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Protective effects of the total saponins from *Dioscorea nipponica* Makino against carbon tetrachloride-induced liver injury in mice through suppression of apoptosis and inflammation

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ABSTRACT

The present study was to investigate the effects and possible mechanisms of the total saponins from *Dioscorea nipponica* Makino (TSDN) against CCl_4-induced hepato-toxicity in mice. The mice were orally administrated with TSDN for seven days and then given CCl_4 (0.3%, 10 ml/kg i.p.). The results showed that TSDN significantly attenuated the activities of ALT and AST, consistent with hematoxylin–eosin staining. The ALP levels and relative liver weight were significantly decreased by TSDN compared with model group. Moreover, TSDN dramatically decreased MDA, iNOS and NO levels, while the levels of GSH, GSH-Px and SOD were increased. Further investigations showed that TSDN inhibited CCl_4-induced metabolic activation and CYP2E1 expression, down-regulated the levels of MAPKs phosphorylation, NF-κB, HMGB1, COX-2 as well as effectively suppressed the expressions of Caspase-3, Caspase-9, PARP and Bak. Quantitative real-time PCR assay demonstrated that TSDN obviously decreased the gene expressions of TNF-a, IL-1β, IL-6, IL-10, Fas, FasL, Bax as well as modulated Bcl-2 mRNA level. This is the first time to report the protective actions of the TSDN against CCl_4-induced liver damage in mice through suppression of inflammation and apoptosis. This natural product should be developed as a new drug for treatment of liver injury in future.

1. Introduction

Liver disease has been confirmed as one of the most serious health problems in the world [1], which can be caused by many factors including hepatitis virus infection, induction of drugs and toxins, and hepatic ischemia-reperfusion [2]. Carbon tetrachloride (CCl_4), a classical hepatotoxin, has been used extensively to induce liver injury in experimental models [3], which can be prevented or treated through blocking or retarding the processes of inflammation and apoptosis [4].

Nowadays, many hepatoprotective medicines have been widely used, and however, some of them have potential adverse effects [5]. Natural products from medicinal plants have been attracted much attentions as effective and safe alternative treatments for liver diseases [6]. *Dioscorea nipponica* Makino, a famous traditional Chinese medicinal plant, has been widely used in China for relieving cough and asthma, eliminating rheumatic aches, alleviating pain and improving blood circulation [7]. Saponins, the major components of *D. nipponica* Makino, have anti-oxidant, anti-virus, anti-inflammation and anti-cancer activities [8–10]. In our previous study, one major saponin (dioscin) from this herb, has been shown significant effects against alcohol-, CCl_4- and acetaminophen-induced liver injury [9,11,12]. However, there are no papers to report the protective effects of the total saponins from *D. nipponica* Makino (TSDN) against CCl_4-induced liver injury to the best of our knowledge.

Therefore, the aim of the present paper was to investigate the hepatoprotective effects of the total saponins from *D. nipponica* Makino (TSDN) against CCl_4-induced liver injury in mice and then to explore the possible mechanisms.

Abbreviations: ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; Caspase-3, cysteinyl aspartate specific proteinase-3; Caspase-9, cysteinyl aspartate specific proteinase-9; CCl_4, carbon tetrachloride; COX-2, cyclooxygenase-2; CYP2E1, cytochrome P450 2E1; ERK, extracellular signal-regulated kinase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GSH, glutathione; GSH-PX, glutathione-peroxidase; HMGB1, high mobility group box-1 protein; iNOS, nitric-oxide synthase; JNK, Jun N-terminal kinases; MDA, malondialdehyde; NF-κB, nuclear factor-kappa B; NO, nitric oxide; PARP, poly ADP-ribose polymerase; p38, p38 mitogen-activated protein kinase; p-p38, phosphorylation p38 mitogen-activated protein kinase; p-ERK, phosphorylation extracellular signal-regulated kinase; p-JNK, phosphorylation Jun N-terminal kinases; SOD, superoxide dismutase; TNF-α, tumor necrosis factor-alpha; TSDN, total saponins from *Dioscorea nipponica* Makino.

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Table 1
The information of the antibodies used in the present work.

Antibody	Source	Dilutions	Company
GAPDH	Rat	1:2000	Proteintech Group Inc. (Chicago, USA)
NF-κB	Rabbit	1:500	Proteintech Group Inc. (Chicago, USA)
COX-2	Rabbit	1:50	Proteintech Group Inc. (Chicago, USA)
Bak	Rabbit	1:200	Proteintech Group Inc. (Chicago, USA)
PARP	Rabbit	1:200	Proteintech Group Inc. (Chicago, USA)
CYP-2E1	Rabbit	1:200	Proteintech Group Inc. (Chicago, USA)
HMGB1	Rabbit	1:500	Proteintech Group Inc. (Chicago, USA)
Caspase-3	Rabbit	1:200	Proteintech Group Inc. (Chicago, USA)
Caspase-9	Rabbit	1:200	Proteintech Group Inc. (Chicago, USA)
p38	Rabbit	1:500	Bioworld Technology (USA)
p-p38	Rabbit	1:500	Bioworld Technology (USA)
ERK	Rabbit	1:500	Bioworld Technology (USA)
p-ERK	Rabbit	1:500	Bioworld Technology (USA)
JNK	Rabbit	1:500	Bioworld Technology (USA)
p-JNK	Rabbit	1:500	Bioworld Technology (USA)

2. Materials and methods

2.1. Chemicals and materials

The standard protodioscin, methyl protodioscin dioscin and glibenclamide with purities > 98% were purchased from National Institutes of Food and Drug Control of China (Beijing, China). Analytical grades of glacial acetic acid, perchloric acid and vanillin were purchased from Xinyuan Chemical Technology Co., Ltd. (Tianjing, China). CCl_4 was purchased from Kaixing Chemical Industry Co., Ltd. (Tianjin, China). Silymarin was obtained from Sigma Chemical Company (Milan, Italy). Glacial acetic acid, vanillin and perchloric acid with analytical grades were purchased from Xinyuan Chemical Technology Co., Ltd. (Tianjing, China). The detection kits including alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione (GSH), inducible nitric oxide synthase (iNOS), nitric oxide (NO) and malondildehyde (MDA) were all purchased from Nanjing Jiancheng Institute of Biotechnology (Nanjing, China). 4′, 6′-Diamidino-2-phenylindole (DAPI), Tris and SDS were purchased from Sigma (St. Louis, MO, USA). Enhanced Bicinchoninic Acid (BCA) Protein Assay Kit was obtained from Beyotime Institute of Biotechnology (Jiangsu, China). Tissue Protein Extraction Kit was purchased from KeyGEN Biotech. Co., Ltd. (Nanjing, China). In Situ Cell Death Detection Kit, POD (TUNEL) was obtained from Roche (Roche Diagnostic, Mannheim, Gemany). Histostain-TM-Plus and 3, 3′-Diaminobenzidine tetrahydrochloride (DAB) Substrate Kits were purchased from Zhongshan Golden Bridge Biotechnology (Beijing, China).

2.2. Preparation of the crude extract from D. nipponica Makino

The medicinal plant was supplied by Yunnan Qiancaoyuan Pharmaceutical Company Co. Ltd. (Yunnan, China) and identified by Dr. Yunpeng Diao (College of Pharmacy, Dalian Medical University, Dalian, China). A voucher specimen (DLMU, CSL-20120526) was placed in the Herbarium of College of Pharmacy, Dalian Medical University (Dalian, China). The raw materials were ground into powders (20–40 mesh), and the powders (1.0 kg) were extracted with 75% aqueous ethanol for two times (2.5 h for each) under heat and reflux. The extracted solution was combined and evaporated under reduced pressure at 60 °C, and total of 1000 ml brown residue was obtained. Then, 1000 ml distilled water was added into the residue and the mixture was used for subsequent column chromatography.

2.3. Macroporous resin column chromatography

Firstly, suitable macroporous resin for preparation of the total saponins from the crude extract was optimized. Six types of macroporous resins including D318, D101, D315, DM301, X-5 and D1400 supplied by Haiguang Chemical Factory (Tianjin, China) were tested. Amount of 5.0 g macroporous resins were placed into flasks, and 50 ml sample solution was added, and then the flasks were shaken (150 rpm) for 6 h at 25 °C. After that the solutions were removed, and the supernatant was analyzed. In addition, the resins were washed three times with deionized water, and then washed with 50 ml 70% aqueous ethanol. The flasks were shaken for 6 h (150 rpm, 25 °C), and desorption solutions were analyzed. The adsorption capacity and desorption ratio of each resin were calculated based on the following formulas (1) and (2), respectively

$$\text{Adsorption capacity} = \frac{V_0 \times (C_o - C_e)}{W} \quad (1)$$

$$\text{Desorption ratio} = \frac{V_d C_d}{V_0 \times (C_0 - C_e)}. \quad (2)$$

Where adsorption capacity (mg/g) represents the amount of TSDN adsorbed on one gram dry resin; C_0 and C_e are the initial and equilibrium concentrations of TSDN in the solutions (mg/ml), respectively; V_0 is the volume of the initial sample solution (ml) and W is the weight of dry resin (g); V_d is the volume of the desorption solution (ml) and C_d represents the concentration of TSDN in desorption solution (mg/ml).

In the second step, the extracted sample solution (1000 ml) was added into a glass column (6.0 cm × 80 cm, contained 600 g D101 macroporous resin) with 500 ml of bed volume (BV). Initially, distilled water was used to wash the column, then 4.0 BV of 30% aqueous ethanol was used to remove the high polar components, and the adsorbent was finally eluted with 70% aqueous ethanol. Then 70% aqueous ethanol fraction was collected and evaporated under reduced pressure at 60 °C to dryness, and the shallow white powder was produced, which was used for subsequent experiments.

Table 2
The primer sequences used for real-time PCR assay in mice.

Gene	Forward/reverse primer (5′-3′)	GenBank accession
TNF-a	TATGGCCCAGACCCTCACA GGAGTAGACAAGGTACAACCCATC	NM_013693.2
IL-1β	TCCAGGATGAGGACATGAGCAC GAACGTCACACACCAGCAGGTTA	NM_008361.3
IL-6	CCACTTCACAAGTCGGAGGCTTA CCAGTTTGGTAGCATCCATCATTTC	NM_031168.1
IL-10	GCCAGAGCCACATGCTCCTA GATAAGGCTTGGCAACCCAAGTAA	NM_010548.2
Fas	ACATGGACAAGAACCATTATGCTGA CTGGTTTGCACTTGCACTTGGTA	NM_007987.2
FasL	CATGCAGCAGCCCATGAATTAC CTCTAGGCCCACAAGATGGACAG	NM_010177.4
Bax	CAGGATGCGTCCACCAAGAA CGTGTCCACGTCAGCAATCA	NM_007527.3
Bcl-2	TGAAGCGGTCCGGTGGATA CAGCATTTGCAGAAGTCCTGTGA	NM_177410.2
GAPDH	TGTGTCCGTCGTGGATCTGA TTGCTGTTGAAGTCGCAGGAG	NM_008084.2