Hepatoprotective Activities of Huoshan Dendrobium Officinale Kimura et Migo Water Extracts on Carbontetrachloride-induced Hepatotoxicity in Mice

Jichun Han¹,², Defang Li¹,², Xiaoyu Chen¹, Fanqing Meng¹, Bo Wang³, Xinjie Zhang¹, Qiusheng Zheng¹,³

¹Binzhou Medical University, Yantai, China
²Anhui Hushengji Biotechnology Co., Ltd., Luan, China
³Pharmacy School of Shihezi University, Key Laboratory of Xinjiang Endemic Phytomedicine Resources, Ministry of Education, School of Pharmacy, Shihezi, China

Abstract Objective: The objective of this study is to investigate the hepatoprotective effects of water extracts from Huoshan Dendrobium officinale Kimura et Migo (HDW) in a mouse model of carbon tetrachloride (CCl₄)-induced hepatotoxicity. In addition, potential mechanisms of any effects observed will be investigated.

Methods: Mice received HDW pretreatment prior to induction of hepatotoxicity. HDW was administered to mice once daily for a total of 5 days (p.o.) at three dose levels (20, 100 and 500 mg/kg/day). Hepatotoxicity was then induced in Kunming mice using a single injection (s.c.) of CCl₄. CCl₄ was diluted in corn oil and used at a concentration of 10 ml/kg body weight. Levels of superoxide dismutase (SOD), malondialdehyde (MDA), glutathione disulfide (GSH) and glutathione disulfide (GSSG) were analyzed as a readout to measure oxidative stress status. Levels of C-reactive protein (CRP), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6) were measured as a readout of inflammation status. Levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were analyzed as a measure for the degree of hepatic injury. Finally, the liver ultrastructure was examined using optical microscopy.

Results: CCl₄-induced hepatotoxicity was shown to result in increased levels of ALT, AST, MDA, IL-6, CRP and TNF-α, and decreased levels of SOD and a decreased GSH/GSSG ratio in serum. Histopathological examination of liver sections using microscopy revealed necrosis and inflammatory effects due to CCl₄-induced hepatotoxicity. We observed that HDW pretreatment resulted in decreased levels of ALT, AST, MDA, IL-6, CRP and TNF-α, and increased SOD levels and an increased GSH/GSSG ratio. In addition, the hepatic histo-architecture was shown to be normalized.

Conclusion: This study provides support for the use of HDW to protect against toxic liver injury. Furthermore, we show that the hepatoprotective effects of HDW are likely attributed to its antioxidant and anti-inflammatory activities.

Keywords: dendrobium officinale kimura et migo, antioxidant, anti-inflammatory, hepatotoxicity


1. Introduction

The liver is a vital organ in the human body which functions to detoxify exogenous xenobiotics, drugs, viral infections, and alcohol from the body. Liver disease is a primary causes of mortality and morbidity worldwide. Specifically, drug-induced liver toxicity has been shown to be strongly associated with the onset of hepatic dysfunction [1]. Liver damage is a widespread pathology that is often characterized by oxidative stress and a progressive evolution from steatosis to chronic hepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma [2]. In recent years, a great deal of attention has focused on the transformation of chemicals into highly reactive metabolites associated with cellular toxicity. Carbon tetrachloride- (CCl₄-) induced hepatotoxicity has been widely used in animal models to investigate the hepatoprotective effects of natural compounds [3,4].

Oxidative stress is considered to be a contributing mechanism towards the initiation and progression of hepatic damage in a range of different liver disorders. Excess reactive species derived from oxygen and nitrogen or an antioxidant deficiency are known to be causative agents of oxidative stress, ultimately leading to cell death [5]. Thus, antioxidants isolated from plant sources represent a promising therapeutic strategy for the treatment of liver disease. Both flavonoids and polysaccharides possess unique antioxidant properties as well as other pharmacological activities that could play a role in the protection of the liver from CCl₄-induced...
2. Materials and Methods

2.1. Sample Preparation

Huoshan Dendrobium officinale Kimura et Migo was purchased from Sannor Biotechnology in Luan, China. The authenticity of the material was verified by an author of this paper, and further confirmed by a botanist.

2.2. Extract Preparation

Water extract was prepared by adding 20 g of Huoshan Dendrobium officinale Kimura et Migo to 1 L of boiling water. The mixture was then stored at room temperature for 4 hours to enable the infusion process to occur. Following the infusion step, the mixture was filtered through Whatman No.1 filter paper and the extract was dried by Bain-marie. The extract was then stored in a sterile bottle for 4 hours to enable the infusion process to occur. Following the infusion step, the mixture was filtered through Whatman No.1 filter paper and the extract was dried by Bain-marie. The extract was then stored in a sterile bottle.

2.3. Animals and Experimental Groups

Kunming mice weighing 20–30 g were obtained from Xinjiang Medicine University Medical Laboratory Animal Center [License Number: SCXK(xin)2011-0003]. All experimental procedures were approved by the Institutional Animal Care and Use Committee of the National Institute Pharmaceutical Education and Research.

Mice were randomly divided into five groups (n = 12 mice/group): (1) control group, mice that received distilled water for 5 days, (2) the CCl4 group with mice that received distilled water for 5 days and were intraperitoneally (i.p.) administered 10 mL/kg body weight CCl4 diluted with 1:500 corn oil once on day 6 [13], (3) the three HDW + CCl4 groups with mice who were treated with HDW once daily for 5 days (20, 100 or 500 mg/kg/day) followed by a single i.p. dose of CCl4 (10 ml/kg body weight) on day 6.

2.4. Estimation of Biochemical Parameters

Twenty-four hours post-CCl4 administration, animals were anesthetized ether. A total of 1 ml of blood was collected from each mouse via cardiac puncture. The blood was allowed to clot and was then centrifuged at 4000 g for 10 min. Following centrifugation, the serum was isolated and used to assay for ALT and AST using standard enzyme assay kits (Tsz Biosciences, Greater Boston, USA).

2.5. Assay of Oxidative Stress

SOD activity, MDA levels, GSH levels, and GSSG levels in serum were measured using spectrophotometry according to the ELISA assay instructions (Tsz Biosciences, Greater Boston, USA).

2.6. Assay of Inflammation

Tumor necrosis factor-α (TNF-α), C-reactive protein (CRP), and interleukin-6 (IL-6) levels in serum were measured using spectrophotometry by an ELISA assay (Tsz Biosciences, Greater Boston, USA) according to the manufacturer’s instructions.

2.7. General Histology Survey of Livers

Mice were sacrificed by cervical dislocation. The livers were then excised, washed in phosphate buffer, and dried using tissue paper. Each mouse liver was fixed in 10% formaldehyde and preserved at normal temperature (20°C-25°C). A small piece of hepatic tissue (2 mm × 1 mm × 1 mm) was obtained and fixed in 0.1 mM phosphate buffer (pH 7.2) containing 3% glutaraldehyde and 1.5% paraformaldehyde at 4°C. This piece of hepatic tissue was then further cut into smaller 1 mm3 sized pieces, and subsequently fixed in the abovementioned solution for 4 hours. The piece was then rinsed with phosphate buffer and fixed in 1% osmic acid at 4°C for 1.5 hours. Finally, the tissue was dehydrated using alcohol followed by treatment with dimethylbenzene. The sample was then embedded in epoxy resin 618. The tissue was first located by semi-thin sectioning, and then sliced into ultrathin sections (60 nm). Slides were examined under a microscope (Olympus, Japan) at 100 × magnification in order to observe any histopathological changes.

2.8. Statistical Analysis

Data are presented as the mean ± SD from at least three independent experiments. Statistical significance was
evaluated using ANOVA followed by Student’s t-test. Statistical significance was considered at p < 0.05. Statistical analysis was performed using SPSS (IBM SPSS, International Business Machines Corporation, Armonk, NY, USA).

3. Results

3.1. Effects of HDW on Serum ALT and AST Activities

As shown in Figure 2, CCl4–induced hepatotoxicity in rats resulted in a marked increase in levels of liver function serum markers. Specifically, ALT (251.61±17.18 U/L) (Figure 1A) and AST (166.21±8.15 U/L) levels were observed to be increased compared to the control group (p < 0.01, Figure 1B). We show that the increased levels of liver function markers observed with CCl4-induced hepatotoxicity returned to control levels with the ameliorative effect of HDW treatment. Significant hepatoprotective effects were observed in animals treated with HDW at 100 mg/kg (p < 0.05) and 500 mg/kg (p < 0.01), with a dose-dependent effect of HDW observed.

3.2. HDW Alleviated Oxidative Stress of Hepatotoxicity Induced by CCl4

As shown in Table 1, oxidative stress markers in liver homogenates from mice revealed that CCl4-induced hepatotoxicity resulted in a significant decrease in activity levels of oxidative stress marker enzymes in serum, such as SOD (24.07 ± 2.56 U/mL) and GSH (412.13 ± 20.70 ng/L) in comparison to the toxic control group (SOD, 51.19±4.53 U/mL and GSH, 789.84±18.64 ng/L). In addition, MDA (10.00 ± 0.35 nmol/mL) and GSSG (9.67 ± 0.22 nmol/mL) levels was observed to be significantly increased in CCl4-intoxicated mice compared to control animals.

![Figure 1](image1.png)

**Figure 1.** Prophylactic effect of HDW on the restoration of liver function markers in CCl4-intoxicated mice. Values are expressed as mean ± SD (n = 8/group). #p < 0.05 and ##p < 0.01 compared to the normal control group, *p < 0.05 and **p < 0.01 compared to the CCl4 treatment group

![Figure 2](image2.png)

**Figure 2.** Effect of HDW on the levels of IL-6, TNF-α and CRP in mice subjected to CCl4-induced hepatotoxicity (values are presented as mean ± SD, n = 8). #p < 0.05 and ##p < 0.01 compared to the normal control group, *p < 0.05 and **p < 0.01 compared to the CCl4 treatment group.
Table 1. The effect of HDW pretreatment on oxidative stress parameters of mice in CCl₄-induced hepatotoxicity, Values are expressed as mean ± SD (n = 8/group)

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD(U/mL)</th>
<th>MDA(nmol/mL)</th>
<th>GSH (ng/L)</th>
<th>GSSG(nmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>51.19 ± 4.53</td>
<td>6.66 ± 0.15</td>
<td>789.84 ± 18.64</td>
<td>7.47 ± 0.33</td>
</tr>
<tr>
<td>CCl₄</td>
<td>24.07 ± 2.56##</td>
<td>10.00 ± 0.35##</td>
<td>412.13 ± 20.70##</td>
<td>9.67 ± 0.22##</td>
</tr>
<tr>
<td>20 mg/kg</td>
<td>27.13 ± 2.75</td>
<td>9.52 ± 0.33</td>
<td>459.70 ± 23.35</td>
<td>9.49 ± 1.00</td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>32.78 ± 3.34*</td>
<td>8.76 ± 0.19*</td>
<td>535.91 ± 73.25*</td>
<td>8.70 ± 0.96*</td>
</tr>
<tr>
<td>500 mg/kg</td>
<td>43.49 ± 2.78**</td>
<td>7.80 ± 0.46**</td>
<td>659.10 ± 25.21**</td>
<td>7.73 ± 0.61**</td>
</tr>
</tbody>
</table>

*p < 0.05 and ##p < 0.01 compared to the normal control group, *p < 0.05 and **p < 0.01 compared to the CCl₄ group.

Compared with CCl₄ treatment group, the group pretreated with HDW (100 or 500 mg/kg) showed a significant ameliorative effect with a recovery observed in both SOD (32.78 ± 3.34 U/mL and 43.49 ± 2.78 U/mL) and GSH (535.91 ± 79.25 ng/L and 659.10 ± 25.21 ng/L) levels (p < 0.01). In addition, increased levels of MDA and GSSG in the CCl₄-treatment group were observed to be lowered closer to control levels in the case of animals which received a 500 mg/kg HDW pretreatment (7.8 ± 0.46 nmol/mL MDA and 7.73 ± 0.61 nmol/mL GSSG). Finally, in animals that received a 100 or 500 mg/kg HDW pretreatment, levels of oxidative stress marker enzymes were observed to be restored to levels close to that of control animals.

3.3. HDW Attenuated Inflammation of Hepatotoxicity Induced by CCl₄

Inflammation is a key mechanism which underlies hepatotoxic injury. We measured levels of inflammatory cytokines in serum (IL-6, CRP and TNF-α) associated with hepatotoxic injury in order to identify potential mechanisms underlying the hepatoprotective activity of HDW. We observed significantly lower levels of IL-6 in animals pretreated with HDW at 100 mg/kg (98.55 ± 1.08 pg/mL) (p < 0.05) and 500 mg/kg (87.60 ± 1.26 pg/mL) (p < 0.01) compared to CCl₄-treated animals (115.95 ± 8.21 pg/mL) (Figure 2A). CRP levels were also observed to be significantly decreased in animals pretreated with HDW at 100 mg/kg (1983.15 ± 128.33 μg/L) (p < 0.05) and 500 mg/kg (1073.15 ± 91.46 μg/L) (p < 0.01) compared to that of the I/R group (2284.95 ± 186.55 μg/L) (Figure 2A). TNF-α activity was also observed to be significantly decreased in animals pretreated with HDW at 100 mg/kg (396.63 ± 26.50 ng/L) and 500 mg/kg (321.63 ± 18.03 ng/L) compared to that of the CCl₄ group (496.83 ± 24.93 ng/L) (p < 0.05, p < 0.01) (Figure 2C).

3.4. Estimation of Histopathological Parameters

The histological profile of liver sections from control animals showed normal hepatic architecture consisting of a well-preserved cytoplasm, a prominent nucleus, a central vein, and a compact arrangement of hepatocytes with no fatty lobulation (Figure 3A). In contrast, we observed hydropic changes in centrilobular hepatocytes and cell necrosis surrounded by neutrophils in liver sections from CCl₄-treated animals. In addition, congestion of the central vein and sinusoids was observed along with infiltration of inflammatory cells within sinusoids mainly in the central zone (Figure 3B). Liver sections of mice that were administered 20 mg/kg HDW appeared similar to sections from CCl₄-treated animals, with hydropic changes in centrilobular hepatocytes, cell necrosis...
surrounded by neutrophils, and inflammatory cells infiltrating sinusoids mainly in the central zone (Figure 3C). However, in the case of liver sections from mice that were administered 100 mg/kg HDW, we observed only mild necrosis and a mild presence of inflammatory cells (Figure 3D). Animals that were administered with 500 mg/kg HDW were observed to exhibit significant liver protection against CCl₄-induced liver damage. This was evident by the presence of hepatic cords and the absence of both inflammatory cells and necrosis (Figure 3E).

4. Discussion

In this study, the hepatoprotective effects of HDW were examined in a mouse model of CCl₄-induced liver toxicity. We show that HDW treatment results in a suppression of the CCl₄-induced increase in MDA, IL-6, CRP, GSSG and TNF-α levels, and a concomitant decrease in SOD activity and GSH levels attributed with the CCl₄-induced hepatotoxicity model. Thus, the hepatoprotective effects of HDW could be attributed to its antioxidant and anti-inflammatory properties.

CCl₄ is a well-established hepatotoxin known to induce toxic liver injury. This system is used widely to study the cellular mechanisms that underlie oxidative damage in animal systems [14]. Carbon tetrachloride is metabolized by cytochrome P4502E1 (CYP2E1) to form trichloromethyl (CCl₃-) and trichloromethyl peroxy (CCl₃OO-) radicals that are largely associated with CCl₄-induced hepatic damage [15]. These radicals bind covalently to macromolecules within the cell, with a known preference towards polyunsaturated fatty acids (PUFA) within the cell membrane. This leads to the generation of fatty acid free radicals, known to initiate autocatalytic lipid peroxidation which ultimately causes a loss in membrane integrity and the leakage of microsomal enzymes. This process is characterized by elevated levels of serum marker enzymes, such as AST and ALT, following administration of CCl₄ in animals [16,17]. Here, we show that levels of serum marker enzymes (AST and ALT) are significantly elevated in animals treated with CCl₄. The administration of HDW was observed to reduce the toxic effect of CCl₄ by restoring the serum marker enzyme levels to that of control levels.

ROS generation has been described to be one of the major factors involved in liver toxicity [18]. The antioxidant-like molecules, SOD and GSH, play important roles as defense mechanisms against the damaging effects of reactive oxygen species (ROS) and free radicals in biological systems [19]. We observed increased levels of MDA and GSSG in liver tissue homogenate of CCl₄-treated mice treated in our study. These increased levels of MDA and GSSG are thought to reflect lipid peroxidation and plasma membrane damage caused by oxidative stress [19,20]. Interestingly, we show that HDW treatment in these systems caused SOD and GSH levels to increase back to levels similar to that of control animals, while MDA and GSSG levels were observed to decrease back to levels similar to control animals.

A histopathological profile of a CCl₄-treated mouse liver demonstrated necrosis and an infiltration of inflammatory mediators. Animals treated with HDW showed a significant improvement of CCl₄-induced liver injury, as demonstrated by the presence of normal hepatic cords and an absence of necrosis. In the present study, we found that CCl₄-induced liver injury resulted in an increase in CRP, IL-6, and TNF-α levels in serum. However, HDW treatment was found to reduce the levels of these cytokines. Therefore, we suggest that the suppressed infiltration of inflammatory cytokines with HDW treatment could contribute to its hepatoprotective properties.

5. Conclusion

This study provides evidence that HDW possesses hepatoprotective properties that act to protect against hepatic damage induced by carbon tetrachloride. The hepatoprotective effects of HDW may be attributed to its antioxidant and anti-inflammatory properties.

Acknowledgments

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Conflict of Interests

The authors declare that they have no financial conflict of interests.

References


