韓藥作誌(Korean J. Medicinal Crop Sci.) 34(4) : 238 – 244 (2026)
ISSN(Print) 1225-9306
ISSN(Online) 2288-0186
http://www.medicinalcrop.org
http://dx.doi.org/10.7783/KJMCS.2026.34.4.238

칡 어린순 추출물의 지표성분 분석 및 알코올 대사와 간 기능에 미치는 영향

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Marker Compound Analysis and Effects of *Pueraria lobata* New Vine Extract on Alcohol Metabolism and Liver Function

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Received: 2026 July 11
1st Revised: 2026 August 04
2nd Revised: 2026 August 20
3rd Revised: 2026 August 26
Accepted: 2026 August 26
Published: 2026 August 30

ABSTRACT

Background: This study aimed to characterize the marker compounds of *Pueraria lobata* new vines (PLNV) and evaluate their effects on alcohol metabolism and liver function in alcohol-treated rats.

Methods and Results: Marker compounds in PLNV were analyzed using HPLC-MS/MS, and the effects of PLNV were evaluated in alcohol-treated rats administered with PLNV at 100 or 300 mg/kg. Puerarin, daidzin, and genistin were identified as marker compounds. Serum ethanol concentrations were significantly lower in the PLNV-300 group at 1 h and in both PLNV groups at 3 and 5 h, while serum acetaldehyde concentrations were significantly lower in both groups at all sampling times. Both PLNV groups showed significantly higher hepatic ADH and ALDH activities and lower serum ALT and AST activities than those of the EtOH group (control).

Conclusions: PLNV administration was associated with lower serum ethanol and acetaldehyde concentrations, higher hepatic ADH and ALDH activities, and lower serum ALT and AST activities following alcohol administration.

Key Words **:** *Pueraria lobata*, Alcohol Dehydrogenase, Alcohol Metabolism, Aldehyde Dehydrogenase, HPLC-MS/MS, Liver Function

INTRODUCTION

Pueraria lobata (Willd.) Ohwi, a perennial climbing plant of the family Leguminosae, is widely distributed throughout East Asia, including Korea. Its root, known as Puerariae Radix, has traditionally been used as a medicinal and food resource. Although various parts of *P. lobata* have been investigated for their bioactive constituents and physiological activities, relatively limited information is available on the chemical characteristics and biological activities of its new vines (Park, 2011).

Previous studies have identified various isoflavonoids in *P. lobata*. Among these, daidzin has been reported to selectively inhibit mitochondrial aldehyde dehydrogenase (Keung and Vallee, 1993), while puerarin has been reported to exhibit various biological activities, including antihyperglycemic, hepatoprotective, and neuroprotective effects (Hsu *et al*., 2003; Hwang *et al*., 2007; Gu *et al*., 2010). Puerariae Flos has also been reported to affect alcohol metabolism and alcohol-induced liver injury in experimental animals (Niiho *et al*., 1989; Niiho *et al*., 1990). These findings provide a basis for investigating the marker compounds and alcohol-related effects of the less-studied new vines of *P. lobata*.

Ethanol is primarily oxidized to acetaldehyde by alcohol dehydrogenase (ADH) and subsequently metabolized to acetate

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by aldehyde dehydrogenase (ALDH). Acetaldehyde is a reactive and toxic intermediate associated with alcohol-related cellular and tissue damage (Tuma and Casey, 2003; Lieber, 2005), while excessive alcohol exposure is associated with hepatic injury (Lieber, 1994; Lieber, 2003). Accordingly, natural products affecting alcohol metabolism and related changes in liver function have been investigated as potential functional materials (Lee *et al*., 2004; Lee *et al*., 2012; Seo *et al*., 2022; Han *et al*., 2024).

In the present study, marker compounds of *P. lobata* new vines (PLNV) extract were identified and quantified by HPLC-MS/MS. The effects of PLNV were evaluated in an acute alcohol-treated rat model by measuring serum ethanol and acetaldehyde concentrations, hepatic ADH and ALDH activities, and serum ALT and AST activities. This study aimed to provide basic information on the chemical characteristics of PLNV and its effects on alcohol metabolism and liver function.

MATERIALS AND METHODS

1. Preparation of PLNV extract

PLNV cultivated in Yeongcheon, Gyeongsangbuk-do, Korea, was purchased from a local supplier. The plant material was pulverized, and 100 g of the powder was extracted three times with 70% ethanol at a solvent-to-sample ratio of 20:1 (v/w) for 8 h at room temperature. The combined extracts were filtered through Whatman No. 2 filter paper (Cytiva, Little Chalfont, UK) under vacuum and concentrated at 50 ± 1℃ using a rotary evaporator (N-1110, EYELA, Tokyo, Japan). The concentrate was frozen at −70 ± 1℃ for 24 h, freeze-dried (FDU-2100, EYELA, Tokyo, Japan), and stored at 2 ± 1℃ until use. The extraction yield was 18.7%.

2. HPLC-MS/MS identification and quantification of marker compounds

The marker compounds in the PLNV extract were identified and quantified using a 1290 Infinity II LC coupled to a 6470 Triple Quadrupole LC/MS system (Agilent Technologies, Santa Clara, CA, USA) with an InfinityLab Poroshell 120 EC-C18 column (2.1 × 150 mm, 2.7 μm). The mobile phases were 0.1% formic acid in water (A) and acetonitrile (B), with the following gradient: 10% B (0–3 min), 10–50% B (3–8 min), 50% B (8–9 min), and 10% B (9.1–12 min). The column temperature, flow rate, and injection volume were 40℃, 0.4 mL/min, and 2 μL, respectively. Detection was performed in positive AJS-ESI mode using dMRM, with a nozzle voltage of 500 V, capillary voltage of 3,500 V, source temperature of 300℃, sheath gas temperature of 350℃, sheath gas flow of 11 L/min, and nebulizer pressure of 45 psi. Retention times and dMRM parameters are shown in Table 1. Quantification was performed using calibration curves of the respective standards, and the results were expressed as mg/kg of dried PLNV extract.

Table 1. Optimized dMRM parameters for HPLC-MS/MS analysis of marker compounds in PLNV.

Parameter	Puerarin	Daidzin	Genistin
Retention time (min)	4.73	5.46	6.14
Precursor ion (*m/z*)	417.1	417.1	433.0
Product ion (*m/z*)	297.0 / 267.1	255.1 / 198.9	271.1 / 153.0
Fragmentor (V)	125 / 125	125 / 125	125 / 125
Collision energy (V)	28 / 40	25 / 52	20 / 52
Cell accelerator voltage (V)	4 / 4	4 / 4	4 / 4

For each compound, product ions and their corresponding collision energies are presented in the same order.

3. Experimental animals and experimental design

Six-week-old male Sprague-Dawley rats (80–100 g) were obtained from Raon Bio Co., Ltd. (Yongin, Korea) and housed at 22 ± 2℃ and 50 ± 10% relative humidity under a 12-h light/dark schedule, with ad libitum access to food and water. After 2 weeks of acclimatization, the rats were randomly assigned to four groups (n = 5 per group): NC, EtOH, PLNV-100, and PLNV-300 (Table 2). Blood samples were collected from the lateral tail vein at 1, 3, and 5 h after ethanol administration, and serum was separated by centrifugation at 10,000 × g for 10 min for ethanol and acetaldehyde analyses. At 24 h, blood was collected for serum ALT and AST analyses, and liver tissues were collected and stored at -80℃ for hepatic ADH and ALDH analyses. All procedures were approved by the Institutional Animal Care and Use Committee of Daejeon University (Approval No. DJUARB-2023-022).

Table 2. Experimental groups and treatment conditions.

Group	Sex	n	Alcohol (g/kg)	PLNV (mg/kg)
NC	Male	5	-	-
EtOH	Male	5	3	-
PLNV-100	Male	5	3	100
PLNV-300	Male	5	3	300

4. Administration of PLNV extract and ethanol

The animals were fasted for 20 h before treatment with free access to water. PLNV extract was orally administered once at doses of 100 and 300 mg/kg body weight to the PLNV-100 and PLNV-300 groups, respectively, whereas the NC and EtOH groups received an equivalent volume of distilled water. Thirty minutes later, the EtOH, PLNV-100, and PLNV-300 groups received 30% (v/v) ethanol at 3 g/kg body weight, whereas the NC group received an equivalent volume of distilled water. All treatments were administered by oral gavage.

5. Measurement of serum ethanol concentration

Serum ethanol concentrations were determined using an Ethanol Assay Kit (BIOMAX Co., Guri, Korea). Serum samples (10 μL) or ethanol standards were added to a 96-well microplate and mixed with 90 μL of the reaction mixture. Following a 30 min incubation at room temperature, the reaction was terminated by the addition of 100 μL of stop solution. The absorbance was then recorded at 450 nm using a microplate reader (VersaMax, Molecular Devices, Sunnyvale, CA, USA). Serum ethanol concentrations were calculated using the resulting calibration curve.

6. Measurement of serum acetaldehyde concentration

Serum acetaldehyde concentrations were determined using an EnzyChrom™ Acetaldehyde Assay Kit (EACT-100; BioAssay Systems, Hayward, CA, USA) according to the manufacturer's instructions. After centrifugation, 20 μL of serum was added to each well, followed by 80 μL of working reagent or blank working reagent without Enzyme A for sample-blank correction. After incubation for 30 min at room temperature, absorbance was measured at 565 nm using a microplate reader (VersaMax, Molecular Devices, Sunnyvale, CA, USA). Acetaldehyde concentrations were calculated from a standard curve after sample-blank correction.

7. Measurement of hepatic ADH and ALDH activities

Hepatic ADH and ALDH activities were determined using commercial assay kits (ab102533 and ab155893, respectively; Abcam, Cambridge, UK) according to the manufacturer's instructions. Liver tissues were homogenized in the respective assay buffers and centrifuged at 13,000 × g for 10 min at 4℃. The resulting supernatants were used for the enzyme activity assays. Absorbance was measured at 450 nm using a microplate reader (VersaMax, Molecular Devices, Sunnyvale, CA, USA), and enzyme activities were calculated according to the manufacturer's instructions.

8. Measurement of serum ALT and AST activities

Serum ALT and AST activities were measured using commercial reagent kits (Thermo Fisher Scientific, Vantaa, Finland) and a blood chemistry analyzer (Konelab 20XT, Thermo Electron Co., Finland).

9. Statistical analysis

Data are presented as the mean ± standard deviation (SD). Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test with SPSS version 18.0 (SPSS Inc., Chicago, IL, USA). Serum ethanol and acetaldehyde concentrations were analyzed separately at each time point. Differences were considered statistically significant at $p < 0.05$.

RESULTS

1. Identification and quantification of marker compounds in the PLNV extract by HPLC-MS/MS

The HPLC-MS/MS profiles of the marker compounds in the PLNV extract are presented in Table 3 and Fig. 1. Puerarin, daidzin, and genistin were identified based on their precursor ions, product ions, and retention times. Their contents were 31.18 ± 0.41, 82.61 ± 1.16, and 128.97 ± 0.38 mg/kg of dried PLNV extract, respectively.

2. Effect of PLNV on serum ethanol concentration after acute alcohol administration

Serum ethanol concentrations are shown in Fig. 2. The EtOH

Table 3. HPLC-MS/MS identification and contents of marker compounds in the PLNV extract.

Compound	Precursor ion (*m/z*)	Product ions (*m/z*)	Retention time (min)	Content (mg/kg extract)
Puerarin	417.1	297.0 / 267.1	4.73	31.18 ± 0.41
Daidzin	417.1	255.1 / 198.9	5.46	82.61 ± 1.16
Genistin	433.0	271.1 / 153.0	6.14	128.97 ± 0.38

Values are expressed as mean ± SD of triplicate determinations.

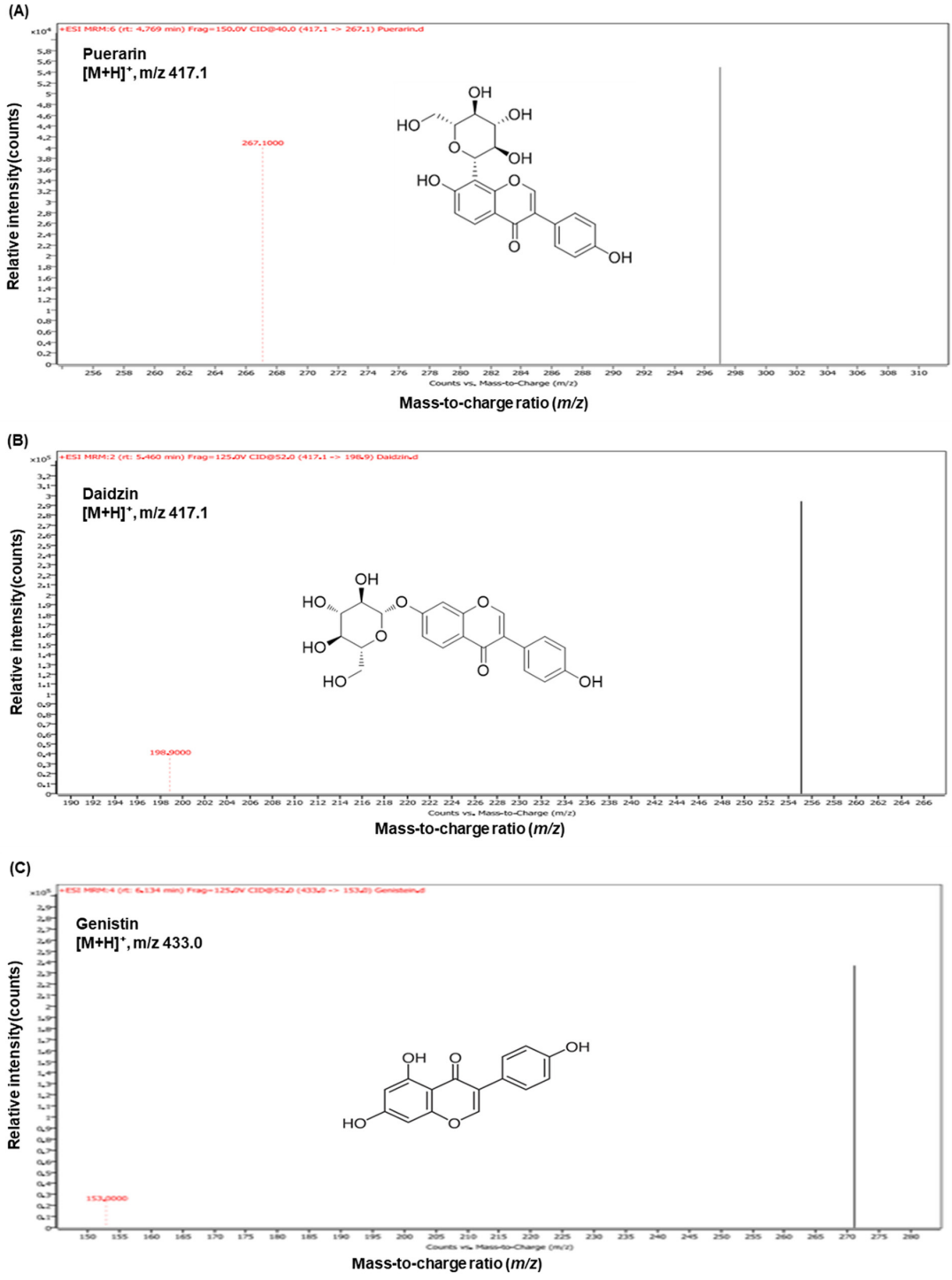

Fig. 1. HPLC-MS/MS product ion spectra of marker compounds identified in the PLNV extract. (A) Puerarin (precursor ion $[M + H]^+$, *m/z* 417.1); (B) daidzin (precursor ion $[M + H]^+$, *m/z* 417.1); and (C) genistin (precursor ion $[M + H]^+$, *m/z* 433.0).

group showed significantly higher concentrations than the NC group at all sampling times ($p < 0.05$). At 1 h, serum ethanol concentrations in the EtOH, PLNV-100, and PLNV-300 groups were 0.14 ± 0.01, 0.13 ± 0.01, and 0.12 ± 0.01%, respectively. At 3 h, the corresponding values were 0.16 ± 0.01, 0.11 ± 0.01, and 0.10 ± 0.01%, respectively. At 5 h, the concentrations were

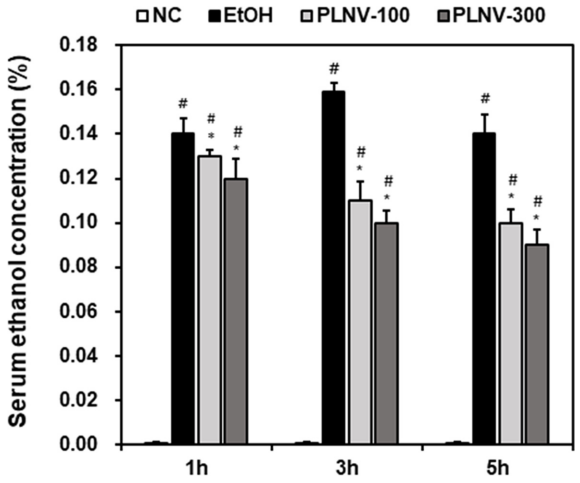

Fig. 2. Effects of PLNV on serum ethanol concentrations in acute alcohol-treated rats. Serum ethanol concentrations were measured at 1, 3, and 5 h after alcohol administration. NC, distilled water; EtOH, distilled water + alcohol (3 g/kg); PLNV-100, PLNV (100 mg/kg) + alcohol (3 g/kg); PLNV-300, PLNV (300 mg/kg) + alcohol (3 g/kg). Data are expressed as the mean ± SD (n = 5). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. $^{\#}p < 0.05$ compared with the NC group; $^{*}p < 0.05$ compared with the EtOH group.

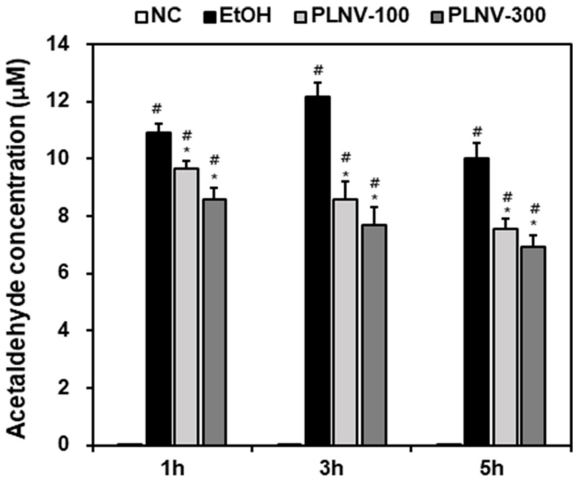

Fig. 3. Effects of PLNV on serum acetaldehyde concentrations in acute alcohol-treated rats. Serum acetaldehyde concentrations were measured at 1, 3, and 5 h after alcohol administration. NC, distilled water; EtOH, distilled water + alcohol (3 g/kg); PLNV-100, PLNV (100 mg/kg) + alcohol (3 g/kg); PLNV-300, PLNV (300 mg/kg) + alcohol (3 g/kg). Data are expressed as the mean ± SD (n = 5). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. $^{\#}p < 0.05$ compared with the NC group; $^{*}p < 0.05$ compared with the EtOH group.

0.14 ± 0.01, 0.10 ± 0.01, and 0.09 ± 0.01%, respectively. Compared with the EtOH group, the PLNV-300 group showed a significantly lower serum ethanol concentration at 1 h, while both PLNV-100 and PLNV-300 groups showed significantly lower concentrations at 3 and 5 h ($p < 0.05$).

3. Effect of PLNV on serum acetaldehyde concentration after acute alcohol administration

Serum acetaldehyde concentrations are shown in Fig. 3. The EtOH group showed significantly higher concentrations than the NC group at all sampling times ($p < 0.05$). At 1 h, the concentrations in the EtOH, PLNV-100, and PLNV-300 groups were 10.90 ± 0.31, 9.66 ± 0.26, and 8.59 ± 0.39 μM, respectively. At 3 h, the corresponding values were 12.16 ± 0.48, 8.59 ± 0.61, and 7.71 ± .63 μM, respectively. At 5 h, the concentrations were 10.01 ± 0.55, 7.54 ± 0.37, and 6.95 ± 0.41 μM, respectively. At each sampling time, both PLNV-treated groups showed significantly lower serum acetaldehyde concentrations than the EtOH group ($p < 0.05$).

4. Effect of PLNV on hepatic ADH and ALDH activities after acute alcohol administration

The effects of PLNV on hepatic ADH and ALDH activities are shown in Table 4. Hepatic ADH and ALDH activities were significantly lower in the EtOH group than in the NC group ($p < 0.05$). Both activities were significantly higher in the PLNV-100 and PLNV-300 groups than in the EtOH group ($p < 0.05$). ADH activity in both PLNV-treated groups and ALDH activity in the PLNV-100 group remained significantly lower than the corresponding activities in the NC group ($p < 0.05$), whereas ALDH activity in the PLNV-300 group did not differ significantly from that in the NC group.

Table 4. Effects of PLNV on hepatic ADH and ALDH activities in acute alcohol-treated rats.

Group[1)]	ADH[2)] (nmol/mg/min)	ALDH[2)] (nmol/mg/min)
NC	5.08 ± 0.10	9.43 ± 0.29
EtOH	3.89 ± 0.12$^{\#}$	7.14 ± 0.25$^{\#}$
PLNV-100	4.37 ± 0.20$^{\#*}$	8.20 ± 0.39$^{\#*}$
PLNV-300	4.80 ± 0.10$^{\#*}$	8.78 ± 0.48*

[1)]NC, normal control; EtOH, alcohol-treated control; PLNV-100, 100 mg/kg PLNV extract plus alcohol; PLNV-300, 300 mg/kg PLNV extract plus alcohol. [2)]ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase. [3)]Data are expressed as the mean ± SD (n = 5). [4)]Statistical significance among groups was evaluated using one-way ANOVA followed by Tukey's multiple comparison test. $^{\#}p < 0.05$ versus the NC group; $^{*}p < 0.05$ versus the EtOH group.

Table 5. Effects of PLNV extract on serum ALT and AST activities in acute alcohol-treated rats.

Group[1)]	ALT[2)] (U/L)	AST[2)] (U/L)
NC	47.72 ± 3.99	132.20 ± 4.0
EtOH	56.50 ± 2.96$^{\#}$	163.06 ± 2.76$^{\#}$
PLNV-100	50.12 ± 2.12*	141.70 ± 4.79*
PLNV-300	49.50 ± 2.55*	140.54 ± 4.68*

[1)]NC, normal control; EtOH, alcohol-treated control; PLNV-100, 100 mg/kg PLNV extract plus alcohol; PLNV-300, 300 mg/kg PLNV extract plus alcohol. [2)]ALT, alanine aminotransferase; AST, aspartate aminotransferase. [3)]Data are expressed as the mean ± SD (n = 5). [4)]Statistical significance among groups was evaluated using one-way ANOVA followed by Tukey's multiple comparison test. $^{\#}p < 0.05$ versus the NC group; $^{*}p < 0.05$ versus the EtOH group.

5. Effect of PLNV on serum ALT and AST activities after acute alcohol administration

The effects of PLNV on serum ALT and AST activities are shown in Table 5. Serum ALT and AST activities were significantly higher in the EtOH group than in the NC group ($p < 0.05$). Both activities were significantly lower in the PLNV-100 and PLNV-300 groups than in the EtOH group ($p < 0.05$), whereas neither activity differed significantly between the PLNV-treated groups and the NC group.

DISCUSSION

In this study, puerarin, daidzin, and genistin were identified and quantified as marker compounds in the dried PLNV extract by HPLC-MS/MS, with contents of 31.18, 82.61, and 128.97 mg/kg, respectively. Genistin was the most abundant among the three compounds, whereas puerarin has been reported as a major isoflavonoid in the roots of *P. lobata* (Wang *et al*., 2016; Wang *et al*., 2021). Differences in isoflavonoid composition among plant parts of *P. lobata* have been reported previously (Wu *et al*., 2009; Mun and Mun, 2015), suggesting that the observed profile may reflect differences in plant parts and extraction conditions.

Ethanol is primarily oxidized to acetaldehyde by ADH and subsequently metabolized to acetate by ALDH (Zakhari, 2006; Edenberg, 2007). Serum ethanol concentrations were significantly lower in the PLNV-300 group at 1 h and in both PLNV-treated groups at 3 and 5 h, while serum acetaldehyde concentrations were significantly lower in both PLNV-treated groups at all sampling times. At 5 h, serum ethanol and acetaldehyde concentrations in the PLNV-300 group were approximately 36.5% and 30.6% lower, respectively, than those in the EtOH group. Similar reductions have been reported following administration of other natural products (Kim *et al*., 2000; Yang, 2010; Sung *et al*., 2014).

Hepatic ADH and ALDH activities at 24 h were significantly higher in both PLNV-treated groups than in the EtOH group. Similar changes in alcohol-metabolizing enzyme activities have been reported with various natural products (Kim *et al*., 2002; Lee *et al*., 2004; Lee *et al*., 2012; Kim *et al*., 2018). Although daidzin has been reported to selectively inhibit mitochondrial ALDH (Keung and Vallee, 1993), the present findings were obtained using the whole PLNV extract rather than isolated daidzin. Therefore, the higher hepatic ALDH activity should be interpreted in the context of the overall composition of the extract. Because hepatic ADH and ALDH activities were measured at 24 h, whereas serum ethanol and acetaldehyde concentrations were assessed at 1–5 h, the differences in measurement timing should be considered when interpreting the relationship between these parameters.

Serum ALT and AST activities were significantly lower in the PLNV-treated groups than in the EtOH group. Similarly, aged black garlic has been reported to attenuate alcohol-induced increases in aminotransferase activities in rats (Kim *et al*., 2011). These findings suggest that PLNV administration attenuated the changes in serum aminotransferase activities following alcohol administration.

Taken together, PLNV administration was associated with lower serum ethanol and acetaldehyde concentrations, higher hepatic ADH and ALDH activities at 24 h, and lower serum ALT and AST activities following alcohol administration. Further studies evaluating these parameters at corresponding time points may help clarify their temporal relationships and the contributions of individual PLNV constituents.

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