Original paper

Hepatoprotective Effect of Ultrafine Powder of *Dendrobium officinale* against Acetaminophen-Induced Liver Injury in Mice

Song-Zhi Kong1†, Guo-Sheng Lin2,3†, Jing-Jing Liu2,3, Ling-Ye Su4,5, Lei Zeng4,5, Dan-Dan Luo4,5, Zi-Ren Su2,3* and Hong-Feng Wang4,5*

1Guangdong Ocean University, Faculty of Chemistry and Environmental Science, Zhanjiang 524088, Guangdong, Peoples R China
2Guangzhou University of Chinese Medicine, Mathematical Engineering Academy of Chinese Medicine, Guangzhou 510006, Guangdong, Peoples R China
3Guangzhou University of Chinese Medicine, Guangdong Provincial Key Laboratory of New Drug Development and Research of Chinese Medicine, Guangzhou 510006, Guangdong, Peoples R China
4Guangdong Provincial Key Laboratory of Silviculture, Protection and Utilization, Guangzhou 510000, Guangdong, Peoples R China
5Guangdong Academy of Forestry, Guangzhou 510520, Guangdong, Peoples R China

Received August 28, 2017 ; Accepted December 8, 2017

The aim of this study was to investigate the hepatoprotective effect of ultrafine powder of *Dendrobium officinale* (UDO) against acetaminophen (APAP)-induced liver injury in mice. ICR mice were orally administered with UDO (100, 200, 400 mg/kg/day) for 14 days before intraperitoneal injection of APAP. The results showed that UDO exhibited a significant dose-dependently preventive effect on APAP-induced hepatotoxicity. Pretreatment with UDO significantly reduced the serum levels of alanine transaminase and aspartate aminotransferase. Histological examination indicated that administration with UDO alleviated inflammation and necrosis as well as ameliorated hepatocyte steatosis, especially at 200 and 400 mg/kg doses. Additionally, UDO markedly recovered the activities of glutathione, catalase and total antioxidant capacity, and decreased the content of malonaldehyde and reactive oxygen species. In summary, the results suggested that pretreatment with UDO exists potently hepatoprotective effects against APAP-induced liver damage, which may be related to the enhanced antioxidant capacity and inhibited lipid peroxidation.

Keywords: *Dendrobium officinale*, ultrafine powder, acetaminophen, liver injury, antioxidant

Introduction

Acetaminophen (APAP), an over-the-counter (OTC) drug, is safely and effectively used as antipyretic and analgesic at appropriate dose (Rumack, 2004). Once APAP exceeds the riskless dose, it can cause hepatic injury, kidney damage, and even death potentially. In USA and UK, APAP has become the main cause of drug-induced liver injury a few decades ago (Larson *et al*., 2005; Lee, 2008). Besides, the pathogenesis of APAP-induced liver injury has been demonstrated on many reports, mainly including depletion of GSH and the increased formation of reactive oxygen species (ROS) (Dahlin *et al*., 1984; Ferret *et al*., 2001). With the
metabolism of cytochrome P450 (CYP), APAP is converted to N-acetyl-p-benzoquinone imine (NAPQI). NAPQI, a toxic product, will further combine with GSH to eliminate from the body, which directly led to the reduction of GSH. Meanwhile excessive APAP will increase the production of ROS (Ferret, et al., 2001). Once the balance between antioxidant defense and ROS production is broken, the body appears oxidative stress and damage (Cohen et al., 1997; Reid et al., 2005). Simultaneously, excessive NAPQI also caused mitochondrial dysfunction, lipid peroxidation, as well as DNA fragmentation (Hinson et al., 2004).

According to the previous report and clinical treatment, N-acetylcysteine (NAC) is used as the world’s most recognized drug for the treatment of APAP-induced liver injury for its poisoning detoxification activity. Nonetheless, NAC is confined to administration time before the evident rise in serum transaminase activities. And it has a therapeutic effect only in the early stage when people or animal was given APAP (Hau et al., 2010; Smilkstein et al., 1988). Therefore, it is vital to discover other novel drugs that can alleviate liver injury induced by APAP.

As a traditional Chinese medicine in China, Dendrobium officinale is the highest grade in the species of the Dendrobium, which has been favored and admired for its unique effects of reinforcing the stomach function and generating body fluid (Wu et al., 2013). In addition, due to the powerful safety, Dendrobium officinale was recognized as homology of medicine and food, which was approved by the China Food and Drug Administration (CFDA) (Lv et al., 2013; Schepetkin et al., 2006). In daily life, Dendrobium officinale is generally used for cooking soup and drinking after mixed with water. And it has been developed into functional food with different forms in the health care industry, mainly including powder, granule, capsule, tablet, extract, et.al (Chen et al., 2013). As a new food technology, ultrafine powder has attracted more and more attention from medical staffs, patients and health people for its advantage of less dosage, carrying conveniently and increasing the dissolution of effective composition (Tang et al., 2011). Besides, the previous research demonstrated that Dendrobium possessed activities of immunomodulation, anti-diabetes, anti-inflammation, neuro-protection, hepato-protection, anti-oxidation, et.al (Ng et al., 2012; Teixeira da Silva et al., 2017). However, the effect of Dendrobium officinale or UDO on hepatic injury induced by APAP has seldom been reported. Coincidentally, Dendrobium huoshanense has hepatoprotective effect and Dendrobium officinale has antioxidative effect, which indicated that Dendrobium officinale may have protective effect on APAP-induced liver injury. Therefore, we used UDO, the most popular and efficient form of Dendrobium officinale, to investigate whether Dendrobium officinale could take effect on the model of APAP-induced liver injury in mice.

Serum liver enzymes alanine transaminase (ALT) and aspartate aminotransferase (AST) were examined as the diagnostic marker to evaluate the condition of liver function. To further explore the associated mechanisms, oxidative indicators of GSH, catalase (CAT) and total antioxidant capacity (T-AOC), malonaldehyde (MDA) and ROS content were also detected.

Materials and Methods

Animals Healthy male ICR mice, weighing 28.0 ± 2.0 g (6 - 8 weeks), were provided by Guangdong Medical Laboratory Animal Center (GDMLAC, Foshan, China) with the license number of SCXK2013-0002. Animals were housed in a standard controlled condition with room temperature of 23 ± 2°C, relative humidity of 50 ± 10% and 12 h light/dark cycle. The mice were fed with food and water ad libitum. They were adapted in above environment for one week before the experiment. All the animal experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals, and were approved by the Institutional Animal Care and Use Committee of Guangzhou University of Chinese Medicine.

Chemicals and reagents Acetaminophen (APAP) was obtained from Shanghai Macklin Biochemical Co., Ltd (Shanghai, China). NAC was bought from Guangzhou Feibo Biological Technology Co., Ltd (Guangzhou, China). Plasma liver enzymes test kits including ALT (C009-2) and AST (C0010-2) were also provided by NJBI. Mouse tissue ROS Elisa kit was bought from Beijing Cheng Lin biological technology co., LTD (Beijing, China). Except as otherwise expressly stated, other chemicals and reagents were used in analytical grade for meeting the needs of the experiment.

Plant material and identification Dendrobium officinale was kindly supplied by the Guangdong Academy of Forestry (Guangzhou, China) and identified by Prof. Ziren Su, a connoisseur from Guangzhou University of Chinese Medicine (Guangzhou, China). Fresh stem of Dendrobium officinale was naturally dried in the open sun and then stored in oven at 50°C for 24 h. After that, Dendrobium officinale was smashed by a ball mill and then sift successively through the mesh size of 80 and 500 (Luo et al., 2017). Finally, the satisfactory particle size of UDO was obtained for the experiment.

Animal grouping and experimental protocols After one week adaptation period, 60 ICR mice were randomly assigned to six groups (n = 10): intact group; vehicle group; positive control group (NAC, 200 mg/kg/day); three treatment groups of UDO (100, 200 and 400 mg/kg/day). And the dose of UDO was selected based on the previous investigation and our preliminary experiment (XY Wang et al., 2014; Yuan et al., 2016). NAC and UDO were uniformly dissolved in distilled water before the gavage per day. APAP (220 mg/kg) was dissolved in warm saline. The intact and vehicle groups were orally administration with distilled water (0.1 mL/10 g/day). The other four groups were administered according to the dose of NAC and UDO, respectively. All gavage
work was kept once a day for a total of 14 days. In day 14, after the last administration for 4 h, all mice were subjected to APAP intraperitoneally, while the intact group was injected with normal saline. After intraperitoneal injection for 12 h, blood was collected by posterior orbital venous plexus. And mice were dissected, and then the liver tissues were removed and washed by saline for three times for biological and histological evaluation.

**Relative organ weight of liver** The animals were weighed on the first day of administration, and then individual body weights were recorded every day. After intraperitoneal injection for 12 h, all the mice weight was collected again. And the liver tissues were weighted simultaneously when the animals were eviscerated. Then the relative organ weight was calculated as the ratio of liver (g) to body weight (g) (Ezejiofor et al., 2014; Liu et al., 2015; Peng et al., 2016).

**Determination of serum levels of ALT and AST** Collected blood was remained static under room temperature for 2 h and then centrifuged at 3,000 rpm 4℃ for 10 min to obtain the serum. The serum levels of ALT and AST were tested by using commercially biochemical kit according to the manufacturer’s instructions. The optical densities were measured by the absorbance at 510 nm and results were shown as U/L.

**Determination of GSH, CAT, T-AOC and MDA levels in liver tissues** Liver tissue was accurately weighed, added with 9 times (g: mL) normal saline and then homogenized by grinder, and centrifuged 10 min 4℃ at the speed of 2500 rpm for obtaining supernatant later. Biochemical kits were used to determine the levels of GSH, CAT, T-AOC and MDA according to the manufacturer’s instructions. Above four results were measured by the absorbance at 405, 405, 520, 532 nm, respectively.

**Determination of ROS in liver tissues** Liver tissue was accurately weighed, added with 9 times (g: mL) Phosphate buffered saline (PBS) and then homogenized by grinder, centrifuged 10 min 4℃ at the speed of 2500 rpm for obtaining supernatant later. The level of ROS in the supernatant was measured by using Mouse tissue ROS Elisa kit (Beijing Cheng Lin biological technology co., LTD, Beijing China) Firstly, standard, blank and testing sample wells were set on the ELISA coated pates, and then added 50μL different concentrations of standard products, sample dilution, mixed solution of 40μL sample dilution and 10μL testing sample, respectively. After closing plate with closure plate membrane, the sample was incubated for 30 min at 37℃. Secondly, liquid was discarded and washing buffer was added to every well, which was still for 30 s and repeated 5 times. Horseradish peroxidase (HRP)-Conjugate reagent was added 50μL to each well, except blank well. Then the above steps of incubation and washing were repeated. Thirdly, Chromogenic Solution was added 100μL to each well and evaded the light preservation for 15 min at 37℃. Finally, stop Solution was added, and the optical densities were promptly measured by the absorbance at 450 nm (Wang et al., 2017).

**Histological examination of liver tissues** The mice liver tissues were cut at random and collected for histological evaluation. 5 slices were selected randomly in each group. Fresh liver tissues were fixed in 4% paraformaldehyde at room temperature, and embedded in paraffin shortly afterwards. Then, each piece of liver tissue was made into ultrathin slices (5µm) by hematoxylin and eosin staining (H&E). Finally, the images of central venous of liver were observed by an optical microscope (E100, Nikon Corporation).

**Determination of inflammation index of TNF-α** The above serum samples were also used for testing the inflammation index of TNF-α, which was determined by using commercial ELISA kits according to the manufactures’ instructions as same as the steps of ROS in detail.

**Statistical analysis** All the experimental data were represented as mean ± standard deviation (SD), and statistical analysis was done with using one-way analysis of variance (ANOVA), followed by the multiple comparisons of Least squared distance (LSD) test. The analysis was performed by Statistical Product and Service Solutions software 20.0 (SPSS Inc., USA). P < 0.05 or P < 0.01 was considered as significant difference.

### Results

**Relative organ weight of liver** The change of the weight of body and liver tissues and relative organ weight were shown in Table 1. Among the six groups, the body weight of the APAP-induced animals decreased while the Intact group showed a steady increase. However, there was an increase in liver weights in the APAP-induced. The UDO slightly increased body weight, while significantly (P<0.05) reduced the organ weights.

<table>
<thead>
<tr>
<th></th>
<th>Body weight (g)</th>
<th>Liver weight (g)</th>
<th>Relative organ weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td>28.69 ± 1.62</td>
<td>1.23 ± 0.09</td>
<td>4.391</td>
</tr>
<tr>
<td>Vehicle</td>
<td>26.80 ± 2.25</td>
<td>1.81 ± 0.07</td>
<td>6.198</td>
</tr>
<tr>
<td>NAC</td>
<td>27.95 ± 2.08</td>
<td>1.45 ± 0.19**</td>
<td>5.186</td>
</tr>
<tr>
<td>UDO (100 mg/kg)</td>
<td>30.03 ± 1.62</td>
<td>1.65 ± 0.16*</td>
<td>5.606</td>
</tr>
<tr>
<td>UDO (200 mg/kg)</td>
<td>31.81 ± 1.37</td>
<td>1.64 ± 0.15*</td>
<td>5.636</td>
</tr>
<tr>
<td>UDO (400 mg/kg)</td>
<td>27.86 ± 1.39</td>
<td>1.36 ± 0.18**</td>
<td>4.717</td>
</tr>
</tbody>
</table>

Data were presented as mean ± SD (n = 10). *p < 0.05, **p < 0.01 versus vehicle group.
Effect of UDO on the serum levels of ALT and AST in mice

Results of the effect of UDO on the serum levels of ALT and AST were shown in Figure 1. Compared with the intact group, the indexes of liver function including ALT and AST were significantly increased in the vehicle group (APAP) ($p < 0.01$). However, the ALT and AST levels of pretreatment with 200 and 400 mg/kg UDO groups were markedly lower than the vehicle group ($p < 0.05$ and $p < 0.01$, respectively), which indicated UDO could ameliorate the deteriorative liver function in mice.

Effect of UDO on levels of GSH, CAT, T-AOC and MDA in liver tissues

Previous research showed that oxidative stress is closely associated with liver injury. As seen from Figure 2, the levels of GSH (A), CAT (B) and T-AOC (C) in the liver from the vehicle group were significantly lower than the intact group ($p < 0.01$, $p < 0.05$ and $p < 0.05$, respectively). Compared with the vehicle group, NAC group also significantly increased the levels of GSH, CAT and T-AOC ($p < 0.01$, $p < 0.05$ and $p < 0.05$, respectively). After pretreatment with 200 and 400 mg/kg UDO, the levels of GSH, CAT and T-AOC were markedly increased (all $p < 0.01$). Furthermore, compared with NAC group, 200 and 400 mg/kg groups significantly increased the level of T-AOC ($p < 0.01$). As shown in Figure 2 (D), MDA level of the vehicle group was significantly higher than the intact group ($p < 0.01$). Beside, NAC group was notably lower than the vehicle group ($p < 0.01$). And UDO pretreatment at dose of 200 and 400 mg/kg significantly attenuated the level of MDA in the liver ($p < 0.05$ and $p < 0.01$). Meanwhile, the level of MDA was down-regulated in 400 mg/kg UDO-treated mice compared with NAC group ($p < 0.01$).

Effect of UDO on levels of ROS in liver tissues

Results of UDO on the mice tissues level of ROS were shown in Figure 3. In comparison with the intact group, the ROS activity in the vehicle group was significantly higher ($p < 0.01$). As shown in Figure 3, UDO significantly decreased ROS level in the liver ($p < 0.05$ and $p < 0.01$).

Effect of UDO on liver histopathology

The histopathologic
Hepatoprotective Effect of *D. officinale* against APAP-induced Liver Injury

Features of H&E liver sections were shown in Figure 4. In the intact group, arranged neatly uniform size of hepatic cells, clear boundaries and no inflammatory cell infiltration were clearly showed. However, the vehicle group presented significantly inflammatory cell infiltration and damaged liver lobular structure. Moreover, around the center of the central vein was appeared focal liver cell degeneration. Compared with the vehicle group, clear cell texture and transparent liquid were observed in liver tissues of NAC group. After pretreatment of three doses of UDO, the condition was improved in the liver histopathology, especially the 400 mg/kg group, which solely showed minor histopathological changes when comparable to the NAC and intact groups. The hepatic lobule of 200 mg/kg group existed a little obscure and the 100 mg/kg group had some inflammatory infiltration.

**Effect of UDO on inflammation index of TNF-α** Effect of UDO on the mice tissues level of TNF-α were shown in Figure 5. In comparison with the intact group, the TNF-α activity in the vehicle group was significantly higher (p < 0.01). However, NAC group was notably lower than the vehicle group (p < 0.01). Interestingly, compared to the Vehicle mice, the results indicated that serum level of TNF-α were significantly decreased by treatment with UDO at the doses of 100, 200 and 400 mg/kg (p < 0.05).

**Discussion**

Liver injury, especially drug-induced damage, is a major health problem which has received increasing attention. Drug-induced liver damage plagued a lot of people, including patients, clinical health counselors, drug manufacturers, drug regulators, etc. However, the mechanisms of a great number of drug-induced liver injuries are remained complicated (Mosedale *et al*., 2017). APAP, an analgesic and antipyretic drug, is the common cause of acute liver failure in USA and western countries (Larson, *et al*., 2005; Lee, 2008). Once the drug dose is beyond the safe range, in addition to hepatotoxicity and nephrotoxicity, other side effects will also occur, such as allergies, asthma and even death badly.
had very high medicinal value in our study, is still used for the first line clinical treatment in cases of APAP overdose (Hau, et al., 2010). However, NAC only played its efficacy in the early stage after ingesting overdoses APAP, which vastly restricted its clinical application. (Smilkstein, et al., 1988). In addition, some traditional Chinese medicine has been reported earlier about the treatment effect in liver injury induced by APAP, such as Schisandra fructus, Panax notoginseng, Swertia pumicea and Cuscuta chinensis (Jiang, et al., 2015; S Wang, et al., 2014; Yen, et al., 2007; Zheng, et al., 2014). These research clues provided us with orientation of looking for a novel drug.

*Dendrobium officinale*, as one traditional Chinese medicine, is famous in China and even the whole Asia. According to medical records, *Dendrobium officinale* had very high medicinal value in nourishing stomach and kidney (Wu, et al., 2013). The active ingredients of *Dendrobium officinale* mainly include polysaccharides, alkaloids, amino acids and trace elements. Pharmacological studies were common in enhancing immunity, anti-oxidation, antidiabetics and anti-tumor (Ng, et al., 2012). Furthermore, the previous research demonstrated that *Dendrobium huoshanense* alleviated ethanol and selenite-induced liver injury and *Dendrobium officinale* has antioxidative effect (Pan et al., 2014; Pan, et al., 2012; XY Wang, et al., 2014). Therefore, *Dendrobium officinale* may be a potential drug or diet supplement for the treatment or prevention in APAP overdose. And UDO was selected to investigate in present study.

In current study, the hepatoprotective effect and underlying pathogenesis of UDO were investigated in APAP-induced liver injury mice. And the animal model was also successfully exhibited. Firstly, the classical indicators of liver function, such as the levels of ALT and AST, were significantly increased in mice after APAP injection when compared with the intact group. Meanwhile, MDA, the end production of lipid peroxidation, and it can be deemed as an indicator of oxidative stress for its role in reaction between free radicals and unsaturated fatty acids (Sener et al., 2006; Zhang et al., 2016) was also markedly increased in mice from the vehicle group. Furthermore, the images of H&E staining indicated that the vehicle group showed fuzzy hepatocellular texture and inflammatory cell infiltration. These results were consistent with the previous study (Fan, et al., 2015; Hau, et al., 2010). More importantly, UDO could protect mice from APAP-induced liver injury in a dose-dependent manner. Firstly, the levels of ALT and AST were obviously reduced after UDO-pretreatment in a dose-dependent manner. Secondly, after administration with UDO, MDA in mice were also significantly lower than the vehicle group. Besides, the images of H&E staining showed that hepatic lobule, nucleus, central veins and thin sinusoids were clearly seen in UDO-treated mice, which demonstrated that administration of UDO dramatically ameliorated the morphology of hepatic cell of APAP-induced liver damage in mice. In addition, TNF-α is a notable marker for inflammatory reactions in liver tissues (J Li et al., 2013; Su et al., 2017). The results of the present study revealed that UDO caused significant decreases in TNF-α levels.

To better understand the effect of UDO on the APAP induced liver injury, the mechanism of UDO was further explored on the basis of above work. Research showed that the mechanism of APAP-induced liver injury could be divided into as follows: the increasing formation of reactive oxygen species, depletion of glutathione and increasing lipid peroxidation (Beger, et al., 2015; Jaeschke, 2003). With the help of enzyme CYP2E1, overdosed-APAP is transformed into NAPQI (Liu, et al., 2016). Immediately, NAPQI is reacted with GSH in vivo and lead to the depletion of GSH, which plays positive roles in including detoxification, antioxidant defense, maintenance of thiol status, and modulation of cell proliferation. Depletion of GSH will lead to hepatic cell damage (Lu, 2009). So GSH is an important index of antioxidant capacity. Meanwhile excessive APAP will increase the production of ROS. ROS is a byproduct of normal oxygen metabolism, including oxygen ions, peroxides, oxygen free radicals, etc. (Ferret, et al., 2001; Jaeschke et al., 2002). Once the ROS level beyond the safe range, some problem might emerge such as damage to cells or genetic structures (Zorov et al., 2014). When ROS accumulated in the body, CAT is the cellular defense against ROS. CAT in vivo mainly metabolizes hydrogen peroxide into water to reduce the damage (Jin et al., 2011; Yang et al., 2017). In addition, T-AOC is a vital marker to incarnate the synthetic ability of antioxidant in whole body (Ghiselli et al., 2000; Li et al., 2012).

According to the above mechanism, APAP-induced hepatotoxicity probably could be summed up as oxidative stress and defense (Tang, et al., 2009). The key part is imbalance between oxidant and antioxidant, which mainly works on free radicals (Lee et al., 2009). The antioxidant defense systems were established by a set of antioxidant enzymes, including GSH, CAT, SOD, etc. (GLi et al., 2013). Such these antioxidant enzymes played a positive role in the defense against free radicals. Previous experimental data has revealed the levels of GSH, CAT and T-AOC were significantly declined when the generation of ROS was markedly agrandized in the liver of APAP-treated mice (Li, et al., 2012; Reid, et al., 2005). Meaningfully, pretreatment UDO significantly added the level of GSH, CAT and T-AOC and reduced the ROS in a dose-dependent manner. The tendency of GSH, CAT, T-AOC and ROS of UDO mice were contrary to the level change of APAP mechanism. These results also potently indicated that UDO might effectively strengthen the defense capability of antioxidant and relieved oxidative damage in liver of APAP-treated mice. Furthermore, all indexes of the 200 mg/kg UDO had a statistical significance (p < 0.05) and 400 mg/kg was particularly optimal (p < 0.01). Consequently, UDO might effectively protect APAP-induced liver injury in a dose-dependent manner. While compared with NAC group, 400 mg/kg UDO significantly increased the level of T-AOC and reduced the level of MDA (all p < 0.01). These demonstrated that the effect of high dose UDO was better than the
positive drug in the aspects of antioxidant ability and lipid peroxidation. Besides, with the literature of *Dendrobium* polysaccharide in the effect of liver protection, suggested that polysaccharides may play a major role in liver protection.

Meanwhile, *Dendrobium officinale* was approved as a homology of medicine and food by the CFDA. Thus, UDO may be a promising health functional food for hepatic protection in the future. Besides, it is worthwhile to further explore UDO hepatic protection mechanism as well as the other pharmacological activities.

**Conclusions**

Pretreatment with UDO has potentially hepatoprotective effects against APAP-induced liver damage. The underlying mechanisms were related to the enhanced defense capability of antioxidant and inhibited lipid peroxidation. Therefore, UDO might have potential for development as diet therapy or health functional food for liver injury.

**Acknowledgements** This work was supported by the special foundation for excellent young teachers of Guangdong Ocean University (No. 2014008), the natural science foundation of Guangdong Province (No. 2015A030310360), Forestry Science and Technology Innovational Specific Project of Guangdong Province (No. 2014KJCX019-02, 2016KJCX006 & 2017KJCX007).

**References**


