-fold, were associated with immune cell response and cytokine expression. KEGG enrichment analysis revealed that

these inflammatory mediators were implicated in inflammation-related diseases, immune-related diseases, and infectious diseases.

Several studies have demonstrated that autophagy can inhibit macrophage activation and reduce proinflammatory cytokines during acute liver damage [25]. In this study, the results showed that AS-IV treatment could enhance the autophagy level in two kinds of monocytes and macrophages. The results also revealed that AS-IV exerted extensive regulatory effects on the expression of autophagy-related genes in liver tissues. A total of 90 genes showed significant changes, including up-regulated genes such as Atg5, Becn1, Atg12, Pten, Htt, Dapk1, Bnip3, Pik3cg, among others [26] [27] [28] [29] [30]. The up-regulation of genes like Dapk1 and Bnip3 can promote autophagy, inhibit inflammation, and reduce apoptosis and tissue damage [31] [32] [33] [34]. The up-regulation of Pik3cg inhibits apoptosis, while the apoptosis-related gene Cyclin-Dependent Kinase Inhibitor 1B (CDKN1B) promotes autophagy through the Mtor1-dependent pathway [35] [36]. Conversely, down-regulated genes were also identified, including Sqstm1, Tnfsf10, Tnf, IFNg, Bax, Bid, Tgfb1, Fas, Nfkb1, Cdkn2a, among others. The decreased expression of Sqstm1 indicates increased autophagy, as it serves as a marker for autophagy. The reduced expression of pro-apoptotic proteins like Tnfsf10, Tnf, IFNg, Bax, Bid, Tgfb1, and Fas [37] [38] [39] [40] [41] suggests a lower level of apoptosis in liver tissue. The down-regulation of Nfkb1 indicates reduced tissue inflammation [42], and the decreased expression of Cdkn2a, an aging marker, suggests decreased apoptosis. In conclusion, the mechanism of action of AS-IV in the treatment of acute liver failure involves the regulation of autophagy levels, which may modulate inflammation by enhancing autophagy and inhibiting apoptosis.

Metabolites are closely related to inflammation, because most of the metabolites were decreased with LPS treatment in THP-1 [43]. Hence, the increased expression levels of inflammatory mediators in LPS-stimulated monocytes in vitro may be associated with the reduced expression levels of most metabolites. The results in this study suggest that AS-IV may up-regulate the expression of certain metabolites, such as L-Methionine in the THP-1 cell culture medium, which could potentially exert an anti-inflammatory effect. Previous results showed that the increased levels of the metabolite L-Methionine may indicate a reduction in inflammatory response, oxidative stress, and improved energy metabolism in acute kidney injury (AKI) caused by Cisplatin (CDDP) [44]. In non-alcoholic fatty liver models of rats fed a high-fat fructose diet, L-methionine supplementation has been shown to improve liver pathology by regulating lipogenesis, inhibiting the release of pro-inflammatory cytokines, and activating the SIRT1/AMPK pathway [45]. Furthermore, both L-Met and DL-Met supplementation have demonstrated comparable protective effects on parameters of intestinal health and function, including intestinal morphology and antioxidant status [46]. Based on the reported anti-inflammatory effects of L-methionine in the literature mentioned above, we hypothesized that AS-IV exhibits anti-inflammatory effects

through the up-regulation of L-methionine in the cell culture medium of THP-1 cells induced by LPS.

Overall, the results obtained in this study hold promise for the clinical application of AS-IV in the treatment of liver failure and provide valuable insights for the development of safer and more effective modified compounds in the future.

Author Statement

Ying Yang formulate the overarching research goals and aims; Ying Yang and Meng Hong performed the research, analyzed the data and write the manuscript; Wenwen Lian performed the research and analyzed the data; Ying Yang and Zhi Chen acquire the financial support for the project leading to this publication. All authors read and approved the final manuscript.

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Conflicts of Interest

The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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