Hyperin attenuates inflammation by activating PPAR- γ in mice with acute liver injury (ALI) and LPS-induced RAW264.7 cells

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Abbreviations

HP Hyperin

ALI acute liver injury

LPS lipopolysaccharide

PPAR- γ peroxisome proliferatoractivated receptor gamma

23	ERK	extracellular signal-regulated kinase
24	TNF- α	tumor necrotic factor alpha
25	IL-6	Interleukin-6
26	AST	aspartate transaminase
27	ALT	alanine transaminase
28	siRNA	small interfering RNA
29	ELISA	Enzyme-linked immunosorbent assay
30	PCR	Polymerase chain reaction

31 **Abstract**

32 Hyperin(HP), a flavonoid compound found in various plants like Ericaceae, Guttifera
33 and Celastraceae. The present study has revealed that HP has a variety of
34 pharmacological effects including anti-oxidant, anticancer, and anti-coagulant,
35 especially anti-inflammatory. However, the potential molecular mechanism of
36 anti-inflammatory is still unrevealed. In this study, HP not only significantly
37 attenuated inflammation in C57BL/6J mice with acute liver injury (ALI), but also
38 reduced the expression of TNF- α and IL-6 in lipopolysaccharide (LPS)-induced
39 RAW264.7 cells. Furthermore, our findings showed that HP remarkably induced the
40 expression of PPAR- γ *in vivo* and *in vitro*. Interestingly, compared with the HP
41 treatment group, a specific blocking agent of PPAR- γ T0070907 and PPAR- γ small
42 interfering (si)-RNA-mediated silencing in RAW264.7 cells were used to evaluate the
43 involvement of HP in alleviating LPS-induced inflammation. More importantly,
44 over-expression of PPAR- γ had an opposite effect on the expression of TNF- α and

IL-6 in LPS-induced RAW264.7 cells after treatment with HP. In addition, HP remarkably inhibited the expression of P-ERK1/2 and P-P38 MAPK. Taken together, all above results indicate that HP may serve as an effective modulator of PPAR- γ , further down-regulating ERK1/2 and p38 MAPK during the pathogenesis of inflammation.

Keywords: Hyperin; PPAR- γ ; ERK1/2; P38 MAPK; acute liver injury (ALI)

1. Introduction

Acute liver injury (ALI) is associated with an unacceptably high degree of morbidity and mortality. Furthermore, ALI can cause permanent structural damage, with progressive fibrosis and long-term repercussions for liver function [1]. Inflammation reaction is a significant development of the initiation and exacerbation of ALI [2]. Inflammation is a fundamental mechanism of diseases caused by microbial, autoimmune and metabolic factors [3]. Liver inflammation causes fibrillation and disability. It has been reported that liver macrophages play an important role in modulating inflammation through phagocytosis, cytokine production, and antigen presentation [4, 5]. Inflammatory cytokines can also induce production of other inflammatory mediators [6]. It widely accepted that liver inflammatory cytokines such as TNF- α and IL-6 play a significant role in the pathogenesis of liver inflammation [7].

Moreover, LPS stimulates liver macrophages to induce MAPK activation, which finally induces the release of TNF- α and IL-6 [8]. Accumulating evidence suggests that neutralization of LPS-induced inflammatory mediators appears to be useful in the treatment of liver inflammation [9]. Moreover, PPAR- γ has been proved to have anti-inflammation effects in various macrophage models, inhibiting the expression of many pro-inflammation genes, including, IL-6, TNF- α and IL-12 [10, 11].

Plant species of the genus *Hypericum* are famous for their use in traditional medicine because of their therapeutic efficacy [12]. Hyperin (HP) is one of the chief flavonoid components of Ericaceae, Guttifera, Leguminosae and Celastraceae. It has been reported that HP has obvious anti-inflammatory properties [3]. However, the potential molecular mechanism is still unrevealed. *In vivo*, HP was found to protect the heart against ischemia and reperfusion lesions [13]. Nevertheless, studies that their protective effect on acute liver lesions remains few. Thus, we investigated the anti-inflammatory effects of HP and the potential mechanisms on C57BL/6J mice with ALI and LPS-induced RAW264.7 cells.

2. Materials and Methods

2.1. Materials and Reagents

Hyperin (HPLC $\geq$ 98%) was purchased from Preferred (Chengdu, China). LPS (*Escherichia coli* 055:B5), and dimethyl sulfoxide (DMSO) and T0070907 (T8703) were purchased from Sigma Chemical (St. Louis, MO, USA). Mouse

monoclonal antibodies β -actin was purchased from Santa Cruz (California, USA) and Rabbit ERK1/2, P-ERK1/2, P38, P-P38 polyclonal antibody were purchased from Bioss (Beijing, China). Rabbit TNF- α and IL-6 polyclonal antibody were purchased from abcam (Cambridge, UK). Enzyme Linked Immunosorbent Assay (ELISA) kit was acquired from Hushang Biotechnology Co. Ltd (Shanghai, China). ALT and AST activity assay kit were purchased from Jiancheng (Nanjing, China).

2.2. *Animal treatment*

C57BL/6J mice were provided by the Experimental Animal Center of Anhui Medical University. Animal experiments were conducted under principles in good laboratory animal care, and approved by ethical committee for Laboratory Animals Care and Use of Anhui Medical University. C57BL/6J mice were injected intraperitoneally with a single dose of 1% CCl₄ (10 ml/kg in oil), to induce acute liver injury by CCl₄ after gavaging different concentrations of HP(50 mg/kg, 100 mg/kg, 200mg/kg and 400mg/kg) for three days . Control groups received the same volume of vehicle (oil) intraperitoneally.

2.3. *ALT/AST activity assay*

ALT and AST levels in serum from C57BL/6J mice with the acute liver injury, were assayed using ALT and AST activity assay kits. The absorbance at 510 nm was obtained with a Multiskan MK3 (Biotek , USA).

2.4. Histopathology

The middle portion of the left lobe of the liver of each C57BL/6J mice was excised and sectioned and then perfused in 4% paraformaldehyde for at least 24 h. After fixation, the tissues were embedded in paraffin, and 5µm thick sections were then stained with hematoxylin and eosin (H&E) to observe the tissue damage by light microscopy.

2.5. Isolation of liver macrophages

Liver Macrophages cell were isolated following a method established previously [14]. Briefly, a 20-G catheter was put through mouse the portal vein, and the inferior vena cava were cut. The liver was per fused with PB (dilute 40 ml of PBC [dissolve NaCl (103.75 g), KCl (6.25 g) and Hepes (28.70 g) in H₂O (350 ml) while stirring. When all has been dissolved add 1M NaOH (75 ml). Add H₂O to a total volume of 500 ml.] by milliQ water to a total volume 1 litre), followed by a digestion buffer [1×PBC, supplemented with type IV collagenase (Sigma, St. Louis, MO, USA), Pronase (Sigma, St. Louis, MO, USA) and 4.76 mM CaCl₂. After digestion, the liver was disrupted in BSA solution. Single cells were passed through 200-mesh sieve cell strainer, and the cells were fractionated using 25% Percoll and 50% Percoll (Sigma, St. Louis, MO, USA).

2.6. Cell Culture

RAW264.7 cells were obtained from Cell Bank of Academy of Sciences

(Shanghai, China). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM; HyClone, USA) with supplemented with 10 % (vol/vol) heat-inactivated fetal bovine serum (Bovine, China) at 37°C humidified incubator under 5% CO₂. Cells were cultured by adding different concentrations of HP for 48 h, or adding 1000 ng/ml LPS for 6h and then adding different concentrations of HP (5μM, 10μM, 20μM, 50μM and above) for 48 h in the suitable conditions.

2.7. RNA Interference Analysis

Total RNA was extracted from liver Macrophages and RAW264.7 by using TRIzol (Invitrogen) according to the manufacture's instruction, and reverse transcribed to cDNA using TAKARA kit (Japanese). The reaction mixture was prepared according to the manufacture's instruction using SYBRGreen q-PCR Master Mix (QIAGEN). The mRNA expression of mouse PPAR-γ, TNF-α and IL-6 were detected by the quantitative real-time PCR (q-PCR). The primers used were listed as following: GAPDH (forward: 5'-GGACCTCATGGCCTACATGG-3'; reverse: 5'-TAGGGCC TCTCTTGCTCAGT-3'), PPAR-γ (forward: 5'-CTGCATGTGATCAAGAAGAC-3'; reverse: 5'-AGTGCAATCAATAGAAGGAAC-3'). PCR was performed 95°C for 10 minutes followed by 40 cycles at 95°C for 15 seconds and at 60°C about 1 minute by using Thermo Step One. Reactions were conducted three times, and threshold cycle values were normalized to GAPDH gene expression. The specificity of the products was determined by melting curve analysis. Relative mRNA expression of target genes were obtained by normalized to control group and the level of GAPDH.

2.8. Western Blot

Liver Macrophages and RAW264.7 cells were lysed in protein extraction solution for Western (Beyotime, China). Protein concentration of the extract was determined by using NanoDrop 2000 Spectrophotometer (Thermo scientific, USA). Equal amounts of protein were electrophoresed on SDS-PAGE and blotted onto PVDF membranes (Millipore Corp, Billerica, MA, USA). After blockade of nonspecific protein binding, nitrocellulose blots were incubated for 6 h with primary antibodies diluted in primary antibody dilution buffer (Beyotime, China). The primary antibodies recognizing PPAR- γ , ERK1/2, P-ERK1/2, P38, P-P38, TNF- α , IL-6 and β -actin were used 1:500, 1:500, 1:500, 1:1,000, 1:1,000, 1:300, 1:300 and 1:3000, respectively. The membranes were then incubated in TBST containing 5 % skim milk at 37°C for 3 h, and with specific primary antibodies at 4°C overnight. The next day, the membranes were washed 3 times with TBS/Tween20 (0.075%), followed by incubation with HRP-conjugated secondary antibodies (1:8000–1:10000) at 37°C for 1 h. After washing three times with TBST (Boster, China), proteins were visualized with ECL-chemiluminescent kit (ECL-plus, Thermo Scientific, USA).

2.9. Cytokine assay by ELISA

RAW264.7 cells (2×10^5 /well) were plated in 24-well culture plates for 12 h. Then, the cells were treated with HP 42 h before LPS treatment. Six hours later, the culture medium was collected and the concentrations of TNF- α and IL-6 in the culture

medium were measured with an ELISA kit (Hushang Biotechnology Co. Ltd Shanghai, China) according to manufacturer's instructions.

2.10. Statistical Analysis

Statistical analysis was completed with the Statistical package for Social Sciences (SPSS Inc., Chicago, IL, USA, version 13.0). These results give a summary of the data from at least-three experiments, and are presented as the mean $\pm$ SEM. Statistical evaluation of the results was performed by one-way ANOVA. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Anti-inflammatory effects of HP in vivo

To investigate the effect of HP on mice with ALI, histopathological study was performed. H&E staining showed that CCl₄ (1%) injection caused remarkable ALI [15] including hepatocyte necrosis and inflammatory cells infiltrations as compared with the control group and counting of inflammatory cell accumulation by H&E staining in Fig. 1A. Meanwhile, the serum levels of ALT (Fig.1B) and AST (Fig.1C) were markedly elevated in mice with ALI. Western blot analysis showed that the expression of TNF- α and IL-6 was obviously increased in liver macrophages isolated from mice with ALI (Fig.1D). These results suggest that mice with ALI have remarkably inflammatory reaction.

Next, HP (50 mg/kg, 100 mg/kg, 200mg/kg and 400mg/kg) efficiently prevented

both hepatocyte destructions and inflamed cell infiltrations in the hepatic tissue, as shown by H&E staining compared with model group, respectively (Fig.1A). Meanwhile, pretreatment with HP (200mg/kg) significantly attenuated the increase of serum ALT (Fig.1B) and AST (Fig.1C) levels compared with that in the model group, respectively. Furthermore, the expression of TNF- α and IL-6 was remarkably reduced in isolated liver macrophages (Fig.1D). In conclusion, these results suggest that HP have an obvious anti-inflammatory effect *in vivo*.

3.2. Anti-inflammatory effects of HP *in vitro*

The secretion of inflammatory cytokines is a pivotal cellular response to infection [6, 7]. Thus, the effect of HP on the expression of TNF- α and IL-6 was examined. RAW264.7 cells were treated with 1000ng/ml LPS for 6 hours and then added different concentrations of HP for 48 hours. As shown in Fig. 2A and 2B, at low dose concentrations (5 μ M, 10 μ M, 20 μ M), HP had no significant anti-inflammatory effect on the expression of TNF- α and IL-6 compared with the LPS-induced cells. However, higher dose concentrations (50 μ M and above) of HP showed a significant anti-inflammatory effect on LPS-induced RAW264.7 cells. In addition, western blot analysis showed that expression of TNF- α and IL-6 were remarkably decreased in LPS-induced RAW264.7 cells after the treatment with HP (50 μ M) (Fig. 2C). Thus, 50 μ M HP was employed in the following experiments.

3.3. HP activates PPAR- γ expression in mice with ALI and LPS-induced RAW264.7

cells

It has been reported that PPAR- γ reduces the systemic inflammatory response [16, 17]. Therefore, the effect of HP on PPAR- γ activation in mice with ALI and LPS-induced RAW264.7 cells was investigated. The results of western blot showed that HP promoted the protein expression of PPAR- γ in liver macrophages isolated from ALI mice (Fig. 3A). Moreover, the effects of HP on the expression of PPAR- γ in LPS-induced RAW264.7 cells were investigated. The results showed the expression of PPAR- γ was observably increased in LPS-induced RAW264.7 cells treated with HP (Fig. 3B). Thus, our findings suggest HP can up-regulate PPAR- γ in mice with ALI and LPS-induced RAW264.7 cells.

To explore the mechanism underlying HP has anti-inflammatory activities by up-regulating PPAR- γ , we used T0070907 and PPAR- γ siRNA to reduce PPAR- γ expression in LPS-induced RAW264.7 cells. More significantly, ELISA analysis (Fig.3E,F) showed the expression of TNF- α and IL-6 was observably increased, which indicated the anti-inflammatory effect of HP *in vitro* was remarkably weakened. However, after treating with HP, western blot and q-PCR (Fig.3C,D) analysis showed PPAR- γ expression were remarkably increased, as well TNF- α and IL-6 production was observably reduced. Conversely, over-expression of PPAR- γ (Fig.3G), the anti-inflammatory effects of HP was more pronounced. The expression of TNF- α and IL-6 was reduced remarkably could proved this finding (Fig. 3H).

Taken together, our results indicated that HP had an anti-inflammatory effect by activating PPAR- γ *in vivo* and *in vitro*.

3.4. HP suppresses P-ERK1/2 and P-P38 expression *in vivo* and *in vitro*

It has been reported that MAPK is involved in the regulation of inflammatory mediator production, especially P-ERK1/2 and P-P38 MAPK activities [18, 19]. Thus, the effect of HP on P-ERK1/2 and P-P38 expression *in vivo* and *in vitro* was investigated. The result of western blot showed that HP significantly inhibited the expression of P-ERK1/2 and P-P38 in liver macrophages isolated from mice with ALI (Fig. 4A) and LPS-induced RAW264.7 cells (Fig. 4B). Thus, our findings suggested HP could attenuate P-ERK1/2 and P-P38 expression.

3.5. The expression of P-ERK1/2 and P-P38 MAPK are attenuated by up-regulating PPAR- γ in LPS-induced RAW264.7 cells

To further explore the effect of PPAR- γ on P-ERK1/2 and P-P38 MAPK activity, firstly, the cells were subjected to 25 μ M of T0070907 for 6 h before the treatment with LPS. The results of western blot (Fig. 5A) revealed that the up-regulation of PPAR- γ remarkably suppressed P-ERK1/2 and P-P38 MAPK expression in LPS-induced RAW264.7 cells. Secondly, PPAR- γ siRNA in RAW264.7 cells was used to evaluate the involvement of MAPKs (P-ERK1/2 and P-P38) in relieving LPS-induced inflammation. Cells were pretreated with PPAR- γ siRNA and then subjected to LPS for 6 h. Results of western blot (Fig. 5B) revealed that after the cells were treated with LPS and PPAR- γ siRNA, a significant increase in the expression of P-ERK1/2 and P-P38 was observed. Finally, over-expression plasmid of PPAR- γ

(pEX-2-PPAR- γ) was used to assess the involvement of MAPKs (P-ERK1/2 and P-P38) in alleviating LPS-induced inflammation in RAW264.7 cells. Due to the up-regulation of PPAR- γ , the expression of P-ERK1/2 and P-P38 had an obvious drop (Fig. 5C). These findings showed that PPAR- γ could modulate MAPK pathway including P-ERK1/2 and P-P38 signaling in LPS-induced RAW264.7 cells.

4. Discussion

Inflammation is a physiological reaction aiming at defending the organism against initiating the healing process and injurious stimuli in order to recover tissue homeostasis [20]. ALI induced inflammation has been recognized as an important pathological outcome of clinical syndromes including severe coagulopathy, and high rates of mortality [21]. Inflammation is a major component of the initiation and exacerbation of ALI [22]. It has been reported that macrophages are pivotal cells of the immune system that are constituted in response to an infection or accumulating damaged or dead cells, which play a significance role in the maintenance, initiation and resolution of inflammation. Furthermore, conclusive evidence indicated the involvement of pro-inflammatory cytokines such as tumor necrosis factor (TNF)- α , interleukin-6 (IL-6) in ALI induced inflammation response. Accumulating evidences suggest that inhibition of these inflammatory mediators has therapeutic potential in the treatment of ALI [23]. In this study, our results showed that HP remarkably inhibited TNF- α and IL-6 expression in isolation of liver macrophages from C57BL/6J mice with ALI and LPS-induced RAW264.7.

Hyperin, a bioactive component isolated from Ericaceae, Guttifera and Celastraceae, has been described to exhibit a remarkable anti-inflammatory and immunoregulatory effects [24]. However, there are only few correlational studies available related to HP, especially anti-inflammatory effects studies. Thus, we investigated the anti-inflammatory effects of HP in isolation of liver macrophages from C57BL/6J mice with the ALI and LPS-induced RAW264.7.

PPAR- γ is the most ample PPAR subtype in white adipose tissue, and its main function is an administrator of insulin sensitivity, fatty acid uptake and glucose homeostasis [25]. It has been regarded as the receptor of the thiazolidinedione, a resultant chemical among anti-diabetic drugs, and in consequence, it could be targeted by many drug candidates [26]. However, PPAR- γ specific agonists exhibit long-term side effects on the health of certain patients, such as increased fluid retention, body weight and increased risk of cardiac failure [18]. Although not explicitly demonstrated, it is very likely that the side effects result from high doses of full agonists, such as Pioglitazone [27], Bezafibrate [28] and metaglidasen [29]. Therefore, partial enhancing PPAR- γ may be a better way to overcome the extreme side effect caused by using full agonists. Moreover, studies indicated that many natural compounds could activate PPAR- γ and produced slight even no side effects [30-32]. More importantly, PPAR- γ has been known to have remarkably anti-inflammatory activities [16, 17]. So, we can find a natural compound instead of chemical agonists to produce anti-inflammatory effects by activating PPAR- γ . Thus, we focus on that the effect of Hyperin regulated PPAR- γ . In this study, our results showed that HP inhibited

inflammatory response in C57BL/6J mice with ALI and LPS-induced RAW264.7 by promoting the expression of PPAR- γ , it showed that HP might act as an agonist of PPAR- γ to activating PPAR- γ to relieve inflammatory response.

MAPKs, mediate the signal transduction involved in cell proliferation, differentiation, transformation, survival, and death [33]. It has been known that MAPK signaling pathways play critical roles in the regulation of inflammatory mediators [34, 35]. Especially, ERK1/2 and p38 MAPK signaling pathways serve as main signaling pathways in inflammatory response [18, 19]. It has been reported that stimulating of RAW264.7 by LPS induces phosphorylation and activation of ERK1/2 and p38 MAPKs [11, 36]. In the present study, we found that blocking of PPAR- γ by T0070907 or PPAR- γ siRNA or over-expression plasmid of PPAR- γ , PPAR- γ regulating ERK1/2 and p38 MAPK expression could be proved. Taken together, these results suggested that PPAR- γ subsequently inhibited LPS-induced ERK1/2 and p38 MAPK activation in RAW264.7.

5. Conclusion

Our work explored the potential therapeutic effect of a flavonoid compound HP, most likely through activating PPAR- γ to suppress inflammation. In addition, inhibition of PPAR- γ by T0070907 or PPAR- γ siRNA, the anti-inflammatory effects of HP was reversed, the results showed that HP up-regulated the expression of PPAR- γ in a dose-dependent manner. Our findings demonstrated that HP inhibited CCl₄-induced and LPS-induced inflammatory cytokines TNF- α and IL-6 production

in vivo and *in vitro*. Administration of HP significantly attenuated inflammation and regulated the expression of PPAR- γ in C57BL/6J mice with ALI. Moreover, we have proven HP indeed inhibited MAPK signaling pathways activation. The mechanism of HP action involves activation of PPAR- γ functions as it enhances the activities of PPAR- γ and promotes the expression level of its target genes. Importantly, our study suggests that activation of PPAR- γ is indispensable for HP to regulate ERK1/2 and p38 MAPK activation in LPS-induced RAW264.7 cell (Fig. 6).

Collectively, our findings may support a novel role of HP, as an effective modulator of PPAR- γ expressional activity and a potential therapeutic choice in dampening inflammation and inflammatory diseases.

Conflict of interest

None.

Acknowledgments

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A **B** **C** **D**

A

B

C

Figure legend:

Fig.1. H&E staining were conducted and examined under microscopy ($\times 100$) and counting of inflammatory cell accumulation by H&E staining (A). CCl₄ (1%) injection dramatically increased serum ALT(B) and AST(C), whereas HP pretreatment significantly attenuated these increases of both serum AST and ALT levels as compared with the model group. HP reduced TNF and IL-6 expression markedly compared with the model group (D). All values are expressed as mean $\pm$ SEM. [#]P < 0.05, ^{##}P < 0.01 compared with control group, *P < 0.05, **P < 0.01 compared with the model group.

Fig.2. TNF- α (A) and IL-6 (B) production was measured by ELISA assay. Cells were cultured with different concentrations of HP (5 μ g/ml, 10 μ g/ml, 50 μ g/ml, 100 μ g/ml) for 48 h. HP inhibited TNF- α and IL-6 production. HP (50 μ g/ml) was relatively appropriate concentration. All values are expressed as mean $\pm$ SEM. ^{##}P < 0.01 compared with control group, *P < 0.05, **P < 0.01 compared with LPS-induced group. TNF- α and IL-6 production was measured by western blot (C). All values are expressed as mean $\pm$ SEM. [#]P < 0.05, ^{##}P < 0.01 compared with control group, **P < 0.01 compared with LPS-induced group.

Fig.3. Western blot analysis(A) showed that HP promoted the protein expression of PPAR- γ in liver macrophages isolated from ALI mice. RAW264.7 cells were cultured

with LPS or LPS plus HP (50 μ g/ml) for 48 h. The relative protein levels and mRNA levels of PPAR- γ in the RAW264.7 cells or the primary peritoneal macrophages from C57/B6 mice with ALI were measured by q-PCR and western blot (B), GAPDH and β -actin served as a control. (All values are expressed as mean $\pm$ SEM. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared with the control group; $^*P < 0.05$, $^{**}P < 0.01$ compared with LPS-induced group) Western blot and q-PCR (C) analysis showed PPAR- γ expression had a remarkable drop. On the contrary, ELISA analysis (D) showed the expression of TNF- α and IL-6 was increased observably ($^{\#\#}P < 0.01$, compared with the control group; $^*P < 0.05$, $^{**}P < 0.01$ compared with LPS-induced group, $^{\&}P < 0.05$ compared with the LPS plus T0070907 group). As showed in E, the expression of PPAR- γ was obviously reduced. Oppositely, the production of were obviously increased by ELISA analysis (F). ($^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared with LPS plus Nc group, $^*P < 0.05$, $^{**}P < 0.01$ compared with the LPS plus TNF- α and IL-6 PPAR- γ siRNA group). The expression of PPAR- γ mRNA and protein (G) were significantly increased after when the cells were treated with pEX-2-PPAR- γ . On the contrary, ELISA analysis (H) showed that the production of TNF- α and IL-6 were remarkably reduced in RAW246.7. ($^{\#}P < 0.05$ compared with the LPS plus pEX-2 group, $^*P < 0.05$, compared with LPS plus pEX-2- PPAR- γ group, $^{\&}P < 0.01$, compared with the LPS plus HP plus pEX-2 group)

Fig.4. Western blot analysis (A) showed that HP inhibited the expression of P-ERK1/2 and P-P38 protein in isolation of liver macrophages. HP inhibited the expression of

P-ERK1/2 and P-P38 by western blot and q-PCR (B) analysis in RAW264.7 cells. (All values are expressed as mean $\pm$ SEM. [#]P < 0.05, ^{##}P < 0.01 compared with the control group, *P < 0.05, **P < 0.01 compared with the CCl₄-induced group, LPS-induced group).

Fig.5. Western blot (A) analysis showed that the down-regulation of PPAR- γ obviously promoted P-ERK1/2 and P-P38 expression in RAW264.7 cells (All values are expressed as mean $\pm$ SEM. ^{##}P < 0.01 compared with the control group, *P < 0.05, **P < 0.01 compared with LPS-induced group). While, Western blot (B) analysis showed P-ERK1/2 and P-P38 were activated remarkably after cells were treated with PPAR- γ si-RNA for 12 h. ([#]P < 0.05, ^{##}P < 0.01, compared with LPS plus Nc group). The expression of P-ERK1/2 and P-P38 were obviously reduced because of the up-regulation of PPAR- γ by pEX-2- PPAR- γ (C). ([#]P<0.05 compared with the pEX-2 group, *P < 0.05, **P < 0.05 compared with LPS plus pEX-2 group).

Fig.6. HP activated target genes of PPAR- γ and up-regulated the expression of PPAR- γ in a dose-dependent manner in LPS-induced RAW264.7 cell. Then, it could inhibit the production of TNF- α and IL-6 through suppressing ERK1/2 and p38 MAPK activation.