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Protective effect of Chrysophanol on LPS/D-GalN induced hepatic injury through RIP140/NF-κB pathway

Wenjiao Jiang, †[a] Rui Zhou, †[b] Peijin Li,[a] Yilin Sun,[a] Qianfeng Lu,[a] Yue Qiu,[c] Jinglei Wang,[d] Jingyan Liu,[b] Kun Hao*[a] and Xuansheng Ding *[ae]

[a]State Key Laboratory of Natural Medicines, China Pharmaceutical University, Nanjing 210009, China
[b]Department of Physiology and Pharmacology, China Pharmaceutical University, Nanjing 210009, China
[c]School of Pharmacy, China Pharmaceutical University, Nanjing 210009, China
[d]School of Life Sciences, China Pharmaceutical University, Nanjing 210009, China
[e]Department of Clinical Pharmacy, China Pharmaceutical University, Nanjing 210009, China

† Wenjiao Jiang and Rui Zhou contributed equally to this work

To whom correspondence should be addressed at: haokun@cpu.edu.cn (K.Hao), dxs0162@sina.com (X. Ding).

Abstract

Chrysophanol(Chr), one of the major anthraquinone which are extracted from Rhubarb, has been reported to possess various pharmacological properties including anti-cancer, antimicrobial and anti-inflammatory effects. The purpose of this current study was to investigate the possible protective effects of chrysophanol for the intervention of lipopolysaccharide (LPS)/D-galactosamine (GalN)-challenged acute liver injury in mice. LPS (50 μg/kg) and D-GalN (800 mg/kg) were injected i.p. 1 h after chrysophanol (1,10mg/kg) pretreatment on day 7. The results demonstrated that chrysophanol showed a trend for protection against acute liver injury, as evidenced by the alleviations of hepatic pathological damage, serum ALT and AST levels, as well as the serum and hepatic productions of tumor necrosis factor-a(TNF-α), interleukin-6(IL-6), IL-10. Moreover, the treatment with Chr attenuated the levels of malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) and glutathione peroxidase (GSH-Px) in hepatic tissues. Chrysophanol also significantly attenuated hepatic iNOS expression by inhibiting RIP140 and NF-κB activation, which could observed through western blotting analysis. Simultaneously, in this present study, treatment of chrysophanol decreased the ratio of Bax/Bcl-2, caspase-3 and caspase-8 expressions, which suggested its anti-apoptotic property. Taken together, the conclusion mirrored by our results was that chrysophanol prevented against LPS/D-GalN-induced liver injury through the induction of antioxidant defense, suppression of apoptosis and reduction inflammatory response via inhibiting RIP140/NF-κB pathway.

Key word: Chrysophanol, Lipopolysaccharide (LPS), Acute liver injury, RIP140, NF-κB

1. Introduction

Liver injury, a common clinical disease associated with high mortality, is attributed to some susceptibility factors such as alcohols, chemicals and viruses [1]. In clinical study, endotoxemia or sepsis become one of the syndrome investigated frequently among the patients leading to liver failure [2]. Unfortunately, effective pharmacological interventions for treating acute liver injury still remain poor. Therefore, the discovery and development of efficient drugs aimed at liver therapy is needed urgently. In addition, exposure to LPS/D-GalN by intraperitoneal instillation is a well-recognized model to mimic fulminant hepatic failure in patients [3]. The interaction of LPS with hepatocytes was found to be highly correlated positively with the regulation of pro-inflammatory mediators [4, 5]. LPS/D-GalN mixture at the dose of LPS(50 μg/kg body weight) and D-GalN(800 mg/kg body weight) dissolved in PBS was injected intraperitoneally to induce acute hepatic injury.

LPS/D-GalN stimulation results in the overexpression of Receptor-interacting protein 140 (RIP140). RIP140 promotes NF-κB to produce various cytokines as exemplified by tumor necrosis factor-alpha (TNF-α) which have been proved to play essential role in the pathogenesis of liver injury [6, 7]. IL-10 is a crucial anti-inflammatory mediator in inhibiting other pro-inflammatory cytokine production [8]. Moreover, LPS can stimulate the production of the inflammatory factors, nitric oxide (NO) inducible nitric oxide synthase (iNOS), respectively [9]. Hence, we propose that the repression of pro-inflammatory mediators through a variety of diverse routes could exert a potent preventive effec on the treatment or prevention of acute liver injury.

Nature products have been widely applied to treat various diseases [10, 11]. Chrysophanol(Chr), one of the major anthraquinone isolated from Rhubarb, is attracting the growing number of attention due to its numerous biological activities, such as anti-inflammatory activity [12] and anti-microbial activity [13]. As an important member of traditional Chinese medicines, Rheum palmatum is used for patients with liver and gasteroenteritic diseases [14]. However, there is no report to explain the pharmacological effect of Chrysophanol on hepatic injury or the underlying mechanism. In the present study, we sought to evaluate the hepatoprotective efficiency of Chr on LPS/D-GalN-induced acute hepatic injury in mice and elucidate the potential mechanism.

2. Materials and Methods

2.1. Chemicals and reagents

Chrysophanol (Chr), Dexamethasone (Dex) were purchased from the National Institutes for Food and Drug Control (Beijing, China). LPS (Escherichia coli055:B5) and D-galactosamine were provided by Sigma Co. Mouse TNF-α, IL-6 enzyme-linked immunosorbent assay (ELISA) kits were obtained from Biolegend Inc. (San Diego, CA, USA). The alanine aminotransferase (ALT) and aspartate aminotransferase (AST) detection kits were produced by the Jiancheng Bioengineering Institute of Nanjing (Nanjing, Jiangsu, China). The levels of MDA, SOD, CAT, GSH, GSH-Px were evaluated using commercially available kits subscribed from Jiancheng Institute of Biotechnology(Nanjing, China). Primary antibody against RIP140(sc-8997) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Primary antibodies against IκB(#4814), p-IκB(#2859), NF-κB(#9936), p-NF-κB(#9936), Bcl-2(#2870), Bax (#14796), caspase-3(#9662), caspase-8(#4927), caspase-9(#9504), iNOS(#2982), horseradish peroxidase-conjugated anti-rabbit antibody or horseradish peroxidase-conjugated anti-mouse antibody (#9936) were provided by Cell Signaling Technology (Beverly, MA, USA).

2.2. Animals and treatment

Six-week-old male BALB/c mice (20 ± 2 g) obtained from Jiangning Qinglongshan Animal Cultivation Farm (Nanjing, China) were allowed to adapt to their new condition for at least 5 days prior to use. Animals were housed in an environmentally controlled room and had free access to food or water. All the experimental procedures were performed in accordance with protocols approved by China Pharmaceutical University (No. CPU-TCM-2013012) Medicine Animal Care and Use Committee.

Fifty mice were randomly divided into five groups: control group, LPS/D-GalN group, Dex (5 mg/kg) group, and chrysophanol (1,10 mg/kg) + LPS/D-GalN group. Chr (1,10 mg/kg), Dex (5

mg/kg) were administrated intragastrically (i.g.) daily for 7 consecutive days. The dose of chrysophanol was determined based on previous preliminary experiments [15]. Then LPS (50 μg/kg) and D-GalN (800 mg/kg) dissolved in PBS were injected intraperitoneally (i.p.) 1 h after the last drug administration on day 7 in order to induce acute hepatic injury. Control group received an equal volume of PBS for being consistent with LPS/D-GalN stimulation. The serum and hepatic tissue samples were obtained from mice 8 h after LPS/D-GalN injection.

2.3. Serum aminotransferase levels

The activities of ALT and AST were determined to access the magnitude of hepatic damage. At the end of the experiment, serum samples were harvested for detection. ALT and AST activities were determined with commercial test kits supplied by Nanjing Jiancheng Bioengineering Institute according to the manufacturer's protocol.

2.4. Cytokine assay

The levels of the inflammatory cytokines including TNF-α, IL-6, IL-10 in serum and liver tissues was collected 8 h after LPS/D-GalN treatment and determined using ELISA kits obtained from Biolegend (San Diego, CA, USA) according to the instructions.

2.5. Evaluation of Oxidative Stress in liver tissues

The indicators of lipid peroxidation including MDA, SOD, CAT, GSH and GSH-Px were used to elucidate anti-oxidative property of Chr in the liver injury. Liver samples were homogenized with cold normal saline prepared to make 1:10 (w/v) homogenates followed by centrifugation at 12,000 × g (4 °C) for 20 min. The supernatants was collected into tubes and stored at -80 ℃. The protein contents were measured with a BCA protein assay kit. Hepatic levels of MDA, SOD, CAT, GSH and GSH-Px were determined with the commercial detection kits (Jiangcheng Institute of Biotechnology, Nanjing, China) according to the manufacturer's instructions.

2.6 Histopathological examination

Hepatic tissues were collected for histological evaluation at 8 h after LPS/D-GalN treatment and were fixed with 10% neutral-buffered formalin for 48 h. Briefly, the samples were dehydrated in graded alcohol, deparaffinized with xylene, embedded in paraffin wax and sliced at a thickness of 4 μm. Then the sections were stained with hematoxylin and eosin (H&E) according to the regular staining method. After washing with PBS, the slides were dehydrated through graded alcohols, cleared in xylene and covered with neutral balsam. Histopathological evaluation was performed under a light microscope by two pathologists in blinded manners.

2.7 Western Blot assay

Extraction of cytoplasmic proteins was accomplished using protein extract kit (Thermo) according to the manufacturer's protocol. BCA protein assay kit was used to determine protein concentrations. Protein extracts were divided by electrophoresis on 8-12 % sodium dodecyl sulfate polyacrylamide gel and transferred to polyvinylidene difluoride membranes. The membranes were blocked using 5% nonfat milk for 2 h at room temperature. Primary antibodies, including antibodies against RIP140(1:1000), IκB(1:1000), p-IκB(1:1000), cytosolic NF-κB(1:1000), cytosolic p-NF-κB(1:1000), Bcl-2 (1:1000), Bax (1:1000), caspase-3(1:1000), caspase-8 (1:1000), caspase-9 (1:1000), iNOS(1:1000) were incubated with primary antibodies overnight at 4 ℃. Thereafter, the membranes were washed and treated with horseradish peroxidase-conjugated secondary antibodies. Blots were washed and visualized with an enhanced chemiluminescene (ECL) western blotting detection system.

2.8 Statistical analysis

The data are presented as mean values ± SDs. Differences between groups were analyzed by one-way analysis of variance (ANOVA) with Tukey multiple comparison test using graphpad prism, $p<0.05$ was considered as significant difference.

3 Results

3.1. Effects of Chr on serum AST and ALT activities

The AST and ALT activities in serum were determined to assess acute liver injury. As shown in Fig. 1, both serum transaminase were still maintain low levels in control group. Exposure to LPS and D-GalN in mice significantly increased the activities of AST(from 123.12 ± 24.61 to 898.34 ± 159.20 IU/L) and ALT(from 130.90 ± 25.21 to 1681.31 ± 379.79 IU/L) compared with those in control group. However, pretreatment with Chr(1 mg/kg, 10 mg/kg) effectively suppressed the levels of AST(to 607.26 ± 135.30 IU/L, to 471.21 ± 65.10 IU/L, respectively) and ALT(to 1286.01 ± 210.10 IU/L, to 1177.14 ± 173.55 IU/L, respectively) in LPS/D-GalN-induced animals. Moreover, administration of Dex also reduced the activities of AST(to 434.43 ± 78.67 IU/L) and ALT(to 1055.20 ± 188.15 IU/L). Our data suggested that Chr could ameliorate transaminase activity in acute liver injury caused by LPS/D-GalN challenge.

3.2. Effects of Chr on inflammatory cytokines generations

The effects of Chr on hepatic and serum cytokines including TNF-α, IL-6 and IL-10 were assayed using ELISA kits. As revealed in Fig. 2, the TNF-α(from 168.95 ± 53.01 to 1909.60 ± 117.43 pg/ml), IL-6(from 181.57 ± 6.26 to 3004.47 ± 150.62 pg/ml) and IL-10(from 35.53 ± 3.65 to 714.66 ± 129.88 pg/ml) levels in the serum samples presented an apparent increase by LPS/D-GalN. Pretreatment with Chr(1 mg/kg, 10 mg/kg) significantly inhibited the serum contents of TNF-α(to 1659.84 ± 112.60 pg/ml, to 1383.04 ± 73.01 pg/ml, respectively) and IL-6(to 2607.76 ± 139.03 pg/ml, to 2024.95 ± 216.77 pg/ml, respectively) compared with those in LPS/D-GalN group. While administration of Chr(1 mg/kg, 10 mg/kg) dramatically increased the IL-10 content in serum (to 801.93 ± 184.63 pg/ml, to 879.39 ± 128.14 pg/ml, respectively).

Additionally, stimulation with LPS/D-GalN elevated the levels of TNF-α (from 62.41 ± 12.11 to 791.25 ± 116.85 pg/ml), IL-6(from 78.39 ± 5.99 to 861.44 ± 64.04 pg/ml) and IL-10(from 55.34

± 5.75 to 448.38 ± 97.35 pg/ml) in liver tissues. As expected, treatment with Chr(1 mg/kg, 10 mg/kg) effectively decreased the contents of TNF-α(to 650.32 ± 68.60 pg/ml, to 582.55 ± 76.96 pg/ml, respectively) and IL-6(to 830.64 ± 35.18 pg/ml, to 632.17 ± 58.22 pg/ml, respectively) compared with those in model group. Nevertheless, Chr(1 mg/kg, 10 mg/kg) could increase the level of IL-10 (to 530.35 ± 80.08 pg/ml, to 539.54 ± 61.83 pg/ml, respectively). Our experimental data indicated that Chr was capable of attenuating serum and hepatic inflammatory conditions in LPS/D-GalN-induced liver injury(Fig. 2).

3.3. Effects of Chr on lipid peroxidation

MDA, SOD, CAT, GSH, GSH-Px are known as a biochemical marker of lipid peroxidation. As illustrated in Fig. 3, LPS/D-GalN challenge decreased the serum levels of SOD(from 53.86 ± 12.38 to 27.74 ± 5.01 U/mg prot), CAT(from 10. 43 ± 1.48 to 3.16 ± 0.53 U/mg prot), GSH(from 29.69 ± 4.75 to 10.83 ± 2.25 nmol/mg prot), GSH-Px(from 38.01 ± 5.74 to 20.55 ± 3.98 U/mg prot) and increased the MDA content(from 1.53 ± 0.35 to 7.24 ± 0.85 nmol/mg prot). However, treatment with Chr(1 mg/kg, 10 mg/kg) remarkably elevated the levels of SOD(to 36.70 ± 3.80, to 40.40 ± 4.21 U/mg prot, respectively), CAT(to 6.64 ± 0.77, to 7.12 ± 0.60 U/mg prot, respectively), GSH (to 17.50 ± 2.95, to 21.58 ± 3.27 nmol/mg prot, respectively), GSH-Px(to 24.21 ± 6.82, to 30.00 ± 5.57 U/mg prot, respectively) and reduced the MDA content(to 6.04 ± 0.41 nmol/mg prot, respectively). In addition, administration of Dex also increased the levels of SOD(to 43.52 ± 5.65 U/mg prot), CAT(to 7.91 ± 0.87 U/mg prot), GSH (to 20.81 ± 3.15 nmol/mg prot), GSH-Px(to 32.19 ± 3.72 U/mg prot) and reduced the MDA content(4.21 ± 0.55 nmol/mg prot). The analytical data suggested that Chr might alleviated the oxidative stress in LPS/D-GalN-induced mice.

3.4. Effect of Chr on LPS/D-GalN-induced histopathologic changes in liver tissues

H&E staining assay was performed to evaluate the histopathological condition of LPS/D-GalN-induced liver injury and our results supported the findings of biochemical analysis parts above. Liver sections from control group showed normal liver architecture and cellular structure. LPS/D-GalN challenge contributed to remarkable histological changes containing

242 extensive vacuolization and vast areas of cellular necrosis with inflammatory cell infiltration. By
243 contrast, the treatment with Chr(1,10 mg/kg) effectively attenuated the development of
244 histopathological changes (Fig. 4).
245
246 **3.5. Effect of Chr on LPS/D-GalN-induced apoptosis in liver**
247
248 The magnitude of apoptotic-related protein expressions was analyzed via western blotting to
249 explore the underlying mechanism of Chr on the inhibition of LPS/D-GalN-induced hepatocyte
250 apoptosis. As observed in Fig. 5E-5I, LPS/D-GalN markedly up-regulated the expression of
251 Bax(to 1.68 fold), caspase-3(1.43 fold), caspase-8(to 1.44 fold), caspase-9(to 1.52 fold) and
252 down-regulated the expression of Bcl-2(to 0.47 fold). On the contrary, treatment with Chr(1 mg/kg,
253 10 mg/kg) inhibited the protein levels of Bax(from 1.68 to 1.52 and 1.38, respectively),
254 caspase-3(from 1.43 to 1.29 and 1.25, respectively), caspase-8(from 1.44 to 1.33 and 1.24,
255 respectively), caspase-9(from 1.52 to 1.39 and 1.31, respectively) and increased the levels of
256 Bcl-2(from 0.47 to 0.56 and 0.71, respectively) compared with those in LPS/D-GalN group.
257 Besides, administration of Dex down-regulated the expressions of Bax(from 1.68 to 1.37),
258 caspase-3(from 1.43 to 1.21), caspase-8(from 1.44 to 1.23), caspase-9(from 1.52 to 1.30) and
259 increased the levels of Bcl-2(from 0.47 to 0.77) compared with those in LPS/D-GalN group.
260
261 **3.6. Effects of Chr on RIP140, NF-κB and iNOS activation**
262
263 To elucidate the anti-inflammatory mechanism of Chr on LPS/D-GalN -induced acute hepatic
264 injury in mice, the protein expressions of RIP140, iNOS and phosphorylation of IκB, NF-κB were
265 detected. The protein levels of RIP140(to 1.49 fold), p-IκB(to 1.42 fold), p-NF-κB(to 1.69 fold)
266 and iNOS(to 1.42 fold) were upregualted in LPS/D-GalN group compared with those in model
267 group. By contrast, Chr(1 mg/kg, 10 mg/kg) significantly exerted a suppressive effect on
268 LPS/D-GalN -induced phosphorylated IκB (from 1.42 to 1.25 and to 1.21, respectively) and
269 NF-κB (from 1.69 to 1.45 and to 1.37, respectively) promotions. Simultaneously, Chr(1 mg/kg, 10
270 mg/kg) administrations were demonstrated to have inhibited effects on the expressions of
271 RIP140(from 1.49 to 1.39 and to 1.33, respectively) and iNOS(from 1.42 to 1.27 and to 1.22,

respectively). Meanwhile, Dex could be observed to alleviated the expressions of RIP140(from 1.49 to 1.28), p-IκB(from 1.42 to 1.14), p-NF-κB(from 1.69 to 1.34) and iNOS(from 1.42 to 1.17). (Fig. 5B, 5C, 5D, 5J).

4. Dicussion

Chrysophanol extracted from Rhubarb has been recently recognized as an efficient anti-inflammatory regent in the prevention/treatment of disease triggered by pathological conditions, such as diabetes [16], oxidative stress [17], and ischemia–reperfusion injury [18]. The present study was designed to demonstrate the preventive effect of Chr protecting from acute liver injury stimulated with LPS/D-GalN. Besides, the potent underlying mechanisms of Chr on oxidative stress, hepatocellular apoptosis and inflammatory response were explored.

AST and ALT activities were biochemical markers to reveal the degree of early acute hepatic injury [19]. The data were consistent with previous reports which proved that the administration of LPS/D-GalN conduced to an obvious increase in serum AST and ALT levels [20]. Furthermore, our results were supported by H&E examination where pathological changes occurred including extensive vacuolization and vast areas of cellular necrosis with inflammatory cell infiltration. The above findings implied the occurrence of liver injury. On the contrary, pretreatment with Chr recovered the hepatic function, which was supported by the ameliorations of pathological conditions and transaminase activities.

The imbalance between free radical production and the antioxidant activity in liver damage would contribute to the consequence of oxidative stress and lipid peroxidation [21]. LPS/D-GalN challenge alters the antioxidant system in liver tissue and conduce to biological lipid peroxidation. It is reported that the activities of antioxidant enzyme including SOD, CAT, GSH, GSH-Px are decreased in response to LPS/D-GalN stimulation [22, 23]. Additionally, MDA, the end product from lipid breakdown, is recognized as a reliable index sensitive to oxidative stress [24]. Our present data showed that treatment with Chr significantly reduced the content of MDA, and restored the activities of SOD, CAT, GSH, GSH-Px. These analytical results suggested that the protective

effect of Chr might be attributed to its anti-oxidative activity.

LPS/D-GalN administration could provoke hepatocellular apoptosis in mice liver. LPS/D-GalN elevates the permeability of cells to enhance caspase cascade and directly induces massive apoptosis of hepatocytes [25]. The harmful stimulant activates caspase-9 through a variety of apoptotic signaling pathway and promotes effector caspase-3, which eventually triggers apoptotic process [26]. In this study, Chr inhibited the LPS/D-GalN-activated caspase-3, caspase-8 and caspase-9 expressions. Meanwhile, Bcl-2 family proteins that are involved in the regulation of apoptotic signaling system contain two different types, anti-apoptotic proteins(Bcl-2) and pro-apoptosis proteins(Bax) [27]. Bcl-2 can promote cell survival by suppressing apoptosis while Bax has opposite effect [28]. In the present study, compared with model group, treatment with Chr apparently suppressed the pro-apoptotic Bax level and enhanced the anti-apoptotic Bcl-2 level in LPS/D-GalN-challenged mice. Thus, the inhibitions of activated caspase-3, caspase-8, caspase-9 as well as the decreased ratio of Bax/Bcl-2 demonstrated the inhibitory effect of Chr treatment on LPS/D-GalN-induced liver injury via apoptotic pathway.

The anti-inflammatory effect of Chr was evaluated in this study, as clearly evidenced by marked inhibition of the pro-inflammatory factors release. Recent studies illustrated a potential relationship between metabolic disorders and inflammation in the pathological process, where receptor-interacting protein 140 (RIP140) and inflammation trigger NF-κB play an important role [29]. RIP140 serves as a coactivator for NF-κB, which consequently increases the protein expressions of relevant molecules and regulates a series of mediators to enhance their inflammatory responses [30, 31]. Po et al proved the close association between RIP140 and NF-κB with RIP140 SiRNA[32]. Besides the phosphorylation of IκB triggers the activition of NF-κB which leads to the transcriptions of and the generations of inflammatory cytokines [33, 34]. Our western blotting assay also indicated the up-regulation of RIP140 and NF-κB after LPS/D-GalN challenge in the current study. On the contrary, Chr reduced RIP140 and NF-κB levels, which indicated its protective role against LPS/D-GalN-induced inflammatory response. Meantime, overexpression of inflammatory mediators was implicated in the pathogenesis of inflammatory diseases [35]. Consistently, the inhibition of NF-κB pathway leads to inhibit the expressions of pro-inflammatory cytokines [36]. Evidence has emerged indicating that pro-inflammatory cytokines were the important modulators of liver injury by initiating, amplifying, and perpetuating the inflammatory response in the liver [37].

IL-10 is an anti-inflammatory cytokine which inhibits the synthesis and secretion of numerous pro-inflammatory cytokines hepatic injury caused by LPS/D-GalN [38]. The model group displayed the upregulations of TNF-α, IL-6 and IL-10 in serum and hepatic. Both the elevations of pro-inflammatory and anti-inflammatory cytokines indicated that LPS/D-GalN triggered the acute inflammation with the overproduction of anti-inflammatory cytokine which could not suppressed the excessive anti-inflammatory cytokines. While treatment with Chr reduced the contents of TNF-α, IL-6 and increased the IL-10 content, suggesting that Chr exhibited the beneficial effect through the inhibition of pro-inflammatory cytokines and promotion of anti-inflammatory mediator. Furthermore, the inhibitory effect of Chr on inflammatory responses could be reflected directly by iNOS expression as well. It is widely known that LPS/D-GalN challenge contributes to the high level of iNOS and then results in the overproduction of NO [39]. We also confirmed the involvement of iNOS in Chr-suppressed inflammation. Our data indicated that the down-regulation the expressions of RIP140, iNOS and NF-κB-driven inflammatory cytokines in mice. In summary, it was ascertained that Chr exerted protective activity in the treatment of LPS/GalN-challenged hepatic inflammation through the suppression of RIP140/NF-κB pathway.

Based on the above, the results of the present study revealed that chrysophanol could effectively attenuate LPS/GalN-induced liver injury in vivo. The potential mechanism might be involved in the ameliorations of iNOS, TNF-α and IL-6 production via the blockade of RIP140/ NF-κB pathway. In addition, the inhibitory effect of Chr on LPS/GalN liver injury was also found to be correlated with the attenuations of oxidant stress and hepatocellur apoptosis. Further studies are necessary to test the clinical application of Chr.

Conflict of interest statement

There is no conflict of interest among authors.

Acknowledgments

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Fig 1. Effects of Chrysophanol on ALT and AST levels in mice with LPS/D-GalN-induced acute liver injury. Mice were intragastrically (i.g.) administrated with Chr (1,10 mg/kg) and Dex (5 mg/kg) 1 h prior to LPS/GalN treatment. Serum samples were obtained 8 h after challenge and serum transaminase activity was determined. Values are expressed as means ± SDs (n=10). The experiment was independently repeated three times. Compared with control: ##P<0.01,; compared

with model: * P<0.05, **P<0.01.

Fig 2. Chrysophanol suppressed LPS/GalN-induced production of inflammatory cytokines. Mice were treated with vehicle or Chr(1,10 mg/kg) at 1 h before LPS/D-GalN exposure. Serum and liver tissues was collected 8 h after LPS/GalN stimulation for the determination of inflammatory cytokines generations including TNF-α, IL-6, IL-10 levels in serum and hepatic Data are expressed as means ± SDs (n=10). Compared with control: ##P<0.01,; compared with model: * P<0.05, **P<0.01.

Fig 3. Chrysophanol decreased hepatic levels of MDA, SOD, CAT, GSH, GSH-Px in LPS/D-GalN simulated mice. Mice were intragastrically (i.g.) given with Chr (1,10 mg/kg), Dex (5 mg/kg) 1 h followed by LPS/GalN challenge on day 7. Liver samples were harvested at 8 h after LPS/D-GalN injection for the assay of hepatic MDA contents. Values are presented as means ± SDs (n=10). Compared with control: ##P<0.01,; compared with model: * P<0.05, **P<0.01.

Fig 4. The pathological status in liver sections observed using H&E staining in mice pretreated with or without Chr (1,10 mg/kg).Mice were intragastrically (i.g.) treated with Chr (1,10 mg/kg), Dex (5 mg/kg) and vehicle 1 h before the presence of LPS/D-Gal challenge. Livers from each group were excised at 8 h after LPS/D-GalN exposure and the liver sections were stained with hematoxylin-eosin(H&E) for morphological assess. The representative liver sections chosen from each group are shown above. (A) the liver section from control mice. (B) the liver section from the mice administered with LPS/D-GalN. (C) the liver section from the mice administered with dexamethasone (5 mg/kg) and LPS/D-GalN. (D) the liver section from the mice administered with Chr (1 mg/kg) and LPS/D-GalN. (E) the liver section from the mice administered with Chr (10 mg/kg) and LPS/D-GalN.

Fig 5. Effects of Chr on liver expressions of RIP140(B), bcl-2(E), Bax(F), caspase-3(G), caspase-8(H), caspase-9(I), iNOS(J) and phosphorylations of IκB(C), p65 NF-κB(D). Mice were given i.g. with Chr(1, 10 mg/kg) Dex (5 mg/kg) 1 h prior to LPS/D-GalN administration. Liver tissues were collected from mice sacrificed 8 h after LPS/D-GalN injection. Cytosolic and nuclear

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517 means ± SDs (n=10). Compared with control: ##P<0.01,; compared with model: * P<0.05,
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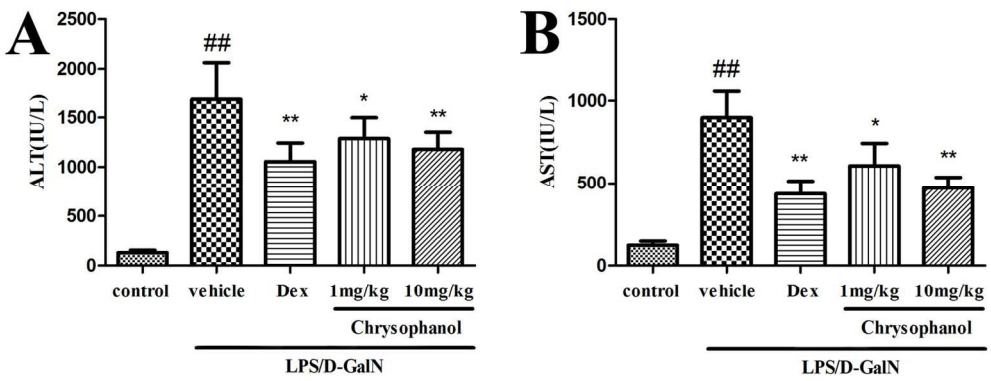

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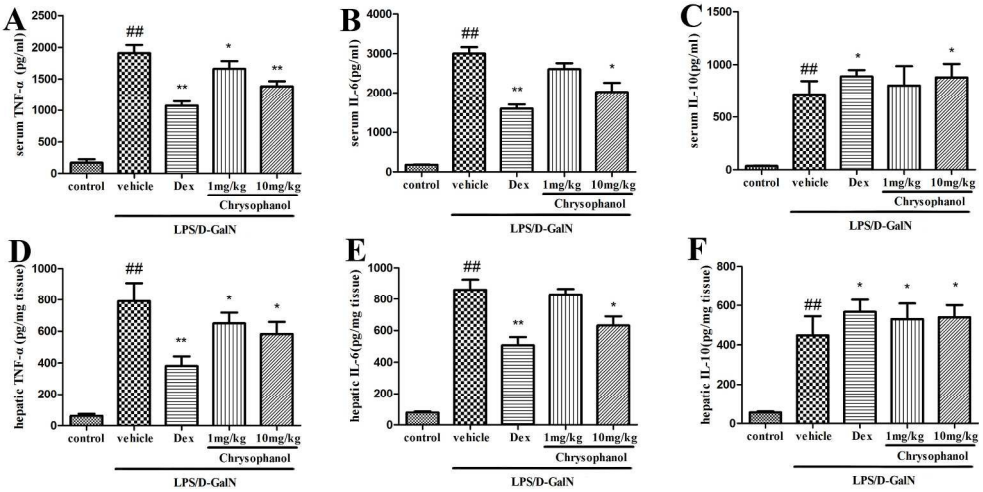

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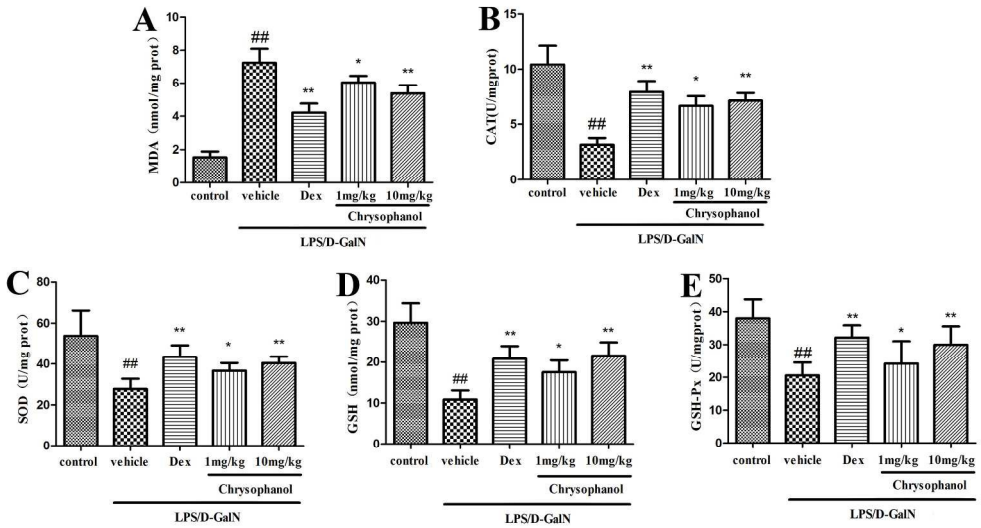

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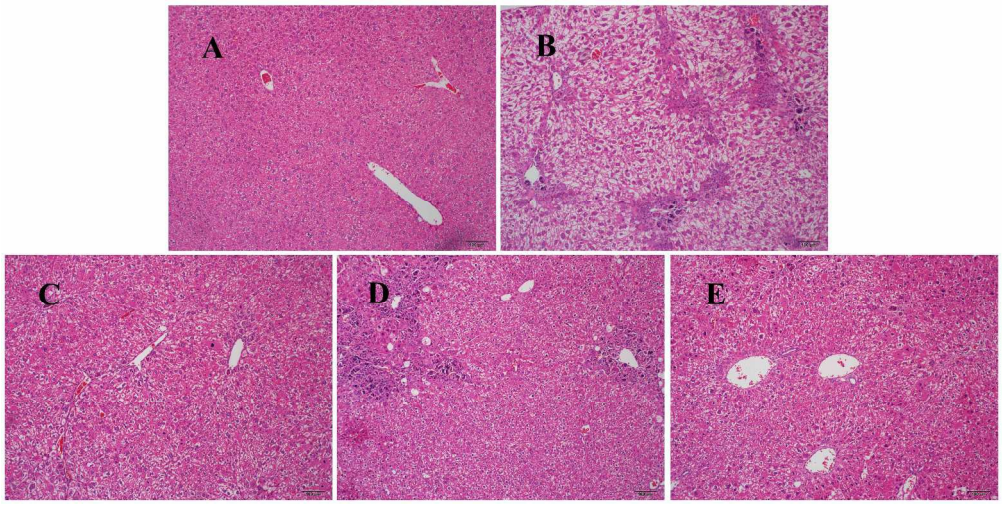

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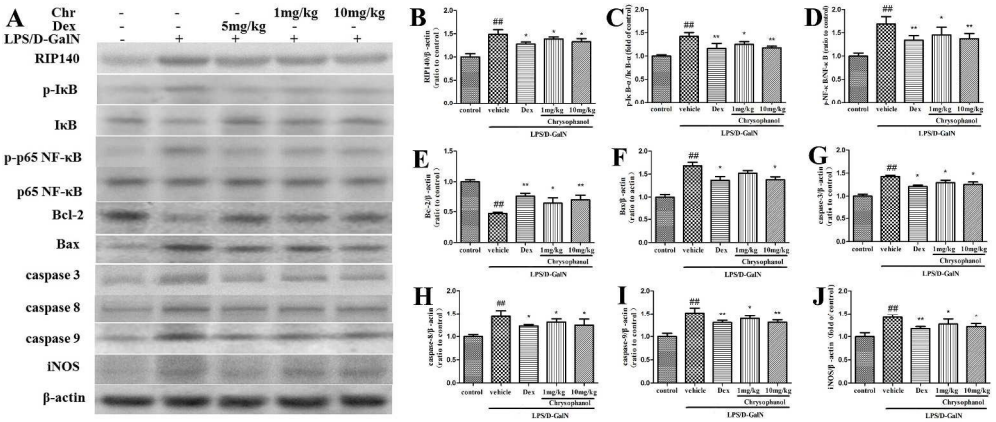

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