RETRACTED ARTICLE: Amygdalin reduces lipopolysaccharide-induced chronic liver injury in rats by down-regulating PI3K/AKT, JAK2/STAT3 and NF-κB signalling pathways

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ABSTRACT

This study was aimed to evaluate the anti-inflammatory potential of AG on lipopolysaccharide (LPS)-induced liver injury and investigate the underlying mechanism. Administration of LPSs in the rat produced rat liver injury model which was ascertained at histological and molecular levels. Those models were treated with a range of doses of LPSs (0.5, 1.0 and 1.5 mg/kg body weight), followed by measurement physical parameter and function of the liver. Within the max treatment doses, no toxicity was shown but protective effects of AG were evidenced by regulation of physical parameters and functions of the liver. Interestingly, nuclear factor kappa B (NF-κB) levels and inflammatory factors were down-regulated by AG. Furthermore, the histopathological analysis demonstrated that AG promoted recovery from dysfunction of liver tissue in the rats, which was further confirmed by observing expression changes of inflammation-associated proteins. Particularly, alteration in the PI3K/AKT and JAK2/STAT3 signalling pathway protein expression were regulated by AG in a dose-dependent manner, indicating the mechanism underlying the relief effect of AG in liver injury. Our study demonstrated the potential of AG in the management of complications related to liver injury.

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Introduction

Chronic liver injury (CLI) is a severe complication commonly occurred in liver diseases, which causes approximately 80–90% dysfunction of liver cells [1,2]. Patients with CLI are prone to hepatic encephalopathy and suffer from impaired ability to produce protein. A wide range of symptoms may be manifested with CLI, from cerebral oedema and multi-organ dysfunction, which may lead to coma or even death [3]. About 60% of all CLI patients developed systemic inflammation regardless of their infection status [4], which are prone to end up with failure of multiple organs. Although the pathogenic aetiology remains elusive, such systemic multi-organ failure may result from a sequence of unfavourable conditions, such as inflammation induced by oxidative stress and apoptosis.

The Janus kinase 2/signal transducer and activator of transcription 3 (JAK2/STAT3) pathway are highly implicated in the mechanism underlying inflammatory response to tissue injury [5]. The SOCS3, also known as suppressor of cytokine signalling 3, was found to inhibit the activation of JAK2/STAT3 pathway during nephropathic lesion complicated with diabetes [6]. It is reasonable to infer that SOCS3 dysregulation and JAK2/STAT3 activation play an important role in diabetes-induced tissue injury. However, no specific experiments about hepatic JAK2/STAT3 signalling pathway and SOCS3 and tissue inhibitors of metalloproteinase-1 (TIMP-1), which is dysregulated in metabolic syndrome and associated with macro and microvascular complications in patients, expression are displayed in rat models of liver dysfunction [6,7].

Lipopolysaccharide (LPS), a product derived from the cell wall of Gram-negative bacteria, was demonstrated to elicit serious global problem of sepsis and activate response of immunological system [8,9]. The uncontrolled response of the immune system to LPS could result in septic shock and systemic inflammatory [10], by which, it has been used for establishing injury model in liver, lung and kidneys, etc. [11–13]. In addition, inflammatory cells, cytokines and chemical mediators are highly implicated in pathological mechanisms underlying liver injury directly induced by LPS [14]. For instance, Kupffer cells have an abundant expression of nuclear factor kappa B (NF-κB), a type of transcription factor that could dominate the transcription of a bunch of inflammatory genes, such as TNF-α [15]. Endotoxin could activate NF-κB, which prompt us to hypothesize that targeting NF-κB...
in Kupffer cells may relieve the systemic damages by alcohol lipid disease [16,17]. Therefore, we sought to identify the link between LPS-induced injury and NF-κB signaling pathway.

Amygdalin (AG), a natural product derived from the aromatic amino acid phenylalanine, has a lot of cell-protective functions in many cell lines [18–20]. Previous studies have suggested that AG has multiple pharmacological effects, including anticancer, antibiotic, and anti-inflammation activities [21,22]. Recent studies [23] have reported that AG markedly improved glucose tolerance, and ameliorates insulin action in obese and/or diabetic patients by activating a signaling pathway related to glucose metabolism. In addition, a large body of evidence demonstrates that AG plays an important role in enhancing insulin sensitivity and reducing hyperlipidaemia [24]. Therefore, it is reasonable to speculate that this mechanism has the potential to treat acute liver injury in patients. However, the underlying mechanism of AG on improving liver injury or dysfunction remains unclear. Therefore, further study is needed to elucidate the underlying metabolism.

Materials and methods

Animal model and treatment

A total of 60 male Sprague–Dawley rats (180–220 g) were purchased from the Experimental Animal Center of Nanjing Medical University. The establishment of animal model was conducted as previously described [25]. Rats were raised at room temperature under 55 ± 5% humidity and a normal 12-h light/12-h dark cycle with the lights on, giving standard chow and water randomly. After one week of acclimatization, rats were subject to LPS or AG administration. All procedures were designed to minimize the suffering of the animals and decrease the number of animals used and strictly complied with the China Council on Animal Care. Rats were deprived of food overnight. LPS was purchased from Sigma-Aldrich, St. Louis, MO and prepared in PBS at a concentration of 10 mg/ml. AG was purchased from National Drug Research Institute, Beijing, China and prepared in PBS at a concentration of 50 mg/Kg. Rats were randomly divided into five groups as follows: (1) Control group; (2) 2.5 mg/Kg LPS; (3) 2.5 mg/Kg LPS + 0.5 mg/Kg AG; (4) 2.5 mg/Kg LPS + 1.0 mg/Kg AG and (5) 2.5 mg/Kg LPS + 1.5 mg/Kg AG. After 1 week of adaption, rats were orally treated with AG every other day for 60 d. From the 30th day, 2.5 mg/Kg LPS was administered by a single i.p. injection every 3 d for remaining 30 d. Citrate buffer was administered in normal control animal. Before blood collection, rats were deprived of food overnight. Blood was sampled from the tail of rats, which was stored in pre-ice tubes. Serum was derived by applying 4000×g to centrifugation machine under 4 °C for 6 min. Liver tissues were quickly resected from the rats and weighed for further calculation. All serum and tissue samples were stored at liquid nitrogen for subsequent experiments.

Biochemical assay

The serum content of metabolic enzymes such as alkaline phosphatase (ALP), aspartate transaminase (AST), alanine transaminase (ALT) and endotoxin (LPS) serves as an indicator of liver damage. Therefore, the above enzymes were determined to evaluate hepatic function. Quantification of enzymes was performed using common biochemical kits (Nanjing Jiancheng Biotech, Nanjing, China).

Histological assessment of the liver

After fixation in 10% neutral buffered formalin, the rat liver tissues were then embedded in paraffin. Liver histological assessment was performed as previously described [26]. A rotary microtome was employed to cut the samples into 7 μm thick sections, which were then mounted on APES coated glass slides. After deparaffinization in xylene and rehydration gradually in water containing decreasing concentrations of alcohol, the haematoxylin-eosin reagent was used to stain the specimens. Each slide was evaluated by histopathologist blinded to the functional status of the liver. The sections were investigated by light microscope.

Measurement of pro-inflammatory cytokines

After weighing of liver samples, a mixture of protease inhibitors was added to the samples. The mixture is composed of 0.002% sodium azide, 1.0 mg/ml BSA, 2 mM PMSF, 10 nM EDTA, and 0.1 mg/ml soybean trypsin inhibitor, dissolved in isotonic PBS (pH 7.0). Tissues were homogenized and then lysed to release the pro-inflammatory cytokines. The lysates were incubated at 4 °C for 3 h. Centrifugation at 10,000×g for 10 min was applied twice to extract the supernatant for protein quantification. ELISA kits (R&D Systems, Minneapolis, MN) was used to quantify IL-1β, IL-6, TNF-α, IL-1, IL-8 and IL-18 levels in the liver according to the manufacturer’s manuals [27].

Real-time quantitative PCR (RT-qPCR) analysis

Trizol reagent (Invitrogen, Carlsbad, CA) was used to extract total RNA from each rat liver according to manufacturer’s manual. mRNA expressions of IL-1β, IL-6, TNF-α, IL-1, IL-8 and IL-18, PI3K, AKT, m-TOR, SOCS3, TIMP-1, STAT3, IKKβ, IκBα, and JAK2 were evaluated by RT-qPCR. Briefly, SuperScript First-Strand Synthesis kit (RT-PCR; Invitrogen, Carlsbad, CA) was used to reversely transcribe the cDNA. Primers were designed and synthesized by Biogenes Biotechnology, and carefully checked in GenBank to ensure low similarities in between. SYBR Green PCR master mix (Applied Biosystems, Foster City, CA) was added to the PCR mixtures and then subject to programmed PCR cycles. The fluorescence produced during the PCR reaction was read by BioRad iCycler IQ Detection System. In parallel with RT-PCR of target genes, rGAPDH levels were also quantified as an internal control. Normalization and fold change for each of the genes were
Western blot

Frozen rat liver tissues were firstly homogenized in 1.5 ml RIPA buffer and the mixture was then centrifuged at 12,000 rpm for 15 min. Bradford method was applied to measure the protein level, before incubation in boiling water for 5 min. SDS-PAGE electrophoresis was performed on the total protein, which was then transferred electrophoretically to polyvinylidene difluoride (PVDF) membrane. The background protein was blocked by 5% skimmed milk for 3 h and primary antibodies were added to the membrane to incubate overnight at 4°C. HRP-conjugated goat anti-rabbit IgG (1:5000) was added to the immunoreactive bands. Immunoreactive bands were visualized using photoperoxidase Western blot detection system and quantified by densitometry using Molecular Analyst Software. Primary antibodies used were rabbit polyclonal antibodies against rP3K (1:2000), rAKT (1:2000), phosphorylation of rAKT (1:2000) and r-mTOR (1:1000), rSOS3 (1:3000), rTIMP-1 (1:2000), rα-SMA (1:2000), rCOL1A1 (1:10000), phosphorylation of rSTAT3 (1:2000), rJAK2 (1:5000), rJAK2 (1:1000), phosphorylation of rNF-κB (1:1000), rNF-κB (1:1000), rIKKβ (1:1000), rIXB (1:1000), rLIL-1 (1:1000), rLIL-18 (1:5000) and rat GAPDH (1:5000). All the antibodies were purchased from Abcam Co. Ltd, Cambridge, UK.

Statistical analysis

Numerical data were presented as the mean ± standard error of the mean deviation (SEM). One-way analysis of variance (ANOVA) was performed to detect the difference of multi comparison, and Student–Newman–Keuls’s test was then employed to assess the difference between two treatments. A p-value <.05 was considered significant. GraphPad Prism 4 (GraphPad Software, Inc. San Diego, CA) was used to derive statistical graphs.

Table 1. Primer sequences of RT-PCR analysis.

| Gene     | Forward primers (5′–3′) | Reverse primers (5′–3′) |
|----------|-------------------------|-------------------------|
| GAPDH    | CATTCAAGCCGAGCCAGAGG    | ACATACTGACAGCCAGCATACC |
| IL-1β    | CAAGGCTCTACCTCTGATGCA    | CTTAGGCTCCTTCTGCACTTG  |
| IL-6     | CAGAAGCAGCCAGGGCCA      | ACGCTGGCGACTTTCCTCA    |
| TNF-α    | GCTGCAAGAAGGAGGAGCT     | CTGCACTGGCGCTCCTCTG   |
| IL-1     | AGCACAAGAAGAGGAGCTCC    | CGTCACTGGCTGATGAGTG   |
| IL-8     | CAAAGACAGAAGTCTGAGCC    | GTGTCAGGAGCTGATGAGTG  |
| IL-18    | GACAGCAAGTGATGGGAGCT    | GTGTCAGGCTGCTGGCTGT   |
| PI3K     | AGCAACAAGAGGGATACCTGCC  | GTGTCAGGCTGCTGGCTGT   |
| AKT      | CAGAAGAAGGATGGCCC       | GTGTCAGGCTGCTGGCTGT   |
| m-TOR    | AGCTTACAGAAGGAGGAGCT    | GTGTCAGGCTGCTGGCTGT   |
| TIMP-1   | AGCCCTCCCTCTGACTATACCT  | ATTGAAAAACACACATCCTCA |
| STAT3    | CTTGGAACAGATCTGGAAAAA   | AAGGAAGCTGGAGAAGAGTG  |
| IKKβ     | AACGCTTCAGAATGGCCGT     | CTGGAAGCAGATTGGAGAAA  |
| IκBα     | CTGCCCTCGAGCCAGACA      | GTAGCAAGTTGCTGAGAGGT  |
| JAK2     | CAGAGCTCTTCTGATCAGTTG   | CCTTATGCTGCTGACCTTG  |

Results

Effects of amygdalin on body weight, relative liver weight and endotoxin levels of serum and liver in LPS-induced chronic liver injury rats

Body weight, relative liver weight and endotoxin concentration in liver and serum are shown in Figure 1. In LPS-induced liver injury rats, body weights were decreased, while relative liver weight, endotoxin levels of serum and liver were significantly increased than the normal control group. Oral administration of AG suggested that AG down-regulated LPS-induced high levels of inflammatory factors in the liver of rats.

Effects of amygdalin on hepatic steatosis and alleviates the hepatic injury in LPS-induced rats

As summarized in Figures 1 and 2, the relative weight of the liver, steatosis and inflammation scores were significantly elevated in the LPS-treated rats compared to the normal control group (Figure 2). Moreover, LPS-treated rats developed liver injury as indicated by increased serum and tissues levels of ALT, AST and ALP (Figure 3(A–F)), which were restored by 0.5, 1.0 and 1.5 mg/kg AG. The increased cell proliferation, α-SMA expression and ECM production were major events occur during phenotypic alterations in response to liver damage [28] and staining of α-SMA and collagen proteins were routinely measured to indicate liver injury [10,29]. Thus, α-SMA and COL1A1, a product of ECM, were measured to further confirm the successful establishment of liver injury rat model as well as the alleviation of AG on the hepatic injury. Quantification showed that COL1A1 and α-SMA expression were significantly elevated in LPS-induced rats, and immunostaining of them suggests fibrosis in LPS-induced rats (Figure 3(G–I)), which was restored by AG administration, indicating that liver injury was successfully established. These data suggest that pre-treatment of AG effectively prevented these changes in LPS-induced liver injury rats.

Effects of amygdalin on hepatic inflammation in LPS-induced liver injury rats

Since inflammation is involved in liver injury, we measured hepatic inflammatory factors of the liver in LPS-induced liver injury. The results showed that in our study inflammatory cytokines including IL-1β, IL-6, IL-18, IL-1, IL-8 and TNF-α mRNA levels and protein levels in liver tissue were up-regulated in LPS-induced rats with a significant statistical difference (Figure 4(A–L)). AG successfully reversed LPS-induced up-regulation of IL-1β, IL-6, IL-18, IL-1, IL-8 and TNF-α levels in rats in a typical dose-independent manner. These results suggested that AG down-regulated LPS-induced high levels of inflammatory factors in the liver of rats.
Effects of amygdalin on the PI3K/AKT and JAK2/STAT3 pathway in LPS-induced liver injury

The PI3K/AKT and JAK2/STAT3 pathway are suggested to be an essential pathogenic mechanism leading to liver dysfunction [30]. From the results of our study, the q-PCR (Figure 5) and western blot (Figure 6A–G) analysis demonstrated that the expression levels of liver PI3K ($p < .01$), AKT ($p < .01$), mTOR ($p < .05$), SOCS3 ($p < .01$), TIMP-1 ($p < .01$), STAT3 ($p < .01$) and JAK2 ($p < .01$) were elevated in LPS-treated rats. We found that this over-expression protein of liver PI3K, AKT, mTOR, TIMP-1, STAT3 and JAK2 in LPS-treated rats was suppressed remarkably by the treatment of 1.5 mg/kg AG
(p < .05), which indicates that the suppression of AG on PI3K/AKT and JAK2/STAT3 pathway may elucidate the protective function of AG.

**Effects of amygdalin on the NF-κB pathway in LPS-induced liver injury**

In LPS administrated inflammatory responses, activation of NF-κB pathway could be significantly observed in many animal models. Thus, in this part, IKKβ, IκBα, p-NF-κB, IL-1β and IL-18 protein levels were analyzed to determine the role of NF-κB pathway in LPS-induced liver injury and protective effects of AG on inhibition of activation of NF-κB. mRNA quantification showed that LPS injection could enhance the up-regulation of NF-κB pathway related IKKβ and IκBα in tissue, suggesting the NF-κB pathway was significantly activated under LPS administration in liver injury (Figure 7). Consistently, protein quantification further indicated that LPS could up-regulate the protein levels expression of the NF-κB pathway (Figure 8(A–E)). Of note, AG treatment has the ability to suppress activation of NF-κB, thereby inhibiting pro-inflammatory cytokines including IL-18 and IL-1β production. AG inhibits the NF-κB pathway in a dose-independent manner.

**Discussion**

AG is a natural compound which was originally extracted from herbs, which has been reported to possess pharmacological properties in reducing glucose levels and antimicrobial activities [31,32]. Given the antibiotic function of AG reported in previous studies, we postulated that it might alter whole body inflammation response to ameliorate the liver dysregulation and improve inflammatory response for the LPS-induced liver injury by direct and/or indirect regulation of NF-κB and PI3K/AKT and signalling pathway in tissues. In this study, we showed that AG relieved liver inflammation response by regulating key factors implicated in the onset of liver injury. Many types of research have indicated that PI3K/AKT signalling pathway is responsible for inflammatory responses in diabetic mice, regulating insulin sensitivity, obesity as well as diabetes [30,33]. In accordance with a previous study [2], our study demonstrated the elevation of protein expression of PI3K/AKT signalling pathway in LPS-induced rat liver, affecting NF-κB signalling pathway significantly and resulting in changes of cell transcription that finally develops hepatic dysfunction. These results were similar to elevation of hepatic AST, ALT and ALP, which further indicated liver injury in LPS-induced rats. Moreover, this study
suggests that in rats with liver injury, phosphorylated JAK2 could activate the transcription factor STAT3 [34,35]. In parallel with JAK2/STAT3 activation, SOCS3 was overexpressed in LPS-induced rats. SOCS3 is an intracellular negative regulator of JAK2/STAT3 pathway. We found that JAK2, STAT3 and SOCS3 were up-regulated in the liver of LPS-induced liver injury rats, indicating JAK2/STAT3 plays an important role in liver injury during the progression of LPS administration. AG was shown to alter PI3K, AKT, JAK2, STAT3, TIMP-1 and SOCS3 protein levels as well as serum and hepatic ALP, ALT and AST levels in LPS-induced rat liver injury.
Figure 5. Amygdalin inhibits PI3K/AKT, JAK2, STAT3 pathway related indicators mRNA expression. q-PCR analysis for the mRNA levels expression of PI3K, AKT, m-TOR, SOCS3, TIMP-1, STAT3 and JAK2 in liver tissues. These results are expressed as mean ± SEM, n = 10. **p < .01 compared to control group, #p < .05 and ##p < .01 compared to LPS group.

Figure 6. Amygdalin inhibits PI3K/AKT, JAK2, STAT3 pathway related indicators protein levels expression. (A-G) Western blot analysis for the protein levels expression of PI3K, AKT, m-TOR, SOCS3, TIMP-1, STAT3 and JAK2 in liver tissues. These results are expressed as mean ± SEM, n = 10–12. **p < .01 compared to control group, #p < .05 and ##p < .01 compared to LPS group.
The molecular mechanisms underlying resistance to apoptosis have long been implicating PI3K/AKT signalling and related pathways [36]. The over-activation of PI3K/AKT signalling has been found in focus tissues in patients affected by various diseases and phosphorylated AKT could stimulate the expression of apoptosis-related proteins, such as Bax and Caspase-9, and necrosis factor NF-κB, together with leading to initiation of resistance to apoptosis [37,38]. Despite the role of LPS in inducing liver injury remains elusive, the interactions between LPS activated molecules and apoptosis-related proteins amount to a postulation indicating the possible sequence of biological events. In this study, we demonstrated a reduced expression of PI3K and phosphorylated AKT in AG-treated cells, suggesting that AG exerted an inhibitory effect on AKT activation. Although further studies may be warranted to elucidate the detailed biological events, the inhibition of AG may be functional in promoting the restoration of LPS-induced liver injury.

Deep into the molecular level, LPS treatment significantly increased liver levels of IL-1β, TNF-α and IL-6 in the liver of rats. Considering the essential role of TNF-α in liver injury and IL-6, involving substantial inflammation responses, we supposed that promotion of cytokines production, such as IL-1β and IL-6, as a sign of overactive inflammation responses may result from LPS treatment, which preliminarily establishes the link between LPS and liver injury [39,40]. Furthermore, AG has been reported as an activator of NF-κB

Figure 7. Amygdalin inhibits NF-κB signalling pathways indicators expression in LPS-induced liver injury. q-PCR analysis for the mRNA levels expression of IKKβ and IκBα. These results are expressed as mean ± SEM, n = 10–12. **p < .01 compared to control group, #p < .05 and ##p < .01 compared to LPS group.

Figure 8. Amygdalin inhibits NF-κB signalling pathways in LPS-induced liver injury. (A–E) Western blot analysis for the protein levels expression of IKKβ, NF-κB, p-NF-κB, IL-1β, IL-18 and IκBα in liver tissues. These results are expressed as mean ± SEM, n = 10–12. **p < .01 compared to control group, #p < .05 and ##p < .01 compared to LPS group.
translocation by binding to the promoter sites of NF-κB thereby triggering activation of TNF-α transcription [41]. Of note, these results were recovered by the administration of AG, which down-regulated the expression of IKKβ, IkBα, p-NF-κB, IL-1β and IL-18, as well as serum IL-6 and TNF-α, suggesting that amygdalin serves as a protective role in liver injury via its anti-inflammation effects of regulating NF-κB signalling pathway.

The present results showed that AG consumption reversed most of the pathological changes of the LPS-induced liver injury of rats. The beneficial effects of AG may be due to its strong anti-inflammatory abilities and improvement of hepatic dysfunction through inhibiting PI3K/AKT, JAK2/STAT3 and NF-κB signalling pathways. Therefore, AG possessed a significantly protective effect for CLI.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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**References**

[1] Zhan W, Shang J. Amygdalin alleviates chronic liver injury by down regulating PI3 K/AKT, JAK2/STAT3 and NF-κB signalling pathways in lipopolysaccharide-induced rats. Hepatol Int. 2017;11:1.

[2] Zhong W, Qian K, Xiong J, et al. Curcumin alleviates lipopolysaccharide induced sepsis and liver failure by suppression of oxidative stress-related inflammation via PI3K/AKT and NF-κB related signaling. Biomed Pharmacother. 2016;83:302–313.

[3] Aturi DK, Prakash R, Mullen KD. Pathogenesis, diagnosis, and treatment of hepatic encephalopathy. J Clin Exp Hepatol. 2011;1:77–86.

[4] Bertolletti A, Ferrari C. Kinetics of the immune response during HBV and HCV infection. Hepatology. 2003;38:4–13.

[5] Capiralla H, Vingtdeux V, Venkatesh J, et al. Identification of potent small-molecule inhibitors of STAT3 with anti-inflammatory properties in RAW 264.7 macrophages. FEBS J. 2012;279:3791–3799.

[6] Haghikia A, Stapel B, Hoch M, et al. STAT3 and cardiac remodeling. Heart Fail Rev. 2011;16:35–47.

[7] Wen J, Friedman JR. mIR-122 regulates hepatic lipid metabolism and tumor suppression. J Clin Invest. 2012;122:2773–2776.

[8] Stoyanoff TR, Todaro JS, Aguirre MV, et al. Amelioration of lipopolysaccharide-induced acute kidney injury by erythropoietin: involvement of mitochondria-regulated apoptosis. Toxicology. 2014;318:13–21.

[9] Yeh CH, Yang JJ, Yang ML, et al. Rutin decreases lipopolysaccharide-induced acute lung injury via inhibition of oxidative stress and the MAPK-NF-κB pathway. Free Radic Biol Med. 2014;69:249–257.

[10] Pei Z, Wang J. Propofol attenuates LPS-induced tumor necrosis factor-α, interleukin-6 and nitric oxide expression in canine peripheral blood mononuclear cells possibly through down-regulation of nuclear factor (NF)-κB activation. J Vet Med Sci. 2015;77:139–145.

[11] Yao H, Hu C, Yin L, et al. Dioscin reduces lipopolysaccharide-induced in inflammatory liver injury via regulating TLR4/MyD88 signal pathway. Int Immunopharmacol. 2016;36:132–141.

[12] Yao H, Sun Y, Song S, et al. Protective effects of dioscin against lipopolysaccharide-induced acute lung injury through inhibition of oxidative stress and inflammation. Front Pharmacol. 2017;8:120.

[13] Qi M, Yin L, Xu L, et al. Dioscin alleviates lipopolysaccharide-induced inflammatory kidney injury via the microRNA let-7i/TLR4/MyD88 signaling pathway. Pharmacol Res. 2016;111:509–522.

[14] Sindram D, Porte RJ, Hoffman MR, et al. Synergism between platelets and leukocytes in inducing endothelial cell apoptosis in the cold ischemic rat liver: a Kupffer cell mediated injury. FASEB J. 2001;15:1230.

[15] Xie X, Ye Y, Zhou L, et al. Kupffer cells promote acute rejection via induction of Th17 differentiation in rat liver allotransplants. Transplant Proc. 2010;42:3784–3792.

[16] Tiegs G, Lohse AW. Immune tolerance: what is unique about the liver. J Autoimmun. 2010;34:1–6.

[17] Jones C, Badger SA, Hopper M, et al. Hepatic cytokine response can be modulated using the Kupffer cell blocker gadolinium chloride in obstructive jaundice. Int J Surg. 2013;11:46–51.

[18] Chang H, Yang H, Lee T, et al. Armeniace semen extract suppresses lipopolysaccharide-induced expressions of cyclooxygenase-2 and inducible nitric oxide synthase in mouse BV2 microglial cells. Biol Pharm Bull. 2005;28:449–454.

[19] Fukuda T, Ito H, Mukainaka T, et al. Anti-tumor promoting effect of glycosides from *Prunus persica* seeds. Biol Pharm Bull. 2003;26:271–273.

[20] Chang H, Shin M, Yang H, et al. Amygdalin induces apoptosis through regulation of Bax and Bcl-2 expressions in human DU145 and LNCaP prostate cancer cells. Biol Pharm Bull. 2006;29:1597–1602.

[21] Jeong S, Lim H, Seo C, et al. Anti-inflammatory actions of herbal formula gyejibokryeong-hwan regulated by inhibiting chemokine production and STAT1 activation in HaCat cells. Biol Pharm Bull. 2015;38:425–434.

[22] Perez JJ. Amygdalin analogs for the treatment of psoriasis. Future Med Chem. 2013;5:799–808.

[23] Zhao F, Yang Z. Amygdalin attenuates atherosclerosis progress through inhibiting of toll-like receptors expression and activity. J Anim Vet Adv. 2012;11:1613–1621.

[24] Paolletti I, De Gregorio V, Baroni A, et al. Amygdalin analogues inhibit IFN-γ signalling and reduce the inflammatory response in human epidermal keratinocytes. Inflammation. 2013;36:1316–1326.

[25] Kang LL, Zhang DM, Ma CH, et al. Cinnamaldehyde and allopurinol reduce fructose-induced cardiac inflammation and fibrosis by attenuating CD36-mediated TLR4/6/IRAK4/1 signaling to suppress NLRP3 inflammasome activation. Sci Rep. 2016;6:27460.

[26] Wang C, Pan Y, Zhang Q, et al. Quercetin and allopurinol ameliorate kidney injury in STZ-treated rats with regulation of renal NLRP3 inflammasome activation and lipid accumulation. PLoS One. 2012;7:e38285.

[27] Wang W, Wang C, Ding X, et al. Quercetin and allopurinol reduce liver thioredoxin-interacting protein to alleviate inflammation and lipid accumulation in diabetic rats. Br J Pharmacol. 2013;169:1352–1371.

[28] Zhang H, Han X, Yin L, et al. Fibrosis. Biomed Res Int. 2015;2015:1–11.

[29] Yin L, Qi Y, Xu Y, et al. Dioscin inhibits HSC-T6 cell migration via adjusting SDC-4 expression: insights from iTRAQ-based quantitative proteomics. Front Pharmacol. 2017;8:1–10.

[30] Chen K, Li G, Geng F, et al. Berberine reduces ischemia/reperfusion-induced myocardial apoptosis via activating AMPK and PI3K-Akt signaling in diabetic rats. Apoptosis. 2014;19:946–957.

[31] Minaiyan M, Ghannadi A, Asadi M, et al. Anti-inflammatory effect of *Prunus armeniaca* L. (Apricot) extracts ameliorates TNBS-induced ulcerative colitis in rats. Res Pharm Sci. 2014;9:225–231.

[32] Song Z, Xu X. Advanced research on anti-tumor effects of amygdalin. J Cancer Res Ther. 2014;10:3.
[33] Yan X, Qiu W, Jia B, et al. Myocardial protection by interferon-γ late preconditioning during cardiopulmonary bypass-associated myocardial ischemia-reperfusion in pigs. Oncol Rep. 2013;30:2145–2152.

[34] Constantinescu SN, Girardot M, Pecquet C. Mining for JAK-STAT mutations in cancer. Trends Biochem Sci. 2008;33:122–131.

[35] Minakhina S, Tan W, Steward R. JAK/STAT and the GATA factor Pannier control hemocyte maturation and differentiation in Drosophila. Dev Biol. 2011;352:308–316.

[36] Elmore SA. Apoptosis: a review of programmed cell death. Toxicol Pathol. 2007;35:495–516.

[37] Jin Z, Eldeiry WS. Overview of cell death signaling pathways. Cancer Biol Ther. 2005;4:139–163.

[38] Shin DY, Kim G, Lee JH, et al. Apoptosis induction of human prostate carcinoma DU145 cells by diallyl disulfide via modulation of JNK and PI3K/AKT signaling pathways. Int J Mol Sci. 2012;13:14158–14171.

[39] Seki E, De Minicis S, Osterreicher CH, et al. TLR4 enhances TGF-beta signaling and hepatic fibrosis. Nat Med. 2007;13:1324–1332.

[40] Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. Cell. 2006;124:783–801.

[41] Simone RE, Russo M, Catalano A, et al. Lycopene inhibits NF-κB-mediated IL-8 expression and changes redox and PPARγ signaling in cigarette smoke-stimulated macrophages. PLOS One. 2011;6:e19652–63.