International Immunopharmacology 34 (2016) 44–52

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Protective effect of wedelolactone against CCl_4-induced acute liver injury in mice

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ARTICLE INFO

Article history:
Received 24 November 2015
Received in revised form 7 January 2016
Accepted 2 February 2016
Available online xxxx

Keywords:
Wedelolactone
Acute liver injury
Hepatoprotective action
Oxidative stress
Inflammatory response
Apoptosis

ABSTRACT

Eclipta, a traditional Chinese medicine, has been used to treat liver disease for centuries. However, the chemical basis and biological mechanisms of *Eclipta* remain elusive. The current study aims to investigate the hepatoprotective effect of wedelolactone (WEL), a major coumarin in *Eclipta*, using C57BL/6 mice with carbon tetrachloride CCl_4-induced acute liver injury (ALI). Our data showed that WEL markedly decreased the CCl_4-induced elevation of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities, and improved hepatic histopathology changes. WEL also significantly decreased the content of MDA in liver tissues, meanwhile increased the activities of antioxidant enzymes SOD and GSH-Px. In addition, WEL reduced the protein expression of TNF-α, IL-1β and IL-6, as well as mRNA expression. Western blot results revealed that WEL repressed phosphorylation of extracellular signal-regulated kinase (ERK) and translocation of NF-κB p65 from cytoplasm to nucleus and enhanced the phosphorylation of *c*-Jun. *N*-terminal kinase (JNK). Moreover, results showed that WEL significantly inhibited CCl_4-induced hepatocytes apoptosis, markedly suppressed the down-regulation of Bax and active Caspase-3 expression and accelerated the expression of Bcl-2. Overall, the findings indicate that WEL exhibits a protective effect against CCl_4-induced ALI in mice by enhancing the antioxidative defense system, suppressing the inflammatory response and cell apoptosis of liver.

1. Introduction

The liver plays a major role in regulating various physiochemical functions of our body including metabolism, glycolysis and scavenging free radicals [1,2]. Due to these critical physiochemical functions, liver injuries need to be rapidly and efficiently remedied [3]. Toxic substances such as alcohol, acetaminophen and CCl_4 can induce liver injury. Since the similar mechanism of CCl_4-induced liver injury to liver diseases, it is widely used in experimental hepatopathy [4–6].

Although there is an increasing need for medicines to protect the liver from damage, modern medicine is still lack of reliable liver-protective drugs. Therefore, numerous traditional herbals have been studied to evaluate their hepatoprotective efficiency. Wedelolactone (WEL) (Fig. 1) is a major coumarin isolated from *Eclipta*, which is used to prevent liver damage due to different etiology factors including infectious agents, hepatotoxic chemicals, jaundice and inflammation in many Asian countries [7]. WEL has been reported to treat septic shock, hepatitis and venom poisoning [8–10]. Modern researches have shown that WEL has diverse pharmacological effects like antihepatotoxicity, anti-androgen and anti-immunodeficiency activities [11–13]. To our knowledge, the effect of WEL on acute liver injury remains unexplored.

This is the first study to investigate the protective effect of WEL treatment against acute liver injury using animal experimental systems. The main purpose of this study was to investigate the potential role of WEL in reducing inflammation and apoptosis in the liver of mice induced by CCl_4 which could provide helpful information on the efficacy of WEL's protection against CCl_4-induced acute liver injury.

2. Materials and methods

2.1. Animals

C57BL/6 mice (male, 4 to 5 weeks old, 18–22 g) were obtained from Laboratory Animal Centre of the Fourth Military Medical University [Permit Number: SCXK (Army) 2007-007]. Animal care and procedures were approved by the Institutional Animal Care and Use Committee. All mice were placed (8 per cage) in clean, pathogen-free polycarbonate cage in the animal care facility, and were fed a standard animal diet and water ad libitum under controlled temperature conditions with 12-h light–dark cycles.

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http://dx.doi.org/10.1016/j.intimp.2016.02.003
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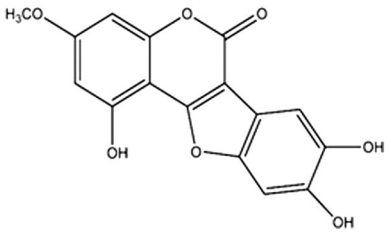

Fig. 1. The chemical structure of wedelolactone.

2.2. Instruments and reagents

WEL purchased from Xi'an Snout Co., Ltd. (Xi'an, China) was more than 95% pure by high performance liquid chromatography (HPLC) analysis. CCl_4 was purchased from Tianjin Kemiou Chemical Reagent Co., Ltd. (Tianjin, China). The ELISA kits of TNF-a, IL-1β and IL-6 were obtained from Wuhan Colorful gene Biotechnology Co., Ltd. (Wuhan, China). The detection kits used for the determination of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities, superoxide dismutase (SOD) activity, glutathione peroxidase (GSH-Px) activity and malondialdehyde (MDA) contents were purchased from Nanjing Jiancheng Institute of Biotechnology (Nanjing, China). Antibodies specific for p38, *p*-p38, *c*-Jun. *N*-terminal kinase (JNK), *p*-JNK, extracellular signal-regulated kinase (ERK), *p*-ERK, COX-2, iNOS, NF-κB, Bax, Bcl-2, active caspase-3 were purchased from Cell Signaling Technology (Beverly, MA). Trizol reagent was purchased from Invitrogen (Carlsbad, CA, USA). The TaKaRa PrimeScript RT reagent kit was purchased from Takara Biotechnology Co., Ltd. (Dalian, China). TUNEL detection kit was procured from Roche (Roche Diagnostic, Mannheim, Germany). The FastStart Universal SYBR Green Master and RT -PCR Kit were purchased from Thermo Fisher Scientific (CA, USA).

2.3. Experiment scheme

In the experiment, acute liver injury was induced by intraperitoneal injection of CCl_4 (0.4 ml CCl_4 dissolved in 100 ml oil, 10 ml/kg). A total of 48 male C57BL/6 mice were randomly divided into six groups as follows: (I) the control group: double distilled water(DDW) for 7 days; (II) the WEL control group: WEL(220 mg/kg) for 7 days; (III) the model group: DDW for 7 days then challenged with CCl_4; (IV) the CCl_4 plus WEL group: WEL(55 mg/kg) for 7 days then challenged with CCl_4; (V) the CCl_4 plus WEL group: WEL(110 mg/kg) for 7 days then challenged with CCl_4;(VI) the CCl_4 plus WEL group: WEL(220 mg/kg) for 7 days then challenged with CCl_4. WEL or DDW was administered orally once daily for 7 consecutive days. An hour after the last dose of WEL and DDW, CCl_4 was given by intraperitoneal injection. Mice were sacrificed 24 h after CCl_4 administration and blood samples were collected from the eyeballs. Serum was separated by centrifugation at 4 °C, 4000 × g for 15 min. The liver was removed immediately from each mouse. Liver tissues used for biochemical analysis were dispersed into 10% homogenate in phosphate buffered saline(PBS, pH = 7.4) and the supernatants were separated by centrifugation at 4 °C, 6000 × g for 10 min, then kept at −80 °C until analyzed.

2.4. Measurement of liver enzymes and liver index

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined using a commercially available kit. Mouse body weight was measured before killed and liver weight was measured immediately after killed. Liver index was expressed as (liver weight / body weight) × 100%.

2.5. Cytokine measurement in hepatic tissue

Levels of liver tissue TNF-α, IL-1β and IL-6 were measured with commercial ELISA kits following the instructions of the manufacturer.

2.6. Measurement of MDA levels and GSH-Px, SOD activities

MDA levels and GSH-Px, SOD activities in liver homogenate were measured using commercial reagent kits according to the instruction manuals.

2.7. Western blot assay

At 24 h after CCl_4 intraperitoneal injection, the liver tissues were homogenized to prepare whole protein extracts. Protein concentration of the supernatants was determined by BCA protein assay kit. Western blot analysis was performed with p38, *p*-p38, JNK, *p*-JNK, ERK, *p*-ERK, NF-κB(p65), COX-2, iNOS, active caspase-3.

2.8. Quantitative real-time PCR

Total RNA was extracted from liver tissues using Trizol reagent, and was used for reverse transcription according to the manufacturer's instruction. Quantitative real-time PCR was carried out using SYBR Green PCR Master Mix in a total volume of 10 μL on Step One Plus Real-Time PCR System (Applied Biosystems) as follows: 94 °C for 3 min, 40 cycles of 94 °C for 20 s, 55 °C for 20 s and 72 °C for 35 s. ABI Prism 7500 SDS Software was used for the analysis of quantitative RT-PCR. The sequences (5′ to 3′) of the primers were shown in Table 1.

2.9. Histopathological and immunohistochemical examination

The liver tissues were excised and fixed in formalin solution for histopathological and immunohistochemical examination. Sections (5 μm thick) were cut and stained with hematoxylin and eosin (H&E) for general histological examination under a light microscope and photographed at 400× magnification. For immunohistochemistry, 5 μm thick sections were used. Briefly, deparaffinized liver slices were incubated overnight with the antibodies against Bax and Bcl-2 and then counterstained with hematoxylin, dehydrated using graded alcohols and xylene, and mounted on microslide. The immunohistochemical staining was analyzed by light microscopy across 10 high-power fields (200×).

2.10. TUNEL assay

Wax-embedded liver tissue sections were prepared for terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay, which was performed using the In Situ Cell Death Detection Kit according to the manufacturer's instructions. Briefly, we dewaxed and rehydrated tissue sections by xylene and a graded series of ethanol, incubated tissue sections for 30 min at 37 °C, and then incubated them with 50 μL TUNEL reaction mixture in dark for 1 h at 37 °C. After that step, sections were incubated with 50 μL converter-POD per sample for 30 min. Hematoxylin was used to stain the nucleus, and then the stained cells were analyzed under light microscope.

Table 1
Primer sequences.

Gene	Primer sequences (5′-3′)
TNF-a(F)	GATCCGAGATGTGGAACTGG
TNF-a(R)	AGTTCAGTAGACAGAAGAGC
IL-1β(F)	CAACTGTCCCTGAACTCAAC
IL-1β(R)	TGGATGCTCTCATCTGGACA
IL-6(F)	CAAGAGACTTCCAGCCAGTT
IL-6(R)	TCATTTCCAAGATCTCCCTG

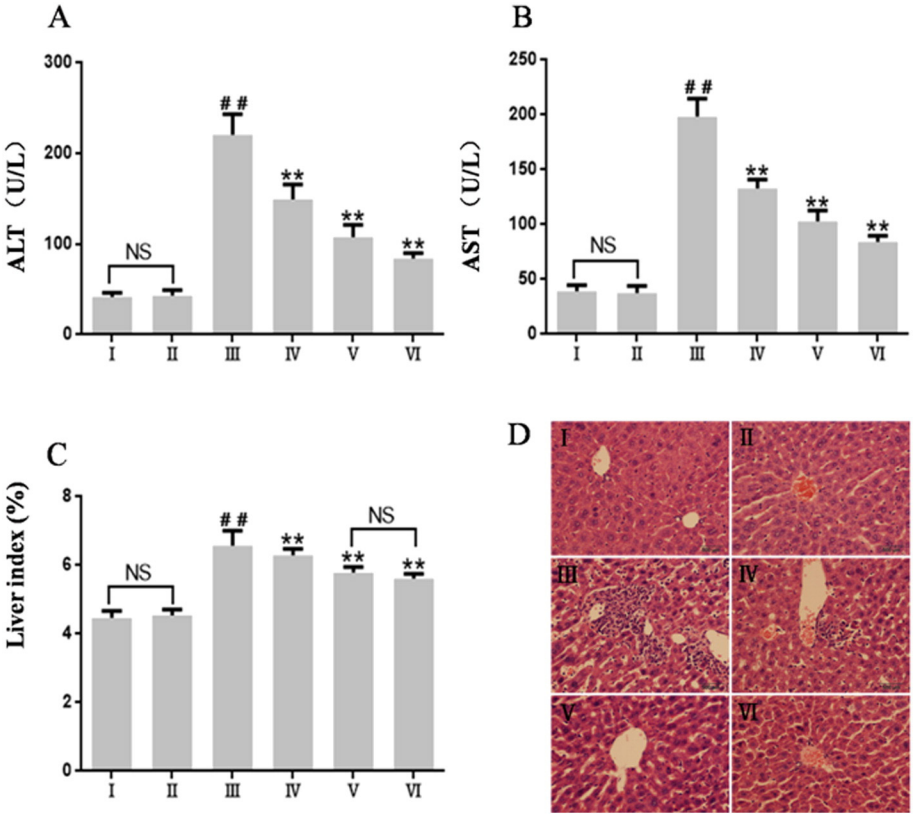

Fig. 2. Pre-administration effects of WEL on CCl_4-induced hepatic injury. The mice were administered with WEL (55, 110, and 220 mg/kg), or distilled water orally once per day for 7 days. After intraperitoneal injection of CCl_4, serum ALT (A) and AST (B) levels were measured. Liver index (C) was expressed as (liver weight / body weight) × 100%. H&E staining (D) were conducted and examined under a microscope (Magnification, 400×). Data were expressed as the mean ± SD ($n = 8$). $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ compared with the normal control group; $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with the CCl_4 group; NS = no significance. Animals were divided into the following groups: I, normal control; II, WEL control; III, model; IV, WEL (55 mg/kg) + CCl_4; V, WEL (110 mg/kg) + CCl_4; VI, WEL (220 mg/kg) + CCl_4.

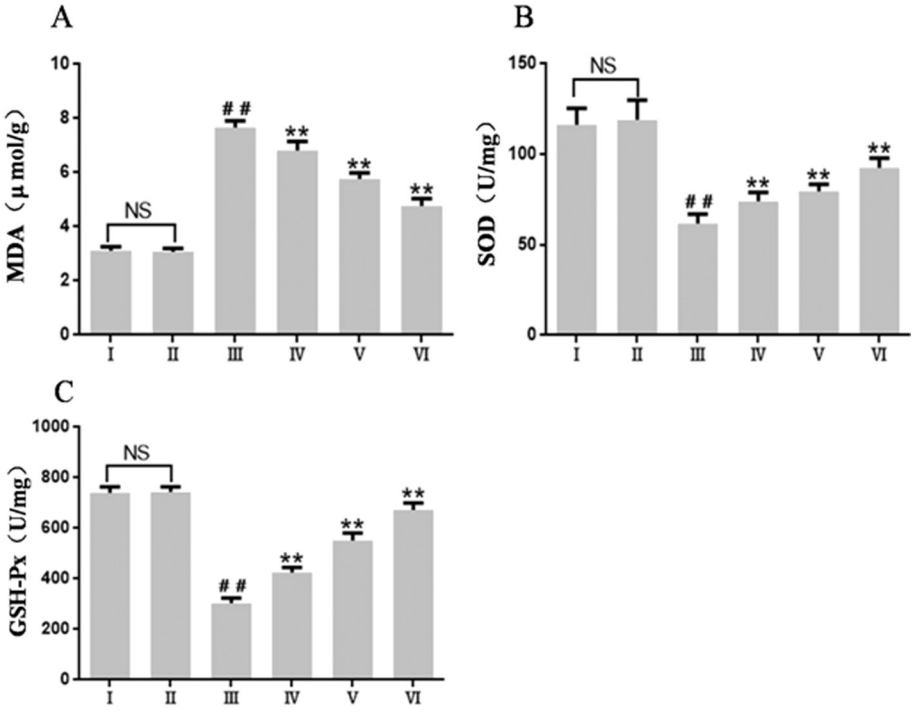

Fig. 3. Effects of WEL on CCl_4-induced liver oxidative stress. The mice were administered with WEL (55, 110, and 220 mg/kg), or distilled water orally once per day for 7 days. After intraperitoneal injection of CCl_4, livers were removed and hepatic tissue levels of MDA (A), SOD (B), and GSH-Px (C) were determined using commercial reagent kits. Data were expressed as the mean ± SD ($n = 8$). $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ compared with the normal control group; $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with the CCl_4 group; NS = no significance. Animals were divided into the following groups: I, normal control; II, WEL control; III, model; IV, WEL (55 mg/kg) + CCl_4; V, WEL (110 mg/kg) + CCl_4; VI, WEL (220 mg/kg) + CCl_4.

2.11. Statistical analysis

Statistical analysis was performed using SPSS 16.0 software (SPSS Inc., Chicago, IL, USA). Comparisons between multiple groups were evaluated using one-way analysis of variance (ANOVA) followed by LSD *t*-test. Data were expressed as mean ± SD and $P < 0.05$ was considered as statistically significant.

3. Results

3.1. WEL protects against acute liver injury induced by CCl_4

Serum activities of ALT and AST are the key biochemical markers of acute liver injury (ALI), which were significantly increased in the CCl_4-treatment group compared with those in normal control group. However, pre-administration of WEL at three different doses for 7 consecutive days significantly prevented the increase of serum ALT and AST activities induced by CCl_4 (Fig. 2A, B). A significant increase of liver index was induced in the mice treated with the CCl_4 alone, which was significantly ameliorated by administration of WEL at a dose of 110 mg/kg or 220 mg/kg. However, the lowest-dose WEL (55 mg/kg)-induced changes in the liver index was weak (Fig. 2C).

3.2. WEL alleviates histopathological changes in liver induced by CCl_4

Histopathological changes in the liver sections stained with H&E were observed under a light microscope to further support the biochemical analysis evidence (Fig. 2D). Normal hepatic cells with well-preserved cytoplasm, prominent nucleus and nucleolus, visible central veins and thin sinusoids were shown in the normal group. There were no statistical differences between the WEL control group and the normal control group. In contrast, severe loss of hepatic architecture and many leukocytes which meant inflammatory infiltration were presented in the liver sections of the model group, but the pre-administration of WEL reversed the hepatic lesions. The maximum protection was seen in the 220 mg/kg WEL group which showed minor histopathology changes, almost comparable to the WEL control group and the normal control group. This histopathological observation was in a good correlation with the biochemical results of serum hepatotoxic biochemical markers.

3.3. WEL suppresses oxidative stress in acute liver injury induced by CCl_4

In order to evaluate the effect of WEL treatment on oxidative stress induced by CCl_4 in liver, levels of liver tissue MDA, activities of GSH-Px and SOD were measured. As an indicator of lipid peroxidation in the liver, the hepatic MDA level markedly increased in the model group

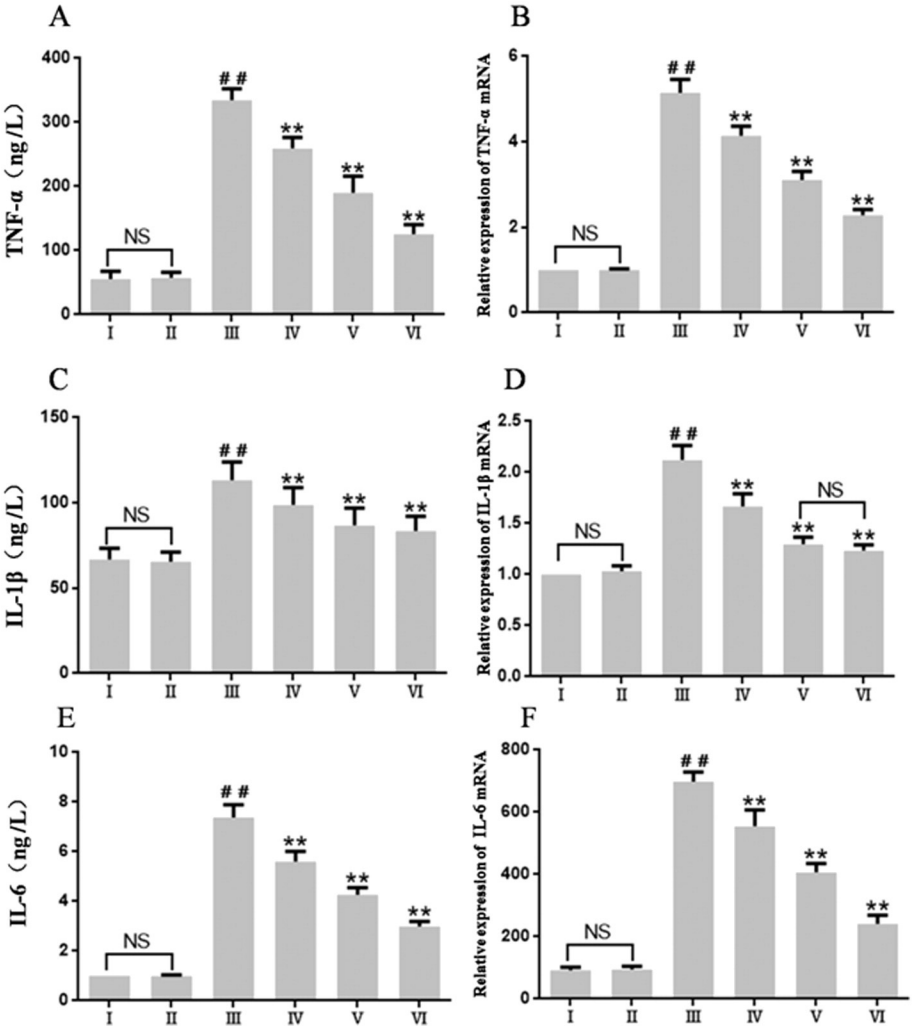

Fig. 4. Cytokine changes in liver tissues. The mice were administered with WEL (55, 110, and 220 mg/kg), or distilled water orally once per day for 7 days. After intraperitoneal injection of CCl4, levels of TNF-α (A), IL-1β (C), IL-6 (E) and levels of liver TNF-α mRNA expression (B), IL-1β mRNA expression (D), IL-6 mRNA expression (F) were determined using a commercial ELISA kit. Data were expressed as the mean ± SD ($n = 8$). $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ compared with the normal control group; $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with the CCl_4 group; NS = no significance. Animals were divided into the following groups: I, normal control; II, WEL control; III, model; IV, WEL (55 mg/kg) + CCl_4; V, WEL (110 mg/kg) + CCl_4; VI, WEL (220 mg/kg) + CCl_4.

compared with the normal control group, while the levels of MDA were significantly reduced in WEL-treatment groups (Fig. 3A). The activities of the antioxidant enzymes in the liver, GSH-Px and SOD, conspicuously decreased in the model group compared with those in the normal control group, whereas the treatment of WEL significantly reversed the decreased activities of GSH-Px and SOD (Fig. 3B, C).

3.4. WEL inhibits pro-inflammatory response in acute liver injury induced by CCl_4

CCl_4 treatment significantly increased expression of the hepatic TNF-α, IL-1β, IL-6 proteins (Fig. 4A, C, E) and mRNAs (Fig. 4B, D, F) compared with those of the normal control group, which meant an induction of a severe inflammatory response. However, the pre-administration of WEL apparently repressed the mRNA and protein expression of hepatic TNF-α, IL-1β and IL-6.

MAPK families play an important role in CCl_4-induced liver inflammatory response [14]. NF-κB is activated by phosphorylation of IκB-α via the activation of MAPKs (such as p38, JNK, ERK) and then migrates into the nucleus which activates the expression of inflammatory cytokines and mediators [15–18]. Thus we examined the effect of wedelolactone on the phosphorylation status of MAPK family proteins ERK, JNK and p38 by Western blot. Pre-treatment with WEL significantly inhibited the phosphorylation of ERK and markedly enhanced the phosphorylation of JNK, but no changes in p38 (Fig. 5B), suggesting the important role of wedelolactone in ERK and JNK signaling. Accumulated evidence showed that the activation of NF-κB was closely associated with inflammation. The transcriptional factor NF-κB plays a key role in the regulation of the expression of iNOS, COX-2 and inflammatory cytokines such as TNF-α [15]. Western blot analysis showed the inhibitory effects of WEL on hepatic COX-2 and iNOS protein expression (Fig. 5A). To further investigate the molecular mechanism of inflammation in the mouse liver, we measured the translational levels of NF-κB p65. NF-κB p65 expression in the nuclear fractions significantly increased in the model group compared with normal control group (Fig. 5A). Accordingly, NF-κB p65 levels in the cytoplasm fractions were significantly reduced in the model group, indicating a translocation of NF-κB p65.

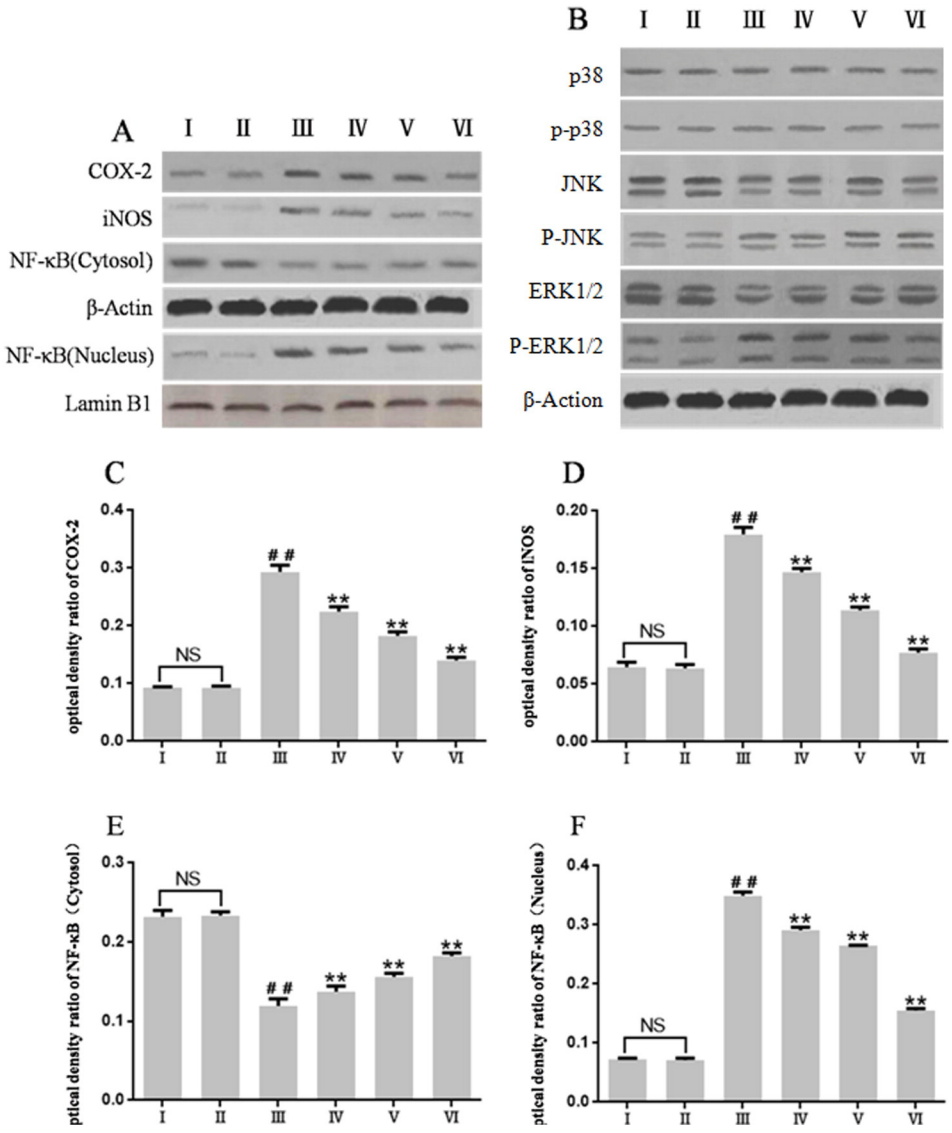

Fig. 5. Pretreatment effects of WEL on CCl_4-induced inflammatory response. (A) Western blot analysis of COX-2, iNOS and NF-κB proteins in response to CCl_4 and WEL; (B) Western blot analysis of p38, *p*-p38, JNK, *p*-JNK, ERK1/2 and *p*-ERK1/2 proteins in response to CCl_4 and WEL; optical density analysis of COX-2 (C), iNOS (D), NF-κB p65 in cytoplasm (E) and nucleus (F)s, p38 (G), *p*-p38 (H), JNK (I), *p*-JNK (J), ERK1/2 (K) and *p*-ERK1/2 (L); data were expressed as the mean ± SD ($n = 8$). $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ compared with the normal control group; $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with the CCl_4 group; NS = no significance. Animals were divided into the following groups: I, normal control; II, WEL control; III, model; IV, WEL (55 mg/kg) + CCl_4; V, WEL (110 mg/kg) + CCl_4; VI, WEL (220 mg/kg) + CCl_4.

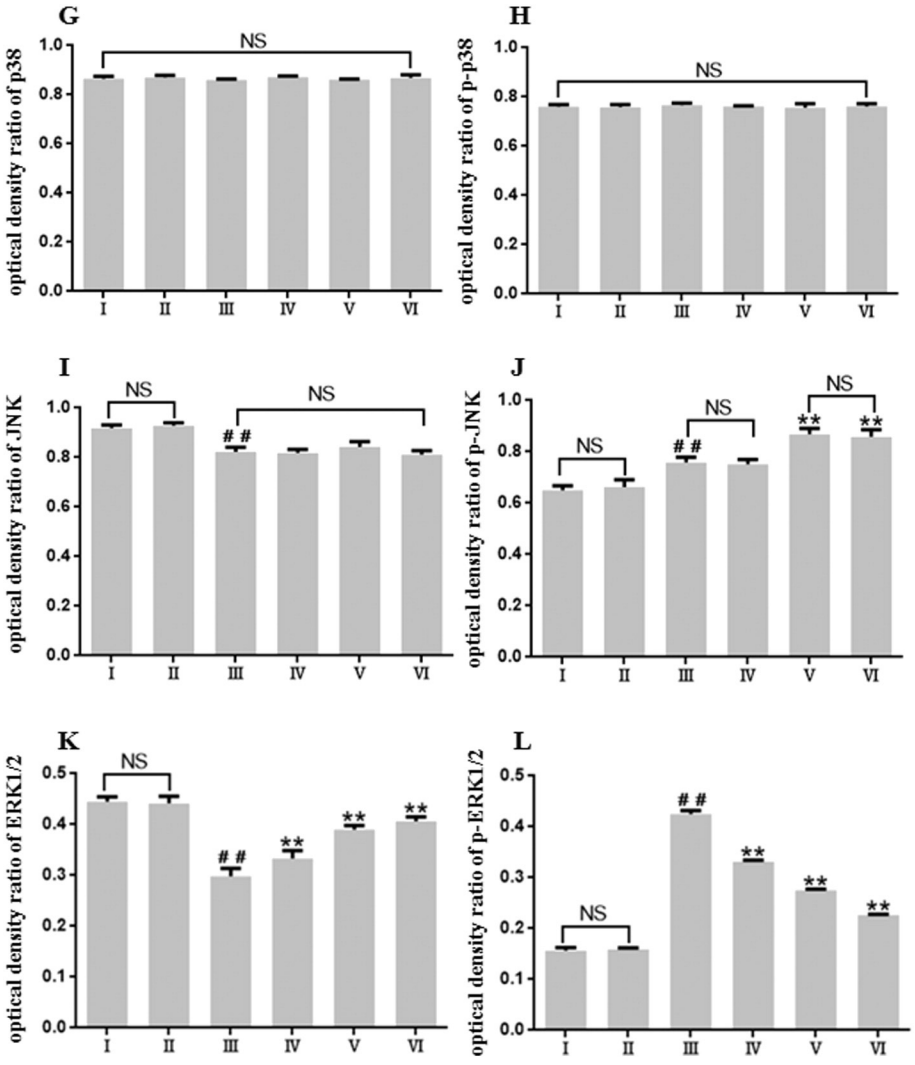

Fig. 5 (*continued*).

However, the pre-administration of WEL markedly inhibited the translocation of NF-κB p65. Herein, we assessed the effect of WEL on JNK, ERK and NF-κB pathway in CCl_4-induced acute liver injury.

3.5. WEL decreases CCl_4-induced apoptosis of hepatocytes

Hepatocytes apoptosis induced by CCl_4 had been reported in the previous studies [19]. TUNEL staining was used to assess the protective ability of WEL against hepatocyte apoptosis induced by CCl_4. The number of TUNEL-positive cells in the liver of the model group significantly increased compared with the normal control group (Fig. 6A, B), which was obviously attenuated by the pre-administration of WEL. The analysis of the active Caspase-3 expression revealed that the pre-administration of WEL inhibited the increased apoptosis induced by CCl_4 (Fig. 6C, D). The expression of Bcl-2 and Bax were determined by immunohistochemical (IHC) staining (Fig. 6E, F). Relative protein levels also indicated that the pre-administration of WEL inhibited the increased hepatocyte apoptosis induced by CCl_4.

4. Discussion

The present study demonstrated the hepatoprotective effect of WEL against CCl_4-induced ALI in animal experimental systems. As the ALI induced by CCl_4 was characterized with liver dysfunction and cell morphology deterioration, the liver histopathological changes and liver function were investigated. Our findings supported that WEL treatment could significantly mitigate liver histopathological changes as evidenced by H&E staining. Meanwhile, as the sensitive indicators of hepatic function and liver injury, high levels of ALT and AST induced by CCl_4 were markedly reversed by the treatment with WEL. Liver index reflects the degree of hepatomegaly. WEL has certain promoting effect on blood circulation to remove blood stasis, especially at a high dose. These play an important role in subsequent pathological and physiological changes. Our results showed that WEL treatment could alleviate hepatic dysfunction in ALI induced by CCl_4.

Oxidative stress has been accepted as a major molecular mechanism in ALI induced by CCl_4, which is responsible for cell membrane damage and the consequent release of marker enzymes of hepatotoxicity [24–26]. Oxidative injury induced by CCl_4 can be monitored by detecting oxidative stress parameters, such as MDA, SOD and GSH-Px. MDA has been used as a biomarker of lipid peroxidation for several decades and the increase of MDA has been considered a key feature in liver injury [27,28]. Our researches revealed that WEL-treatment could significantly prevent the increase of MDA induced by CCl_4 in mice. This effect greatly reduced lipid peroxidation damage and prevented strong inflammatory response. SOD is an endogenous antioxidant enzyme converting superoxide anions into H_2O_2 [29]. GSH-Px is an important enzyme catalyzing the reduction of H_2O_2 and lipid hydroperoxides into water and corresponding alcohols and then terminating the chain reaction of lipid peroxidation [30]. Lipid peroxides or reactive oxygen species (ROS) can

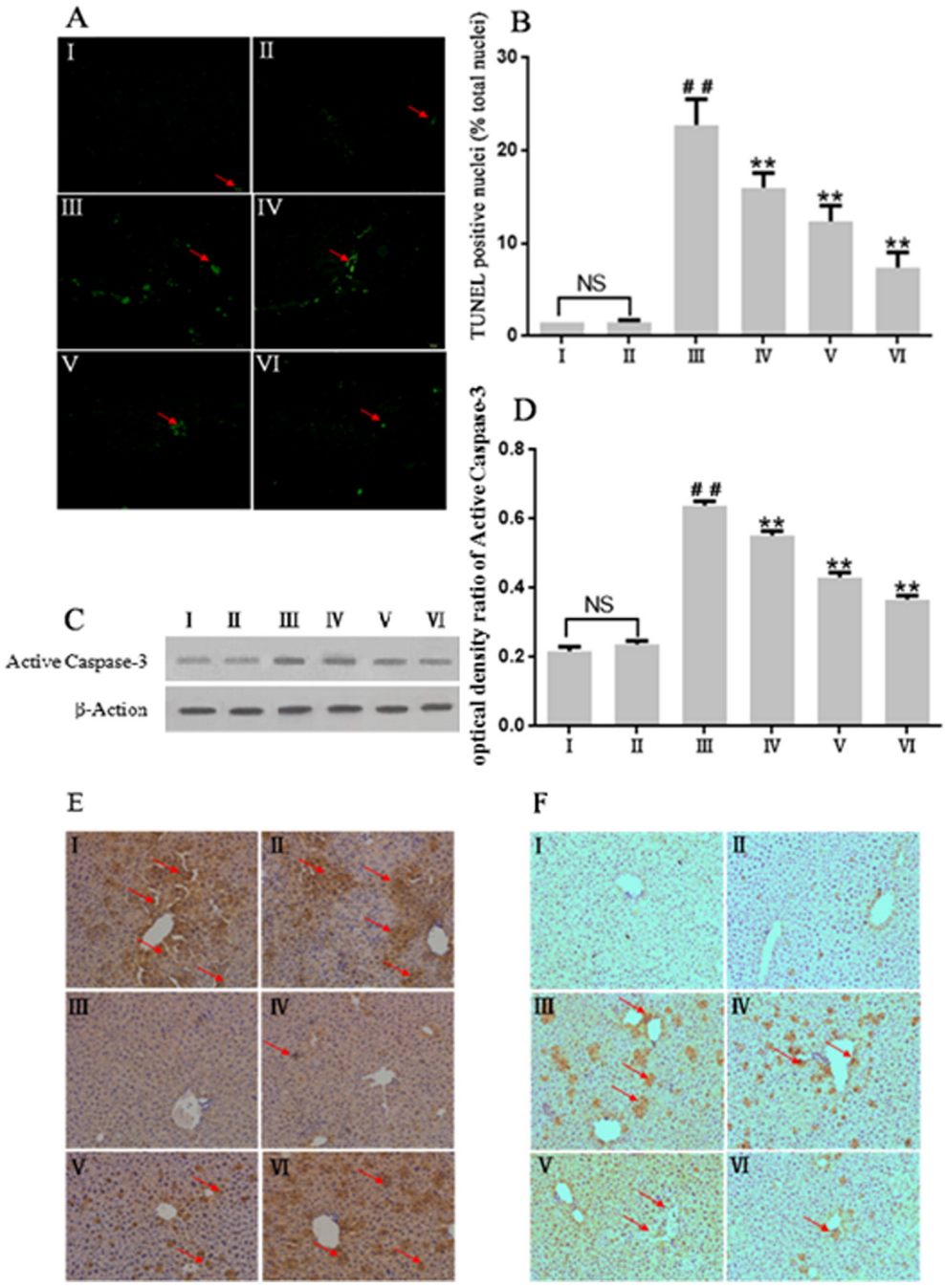

Fig. 6. Pretreatment effect of WEL on CCl_4-induced hepatocyte apoptosis. (A) TUNEL stained liver sections (magnification, 200×): green fluorescence indicated the positive cells (arrows); (B) Statistic analysis of the relative proportion of TUNEL positive cells in the liver of mice;(C) Western blot analysis of active Caspase-3 protein in response to CCl_4 and WEL; (D) Optical density analysis of active caspase-3; (E) IHC staining of Bcl-2 protein in response to CCl_4 and WEL; (F) IHC staining of Bax protein in response to CCl_4 and WEL; Data were expressed as the mean ± SD ($n = 8$). $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ compared with the normal control group; $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with the CCl_4 group; NS = no significance. Animals were divided into the following groups: I, normal control; II, WEL control; III, model; IV, WEL (55 mg/kg) + CCl_4; V, WEL (110 mg/kg) + CCl_4; VI, WEL (220 mg/kg) + CCl_4.

deactivate these antioxidant enzymes [31]. The current results showed that the activities of hepatic SOD and GSH-Px in the liver of CCl_4-treated mice markedly decreased, thereby causing oxidative damage to the liver. However, the pre-administration of WEL significantly elevated the activities of SOD and GSH-Px in the CCl_4-damaged liver, reflecting the ability of WEL to restore and preserve the activities of the two enzymes. As shown in Fig. 3B and C, the activity of GSH-Px had a higher sensitivity than SOD to WEL- treatment. Overall, the protective effect of WEL may be partly due to attenuating oxidative stress in CCl_4-induced ALI.

Inflammatory response was proved to be involved in the process of CCl_4-induced ALI [20]. CCl_4 and excessive ROS induced by CCl_4 probably activated Kupffer cells, which could mediate the hepatic inflammation process by producing TNF-α, IL-1β and IL-6 [21,22]. Nevertheless, levels of pro-inflammatory cytokines which were direct indicators of inflammatory response, were observably prevented the over-production of TNF-α, IL-1β, and IL-6 in mice challenged by CCl_4 in a dose-dependent manner, as well as mRNA over-expression. In order to explore the potential anti-inflammatory mechanism of WEL to ALI mice induced by CCl_4, NF-κB and MAPK signal pathways were investigated. Previous researches had shown that NF-κB regulated the expression of multiple genes involved in inflammatory response, which played a central role in inflammation [23]. Our studies revealed that the translocation of NF-κB p65 from cytoplasm to nucleus markedly increased in ALI liver induced by CCl_4. Then the related proteins, COX-2 and iNOS, were tested

and the expression of the two proteins significantly were upregulated. However, the pre-administration of WEL markedly suppressed the upregulation of COX-2 and iNOS, as well as the translocation of NF-κB p65.

MAPKs have been reported to be essential regulatory roles in both innate and adaptive immune response and play an important role in the transcriptional regulation of iNOS and COX-2 proteins [32,33]. Cytokines production is involved in several intracellular signaling pathways that include three MAPK pathways: ERK, JNK and p38 [33]. Several studies have demonstrated that active compounds could regulate MAPKs activities in vitro [34–37]. Thus, we investigated the effect of WEL on ERK1/2, JNK and p38 in mice. Our results showed that pre-treatment with WEL significantly inhibited the phosphorylation of ERK and markedly enhanced the phosphorylation of JNK, whereas p38 phosphorylation was not affected. These indicated that anti-inflammatory mechanism of WEL was mediated possibly via ERK1/2 and JNK pathway. NF-kB translocation rather than the phophorylation of ERK1/2 and JNK may be involved in WEL reducing cytokine production. However, the role and the underlying mechanism of WEL-induced phosphorylation changes of ERK1/2 and JNK are remained to be further elucidated in vitro.

Previous studies had demonstrated that CCl_4 could cause hepatocyte apoptosis [19]. Therefore, we investigated the effect of WEL on mitochondrial apoptotic pathways including active Caspase-3, Bcl-2 and Bax. Bax is a pro-apoptotic protein existing in the cytosol, but it will translocate to mitochondria induced by apoptosis, whereas Bcl-2 is an anti-apoptotic protein which can inhibit Bax-induced apoptosis [38]. IHC staining revealed that the administration of WEL inhibited the upregulation of Bax expression and restored the downregulation of Bcl-2 expression induced by CCl_4. TUNEL staining proved that CCl_4-treatment significantly increased the rate of apoptosis, which was significantly decreased by administration of WEL. These results indicated that WEL may suppress hepatocyte apoptosis by regulating the expression of the apoptosis-related proteins Bax and Bcl-2 induced by CCl_4.

5. Conclusions

In summary, the present study demonstrates the potent protective effect of WEL against CCl_4-induced acute liver damage, and the protective mechanism might be connected with enhancing the antioxidative defense system, suppressing the pro-inflammatory response and hepatocyte apoptosis. Overall, our study provides evidence of the protective effect of WEL against CCl_4-induced liver damage and suggests WEL as a supportive treatment for ALI.

Abbreviations

WEL	wedelolactone
ALT	alanine aminotransferase
AST	aspartate aminotransferase
H&E	hematoxylin and eosin
SOD	superoxide dismutase
GSH-Px	glutathione peroxidase
MDA	malondialdehyde
ROS	reactive oxygen species
NF-κB	nuclear factor-kappa B
COX-2	cyclooxygenase-2
iNOS	inducible nitric oxide synthase
TUNEL	terminal-deoxynucleotidyl transferase mediated nick end labeling
IHC	Immunohistochemical
CCl_4	carbon tetrachloride
ALI	acute liver injury

Author contributions

The list authors contributed to this work as follows: J.-Y.W., A.-D.W. and Y.L. conceived and designed the experiments, Y.L., D.-M.H. and S.-B.M. performed the experiments and wrote the paper, Y.L., D.-M.H. and S.H. analyzed the data, S.-B.M. contributed materials and analysis tools, X.Z. generated the figures, J.-Y.W., S.W., X.-F.W. and G.W modified the grammar and word faults of the manuscript. All authors read and approved the final manuscript.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgments

The authors are grateful for the grants from the National Natural Science Foundation of China. (No. 81302903).

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