Protective Effects of Methanol Extract and Alisol B 23-acetate of *Alisma orientale* on Acetaminophen-Induced Hepatotoxicity in Rats

Ki Ho Yang\textsuperscript{1,2}, Seong Hee Choi\textsuperscript{3} and Jong Cheol Park\textsuperscript{4,*}

\textsuperscript{1}Research Institute of Korean Oriental Medicine, Sunchon National University, Suncheon, Jeonnam 540-742, Korea
\textsuperscript{2}Susung Oriental Medicine Hospital, 4256 Seongnam-dong, Jungwon-gu, Seongnam, Kyunggi-do 462-829, Korea
\textsuperscript{3}Department of Food Science, Sunmoon University, Asan, Chungnam 336-708, Korea
\textsuperscript{4}Department of Oriental Medicine Resources, Sunchon National University, Suncheon, Jeonnam 540-742, Korea

Abstract – Hepatoprotective effects of methanol extract and alisol B 23-acetate of *Alisma orientale* were studied in acetaminophen (APAP)-treated rats. APAP increased hepatic content of lipid peroxide, which was suppressed by methanol extract and alisol B 23-acetate. The liver of rats treated with APAP had higher P-450, aminopyrine N-demethylase and aniline hydroxylase activities than those of normal control rats. The increases in hepatic drug metabolizing enzymes by the i.p. injection of APAP were significantly alleviated by the administration of methanol extract or alisol B 23-acetate. The injection of APAP also resulted in a substantial reduction of hepatic glutathione content and glutathione S-transferase activity, and the decreases were partially, but significantly, restrained by the oral administration of methanol extract prior to the i.p. injection of APAP. Hepatic activities of glutathione reductase (GR) and \( \gamma \)-glutamylcystein synthetase (\( \gamma \)-GCS) were also decreased significantly in APAP-treated rats. The decreases in hepatic GR and \( \gamma \)-GCS activities by APAP injection were improved partially, but significantly, with administration of methanol extract of *A. orientale*. Treatment with alisol B 23-acetate also improved the hepatic \( \gamma \)-GCS activity significantly, but not GR.

Keywords – Hepatoprotective effect, Alisol B 23-acetate, *Alisma orientale*, Acetaminophen, Lipid peroxide, Hepatic drug metabolizing enzymes

Introduction

*Alisma orientale* is a perennial marshy plant used as an oriental medicine in Korea. In *Dongeui Bogam* written by Huh, Jun, it is described to be cold in nature, sweet and salty in taste without toxicity, and it is effective in facilitating urination and bringing down heat in bladder (Hur, 1994). In traditional oriental medicine it has been used for removing damp spirit of body, smoothing urination, ceasing diarrhea and easing edema. It is also used for curing unconscious emission in men. It has also been used as a folklore remedy for diabetes and as a diuretic (Park \textit{et al.}, 2005).

Several phytochemicals including triterpenoids and sequiterpenoids and hemagglutinating lectin have been isolated from the plant (Matsuda \textit{et al.}, 1999; Nakajima \textit{et al.}, 1994; Oshima \textit{et al.}, 1983; Yoshikawa \textit{et al.}, 1993; Yoshikawa \textit{et al.}, 1997; Park \textit{et al.}, 1995). Alisol compounds isolated from the plant have been found to have hepatoprotective activities against CCl\textsubscript{4} intoxication and hypochoesterolemic effects in mice and rats (Chang \textit{et al.}, 1982; Imai \textit{et al.}, 1970). Some terpene components of the plant have also been reported to have anti-complementary and anti-allergic activities (Lee \textit{et al.}, 2003; Kubo \textit{et al.}, 1997).

Acetaminophen (N-acetyl-p-aminophenol, APAP), a widely used analgesic and antipyretic drug, is safe at therapeutic doses, but when given as large overdoses severe liver injury and acute liver failure may occur in experimental animals and human (Thomas, 1993; Cover \textit{et al.}, 2006). The metabolic events of toxicity have been studied and are believed to be due to the metabolic conversion of APAP to a highly reactive electrophilic intermediate, N-acetyl p-benzoquinonimine (NAPQI), which is generated by cytochrome P-450 mediated oxidases (Jaeschke \textit{et al.}, 2003; Knight \textit{et al.}, 2003). This toxic metabolite is eliminated from hepatocyte by reacting with reduced glutathione (GSH) (Chasseaud, 1979). The relevant decrease in liver GSH is harmful because it is a basic cytosolic antioxidant.

Hundreds of natural resources and plants have been...
used as medicinal stuffs for curing diseases in oriental medicine or as foodstuffs. Recently much attention has been focused on the medicinal plants as targets for research to develop noble medicines. In the present study, the effects of the methanol extract of *A. orientale* and its major compound, alisol B 23-acetate, on lipid peroxidation and the activities of enzymes involved in drug metabolism and glutathione homeostasis were examined in the liver of APAP-treated rats.

**Experimental**

**Plant material** – The aerial part of *A. orientale* was collected from a field in Suncheon, Korea in April, 2004. A voucher specimen (specimen No. NM1104) has been deposited at the Herbarium of the Department of Oriental Medicine Resources at Sunchon National University.

**Extraction and isolation of alisol B 23-acetate** – The air-dried rhizome of *A. orientale* (15 kg) was extracted 3 times with methanol by refluxing for 4 hrs each time and concentrated in vacuo. An aliquot of the methanol extract was partitioned with organic solvents of different polarity to obtain dichloromethane, ethyl acetate, and aqueous fractions. The dichloromethane fraction was subjected to silica gel chromatography (SiO$_2$ fractions. The dichloromethane fraction was subjected to obtain dichloromethane, ethyl acetate, and aqueous fractions. The dichloromethane fraction was subjected to silica gel chromatography (SiO$_2$: 500 g, column: 6.2 cm × 42.5 cm), using a solvent gradient of CH$_2$Cl$_2$-MeOH (20 : 1 → 5 : 1). The effluent of silica gel chromatography was further chromatographed on a Sephadex LH-20 to obtain alisol B 23-acetate (Fig. 1): $^1$H-NMR (CDCl$_3$, 400 MHz) δ: 0.90, 0.94, 0.96, 0.97, 1.07, 1.24, 1.26 (3H each, s), 0.99 (3H, d, $J$ = 6.36 Hz), 1.64 (1H, d, $J$ = 10.70 Hz, H-9), 2.00 (3H, s, OAc), 2.49 (1H, dd, $J$ = 5.69, 13.20 Hz, Ha-12), 2.66 (1H, d, $J$ = 8.52 Hz, H-24), 3.74 (1H, ddd, $J$ = 5.73, 10.72, 10.73 Hz, H-11), 3.91 (1H, ddd, $J$ = 2.71, 8.53, 10.64 Hz, H-23). $^{13}$C-NMR (CDCl$_3$, 100 MHz) δ: 18.38 (C-26), 19.02 (C-29), 19.06 (C-6), 19.09 (C-21), 20.17 (OCOCH$_3$), 22.15 (C-18), 22.83 (C-30), 23.68 (C-17), 24.64 (C-19), 26.83 (C-20), 28.15 (C-16), 28.54 (C-28), 29.65 (C-15), 29.94 (C-1), 32.72 (C-22), 33.18 (C-7), 33.50 (C-12), 35.76 (C-2), 35.93 (C-10), 39.72 (C-8), 45.94 (C-4), 47.48 (C-5), 48.98 (C-9), 50.03 (C-14), 57.45 (C-25), 64.08 (C-24), 69.21 (C-11), 70.52 (C-23), 133.17 (C-17), 137.12 (C-13), 169.02 (OCOCH$_3$) 219.13 (C-3) (Park et al., 2005).

**Fig. 1.** Structure of alisol B 23-acetate isolated from the rhizome of *Alisma orientale.*

**Animals** – Male Sprague-Dawley rats (Daehan BioLink, Eumsung, Korea), weighing 200 ± 10 g, were fed ad libitum with a commercial standard rat diet based on AIN-93G, and maintained at 20 ± 1°C with a 12 hr light/dark cycle. The animals were cared for under the guidelines for the care and use of laboratory animals established by the Institute of Laboratory Animal Resources, U.S.A.

Animals were orally administered daily for 2 weeks with 250 or 500 mg of the methanol extract of the plant/kg of body weight or with 5, 10 or 20 mg/kg body weight of alisol B 23-acetate that had been isolated from the methanol extract. On the last day of oral treatment of the plant materials, rats were injected i.p. with APAP (800 mg/kg) or free with APAP (800 mg/kg). At 24 hr after the injection of APAP, the animals were sacrificed under anesthesia with CO$_2$. Animals were starved for 24 hr before sacrifice.

**Preparation of enzyme sources** – The liver, which had been exhaustively perfused with ice-cold 0.9% NaCl, was homogenized with 4 volumes of an ice-cold 0.1 M potassium phosphate buffer, pH 7.5. An aliquot of the homogenate was used for the determination of lipid peroxide and glutathione contents. The remaining homogenate was centrifuged at 600 × g for 10 min, and the resulting supernatant was centrifuged at 10,000 × g for 20 min. The supernatant was further centrifuged at 105,000 × g for 60 min to obtain the upper fraction as cytoplasm. The pellet was resuspended in the same volume of the 0.1 M potassium phosphate buffer and centrifuged at 105,000 × g for 60 min to obtain the microsomal fraction. The cytoplasmic fraction was used as the enzyme source of glutathione S-transferase, glutathione reductase and γ-glutamylcysteine synthetase, and the microsomal fraction was used to measure the activities of cytochrome P-450, aniline N-demethylase and aniline hydroxylase.

**Assay of serum ALT, AST and SDH** – As a marker of acute liver damage in animals, serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were determined by using a commercial enzyme assay kit AM 101-K (Asan Pharmaceutical Co Ltd., Seoul, Korea) based on the method of Reitman and Frankel (1957). Serum sorbitol dehydrogenase (SDH)