Biochemical and Biophysical Research Communications 432 (2013) 599–605

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Protective effects of *Acanthopanax divaricatus vat. albeofructus* and its active compound on ischemia–reperfusion injury of rat liver

Eun-Jung Lim [a,1], Gyeong-Min Do [a,1], Jae-Ho Shin [b], Oran Kwon [a,*]

[a] Department of Nutritional Science and Food Management, Ewha Womans University, 52 Ewhayeodae-gil, Seodaemun-gu, Seoul 120-750, Republic of Korea
[b] Department of Biomedical Laboratory Science, Eulji University, Seongnam-si, Gyeonggi-do 461-713, Republic of Korea

ARTICLE INFO

Article history:
Received 7 February 2013
Available online 21 February 2013

Keywords:
Acanthopanax divaricatus vat. albeofructus
Acanthoside-D
Ischemia–reperfusion injury
Inflammation
Antioxidation
Hepatotoxicity

ABSTRACT

In the present study, the potential antioxidant and anti-inflammatory effects of *Acanthopanax divaricatus vat. albeofructus* (AE) and acanthoside-D (AD) isolated from AE against hepatic ischemia–reperfusion (I/R) injury were investigated in a rat model. Male Sprague–Dawley rats (200–220 g) were randomized into seven groups: normal controls; sham-operated controls; I/R injury model; I/R injury model with AE pretreatment at 150, 300, and 600 mg/kg body weight; and I/R injury model with AD pretreatment at 600 μg/kg body weight (equivalent to high dose of AE). The AE and AD pretreatments were administered orally for 2 weeks prior to I/R injury surgery. All rats recovered for 1 week with AE and AD treatment after surgery. Compared to the normal control groups, the I/R injury model group without supplemental treatment showed a significantly lower level of serum superoxide dismutase (SOD) and significantly higher levels of tumor necrosis factor-alpha (TNF-α, interleukin (IL)-6, serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), as well as lactate dehydrogenase (LDH) activity. The I/R-induced decrease in SOD and increases in TNF-α and IL-6 were resolved, at least partially, by AE and AD treatments, as evidenced by significantly higher antioxidant activities and significantly lower inflammatory cytokine levels in the treatment groups as compared to the I/R injury model group. The AE and AD treatment groups also showed significantly higher levels of serum IL-10 than I/R injury model group. Histological examination revealed that the AE and AD treated groups had less extensive liver necrosis than I/R injury model group. Concomitantly, AE lowered the I/R-induced increases in AST, ALT, ALP levels and LDH activity. In conclusion, AE and AD are capable of alleviating I/R-induced hepatic injury by inhibiting inflammatory cell infiltration, thereby mitigating the release of inflammatory cytokines and balancing the oxidant–antioxidant status mediated by p38 MAPK and JNK/SAPK signaling.

1. Introduction

Hepatic ischemia is caused by an interruption of the blood supply to an organ or tissue, during which the restricted supply of oxygen and delivery of substrates required for normal metabolism can result in functional and structural injuries. In the liver, ischemia/reperfusion (I/R)-induced injury can provoke activation of the Kupffer cells, which is accompanied by enhanced formation of reactive oxygen species (ROS). Accumulated ROS may initiate oxidative stress, causing cellular injury directly by attacking various molecules and indirectly by activating the p38 mitogen-activated protein kinase (MAPK) and c-Jun NH_2-terminal kinase/stress-activated protein kinase (JNK/SAPK) signaling pathways.

Oxidative stress pathways can stimulate the expression and secretion of several key mediators of inflammation, including tumor necrosis factor-alpha (TNF-α) and the interleukins (ILs) IL-1β and IL-6. The oxidative stress mechanism is composed of several oxygen free radicals, such as superoxide and hydroxyl radicals, as well as hydrogen peroxide (H_2O_2), all of which may cause structural alterations to the cellular and intracellular organelle membranes that contribute to the overall cytotoxic outcome. This mechanism, however, is balanced by the antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px), which exert their protective effects by removing superfluous oxygen-derived free radicals [1,2]. How-

Abbreviations: AD, acanthoside-D; AE, water extract of *Acanthopanax divaricatus vat. albeofructus*; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CAT, catalase; GSH-Px, glutathione peroxidase; JNK, c-Jun NH_2-terminal kinase; MAPK, mitogen-activated protein kinase; ROS, reactive oxygen species; SOD, superoxide dismutase; T-BIL, total bilirubin; TNF-α, tumor necrosis factor-alpha.

* Corresponding author. Fax: +82 2 3277 6860.
E-mail addresses: orank@ewha.ac.kr, orank@gmail.com (O. Kwon).
[1] These authors contributed equally to this work.

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.02.039

ever, not all cases of severe oxidative stress are detrimental to the system. This mechanism is a necessary function by which apoptosis and necrosis are triggered in undesirable or potentially harmful cells, such as cancerous or infected cells, and which, in turn, trigger beneficial inflammatory immune responses [3]. *Acanthopanax* species have a long history of use as medicinal plants. Empirical research over the past several decades has provided evidence of their therapeutic efficacies in treating several chronic disease conditions, such as diabetes mellitus [4], gastric ulcer [5], ischemic heart disease [6], cancer [7], and hepatitis [8]. One of the most commonly used and easily cultivated *Acanthopanax* species, especially in Korea, is *Acanthopanax. divaricatus vat. albeofructus* (AE). This particular medicinal plant has been shown in clinical studies to exert safe and effective anti-inflammatory, anti-rheumatoid arthritis, and anti-diabetic functions [9–11]. Furthermore the principal component of AE, acanthoside-D (AD), has been characterized as possessing anti-inflammatory, anti-fatigue and anti-carcinogenic effects [12,13].

In this study, we investigated the potential protective effects of both AE and its purified active compound AD in hepatic I/R injury. Specifically, we examined the abilities of each to regulate antioxidant enzyme activities and inflammatory cytokine expression through p38 MAPK and JNK/SAPK signaling, using a rat model of I/R injury induced by microvascular clamping.

2. Materials and methods

2.1. Plant materials

A. divaricatus vat. Albeofructus (AE) and acanthoside-D (AD) were provided by Susin Ogapi Co., Cheonan, Korea (Fig. 1). AE was identified by Prof. H. Kim, College of Oriental Medicine, Kyung Hee University. Briefly, the roots and stems (1:4) of AE were dried in hot air, cut, and extracted with water (1:7) under 105 °C for 6 h. The insoluble portion was removed by filtration. The filtrate was vacuum-concentrated, lyophilized, and powdered. AD, syringaresinol di-*O*-β-D-glucoside, was standardized by Korea Health Supplement Institute, Seongnam, Korea (Cat No. ASB-00005065). AD represented 1 mg/g of AE, and its purity was >95%.

2.2. I/R liver injury rat model

Four-week-old male Sprague–Dawley rats (Orient Bio Inc., Gyeonggi-Do, Korea) were acclimatized to the laboratory setting (22.0 ± 2.0 °C, 12 h light/dark cycles, 50 ± 15% humidity, *ad libitum* standard pellet diet [Purina Inc., Gyeonggi-Do, Korea] and water) for 1 week. For experimentation, the rats (200–220 g) were randomized into seven groups, with matching for body weights (bw): CON group, normal controls; SHAM group, sham-operated; I/R group, I/R injury model; AE pretreatment + I/R injury groups, including AE150 (150 mg/kg bw), AE300 (300 mg/kg bw), and AE600 (600 mg/kg bw); and AD pretreatment + I/R injury group (600 μg/kg bw, equivalent to the high dose of AE). Prior to the I/R injury surgery, the rats of the treatment groups received AE or AD (oral, once daily) for 2 weeks. The I/R injury surgery consisted of applying a microvascular clamp to the hepatic portal veins feeding the left and median lobes for 60 min, while the rat was warmed by a 37 °C heating pad [14]. After 1 week of recovery, with oral administration of AD and AE, the animals were fasted overnight, anesthetized, and exsanguinated by cardiac puncture. The blood sample was centrifuged (3000×*g*, 4 °C, 30 min) to separate serum and plasma. Centrifuged blood was stored at −80 °C. The liver, kidney, spleen, and pancreas were removed, weighed, snap-frozen in liquid nitrogen, and stored at −80 °C. This study was preapproved by the Institutional Animal Care and Use Committee of Ewha Womans University (No. 2011-02-057).

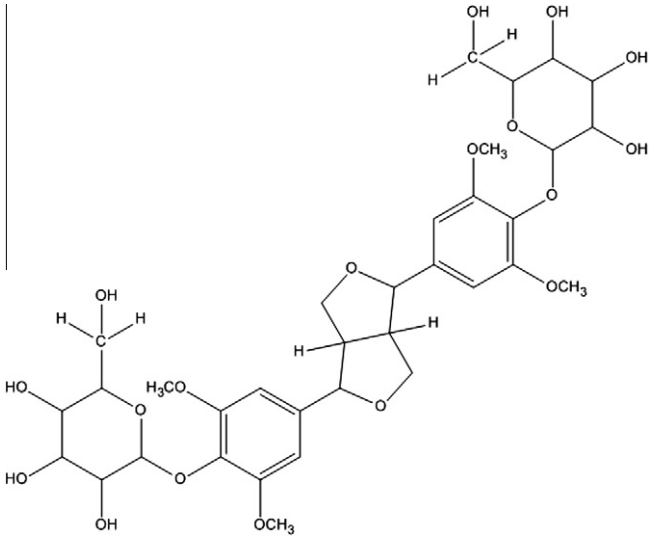

Fig. 1. Structure of acanthoside-D (AD).

2.3. Biochemical assays

Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were measured by Reitman–Frankel colorimetric assay kits (Asan Pharmaceutical, Seoul, Korea). Serum total bilirubin (T-BIL) was quantified by a Jendrassik–Grof enzymatic assay kit (BioAssay Systems, Hayward, CA, USA). Serum alkaline phosphatase (ALP) activity was measured by a colorimetric assay kit (BioVision Inc., Milpitas, CA, USA). Serum lactate dehydrogenase (LDH) activity was determined by enzyme-linked immunosorbent assay (ELISA) detection of pyruvic acid conversion to lactic acid.

2.4. Serum and hepatic antioxidant activities

Commercial kits were used to quantify serum total antioxidant status (TAS; Randox Laboratories Ltd., Antrim, UK), serum superoxide dismutase (SOD) activity (Cayman Chemical Co., Ann Arbor, USA), and serum catalase (CAT) activity (Cayman Chemical Co.). Hepatic GSH-Px activity was determined by ELISA detection of oxidized glutathione produced upon reduction of H_2O_2 [15].

2.5. Cytokine production

Commercial ELISA kits were used to measure serum IL-6 (R&D Systems, Minneapolis, MN, USA), and hepatic TNF-α, IL-6, and IL-10 (DuoSet ELISA development kit; R&D Systems).

2.6. Western blot analysis

Liver proteins were extracted with lysis buffer (Intron Biotech, Seoul, Korea) and quantified by the Bradford method. Total protein (40 μg) was resolved by 10% SDS–PAGE and transferred to polyvinylidene difluoride membranes (Bio-Rad, Hercules, CA, USA). After blocking (room temperature, 120 min) with 3% bovine serum albumin (BSA) and 5% skim milk in Tween/Tris-buffered saline (TBS) and triplicate washes in TBS, the membranes were probed (4 °C, overnight) with rabbit anti-p-p38 MAPK (1:1000; Cell Signaling Technology, Danvers, MA, USA), mouse anti-p-JNK/SAPK (1:2000; Cell Signaling Technology), or mouse anti-β-actin (1:1000; Santa Cruz Biotechnology, Santa Cruz, CA, USA) in 5% BSA + TBS. The immunoreactive antigens were detected (room temperature, 120 min) by horseradish peroxidase-labeled anti-rabbit or

anti-mouse/rabbit IgG (1:2000; Santa Cruz Biotechnology) in 5% BSA + TBS. The immunoreactive bands were quantified by the ChemiDoc XRS + system with Image Lab software (Bio-Rad).

2.7. Histochemical analysis

Formalin-fixed tissue samples were dehydrated in a gradient series (70–100%) of ethyl alcohol, dealcoholized in xylene, embedded in paraffin, and sectioned (5-μm thickness). For histological analysis, the slide-mounted embedded tissues were deparaffinized in xylene, rehydrated in a reverse-gradient series of ethyl alcohol, and stained with hematoxylin and eosin (H&E). Dehydrated and dealcoholized formalin-fixed tissue sections were mounted on slides with Canada balsam and observed by Olympus 51 microscopy (Olympus Optical Co., Tokyo, Japan).

2.8. Statistical analysis

Results are presented as mean ± standard error. Statistical analyses were performed by the Statistical Analysis Systems software, version 9.2 (SAS Institute, Cary, NY, USA). Intergroup differences were analyzed by one-way analysis of variance (ANOVA) with post hoc Duncan's multiple range tests. The threshold for significance was $p < 0.05$.

3. Results

3.1. Effects of AE on serum ALT, AST, T-BIL, ALP, and LDH levels in hepatic I/R injury rats

Serum ALT, T-BIL, ALP, and LDH levels were significantly increased in the untreated I/R group, as compared to the CON group (Table 1). All AE and AD pretreatments significantly decreased the I/R injury-induced increased levels of serum T-BIL and ALP. In addition, the AE and AD pretreatments led to a significant decrease on serum AST compared to I/R. The I/R injury-induced increases in serum ALT and LDH were also significantly reduced by AE300 and AE treatments except for AE600, respectively compared to I/R.

3.2. Effects of AE on serum and hepatic antioxidant activities in hepatic I/R injury rats

Upon I/R injury, the serum TAS was significantly decreased (untreated I/R vs. SHAM; Fig. 2A). However, the low- and mid-dose AE pretreatments significantly increased the I/R injury-induced decrease in serum TAS (AE150 and AE300 vs. untreated I/R). I/R injury also caused a significant decrease in serum SOD activity (untreated I/R vs. CON and SHAM), which was also significantly increased by the low- and mid-dose AE pretreatments (AE150 and AE300 vs. untreated I/R; Fig. 2B). Compared to untreated I/R, the AE pretreatments caused significant increases in both serum CAT activity (vs. AE300 and AE600 pretreatments; Fig. 2C) and hepatic GSH-Px activity (all AE pretreatments; Fig. 2D). The AD pretreatments had no affects on either the serum CAT or hepatic GSH-Px, nor on the I/R injury-induced changes to SOD.

3.3. Effects of AE on inflammatory signaling in hepatic I/R injury rats

All AE and AD pretreatments led to increased levels of phosphorylated p38 MAPK expression in liver (vs. untreated I/R; Fig. 3A); however, only the low- and mid-doses of AE led to increased p-p38/p38 ratio (AE150 and AE300 vs. untreated I/R), with the mid-dose leading to the highest increase. The I/R injury induced an increase in expression of phosphorylated JNK/SAPK in liver (vs. CON), which was decreased by the AE and AD pretreatments (vs. untreated I/R; Fig. 3B). Similarly, the I/R injury caused an increase in the p-JNK/JNK ratio (vs. CON), which the mid-dose AE treatment decreased (AE300 vs. untreated I/R) and the low- and high-dose AE treatments decreased less extensively (AE150 and AE600 vs. untreated I/R).

Hepatic I/R injury induced significant increases in hepatic TNF-α and IL-6 (untreated I/R vs. CON and SHAM). All AE and AD pretreatments significantly reduced the I/R injury-induced increases in hepatic TNF-α and IL-6 (vs. untreated I/R; Fig. 3C and D). Similarly, the I/R injury-induced decrease in hepatic IL-10 level (untreated I/R vs. CON and SHAM) was significantly increased by all AE and AD pretreatments (vs. untreated I/R; Fig. 3E). Moreover, the mid- and high-doses of AE increased the levels of IL-10 to normal (AE300 and AE600 vs. CON). The AE and AD pretreatments all produced significant suppressive effects on the I/R injury-induced increase in serum levels of IL-6 (vs. untreated I/R; Fig. 3F).

3.4. Effects of AE on hepatic morphology in hepatic I/R injury rats

Normal lobular architecture and cell structure were observed in livers from the CON (Fig. 4A) and SHAM (Fig. 4B) groups. In contrast, the untreated I/R-injured livers (Fig. 4C) showed severe hepatic distortion, hemorrhage, and multiple and extensive necrosis, as well as inflammatory cell infiltration, all of which were randomly distributed throughout the parenchyma. The histological features of livers from rats pretreated with AE150 (Fig. 4D) and AE300 (Fig. 4E) were similar to the untreated I/R-injured livers. However, hepatic histology of the I/R-injured livers from rats pretreated with AE600 (Fig. 4F) and AD (Fig. 4G) revealed fewer and smaller areas of necrosis and structural derangement around the central vein, as compared to the untreated I/R livers.

4. Discussion

The antioxidant and anti-inflammatory effects of various *Acanthopanax* species have been previously described in the literature. For example, *A. senticosus* aqueous extracts were shown to reduce oxidative stress via induction of the NF-E2-related factor (Nrf)-2 and other related antioxidant enzymes [6]. *A. radix* extract

Table 1
Protective effects of AE and AD pretreatments on liver function impaired by hepatic I/R in rats.

Group Biomarker	CON	SHAM	I/R	AE150	AE300	AE600	AD
ALT IU/L	13.62 ± 0.73^{c}	13.41 ± 1.24^{c}	19.41 ± 1.09^{a}	$16.92 \pm 0.52^{a,b}$	$15.80 \pm 1.01^{b,c}$	$17.49 \pm 1.13^{a,b}$	$16.30 \pm 1.24^{a,b,c}$
AST IU/L	$30.37 \pm 1.26^{a,b}$	$26.52 \pm 1.14^{b,c}$	34.08 ± 1.03^{a}	29.04 ± 0.95^{b}	$26.45 \pm 1.53^{b,c}$	$26.81 \pm 2.60^{b,c}$	23.14 ± 2.34^{c}
T-BIL mg/L	1.33 ± 0.31^{b}	0.74 ± 0.28^{b}	3.10 ± 0.34^{a}	1.22 ± 0.21^{b}	1.24 ± 0.31^{b}	0.87 ± 0.29^{b}	0.81 ± 0.14^{b}
ALP U/mL	0.19 ± 0.01^{b}	0.17 ± 0.01^{b}	0.31 ± 0.02^{a}	0.18 ± 0.01^{b}	0.16 ± 0.01^{b}	0.17 ± 0.01^{b}	0.17 ± 0.01^{b}
LDH U/L	$161.89 \pm 4.97^{b,c}$	163.05 ± 11.09^{bc}	215.89 ± 14.57^{a}	$155.88 \pm 6.50^{b,c}$	146.89 ± 7.36^{c}	184.54 ± 17.77^{ab}	$176.62 \pm 9.16^{b,c}$

Values with Arabic superscripts are significantly different.

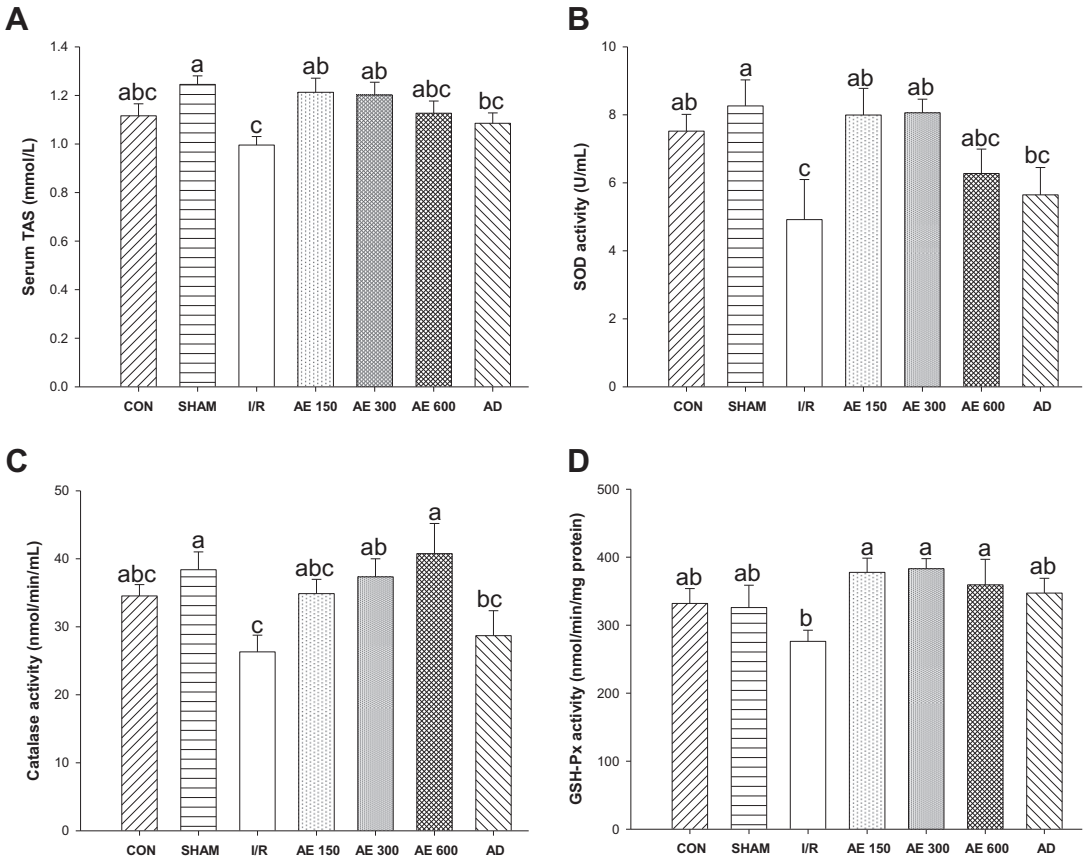

Fig. 2. Effects of aqueous extract of *Acanthopanax divaricatus vat. albeofructus* and acanthoside-D pretreatment on serum total antioxidant status (A), superoxide dismutase (B), catalase (C), and hepatic glutathione peroxidase (D) activities in hepatic I/R-injured rats. Mean values ($n = 8$) are graphed. Values with different Arabic superscripts are significantly different.

was shown to have reactive oxygen radical-scavenging effects under endotoxemic conditions [16]. Finally, *A. senticosus* extract was shown to have anti-inflammatory effects under pathogenic conditions associated with *Porphyromonas gingivalis* infection, whereby the extract suppresses activator protein (AP)-1 and nuclear factor (NF)-κB activity, modulates signal transducer and activator of transcription (STAT) 1 and STAT3, and stimulates heme oxygenase (HO)-1 expression through the activation of MAPK and Nrf-2 signaling pathways [17]. In the present study, the potential beneficial effects of *A. divaricatus vat. albeofructus* and its active component AD were shown in regards to liver function, specifically involving the inflammatory and antioxidant response via p38 MAPK and JNK/SAPK signaling.

I/R injury can be generally characterized as an inflammatory response. The process begins with transcriptional activation of proinflammatory factors that trigger the expression, activity, and secretion of various downstream immunomodulatory factors, such as proinflammatory cytokines, endothelial adhesion molecules, and ROS. Hepatic I/R injury in rats resulted in increased serum levels of ALT, T-BIL, LDH, ALP, and inflammatory cytokines, but decreased antioxidant activities. AE pretreatment protected against I/R injury-induced liver function impairment by mitigating the effects on ALT, T-BIL, LDH and ALP. Although serum ALT is the most frequently used biomarker of hepatotoxicity, it lacks consistent correlation with the histomorphologic data. Thus, additional biomarkers with greater and more consistent specificity for liver function are needed to augment the serum ALT activity marker [18]. Although AST, T-BIL, and ALP are promising candidates for this application, LDH is especially attractive because it has been widely used to evaluate the presence of damage and toxicity of tissue and cells [19].

Epidemiologic studies have identified relationships between oxidative stress and the occurrence of cardiovascular diseases, such as ischemic heart disease [20]. The antioxidant system, which balances the production and destruction of ROS [6], such as superoxide radical (O_2^-) H_2O_2 and hydroxyl radical (·OH), is normally subject to detoxification by other nonenzymatic and enzymatic components, such as SOD, of the antioxidant system to eliminate the ROS and suppress their effects [21]. SOD which converts O_2^- into H_2O_2 is itself detoxified by CAT and GSH-Px [22]. In the present study, the antioxidant properties of AE and AD in I/R-injured liver were found to involve SOD and GSH-Px activities. However, GSH-Px is also known to eliminate other organic peroxides, including lipid peroxides. Therefore, AE and AD may be effective for improving the overall antioxidant capacities of an animal, especially AE, which demonstrated potent enhancement of antioxidant metabolism.

Under conditions of severe oxidative stress, cells undergo apoptotic and necrotic death. The related inflammatory response begins with transcriptional activation of proinflammatory factors, and stimulates downstream mediators, such as TNF-α and IL-6, that facilitate recruitment of leukocytes to the postischemic organ or tissue [23,24]. TNF-α is characterized as the central proinflammatory mediator promoting hepatic damage in response to I/R injury [25]. IL-6 upregulation has also been demonstrated in the I/R liver and is expected to aggravate injury [26]. In the present study, AE

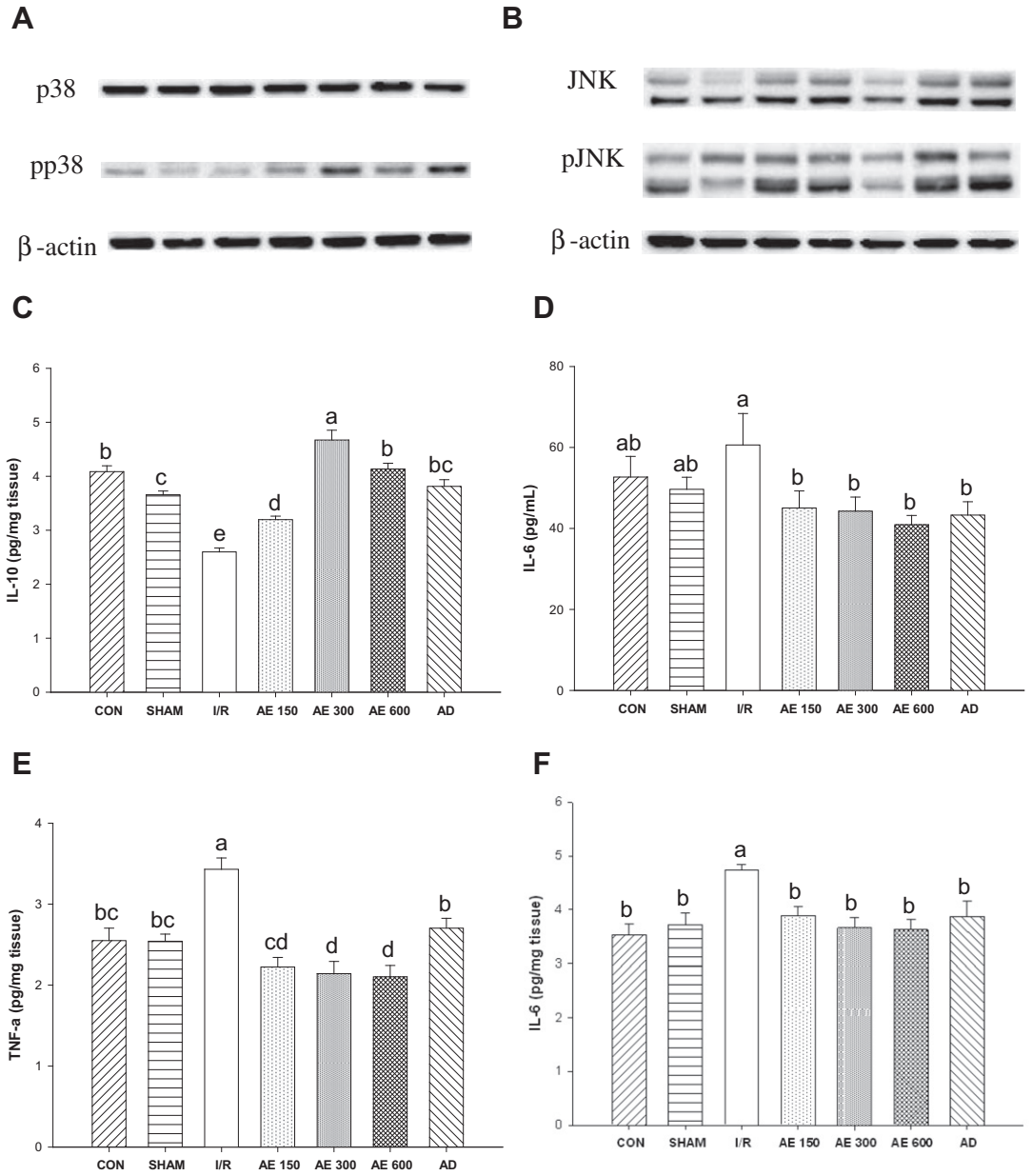

Fig. 3. Effects of aqueous extract of *Acanthopanax divaricatus vat. albeofructus* and acanthoside-D pretreatment on hepatic p38 MAPK (A), JNK/SAPK (B), TNF-α (C), IL-6 (D), and IL-10 (E) and serum IL-6 (F) levels in hepatic I/R rats. Values with different Arabic superscripts are significantly different.

and AD pretreatments significantly reduced the I/R injury-stimulated hepatic TNF-α and IL-6. Furthermore, high-dose AE reduced the extent of I/R injury-induced necrosis and structural derangement around the central vein.

I/R injury-induced oxidative stress damage have been attributed to the activation of MAPK pathways, including JNK/SAPK and p38 MAPK-mediated pathways. Moreover, activation of JNK/SAPK and p38 MAPK is essential to cytokine- and stress-induced apoptosis [27]. Upon TNF-α binding to its cognate receptor, TNF-R, JNK/SAPK and NF-κB are activated [28]. Interaction of p38 MAPK with the JNK-c-Jun pathway in hepatocytes facilitates inhibition of cellular proliferation [29], but overexpression or hyperactivation of c-Jun can lead to uncontrolled proliferation or apoptosis. p38 MAPK also plays dual roles in the antioxidant pathways and anti-inflammatory responses [30], helping to mitigate I/R injury-induced damage and acting as a catalyst for liver damage [31]. The protective and detrimental effects may be mediated by the phosphorylation status of p38 MAPK. In a porcine I/R model, p-p38 MAPK was stimulated by atrial natriuretic peptide treatment, which was also associated with less-extensive tissue damage [32]. In the present study, AE exerted protective effects against liver damage by blocking the I/R injury-induced upregulation of p-JNK/SAPK and downregulation of p38 MAPK.

In conclusion, AE and AD reduced I/R-induced hepatic injury by reducing necrotic areas and/or inflammatory cell infiltration and modulating inflammatory cytokine expression and/or release. Collectively, AE and AD were shown to mitigate oxidative stress and inflammation related to p38 MAPK and JNK/SAPK signaling. AE was found to balance the antioxidant status, through the enzymatic antioxidant system, better than AD.

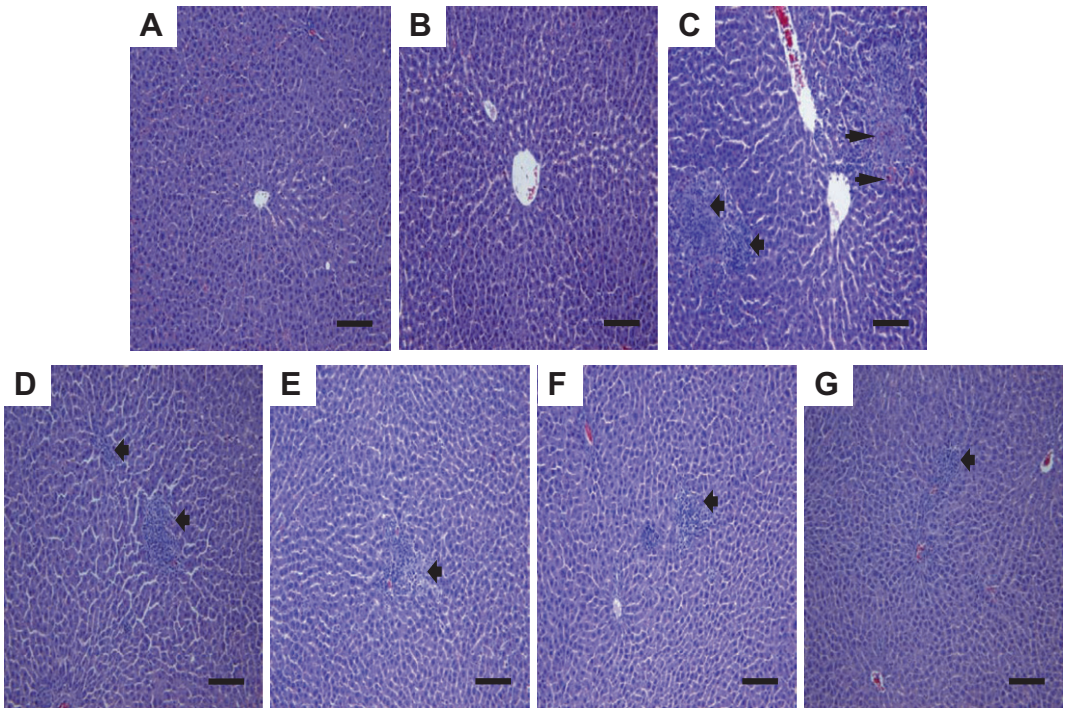

Fig. 4. Effects of *Acanthopanax divaricatus vat. albeofructus* and acanthoside-D administration on hepatic I/R injury-induced histopathologic changes. Representative H&E stained liver samples of rats from (A) CON, (B) SHAM, (C) I/R, (D) AE150, (E) AE300, (F) AE600, and (G) AD groups, Bar, 100 mm. Sharp arrow, hemorrhage; arrows, multiple and extensive necrosis.

Acknowledgments

We thank the Susin Ogapi Co. (Cheonan, Chungcheong-Do, Korea) for its gift of AE and AD. This study was carried out with the support of 'Forest Science & Technology Projects (Project No. S111111L010120)' provided by Korea Forest Service and of Brain Korea 21 Projects by the Korean Ministry of Education, Science and Technology (Project No. 2006-0519-4-7).

References

[1] H. Ozturk, A. Gezici, The effect of celecoxib, a selective COX-2 inhibitor, on liver, Hepatol. Res. 34 (2006) 76–83.
[2] S. Thamilselvan, K.J. Byer, R.L. Hackett, S.R. Khan, Free radical scavengers, catalase and superoxide dismutase provide protection from oxalate-associated injury to LLC-PK1 and MDCK cells, J. Urol. 164 (2000) 224–229.
[3] G.P. Sims, D.C. Rowe, S.T. Rietdijk, R. Herbst, A.J. Coyle, HMGB1 and RAGE in inflammation and cancer, Annu. Rev. Immunol. 28 (2009) 367–388.
[4] D.Y. Sui, Z.Z. Lu, S.H. Li, Y. Cai, Hypoglycemic effect of saponin isolated from leaves of Acanthopanax senticosus (Rupr. et Maxin.) Harms, Zhongguo Zhong Yao Za Zhi 19 (1994) 683–685.
[5] T. Fujikawa, A. Yamaguchi, I. Morita, H. Takeda, S. Nishibe, Protective effects of *Acanthopanax senticosus* Harms from Hokkaido and its components on gastric ulcer in restrained cold water stressed rats, Biol. Pharm. Bull. 19 (1996) 1227–1230.
[6] X. Wang, C.X. Hai, X. Liang, S.X. Yu, W. Zhang, Y.L. Li, The protective effects of Acanthopanax *senticosus* Harms aqueous extracts against oxidative stress: role of Nrf2 and antioxidant enzymes, J. Ethnopharmacol. 127 (2010) 424–432.
[7] H. Hibasami, T. Fujikawa, H. Takeda, S. Nishibe, T. Satoh, T. Fujisawa, K. Nakashima, Induction of apoptosis by *Acanthopanax senticosus* HARMS and its component, sesamin in human stomach cancer KATO III cells, Oncol. Rep. 7 (2000) 1213–1216.
[8] J.X. Nan, E.J. Park, J.B. Nam, Y.Z. Zhao, X.F. Cai, Y.H. Kim, D.H. Sohn, J.J. Lee, Effect of *Acanthopanax koreanum* Nakai (Araliaceae) on D-galactosamine and lipopolysaccharide-induced fulminant hepatitis, J. Ethnopharmacol. 92 (2004) 71–77.
[9] H.S. Kang, Y.H. Kim, C.S. Lee, J.J. Lee, I. Choi, K.H. Pyun, Suppression of interleukin-1 and tumor necrosis factor-α production by acanthoic acid, (−)-pimara-9(11), 15-dien-19-oic acid, and its antifibrotic effects *in vivo*, Cell. Immunol. 170 (1996) 212–221.
[10] E.A. Bae, C.S. Yook, O.J. Oh, S.Y. Chang, T. Nohara, D.H. Kim, Metabolism of chiisanoside from *Acanthopanax divaricatus var. albeofructus* by human intestinal bacteria and its relation to some biological activities, Biol. Pharm. Bull. 24 (2001) 582–585.
[11] S. Lee, B.K. Kim, S.H. Cho, K.H. Shin, Phytochemical constituents from the fruits of *Acanthopanax sessiliflorus*, Arch. Pharmacal. Res. 25 (2002) 280–284.
[12] T. Yamazaki, S. Shimosaka, H. Sasaki, T. Matsumura, T. Tukiyama, T. Tokiwa, (+)-Syringaresinol-di-*O*-beta-D-glucoside, a phenolic compound from *Acanthopanax senticosus* Harms, suppresses proinflammatory mediators in SW982 human synovial sarcoma cells by inhibiting activating protein-1 and/or nuclear factor-kappaB activities, Toxicol. in Vitro 21 (2007) 1530–1537.
[13] S. Nishibe, H. Kinoshita, H. Takeda, G. Okano, Phenolic compounds from stem bark of *Acanthopanax senticosus* and their pharmacological effect in chronic swimming stressed rats, Chem. Pharm. Bull. (Tokyo) 38 (1990) 1763–1765.
[14] C.A. Camargo, J.F. Madden, W. Gao, R.S. Selvan, P.A. Clavien, Interleukin-6 protects liver against warm ischemia/reperfusion injury and promotes hepatocyte proliferation in the rodent, Hepatology 26 (1997) 1513–1520.
[15] J. Mohandas, J.J. Marshall, G.G. Duggin, J.S. Horvath, D.J. Tiller, Differential distribution of glutathione and glutathione-related enzymes in rabbit kidney. Possible implications in analgesic nephropathy, Biochem. Pharmacol. 33 (1984) 1801–1807.
[16] T. Yokozawa, D.Y. Rhyu, C.P. Chen, Protective effects of *Acanthopanax Radix* extract against endotoxemia induced by lipopolysaccharide, Phytother. Res. 17 (2003) 353–357.
[17] H. Soo Kim, S. Young Park, E. Kyoung Kim, E. Yeon Ryu, Y. Hun Kim, G. Park, S. Joon Lee, *Acanthopanax senticosus* has a heme oxygenase-1 signaling-dependent effect on *Porphyromonas gingivalis* lipopolysaccharide-stimulated macrophages, J. Ethnopharmacol. 142 (2012) 819–828.
[18] J. Ozer, M. Ratner, M. Shaw, W. Bailey, S. Schomaker, The current state of serum biomarkers of hepatotoxicity, Toxicology 245 (2008) 194–205.
[19] R. Stentz, R.J. Bongaerts, A.P. Gunning, M. Gasson, C. Shearman, Controlled release of protein from viable *Lactococcus lactis* cells, Appl. Environ. Microbiol. 76 (2010) 3026–3031.
[20] K.F. Gey, P. Puska, P. Jordan, U.K. Moser, Inverse correlation between plasma vitamin E and mortality from ischemic heart disease in cross-cultural epidemiology, Am. J. Clin. Nutr. 53 (1991) 326S–334S.
[21] R. Mittler, Oxidative stress, antioxidants and stress tolerance, Trends Plant Sci. 7 (2002) 405–410.
[22] Y.L. Wu, Y.Z. Jiang, X.J. Jin, L.H. Lian, J.Y. Piao, Y. Wan, H.R. Jin, J. Joon Lee, J.X. Nan, Acanthoic acid, a diterpene in *Acanthopanax koreanum*, protects acetaminophen-induced hepatic toxicity in mice, Phytomedicine 17 (2010) 475–479.
[23] T.L. Husted, A.B. Lentsch, The role of cytokines in pharmacological modulation of hepatic ischemia/reperfusion injury, Curr. Pharm. Des. 12 (2006) 2867–2873.
[24] H. Jaeschke, A. Farhood, C.W. Smith, Neutrophils contribute to ischemia/reperfusion injury in rat liver *in vivo*, FASEB J. 4 (1990) 3355–3359.

[25] M. Abu-Amara, S.Y. Yang, N. Tapuria, B. Fuller, B. Davidson, A. Seifalian, Liver ischemia/reperfusion injury: processes in inflammatory networks-a review, Liver Transpl. 16 (2010) 1016–1032.
[26] S. Tsuchihashi, F. Kaldas, N. Chida, Y. Sudo, K. Tamura, Y. Zhai, B. Qiao, R.W. Busuttil, J.W. Kupiec-Weglinski, FK330, a novel inducible nitric oxide synthase inhibitor, prevents ischemia and reperfusion injury in rat liver transplantation, Am. J. Transplant. 6 (2006) 2013–2022.
[27] S.B. Mkaddem, M. Bens, A. Vandewalle, Differential activation of Toll-like receptor-mediated apoptosis induced by hypoxia, Oncotarget 1 (2010) 741–750.
[28] R.F. Schwabe, D.A. Brenner, Mechanisms of liver injury. I. TNF-α-induced liver injury: role of IKK, JNK, and ROS pathways, Am. J. Physiol. Gastrointest. Liver Physiol. 290 (2006) G583–G589.
[29] L. Min, B. He, L. Hui, Mitogen-activated protein kinases in hepatocellular carcinoma development, Semin. Cancer Biol. 21 (2011) 10–20.
[30] A. Paine, B. Eiz-Vesper, R. Blasczyk, S. Immenschuh, Signaling to heme oxygenase-1 and its anti-inflammatory therapeutic potential, Biochem. Pharmacol. 80 (2010) 1895–1903.
[31] L.A. King, A.H. Toledo, F.A. Rivera-Chavez, L.H. Toledo-Pereyra, Role of p38 and JNK in liver ischemia and reperfusion, J. Hepatobiliary Pancreat. Surg. 16 (2009) 763–770.
[32] K. Kobayashi, K. Oshima, M. Muraoka, T. Akao, O. Totsuka, H. Shimizu, H. Sato, K. Tanaka, K. Konno, K. Matsumoto, I. Takeyoshi, Effect of atrial natriuretic peptide on ischemia-reperfusion injury in a porcine total hepatic vascular exclusion model, World J. Gastroenterol. 13 (2007) 3487–3492.