Protective effect of *Ornithogalum saundersiae* Ait (Liliaceae) against acetaminophen-induced acute liver injury *via* CYP2E1 and HIF-1α

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**[ABSTRACT]** AIM: To investigate the hepatoprotective effect of total saponin from *Ornithogalum saundersiae* Ait (Liliaceae) (OC) against acetaminophen (APAP)-induced acute liver injury *in vivo* and *in vitro*. **METHODS:** Mice were pretreated with OC (300, 200 or 100 mg·kg⁻¹, body weight) or *N*-acetyl-L-cysteine (NAC) (300 mg·kg⁻¹, body weight) for 3 times at 24 h intervals. APAP was administered 2 h after OC last dose. Chang liver cells were incubated with the medium containing OC (50, 100, 200 mg·mL⁻¹) or NAC (10 mmol·L⁻¹) with the presence or absence of APAP (10 mmol·L⁻¹) for 24 h. **RESULTS:** OC showed remarkable hepatoprotective effect 12 h after APAP administration by decreased aspartate aminotransferase and alanine aminotransferase levels, reduced the products of lipid peroxidation, improved the activity of catalase, superoxide dismutase and glutathione peroxidase, inhibited the caspase-3 cleavage and hypoxia inducible factor-1α accumulation *in vivo*. **RESULTS:** OC significantly decreased the activities of metabolism enzyme cytochrome P450 2E1 (CYP2E1) and cyclooxygenase-2 (COX-2) induced by APAP. **CONCLUSION:** OC possesses the ability to protect hepatocyte from APAP-induced liver damage, suggesting that the hepatoprotective mechanism of OC might be related to antioxidation via blocking the CYP2E1, and mediating reactive oxygen species scavenging and accumulation of hypoxia-inducible factor (HIF)-1α.

**[KEY WORDS]** *Ornithogalum saundersiae* Ait; Acetaminophen; Apoptosis; Cytochrome P450 2E1; Hypoxia-inducible factor-1α

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1 Introduction

*Ornithogalum saundersiae* Ait (Liliaceae) (OC) is one of Liliaceae family species and a valuable traditional Chinese herb, which is mainly distributed in the south of Africa, and is commonly used as an anti-inflammatory and anticancer agent to treat liver disease, hepatoma, cholecystitis, etc. Recently it was found that OC showed a strong anti-cancer activity. Now, Fu Fang Wan Nian Qing Jiao Nang (Chinese name, and approval number from the State Food & Drug Administration in China is B20020717) is a commercial Chinese medicine, and the main functional ingredient is OC extract. It was reported that the solid bulb of OC contains lots of alkaloid, saponins and polysaccharides, which can regulate the immune system [1]. Among *Ornithogalum saundersiae* saponins OSW-1 (3β, 16β, 17α-tri-hydroxycholest-5-en-22-one 16-O-(2-O-4-methoxybenzoyl-β-D-xylopyranosyl)-(1→3)-(2-O-acetyl-α-L-arabinopyranoside)) is one of the most efficiently active components isolated from OC. It has been reported that OSW-1 was cytotoxic against several types of malignant cells at nanomolar concentrations, which is approximately 10 to 100 times more potent than such clinically applied anticancer agents as camptothecin, adriamycin and paclitaxel [2]. Total polysaccharides, total saponin, and total alkaloids of OC could significantly inhibit cell viability on different cancer cell lines. Toxicity studies for safety evaluation also verified that OC has no obvious toxicity or side-effects [3]. Previously, we reported that OC could prevent fulminant hepatic failure in mice by suppressing oxidative stress and regulating the expression of HIF-1α [4].

APAP is commonly used as an analgesic and antipyretic agent and has shown to be safe when taken at normal therapeutic doses. However, an overdose can lead to severe liver failure. The hepatotoxicity of APAP is caused by...
N-acetyl-para-benzoquinoneimine (NAPQI), a toxic metabolic product of APAP. NAPQI is rapidly conjugated with glutathione, a sulfhydryl donor. Excessive NAPQI formation or reduction in glutathione stores, which will interact with vital cellular proteins and the lipid bilayer of hepatocyte membranes, results in hepatocellular death and centrilobular hepatic necrosis [5-6]. When conjugated with cellular macromolecules, it can lead to the production of reactive oxygen species (ROS). Increased ROS formation may lead to the development of oxidative stress. The antioxidant enzymes include glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and catalase (CAT), which convert active oxygen molecules into non-toxic compounds and consequently protect the liver against oxidative damage [7]. N-acetylcysteine (NAC) is a thiol-containing compound that has been used for over 30 years as the antidote for APAP toxicity in human [8]. NAC acts as a glutathione (GSH) precursor, promoting GSH synthesis and increasing hepatic GSH stores, eventually detoxifying NAPQI. So we selected NAC as a positive control to evaluate the effect of OC.

Previous studies indicated that inflammation and hypoxia are closely linked in cellular responses [9-11]. Metabolic process of APAP is associated with a series of sever inflammatory response and activates the inflammatory factor. HIF-1α is strictly regulated by the cellular oxygen tension. HIF-1α plays an important role in inflammation and activation of the immune response. Under normoxic conditions, HIF-1α protein is post-translationally hydroxylated on specific proline residues to enable binding to pVHL (von-Hippel-Lindau protein), which targets HIF-1α for ubiquitination and proteasomal degradation. Under hypoxic conditions, proline hydroxylation is blocked, and this leads to ubiquitylation and proteasomal degradation. Under hypoxic conditions, proline hydroxylation is blocked, and this leads to ubiquitylation and proteasomal degradation.

2.2 Materials

APAP and NAC were purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA). The alanine aminotransferase (ALT) and aspartate aminotransferase (AST) Reagent Strips were purchased from Arkray Incorporated (Kyoto, Japan). Caspase-3, HIF-1α, COX-2 and α-tubulin monoclonal antibody were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). CYP2E1 was obtained from Abcam Company (Cambridge, Britain). Malonaldehyde (MDA), glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) were purchased from Nanjing Jiansheng Biology Engineering Institute (Nanjing, Jiangsu, China). DAB (3, 3’-diaminobenzidin) Plus Substrate System was purchased from Lab Vision Corporation (Fremont, CA, USA). Dulbecco’s modified Eagle’s medium (DMEM) (Solarbio Science & Technology Co., Ltd., Beijing, China). Fetal bovine serum (FBS) and 0.5% trypsin-EDTA were purchased from Gibco Co., Ltd. (New York, USA).

2.3 Experimental animals

Male C57BL/6 mice (21–25 g) were obtained from the Animal Division of Jilin University (Jilin, China). Mice were kept in an environmentally controlled room with temperature (24 ± 1) °C and relative humidity (55 ± 1) %. The mice were acclimatized 1 week prior to the experiment. Food and water were freely available. Animal experiments were performed under the latest edition of “Guide to the Care and Use of Laboratory Animals” (National Research Council, 1996).

2.4 Experimental design in vivo

The mice were randomly assigned into six experimental groups, each containing 8 mice. OC (300, 200 or 100 mg·kg–1, body weight) or NAC (300 mg·kg–1, body weight) were orally administered every day for 3 consecutive days. Two hours after the last OC or NAC administration, mice were injected intraperitoneally with APAP (300 mg·kg–1) except normal group. All mice were fasted 16–18 h prior to treatment with APAP dissolved in saline warmed to 40 °C. The animals were sacrificed 12 h after APAP injection. The blood samples were collected from the carotid artery and liver tissues were promptly removed. Serum was separated by centrifugation at 3 000 r·min–1, 4 °C, then stored at –20 °C to analyze ALT and AST levels. The liver tissue samples were divided into two parts. One was immersed in 10% neutral buffered formaldehyde for immunohistochemical examinations and the other was stored at –80 °C for further analysis.

2.5 Serum enzymes and biochemical index

Serum ALT and AST activities 12 h after APAP administration were measured by an Autody chemistry analyzer (Spotchem SP4430, Arkray, Kyoto, Japan). MDA level, GSH, SOD, CAT, GSH-Px activities were determined according to manufacturer’s instructions.
2.6 Histopathology and immunohistochemistry

Liver tissue was fixed in 10% buffered formalin saline, and then dehydrated in graded ethanol and embedded in paraffin max. The tissue was cut into 4 μm sections. Immunohistochemical analysis was deparaffinized and hydrated, and then blocked with endogenous peroxidase. Sections were incubated with goat anti-caspase-3 or goat anti-HIF-1α monoclonal antibody respectively, and followed by Max Vision™ kit and DAB kit. Finally, slides were counterstained with hematoxylin, mounted, observed by light microscopy and examined in a blind fashion. Positive expression area was performed with Image-Pro Plus software (Media Cybernetics, Inc., Georgia, USA).

2.7 Cell culture and treatment in vitro

Chang liver cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS), in a humidified normal atmosphere containing 5% CO2 at 37 °C. The cells were cultured in a 6-well plate in 3 mL complete medium and incubated for 24 h. The cultures were then washed twice in cold phosphate buffered saline (PBS) and cultured in either APAP (10 mmol·L⁻¹) or APAP + OC (50, 100, 200 mg·mL⁻¹) or APAP + NAC (10 mmol·L⁻¹) for 24 h. At the end of the incubation, cells were prepared for Western blotting.

2.8 Cell viability assay

Cell viability was assessed with 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. Chang liver cells were incubated in 96-well plates (2 × 10⁴ cells per well) and treated with OC at various concentrations. MTT solution (0.5 mg·mL⁻¹ in DMEM containing 10% FBS) was added for the last 3 h of incubation. The reduction of MTT to formazan was read at 540 nm using a microplate reader.

2.9 Western blotting analyses

Harvested cells were washed with cold PBS for twice and suspended in a protein lysis buffer containing protease inhibitor (Beyotime, Jiangsu, China). The protein content was determined using a BCA protein assay kit (Beyotime, Jiangsu, China). Fifty micrograms of cellular protein was subjected to SDS-PAGE. The separated proteins were electrophoretically transferred onto the PVDF membrane, blocked with PBST (0.05% tween 20 in PBS) containing 5% skim milk for 1 h at the room temperature. The membrane was incubated overnight at 4 °C with CYP2E1, COX-2 and α-tubulin antibodies; then incubated with HRP conjugated secondary antibodies for 1 h at the room temperature. Protein bands were visualized with enhanced chemiluminescence using WEST-ZOL™ (plus) (nIRON Biotechnology Co., Seongnam, Korea) and exposed with X-ray film. Densities of the immunoreactive bands were analyzed with Quantity One software (Bio-Rad, USA).

3 Statistical Analysis

Statistical analysis was performed by statistical software PRISM 5.0 (Graphpad Software, Inc., San Diego, CA). Data were analyzed using one-way analysis of variance (ANOVA) followed by Turkey’s test. Results were present as mean ± standard error of mean (S.E.M.). Statistical significance was considered at P < 0.05, P < 0.01 and P < 0.001.

4 Results

4.1 Effects of OC on ALT and AST levels

Firstly, we examined the effect of OC on serum of ALT and AST levels. The dose of 300 mg·kg⁻¹ APAP successfully induced liver injury and significantly elevated the activities of ALT and AST 12 h after administration in mice, while in the mice pretreated with different doses of OC or NAC, the levels of ALT and AST lowered to the normal level (Fig. 1A, 1B).

4.2 Effects of OC on lipid peroxidant products and antioxidant levels

MDA is a secondary metabolite of lipid peroxidation. The hepatotoxicity of APAP leads to the development of oxidative stress and increases the products of lipid peroxidation. The MDA content was increased in APAP group (12.52 ± 1.43 nmol·mg⁻¹ protein) compared with the normal group (4.47 ± 0.13 nmol·mg⁻¹ protein). Mice were treated with OC (300, 200, 100 mg·kg⁻¹); liver MDA production were decreased significantly and the contents were 7.39 ± 1.35, 8.82 ± 1.63, 8.89 ± 2.9 nmol·mg⁻¹ protein respectively, and NAC group (5.72 ± 0.93 nmol·mg⁻¹ protein) was closer to the normal (Fig. 2A). GSH plays an important role in the detoxification of APAP. Due to the toxicity, metabolic products of APAP caused GSH depletion. GSH levels in APAP group were decreased markedly (255.78 ± 25.50 mg·g⁻¹ protein). OC pretreatment (300, 200, 100 mg·kg⁻¹) or NAC (300 mg·kg⁻¹), GSH levels were (381.62 ± 30.49, (373.63 ± 41.96), (349.47 ± 38.02), (418.68 ± 21.47) mg·g⁻¹ protein respectively, and NAC group could keep the GSH levels close to the normal (418.68 ± 21.47 mg·g⁻¹ protein) (Fig. 2B). The production of reactive oxygen species in liver was eliminated by antioxidant enzyme to reduce liver damage. APAP-induced levels of CAT, SOD and GSH-Px were decreased approximately by half compared with those of the normal group. However, OC or NAC pretreatment recovered the antioxidant enzyme levels to the normal (Table 1).

4.3 Histopathological examination after OC treatment

Liver tissues from the normal group showed a normal lobular architecture with central veins. However, the liver tissues from the APAP-treated group showed that a large number of inflammatory cells infiltrated to the intralobular and interlobular regions. The liver structure was destroyed and there were more necrotic and fatty degenerated liver cells than the normal. OC treatment reversed the hepatic regions to a great extent, and the liver morphological appearance was similar with that of NAC group (Fig. 1C).

4.4 Immunohistochemistry examination after OC treatment

In view of immunohistochemical results, it was obvious that caspase-3 expressed mainly in cytoplasm and HIF-1α
Fig. 1 Effects of OC on ALT and AST levels and histopathological analysis. The serum and liver tissues were collected 12 h after APAP treatment. (A) Effects of OC on ALT levels. (B) Effects of OC on AST levels. Data were represented as mean ± S.E.M. (n = 8). *** P < 0.001 vs Normal group; ### P < 0.001, ## P < 0.01 vs APAP group. (C) Histopathological analysis of liver sections with hematoxylin and eosin staining. A. Normal B. APAP C. APAP + OC300 D. APAP + OC200 E. APAP + OC100 F. APAP + NAC. All slides are 100 × magnifications. Arrows present the severe inflammatory infiltration hepatocyte.

Fig. 2 Liver MDA and GSH levels. Liver tissues were collected 12 h after APAP treatment. (A) Effects of OC on liver MDA formation. (B) Effects of OC on liver GSH levels. Data were represented as mean ± S.E.M. (n = 8). Compared with normal group, *** P < 0.001 vs normal group; ### P < 0.001, ## P < 0.01 vs APAP group.

mainly in nucleus and cytoplasm, positive staining appearance in buff and yellow brown areas respectively. The hepatotoxicity of APAP increased the expressions of caspase-3 and HIF-1α, but treated with different doses of OC, the positive areas were decreased observably just the same as NAC (Figs. 3A, 3B).

4.5 Effect of OC on CYP2E1 and COX-2 expression

We chose MTT method to assess the toxicity of OC on Chang liver cells. Under different concentrations of OC treatment cell viability was similar to that of the normal. It indicated that OC has no cytotoxicity in Chang liver cell (Fig. 4).

In Chang liver cell line, the expression of CYP2E1...
Table 1 Effect of OC on liver antioxidant enzymes CAT, SOD, GSH-Px levels

<table>
<thead>
<tr>
<th>Group</th>
<th>CAT (U/mg protein)</th>
<th>SOD (U/mg protein)</th>
<th>GSH-Px (U·L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>64.27 ± 4.22</td>
<td>80.51 ± 6.48</td>
<td>956.48 ± 34.46</td>
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<tr>
<td>APAP</td>
<td>38.04 ± 2.48†</td>
<td>46.27 ± 5.05†</td>
<td>687.32 ± 25.01**</td>
</tr>
<tr>
<td>APAP+OC300</td>
<td>65.22 ± 3.34***</td>
<td>77.40 ± 5.07***</td>
<td>851.67 ± 27.81***</td>
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<tr>
<td>APAP+OC200</td>
<td>63.85 ± 5.22**</td>
<td>76.02 ± 6.82**</td>
<td>819.91 ± 34.19*</td>
</tr>
<tr>
<td>APAP+OC100</td>
<td>64.04 ± 6.30**</td>
<td>72.61 ± 5.94**</td>
<td>829.62 ± 16.9**</td>
</tr>
<tr>
<td>APAP+NAC</td>
<td>58.91 ± 3.63#</td>
<td>78.70 ± 3.36##</td>
<td>907.86 ± 21.21###</td>
</tr>
</tbody>
</table>

Data were represented as mean ± S.E.M. (n = 8). ***P < 0.001 vs normal group; **P < 0.01, *P < 0.05 vs APAP group (One-way analysis of variance (ANOVA) followed by Turkey test).

was highly increased with APAP treatment, while the expression of CYP2E1 with OC pretreatment was reduced markedly. However, NAC did not show a significant inhibition on the expression of CYP2E1. The same result appeared in the expression of COX-2, which was induced in macrophages and endothelial cells by proinflammatory cytokines, damaging the liver function [13]. APAP-induced COX-2 expression was increased much more than the normal in Chang liver cell, nevertheless OC and NAC treatment recovered the COX-2 levels. This indicated that OC reversed the inflammatory factor induced by APAP and inhibited CYP2E1 the metabolite enzyme of APAP (Fig. 5).

Fig. 3 Immunohistochemistry with caspase-3 and HIF-1α in APAP-intoxicated Liver. (A) Immunohistochemistry with caspase-3 in APAP-intoxicated Liver. A. Normal B. APAP C. APAP + OC300 D. APAP + OC200 E. APAP + OC100 F. APAP + NAC. Slides stained with caspase-3 are observed under 100 × magnifications. (B) Immunohistochemistry with HIF-1α in APAP-intoxicated Liver. Slides are observed under 400 × magnification. Analysis data was determined by Image - pro plus tool and represented as mean ± S.E.M. of three independent experiments.

Fig. 4 Effect of OC on cell viability. Chang liver cells were incubated with OC (3.125–400 µg) for 24 h. Cell viability was measured using MTT assay. Results are expressed as the percent of control cell viability. Data are presented as mean ± S.E.M. for each dose.

5 Discussions

APAP is a well-known, long trusted, and widely available over-the-counter analgesic and antipyretic drug, however, its overdose causes acute liver damage. The level of serum ALT and AST and histopathological results of liver tissues are the common measures taken to assess APAP toxicity in the experimental setting. In our recent work, 300 mg·kg⁻¹ APAP significantly increased liver serum aminotransferase activities, serious hemorrhagic hepatic necrosis and inflammation appeared in histopathological examination results (Fig. 1A, 1B, 1C). After APAP administration, caspase-3 cleavage was observed from the immunohistochemical...
Fig. 5 Effect of OC on CYP2E1 and COX-2. The cell CYP2E1 and COX-2 protein levels were detected at 24 h after APAP treatment on Chang liver cell. Each immunoreactive band was digitized and expressed as a ratio of α-tubulin levels. The ratio of the normal group band was set to 100%. Values of densitometric analysis were mean ± S.E.M. of three independent experiments. ###P < 0.001 vs control group. ***P < 0.001 vs APAP alone group

data (Fig. 3A). Caspase-3 activation seems to play a key role in the initiation of cellular events during the apoptotic process, which interacts with Fas system, Bel-2 family, NO and finally facilitates apoptosis [14-16]. OC administration not only suppressed the activities of ALT and AST, and decreased the level of caspase-3 cleavage, but also ameliorated the extent of inflammatory infiltration in liver and improved the liver architecture. The results were coincident with the expression of COX-2 in vitro (Fig. 5). COX-2 is one of the key proinflammatory cytokines and well-known for their inflammation and immune response effects. COX-2, an inducible isoenzyme, plays a crucial role in exaggerating inflammation [17]. These results demonstrated that OC might prevent the liver injury caused by APAP overdose. Moreover, regulating the inflammation infiltration and inhibiting COX-2 expression might be related with the hepatoprotective mechanism of OC against APAP.

APAP-induced hepatotoxicity has been linked to lipid peroxidation and ROS production. Oxidative damage mediated by ROS are toxic to cells, because they can react with most cellular macromolecules inactivating enzymes or denaturing proteins, causing DNA damage, and thereby disrupting cellular function and integrity. Similar effects were observed with relatively more production of MDA after APAP administration. MDA, a marker for oxidative stress, is one of reactive species and occurs naturally. ROS can degrade polyunsaturated lipids, and form MDA [18]. GSH plays a key role in the progress of detoxification of APAP, and GSH stores are markedly depleted when liver necrosis begins [19]. GSH deficiency leads to cellular damage followed by severe mitochondrial degeneration. CAT, SOD and GSH-PX are all related with antioxidant system in the internal environment and reduction of hydrogen peroxide and other peroxide. In our study, MDA products were significantly increased after APAP administration (Fig. 2A), while GSH levels, CAT, SOD and GSH-PX activities were depressed, which owed to the hepatotoxicity of APAP (Fig. 2B, Table 1). However, OC pretreatment effectively suppressed these alterations and reversed the levels of oxidative stress. These results indicated that antioxidative activity of OC might be an important effect involved in the pathological process of OC against APAP-induced acute liver injury.

The biotransformation of APAP mainly occurs via conjugation of glucuronide and sulfate by the transferase enzymes that are rarely responsible for toxic metabolite formation and their nontoxic products are generally ready for excretion [20-23]. Toxicity caused by APAP overdose is believed to occur by initial hepatic metabolism through cytochrome P450 enzymes to the highly reactive metabolite NAPQI, which would exacerbate oxidation stress in conjunction with mitochondrial dysfunction, and would especially lead to massive hepatocytes necrosis, liver damage or death. CYP2E1 is the important enzyme in the metabolic process of APAP, which metabolizes numerous small molecules of toxicological interest including ethanol, APAP, halothane, carbon tetrachloride and carcinogens, such as nitrosamines. CYP2E1 also transforms several endogenous substrates including acetone, glycerol, and different fatty acids [24-26] and generates large amounts of ROS, which can damage cellular and mitochondrial components. In order to evaluate the role...
of CYP2E1 in the mechanism of OC against APAP-induced hepatotoxicity, we detected the levels of CYP2E1 in Chang liver cell and found that OC could effectively inhibit its expression (Fig. 5). The result revealed that OC might decrease the metabolism activity of CYP2E1, and further reduce the toxic reactive metabolite of APAP.

The high energy consumption of hepatocytes renders them vulnerable to reductions in oxygen availability. Therefore, hypoxia is an important factor for cell damage in the liver [27]. HIF-1α, an oxygen-sensitive transcription factor, affects cellular response to injury. Hypoxia regulates cell damage, inflammation, liver regeneration, stimulus of angiogenesis and fibrogenesis, and promotes liver carcinogenesis. Most of these events are mediated by HIF-1α [28].

In addition to hypoxia, oxidative stress might also might promote HIF-1α induction [29]. In recent years, it has become evident that disturbances of liver microcirculation, hypoxia, and angiogenesis occur for a long time before the onset of damaged liver [29]. Also, COX-2 expression is known to catalyze the synthesis of prostaglandins which could induce the expression of HIF-1α and the degradation of vHL protein [30]. Immunohistochemical results revealed that the positive expression of HIF-1α was highly increased in APAP-exposed mice. And this increase was significantly attenuated by OC (Fig. 3B), which indicated that hepatocytes might be stricken by hypoxia caused by APAP. Therefore, we could understand that hepatoprotective effect of OC might be associated with the inhibition of HIF-1α. The results were coincident with those in vitro.

**Fig. 6 Potential hepatoprotective mechanism of OC against APAP toxicity.** OC decreased liver injury degree through inhibited caspase-3 expression and effectively attenuated oxidative stress via declined MDA content and raised the activity of antioxidase, that may be due to the inhibition of P450 2E1 activity. At meanwhile, OC showed prominent anti-inflammatory effect during the liver injury process via decreased proinflammatory COX-2 and HIF-1α expression significantly.

The results of the present study indicate that OC markedly prevented acute liver injury caused by APAP and its protective effects were comparable to NAC. Our findings outline a mechanistic understanding of how OC protects liver form APAP-induced toxicity. The potential hepatoprotective effect of OC against APAP might be related with antioxidation, inhibition of HIF-1α. Also, the exact mechanism of OC hepatoprotective effect against APAP requires further research (Fig. 6).

**References**


虎眼万年青通过抑制 CYP2E1 和 HIF-1α 保护对乙酰氨基酚诱导的急性肝损伤
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延边大学长白山生物资源与功能分子教育部重点实验室，延吉 133002

【摘要】目的：通过对乙酰氨基酚体内体外实验建立急性肝损伤模型，观察虎眼万年青总皂苷的肝保护作用。方法：体内实验，小鼠预先口服给予虎眼万年青总皂苷(300, 200, 100 mg·kg–1)或 N-乙酰-L-半胱氨酸(300 mg·kg–1)3次，每次间隔24小时，末次给药后2小时腹腔注射对乙酰氨基酚。体外实验，张氏肝细胞培养在含有虎眼万年青(50, 100, 200 mg·mL–1)或 N-乙酰-L-半胱氨酸(10 mmol·L–1)的培养基中，同时加入对乙酰氨基酚(10 mmol·L–1)培养24小时。结果：虎眼万年青总皂苷在对乙酰氨基酚诱导肝损伤12小时后具有较强的肝保护作用，抑制了血清中谷草转氨酶和谷丙转氨酶的活性，在体内实验中，小鼠预先口服给予虎眼万年青总皂苷(300, 200, 100 mg·kg–1)或 N-乙酰-L-半胱氨酸(300 mg·kg–1)3次，每次间隔24小时，末次给药后2小时腹腔注射对乙酰氨基酚。在体内实验中，小鼠预先口服给予虎眼万年青总皂苷(300, 200, 100 mg·kg–1)或 N-乙酰-L-半胱氨酸(300 mg·kg–1)3次，每次间隔24小时，末次给药后2小时腹腔注射对乙酰氨基酚。在体内实验中，小鼠预先口服给予虎眼万年青总皂苷(300, 200, 100 mg·kg–1)或 N-乙酰-L-半胱氨酸(300 mg·kg–1)3次，每次间隔24小时，末次给药后2小时腹腔注射对乙酰氨基酚。在体内实验中，小鼠预先口服给予虎眼万年青总皂苷(300, 200, 100 mg·kg–1)。在体内实验中，小鼠预先口服给予虎眼万年青总皂苷(300, 200, 100 mg·kg–1)。在体内实验中，小鼠预先口服给予虎眼万年青总皂苷(300, 200, 100 mg·kg–1)。在体内实验中，小鼠预先口服给予虎眼万年青总皂苷(300, 200, 100 mg·kg–1)。在体内实验中，小鼠预先口服给予虎眼万年青总皂苷(300, 200, 100 mg·kg–1)。

【关键词】虎眼万年青；对乙酰氨基酚；凋亡；细胞色素 P450 2E1；缺氧诱导因子-1α

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