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Original Research

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Aqueous extract of *Laurus nobilis* leaf accelerates the alcohol metabolism and prevents liver damage in single-ethanol binge rats

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ABSTRACT

BACKGROUND/OBJECTIVES: Excessive alcohol consumption has harmful health effects, including alcohol hangovers and alcohol-related liver disease. Therefore, methods to accelerate the alcohol metabolism are needed. *Laurus nobilis* is a spice, flavoring agent, and traditional herbal medicine against various diseases. This study examined whether the standardized aqueous extract of *L. nobilis* leaves (LN) accelerates the alcohol metabolism and protects against liver damage in single-ethanol binge Sprague-Dawley (SD) rats.
MATERIALS/METHODS: LN was administered orally to SD rats 1 h before ethanol administration (3 g/kg body weight [BW]) at 100 and 300 mg/kg BW. Blood samples were collected 0.5, 1, 2, and 4 h after ethanol administration. The livers were excised 1 h after ethanol administration to determine the hepatic enzyme activity. The alcohol dehydrogenase (ADH), aldehyde dehydrogenase (ALDH), superoxide dismutase (SOD), and glutathione peroxidase (GPx) activities in the liver tissue were measured.
RESULTS: LN decreased the serum ethanol and acetaldehyde levels in ethanol-administered rats. LN increased the hepatic ADH and ALDH activities but decreased the alanine aminotransferase, aspartate aminotransferase, and gamma-glutamyl transferase activities in the ethanol-administered rats. In addition, LN inhibited lipid peroxidation and increased the activities of SOD and GPx.
CONCLUSIONS: LN modulates the mediators of various etiological effects of excessive alcohol consumption and enhances the alcohol metabolism and antioxidant activity, making it a potential candidate for hangover treatments.

Keywords: *Laurus nobilis*; alcohol dehydrogenase; aldehyde dehydrogenase; superoxide dismutase; glutathione peroxidase

INTRODUCTION

Alcohol has been used for stress relief and in social settings since ancient times. Although mild-to-moderate alcohol consumption has some beneficial physiological and psychological effects, excessive consumption causes liver damage, leading to alcohol-related liver disease.

Conflict of Interest
The authors declare no potential conflicts of interests.

Author Contributions
Conceptualization: Kim J, Kim EJ; Data curation: Kim J, Baek KS; Formal analysis: Jung JI, Kim J, Baek KS; Investigation: Jung JI, Choi YJ; Visualization: Jung JI, Choi YJ; Writing - original draft: Jung JI, Choi YJ, Kim EJ; Writing - review & editing: Choi YJ, Kim EJ.

Excessive alcohol consumption is characterized as binge or frequent drinking. Hangovers are the most common outcome of acute heavy alcohol consumption and have symptoms, such as fatigue, thirst, dizziness, headache, nausea, vomiting, and cognitive impairment [1]. The high prevalence of hangovers is becoming a more serious health issue: more than 75% of people who drink alcohol have reported experiencing a hangover at least once [2]. Frequent hangovers lead to the accumulation of oxidative and inflammatory changes in the liver over long periods, causing severe health problems.

In the liver, alcohol is initially oxidized to acetaldehyde by alcohol dehydrogenase (ADH), an NAD^+-dependent enzyme in the hepatic cytosol. Acetaldehyde is oxidized rapidly to non-toxic acetate and water by mitochondrial aldehyde dehydrogenase (ALDH). Acetate is an acetyl-coenzyme A precursor and energy source [3-5]. Acetaldehyde, a metabolic intermediate in alcohol oxidation, is responsible for the toxic effects of ethanol [6]. Its accumulation in the blood causes hangover symptoms owing to the inactivation and disturbance of ALDH. It also induces alterations in the plasma membrane structure of hepatic cells, leading to liver damage, such as fatty liver or hepatic necrosis [7,8]. In addition, increased reactive oxygen species (ROS) generated during the alcohol metabolism lead to alcohol-induced liver damage because of impaired hepatic antioxidant activity [9]. Therefore, acceleration of the alcohol metabolism is required to relieve hangover symptoms and prevent alcohol-related liver diseases.

Several traditional herbs have been developed to alleviate the harmful health effects of excessive alcohol intake. These herbs were developed based on their ability to accelerate the alcohol metabolism and improve antioxidant activity [10]. On the other hand, they are relatively ineffective against alcohol-induced disorders and have adverse side effects [11,12]. Therefore, more research is needed to develop potent treatments with no adverse side effects.

Laurus nobilis, commonly known as sweet bay, bay laurel, or bay leaf, is a Mediterranean evergreen plant belonging to the *Lauraceae* family. It is widely grown in regions with moderate and subtropical climates [13]. *L. nobilis* leaves are valuable spices and flavoring agents [14]. Moreover, *L. nobilis* leaves are used traditionally as a herbal medicine for their antibacterial and antioxidant properties [14-16]. An *L. nobilis* leaf extract had several pharmacological effects, including antioxidant [17], anti-inflammatory [18], anti-hypertensive [19], anti-diabetic [20], and anti-diarrheal [21] effects.

An aqueous extract of *L. nobilis* leaf attenuated ethanol-induced psychomotor alterations [13] and enhanced the protective effect against the liver toxicity caused by aluminum chloride [22] in rats. Therefore, this study hypothesized that an aqueous extract of *L. nobilis* leaf could alleviate hangover symptoms and ethanol-induced liver damage. Few studies have examined the direct effects of *L. nobilis* on the alcohol metabolism. The potential hepatoprotective effects of *L. nobilis* have been suggested by some studies, given its antioxidant and anti-inflammatory properties [23-25], which can help protect the liver indirectly from alcohol-induced damage. Evidence shows that some constituents of *L. nobilis*, particularly sesquiterpenes, might inhibit alcohol absorption. Yoshikawa *et al.* [26] reported that sesquiterpenes derived from *L. nobilis* had significant inhibitory effects on the blood alcohol levels of rats. They reported that the sesquiterpenes in *L. nobilis* could slow alcohol absorption in the gastrointestinal tract, reducing the blood alcohol concentration. Tinoco *et al.* [27] examined the effects of a hexane extract from *Laurus novocanariensis* (a species closely related to *L. nobilis*) on ethanol metabolism in rats. They reported that the extract suppressed the increase in blood alcohol concentration, indicating that it may influence alcohol absorption,

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metabolism, or both. On the other hand, these studies did not involve *L. nobilis* and the alcohol metabolism directly. Hence, more research is needed to understand the potential role of *L. nobilis* in the alcohol metabolism. To the best of the authors' knowledge, this study is the first to examine the potential utility of *L. nobilis* for hangover relief and the prevention of alcohol-related liver diseases. The current study tested whether the standardized aqueous extract of *L. nobilis* leaf (LN) accelerates the alcohol metabolism and protects against liver damage in single-ethanol binge Sprague-Dawley (SD) rats. LN could improve hangover symptoms and liver damage by lowering the serum acetaldehyde levels by enhancing hepatic alcohol-metabolizing enzymes and improving the biochemical markers of liver damage by enhancing hepatic antioxidant activity. These results suggest a new, natural, and safe intervention for hangovers and liver damage resulting from excessive alcohol consumption, marking a significant contribution to natural remedies for alcohol-induced disorders.

MATERIALS AND METHODS

Preparation of LN

LN was provided by Daehan Chemtech Co., Ltd. (Gwacheon, Korea). The dried leaves of *L. nobilis* were extracted in water at 80°C, followed by filtration. The filtrates were concentrated using a vacuum evaporator and dried using a vacuum dryer. The resulting powder was used as LN.

Determination of isoquercetin in LN

High-performance liquid chromatography (HPLC) was used to determine the phytochemical profile of LN using isoquercetin as the standard compound. The analyses were performed using an e2695 Separations Module (Waters, Milford, MA, USA). Chromatography was conducted on a Symmetry® C18 (250 × 4.6 mm, 5 µm) column at 30°C. The elution solvents were 15% acetonitrile and 85% deionized water containing 0.3% phosphoric acid. The elution was performed in isocratic mode at a 1 mL/min flow rate.

In vitro ADH and ALDH activity measurement

The ADH and ALDH activities of LN were measured using an ADH activity colorimetric assay kit (BioVision, Milpitas, CA, USA) and an ALDH activity colorimetric assay kit (BioVision), respectively, according to the manufacturer's instructions. The ADH and ALDH activities of LN were calculated as the relative activity (%) of the control group not treated with LN.

Ethical statement and animals

The animal care and experimental procedures were performed in accordance with the institutional guidelines for the care and use of laboratory animals to ensure their welfare and minimize distress. All experimental protocols were approved by the Institutional Animal Care and Use Committee of Hallym University (Hallym 2022-32).

Five-week-old male SD rats were purchased from DooYeol Biotech (Seoul, Korea). The rats were housed at the animal research facility of Hallym University and maintained under specific pathogen-free conditions at a temperature of 23 ± 3°C, relative humidity of 50 ± 10%, and a 12-h light/dark cycle. They were fed a standard rodent diet with free access to tap water.

Experimental design and treatment

After a one-week adaptation period, SD rats were randomly divided into 4 groups of 10 rats each: (1) normal control (NC), (2) ethanol control (EC), (3) ethanol + 100 mg/kg body

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weight (BW) LN-treated (LN100), and (4) ethanol + 300 mg/kg BW LN-treated (LN300) groups. After fasting for 18 h, the NC and EC rats were orally administered distilled water as a vehicle. The LN100 and LN300 rats were orally administered LN at doses of 100 and 300 mg/kg BW, respectively. The LN administration concentration was selected based on several supporting studies [26-28]. Previous research has shown that the increased blood alcohol concentration was suppressed in rats administered a single dose of 125, 250, or 500 mg/kg of methanol extract or a single dose of 100 mg/kg of hexane extract from *L. nobilis* [26,27]. Furthermore, Medeiros-Fonseca *et al.* [28] reported that administering hot water extract of *L. nobilis* to mice at a dose of 667 mg/kg for 21 days did not induce toxicity. Based on body surface area conversion, this dosage corresponds to 334 mg/kg in rats. Consequently, for this study, the extract administration dosage range was selected to be a minimum of 100 mg/kg to ensure effect confirmation and a maximum of 334 mg/kg to prevent toxicity in experimental animals. One hour after administration, the EC, LN100, and LN300 rats were administered ethanol by an oral gavage at 3 g/kg BW. Regarding actual alcohol intake, a 5-week-old SD rat weighing approximately 200 g would receive 0.6 g (600 mg) of pure ethanol, mirroring a high intake often associated with binge drinking episodes. The NC rats were administered distilled water as a vehicle. All rats were anesthetized with isoflurane (Vspharm, Hanam, Korea). Blood was then collected from the orbital vein 0.5, 1, and 2 h after ethanol administration and from the heart 4 h after ethanol administration. Serum was obtained from the blood through centrifugation at 3,000 rpm for 20 min at 4°C. The serum was immediately used for ethanol and acetaldehyde analyses.

The hepatic enzyme activity was determined as follows. The rats were administered LN and ethanol as described above. The rats were anesthetized with isoflurane (Vspharm) 1 h after ethanol administration. The blood was collected from the orbital vein, and serum was obtained. The rats were euthanatized by carbon dioxide asphyxiation, and their livers were excised. The livers were subsequently stored at –70°C until the analysis of hepatic enzyme activities.

Measurement of ethanol and acetaldehyde concentration in the serum

The ethanol and acetaldehyde concentrations in serum were measured using an ethanol assay kit (Abcam, Cambridge, UK) and an acetaldehyde assay kit (Megazyme, Wicklow, Ireland), respectively. The measurements were conducted according to the manufacturer's instructions. The area under the concentration-time curve (AUC) of ethanol or acetaldehyde was calculated using the trapezoid rule to evaluate the exposure that integrates the concentration with time.

Measurement of enzyme activity in the serum

The alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyl transferase (γ-GT) activities in the serum were measured using a blood chemistry autoanalyzer (KoneLab 20XT; Thermo Fisher Scientific, Vantaa, Finland).

Measurement of hepatic enzyme activity

For each rat, a 100 mg liver sample was homogenized in 1 mL ice-cold phosphate saline, and the homogenates were centrifuged at 5,000 rpm for 10 min. The supernatants were collected and used as tissue lysates. The protein content of the liver tissue lysates was measured using a BCA protein assay kit (Thermo Fisher Scientific, Waltham, MA, USA). ADH (BioVision), ALDH (BioVision), catalase (Cayman Chemical, Ann Arbor, MI, USA), superoxide dismutase (SOD) (Cayman Chemical), and glutathione peroxidase (GPx) (Cayman Chemical) activities in the liver tissues were measured using relevant assay kits, according to the manufacturer's instructions.

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Measurement of lipid peroxidation in the liver

The liver homogenates were prepared as described above. The level of lipid peroxidation in the liver homogenates was determined by measuring the malondialdehyde (MDA) content using a thiobarbituric acid reactive substances assay kit (Cayman Chemical) according to the manufacturer's protocol.

Statistical analyses

All data are presented as the mean ± SE of mean. Statistical analyses were performed using the Statistical Analysis System for Windows version 9.4 (SAS Institute, Cary, NS, USA). A student's *t*-test was used to analyze the differences between the NC and EC groups. An analysis of variance, followed by Duncan's multiple comparison test, was used to analyze the differences between the treatment groups (EC, LN100, and LN300). *P*-values < 0.05 were considered significant.

RESULTS

Characterization of LN using HPLC

LN (n = 3) was analyzed by HPLC to characterize LN for quality control. **Fig. 1** presents the HPLC results. LN contained more than 1 mg/g of isoquercetin.

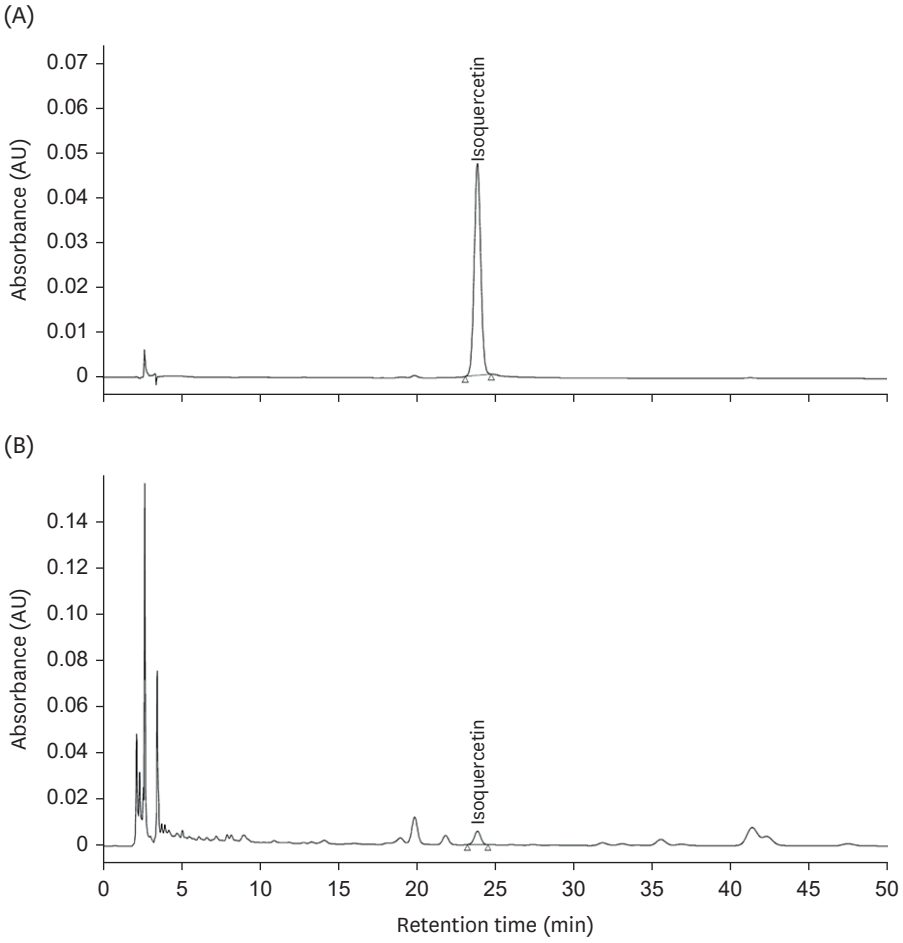

Fig. 1. High-performance liquid chromatography analysis of isoquercetin in LN. (A) Standard, 0.06 mg/mL isoquercetin; (B) 5.07 mg/mL LN.
LN, standardized aqueous extract of *Laurus nobilis* leaf.

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LN increases ADH and ALDH activities *in vitro*

In this study, LN increased the ADH and ALDH activities considerably in a dose-dependent manner. At 1 mg/mL, the ADH and ALDH activities increased by 41.7% and 95.9%, respectively, compared to the untreated control group (0 mg/mL) (**Table 1**).

LN reduces serum ethanol and acetaldehyde concentrations in ethanol-loaded rats

The ethanol and acetaldehyde concentrations increased as the serum ethanol concentrations increased at 0.5, 1, 2, and 4 h in the EC group and were higher than those in the NC group. The serum ethanol concentrations peaked 0.5 h after ethanol administration and decreased gradually thereafter. The serum ethanol concentration in the LN300 group was considerably lower than in the EC group at 0.5, 2, and 4 h after ethanol administration (**Fig. 2A**). Furthermore, the LN treatment reduced the AUC of serum ethanol by 43.4% in the LN300 group compared to the EC group (**Fig. 2B**).

Ethanol administration elevated the serum acetaldehyde concentration in rats. The serum acetaldehyde concentrations peaked 1 h after ethanol administration and decreased gradually thereafter. The serum acetaldehyde concentrations were substantially lower in the LN100 and LN300 groups than in the EC group at 1, 2, and 4 h after ethanol administration (**Fig. 2C**). The AUC of serum acetaldehyde decreased in a dose-dependent manner. Compared to the EC group, the AUC of serum acetaldehyde in the LN100 and LN300 groups decreased by 39.1% and 49.7%, respectively (**Fig. 2D**).

LN enhances hepatic ADH and ALDH activities in ethanol-loaded rats

Ethanol administration increased the hepatic ADH and ALDH activities considerably (**Fig. 3**). The LN treatment significantly increased the ethanol-induced ADH and ALDH activities in the liver. The hepatic ADH (**Fig. 3A**) and ALDH activities (**Fig. 3B**) in the LN300 group increased by 27.5% and 14.7%, respectively, compared to the EC group.

LN prevents liver damage in ethanol-loaded rats

Ethanol administration increased the serum AST, ALT, and γ-GT activities. The LN treatment decreased ethanol-induced AST, ALT, and γ-GT activities significantly in the serum (**Fig. 4**). The AST (**Fig. 4A**), ALT (**Fig. 4B**), and γ-GT (**Fig. 4C**) activities decreased by 14.7%, 14.0%, and 23.9%, respectively, in the LN300 group compared to the EC group.

The MDA content in the liver, an indicator of lipid peroxidation, was substantially higher in the EC group than the NC group. Moreover, it was reduced significantly by the LN treatment in the LN300 group (**Fig. 5**).

Table 1. Effect of LN on the ADH and ALDH activities *in vitro*

LN (mg/mL)	ADH activity (% of control)	ALDH activity (% of control)
0	100.0 ± 2.4^{d}	100.0 ± 2.8^{c}
0.03	105.9 ± 2.5^{cd}	103.7 ± 4.5^{c}
0.1	115.5 ± 1.1^{bc}	112.0 ± 6.2^{bc}
0.3	120.6 ± 2.5^{b}	126.0 ± 4.2^{b}
1	141.7 ± 5.2^{a}	195.9 ± 10.4^{a}

Values are expressed as the mean ± SE of mean.
LN, standardized aqueous extract of *Laurus nobilis* leaf; ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase.
Means without the same letter, $P < 0.05$.

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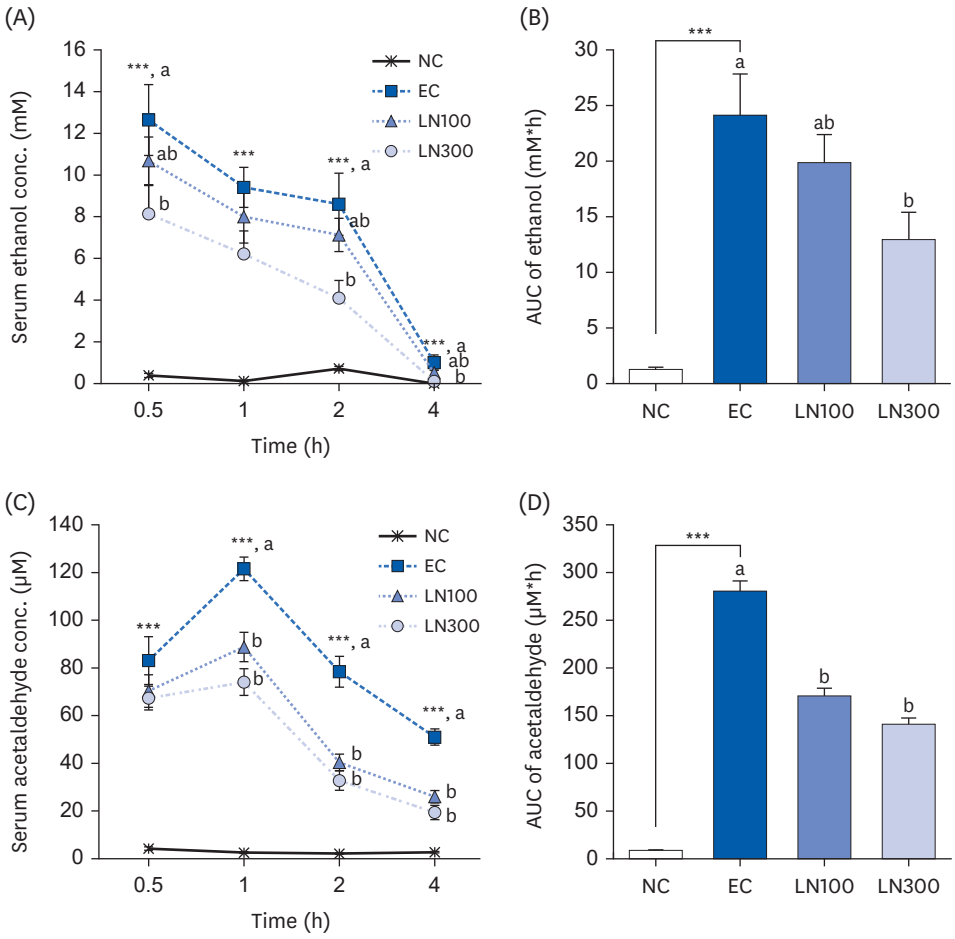

Fig. 2. Effects of LN on the serum ethanol and acetaldehyde concentrations in single-ethanol binge SD rats. SD rats were administered LN orally. After 1 h, the rats were given ethanol at a dose of 3 g/kg body weight by oral gavage. Blood was collected at 0.5, 1, 2, and 4 h after ethanol administration, and serum was obtained from blood. The ethanol (A) and acetaldehyde (C) concentrations in serum were measured using the relevant assay kits. The AUC for serum ethanol (B) and acetaldehyde (D) were calculated. Each bar represents the mean ± SE of mean (n = 10).
LN, standardized aqueous extract of *Laurus nobilis* leaf; AUC, area under the concentration-time curve; NC, normal control group; EC, ethanol control group; LN100, ethanol + 100 mg/kg body weight standardized aqueous extract of *Laurus nobilis* leaf-treated group; LN300, ethanol + 300 mg/kg body weight standardized aqueous extract of *Laurus nobilis* leaf-treated group; SD, Sprague-Dawley.
$^{***}P < 0.001$ indicates a significant difference from the NC group.
Different letters indicate significant differences between the EC, LN100, and LN300 groups at $P < 0.05$.

LN enhances the antioxidant enzyme activity in ethanol-loaded rats

Ethanol administration decreased the hepatic SOD and GPx activities significantly. The LN treatment increased the SOD and GPx activities that had been decreased by ethanol administration in a dose-dependent manner. Compared with the EC group, the SOD and GPx activities were elevated by 68.0% and 53.2%, respectively, in the LN300 group (**Fig. 6A and B**). The hepatic catalase activity was decreased by ethanol administration, but the decrease was not statistically significant. The LN treatment did not affect the catalase activity in the liver (**Fig. 6C**).

DISCUSSION

Five major findings were obtained. 1) LN increases the ADH and ALDH activities in an *in vitro* system, regulating the key mediators of the oxidative pathways in the alcohol metabolism.

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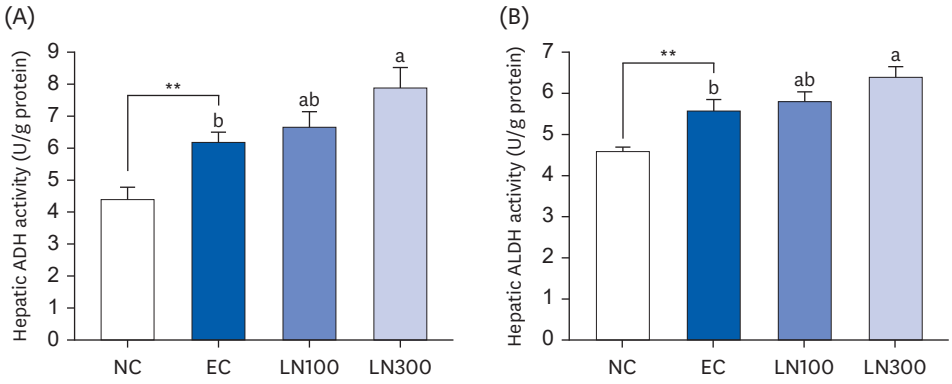

Fig. 3. Effects of LN on hepatic ADH and ALDH activities in single-ethanol binge SD rats. SD rats were administered LN orally. After 1 h, the rats were given ethanol at a dose of 3 g/kg body weight by oral gavage. After 1 h of ethanol administration, the livers were excised from the rats. Hepatic ADH (A) and ALDH (B) activities were estimated using the relevant assay kits. Each bar represents the mean ± SE of mean (n = 10).
LN, standardized aqueous extract of *Laurus nobilis* leaf; ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; NC, normal control group; EC, ethanol control group; LN100, ethanol + 100 mg/kg body weight standardized aqueous extract of *Laurus nobilis* leaf-treated group; LN300, ethanol + 300 mg/kg body weight standardized aqueous extract of *Laurus nobilis* leaf-treated group; SD, Sprague-Dawley.
$^{**}P < 0.01$ indicate significant difference from the NC group.
The different letters indicate significant differences between the EC, LN100, and LN300 groups at $P < 0.05$.

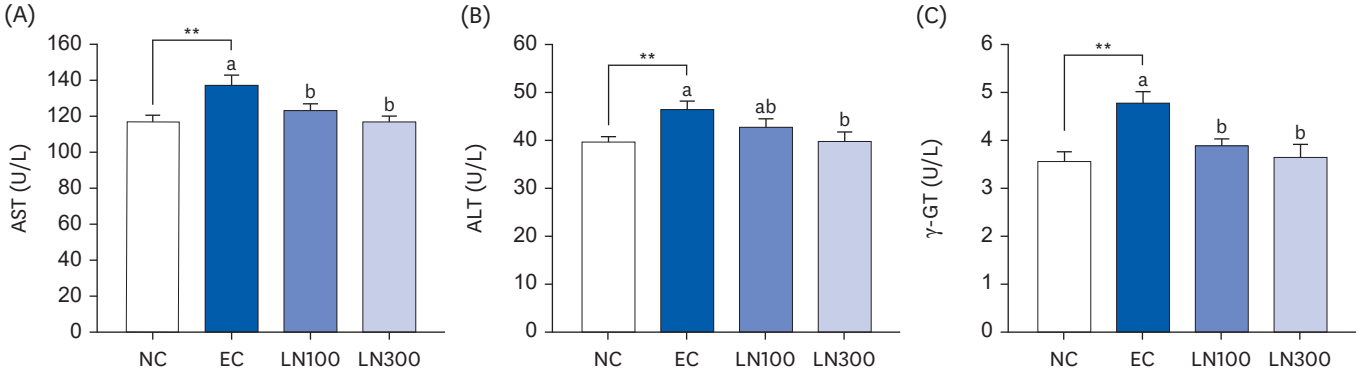

Fig. 4. Effects of LN on the serum AST, ALT, and γ-GT activities in single-ethanol binge Sprague-Dawley rats. The rats were administered LN orally. After 1 h, the rats were given ethanol at a dose of 3 g/kg body weight by oral gavage. After 1 h of ethanol administration, blood was collected, and serum was obtained. The AST (A), ALT (B), and γ-GT (C) activities in serum were measured using a blood chemistry autoanalyzer. Each bar represents the mean ± SE of mean (n = 10).
LN, standardized aqueous extract of *Laurus nobilis* leaf; ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GT, gamma-glutamyl transferase; NC, normal control group; EC, ethanol control group; LN100, ethanol + 100 mg/kg body weight standardized aqueous extract of *Laurus nobilis* leaf-treated group; LN300, ethanol + 300 mg/kg body weight standardized aqueous extract of *Laurus nobilis* leaf-treated group.
$^{**}P < 0.01$ indicates a significant difference from the NC group.
The different letters indicate significant differences between the EC, LN100, and LN300 groups at $P < 0.05$.

2) LN reduced the serum ethanol and acetaldehyde levels in ethanol-administered rats. This suggests that LN modulates the alcohol metabolism and ameliorates the leading cause of alcohol-induced hangovers and organ damage. 3) LN effectively increases the alcohol metabolism by upregulating the hepatic ADH and ALDH activities in ethanol-administered rats, relieving hangovers and protecting the liver from alcohol toxicity. 4) LN reduces the activities of AST, ALT, and γ-GT, indicators of liver damage in the serum of ethanol-administered rats. In addition, LN prevents liver damage by inhibiting MDA production, an indicator of lipid peroxidation, owing to its antioxidant capacity in the liver. 5) LN has potential as a bioactive antioxidant by improving the antioxidant properties by increasing the SOD and GPx activities in ethanol-administered rats. Therefore, LN is a potential hangover treatment. Its mechanism of action involves modulating the mediators of various etiological effects of excessive alcohol consumption and enhancing alcohol metabolism and antioxidant activity.

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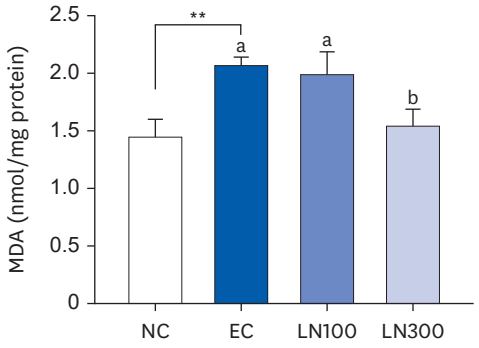

Fig. 5. Effects of LN on hepatic MDA contents in single-ethanol binge Sprague-Dawley rats. The rats were administered LN and ethanol, and the livers were excised as described in **Fig. 3**. MDA content in livers was measured using a thiobarbituric acid reactive substances assay kit. Each bar represents the mean ± SE of mean (n = 10).
LN, standardized aqueous extract of *Laurus nobilis* leaf; MDA, malondialdehyde; NC, normal control group; EC, ethanol control group; LN100, ethanol + 100 mg/kg body weight standardized aqueous extract of *Laurus nobilis* leaf-treated group; LN300, ethanol + 300 mg/kg body weight standardized aqueous extract of *Laurus nobilis* leaf-treated group.
$^{**}P < 0.01$ show significant difference from the NC group.
The different letters indicate significant differences between the EC, LN100, and LN300 groups at $P < 0.05$.

The present study examined the hepatoprotective effects of LN on alcohol-induced liver injury in SD rats. These findings suggest that the LN treatment increases the ADH and ALDH activities in SD rats subjected to acute alcohol toxicity. Hence, LN may play a role in accelerating the alcohol metabolism and reducing alcohol-induced oxidative stress. These results revealed the acute effects of LN treatment following a single episode of heavy drinking, which differs from the chronic effects of alcohol abuse. Previous studies have suggested that long-term chronic alcohol abuse reduces the liver ADH and ALDH activities [29]. Therefore, while these findings add to the body of knowledge regarding the potential hepatoprotective effects of LN, more research is needed to elucidate its impact on chronic alcohol consumption. The study design, in which LN was administered 1 h before alcohol intake, was based on the hypothesis that a LN pre-treatment could prepare the liver and enhance the enzymatic defense system before alcohol-induced oxidative stress. On the other hand, this timing should be carefully justified. Factors, such as the absorption and peak

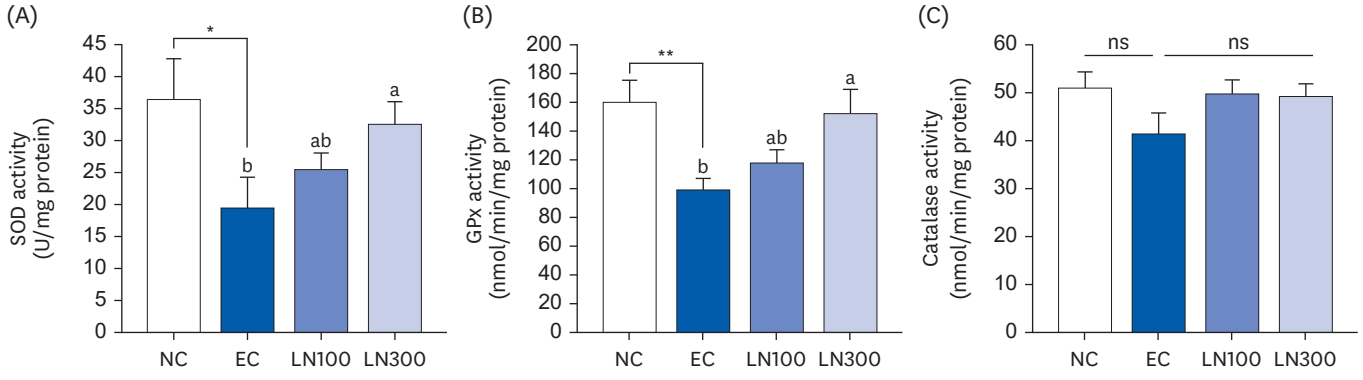

Fig. 6. Effects of hepatic SOD, GPx, and catalase activities in single-ethanol binge Sprague-Dawley rats. The rats were administered with LN and ethanol, and the livers were excised as described in **Fig. 3**. SOD (A), GPx (B), and catalase (C) activities in the liver were measured using the relevant assay kits. Each bar represents the mean ± SE of mean (n = 10).
LN, standardized aqueous extract of *Laurus nobilis* leaf; SOD, superoxide dismutase; GPx, glutathione peroxidase; NC, normal control group; EC, ethanol control group; LN100, ethanol + 100 mg/kg body weight standardized aqueous extract of *Laurus nobilis* leaf-treated group; LN300, ethanol + 300 mg/kg body weight standardized aqueous extract of *Laurus nobilis* leaf-treated group; ns, not significant.
$^{*}P < 0.05$, $^{**}P < 0.01$ indicates a significant difference from the NC group.
The different letters indicate significant differences between the EC, LN100, and LN300 groups at $P < 0.05$.

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concentration time of the active compounds of LN, the alcohol metabolism, and the potential impact of LN on alcohol absorption may influence the observed effects. Future research should consider different LN administration time points to understand the optimal timing for hepatoprotective effects of LN better.

The alcohol metabolism proceeds via oxidative and non-oxidative pathways, the main processes mediated by ADH and ALDH [10]. Alcohol is converted to acetaldehyde and then to acetate by ADH and ALDH, respectively. Acetaldehyde accumulation causes the hangover symptoms and liver damage induced by alcohol consumption [30]. In this study, LN increased the ADH and ALDH activities in a dose-dependent manner *in vitro* (**Table 1**). Furthermore, LN enhanced the hepatic ADH and ALDH activities, even in ethanol-administered rats (**Fig. 3A and B**), highlighting its ability to increase the alcohol metabolism, alleviate hangovers, and protect the liver from alcohol toxicity. In addition, LN decreased the blood ethanol and acetaldehyde concentrations in the LN300 group compared to the control group (**Fig. 2**). Therefore, LN accelerates the alcohol metabolism, suggesting that it ameliorates the leading cause of alcohol-induced hangovers and liver damage. On the other hand, these results are inconsistent with a previous study that found long-term chronic alcohol consumption reduces the hepatic ADH activity and exacerbates the adverse events [31]. Several studies have provided insights into the mechanisms through which antioxidant nutrients, such as those found in LN, might suppress the reduction of the ADH and ALDH enzyme activity often observed with chronic alcohol abuse. Research has shown that ROS generated by the ethanol metabolism can decrease the ADH and ALDH activity by promoting oxidative stress in hepatocytes. This oxidative stress can lead to protein damage, including those of ADH and ALDH enzymes, reducing their functionality [32]. Antioxidant nutrients, including those found in LN, have been shown to counteract this oxidative damage. They work by scavenging ROS, reducing oxidative stress, and preserving the structure and functionality of ADH and ALDH [33].

Moreover, some antioxidants have been reported to upregulate the gene expression of these enzymes, further enhancing their activities [34]. Isoquercetin, a compound found in LN, has such properties. The compound exerts antioxidant activity and modulates gene expression related to the alcohol metabolism, including those of ADH and ALDH [35]. The protective effects observed in this study are seen after acute heavy drinking. Nevertheless, the efficacy of LN or other antioxidants in chronic alcohol abuse, where the liver ADH and ALDH activities are reduced over a longer duration, requires further examination.

The effects of LN on AST, ALT, and γ-GT activities in the liver were examined further. AST and ALT are the most frequently measured indicators of liver disease. The overt signs of liver damage include leakage of cellular enzymes, such as AST and ALT, into the plasma, affecting hepatocyte transport function [36]. Elevated AST levels indicate damage and loss of function in the liver cell membranes. In this study, oral administration of LN reduced the AST, ALT, and γ-GT activities in the serum of ethanol-administered rats. This is consistent with a recent study that examined the effects of aged garlic extract on alcohol-induced liver damage [37]. γ-GT is a membrane enzyme involved in glutathione homeostasis. Glutathione depletion during alcohol consumption induces oxidative stress and increases the intracellular γ-GT levels. Thus, it is used widely for diagnosing alcohol-induced liver disease and as an indicator of oxidative stress [38]. Alcohol-induced oxidative stress generates ROS, which destroy the antioxidant defense system via antioxidant enzymes, such as SOD and GSH, in hepatocytes, depleting the cellular antioxidant capacity. Excessive ROS react with polyunsaturated fatty

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acids in the cell membranes to form lipid peroxides. The product of this reaction is MDA, an indicator of oxidative stress [39]. These results suggest that LN effectively protects against alcohol-induced liver damage by reducing the liver MDA content considerably, an indicator of lipid peroxidation (**Figs. 4** and **5**). This result is also consistent with the changes in antioxidant enzymes. Thus, LN blocks the alcohol-induced factors or acts on the indirect factors through an antioxidant mechanism that improves the antioxidant cellular network.

Another cause of alcohol-induced liver damage is oxidative stress, such as the production of the superoxide anion by cytochrome P450 2E1 (CYP2E1), P450 reductase, mitochondria, and NADPH oxidase [40]. Oxidative stress causes lipid peroxidation and is involved in liver fibrosis [41]. Excessive chronic alcohol consumption activates NADPH oxidase, a dominant mediator of various etiological effects, to produce oxidative stress [42]. Approximately 90% of the alcohol absorbed by the liver is metabolized by ADH to produce acetaldehyde. The remainder is metabolized by CYP2E1 or catalase. ROS produced by CYP2E1, such as hydrogen peroxide and superoxide ions, contribute to the pro-inflammatory profile of alcohol-related liver damage [43]. Alcohol consumption disrupts the balance between the pro- and antioxidant systems of the organism, inducing oxidative stress. Free radicals or ROS attack fats and proteins. Furthermore, they rapidly penetrate and damage the cell membranes, causing alcohol-induced oxidative tissue damage [44]. Thus, effective antioxidant and anti-inflammatory agents or foods could mitigate the detrimental health consequences of excessive alcohol consumption. In this study, LN enhanced the activity of antioxidant enzymes (SOD and GPx) in ethanol-administered rats (**Fig. 6A and B**). These results showed that LN improves hangover symptoms and liver damage by enhancing the liver alcohol metabolizing enzymes to lower the serum acetaldehyde levels and enhance the liver antioxidant activity to improve the biochemical indicators of liver damage. Therefore, LN shows broad promise for preventing and treating hangovers and disorders related to alcohol use.

This study had several limitations. The study was performed on a rat model, which may not translate directly to human physiology because of differences in metabolic rates, lifespan, and genetic variation. In addition, the dosage and administration routes of LN optimized for rat models need to be re-evaluated for human use. Furthermore, while LN upregulated the critical enzymes involved in alcohol metabolism, the precise molecular mechanisms through which LN operates are unclear. This study also focused on the potential use of LN in the context of excessive alcohol consumption and related liver damage without investigating potential interactions of LN with other medications or health conditions.

Therefore, future investigations should incorporate well-designed clinical trials to gauge the efficacy of LN in preventing and treating hangovers and liver damage in humans. This would involve identifying the optimal dosage, discerning potential side effects, and assessing the interactions with other drugs and health conditions. Moreover, it is necessary to gain a better understanding of the precise mechanisms underlying the beneficial effects of LN. The study also used HPLC to confirm that LN contained isoquercetin, a compound with antioxidative properties (**Fig. 1**). Treatment with an *Angelica keiskei Koidzumi* leaf extract containing a large amount of quercetin-based compounds increased the expression of antioxidant enzymes reduced by ethanol, suppressing ethanol-induced oxidative stress [45]. Quercetin derivatives, luteolin, protocatechuic acid, and various phenolic compounds and chalcones protect against oxidative stress, a major factor in alcohol-induced hepatocellular damage [46-49]. In another study, the polyphenols of *Ecklonia cava* inhibited the CYP2E1 enzyme activity, and ROS production was suppressed by this effect [50]. On the other hand, the present study did not

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include an isoquercetin treatment group. The rationale for this design decision was to evaluate the overall effects of LN, considering that the hepatoprotective properties might result from a synergistic interaction among the multiple bioactive compounds present in the extract rather than a single compound. Future studies might include an isoquercetin-only treatment group or other bioactive compounds to delineate the effects of the individual compounds.

In conclusion, LN has promise in alleviating hangovers and alcohol toxicity in the liver of ethanol-administered rats by upregulating the hepatic ADH and ALDH activities, the key enzymes necessary for the alcohol metabolism. The extract also reduces the hepatic AST, ALT, and γ-GT activities in the serum of these rats and protects against liver damage by inhibiting MDA production. LN has potential as a bioactive antioxidant because it enhances the activities of the antioxidant enzymes SOD and GPx in ethanol-administered rats. As a result, LN is a potential candidate for hangover treatments by regulating various mediators of the detrimental effects of excessive alcohol consumption and enhancing the alcohol metabolism and antioxidant activity. Nevertheless, further studies will be needed to understand the mechanisms involved and determine the optimal timing of LN administration relative to alcohol intake. Therefore, clinical studies are required to determine the clinical efficacy of LN as an effective natural product for preventing and treating hangovers and liver damage. Pending successful clinical studies, the applications of LN could extend to therapeutic protocols for managing alcohol-related disorders and other health areas associated with oxidative stress, significantly impacting public health.

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