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Phytocompounds from *Vitis kelungensis* stem prevent carbon tetrachloride-induced acute liver injury in mice

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ABSTRACT

Vitis thunbergii, *Vitis flexuosa*, and *Vitis kelungensis* are three common wild grapes in Taiwan and have been used as a traditional medicine for inflammatory disorders. However, the potential bioactivities of these wild grapes have not been studied to date. In this study, anti-inflammatory activities and hepatoprotective properties of these three wild grapes were assessed by *in vitro* and *in vivo* assays, respectively. Results revealed that the methanolic extract of *V. kelungensis* stem (VKS) strongly suppressed NO production in lipopolysaccharide (LPS)-stimulated murine macrophages. And among all fractions derived from VKS, the EtOAc fraction exhibited the best inhibitory activity. In addition, VKS and its major resveratrol derivatives, (−)-ε-viniferin and 2-(4-hydroxyphenyl)-2,3-dihydrobenzo[*b*]furan-3,4,6-triol, can prevent CCl_4-induced liver injury and aminotransferase activities in mice, which were comparable to that of silymarin or resveratrol, the natural remedies for liver diseases.

1. Introduction

A number of researchers have pointed out that carbon tetrachloride (CCl_4) causes acute and chronic hepatic damage due to free radical generations (Hung, Fu, Shih, Lee, & Yen, 2006; Liao et al., 2007; Song & Yen, 2003; Tung et al., 2009). CCl_4 is metabolized by cytochrome P450 2E1 (CYP2E1) to become a trichloromethyl radical ($^{\cdot}CCl_3$) and a proxy trichloromethyl radical ($^{\cdot}OOCCl_3$), which are assumed to initiate free radical-mediated lipid peroxidation, leading to the accumulation of lipid-derived oxidation products that cause liver injury (Liao et al., 2007; Poli, Albano, & Dianzani, 1987; Recknagel, Glende, Dolak, & Waller, 1989; Shyur et al., 2008). A number of studies have shown that various herbals and natural remedies could protect liver against CCl_4-induced oxidative stress by altering the levels of increased lipid peroxidation and enhancing the decreased activities of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT) and glutathione-*S*-transferase (GST), as well as the decreased level of hepatic-reduced glutathione (GSH) (Hsiao et al., 2003; Hung et al., 2006; Lu & Liu, 1992; Song & Yen, 2003; Tung et al., 2009).

In Taiwan, *Vitis thunbergii*, *Vitis flexuosa*, and *Vitis kelungensis* are three common wild grapes, and they are traditionally used as medicinal plants. Aqueous extracts of wild grape stems are used in Taiwan for the treatment of diarrhoea, fracture, injury, jaundice, and hepatitis (Huang, Tsai, Shen, & Chen, 2005). It is well-known that plants in the genus *Vitis* commonly contain oligomers of resveratrol (Huang et al., 2005). Recently, many studies have shown that resveratrol possesses various pharmacological effects, such as cardiovascular protection (Berrougui, Grenier, Loued, Drouin, & Khalil, 2009) and chemoprevention of cancer (Jang et al., 1997). In addition, resveratrol has also been found to prevent or cure hepatotoxin (e.g., alcohol or CCl_4)-induced liver diseases, such as acute hepatitis and hepatic fibrosis (Ajmo, Liang, Rogers, Pennock, & You, 2008; Chávez et al., 2008; Rivera, Shibayama, Tsutsumi, Perez-Alvarez, & Muriel, 2008; Vitaglione et al., 2009). Thus, the objective of this study was to evaluate the potential protective effects of crude extract and its major resveratrol derivatives from the stem extract of *V. kelungensis* in CCl_4-induced acute liver injury in mice. Furthermore, the contents of major resveratrol derivatives from *V. thunbergii*, *V. flexuosa*, and *V. kelungensis* stems, in different seasons, were also addressed in this study.

2. Materials and methods

2.1. Chemicals

Lipopolysaccharide (LPS), Griess reagent, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), silymarin, and resveratrol were purchased from Sigma Chemical Co. (St. Louis, MO). Dimethyl sulfoxide (DMSO) was purchased by Acros (Belgium). The other chemicals and solvents used in this experiment were of the highest quality available.

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2.2. Plant material

Fresh stems of *V. thunbergii, V. flexuosa*, and *V. kelungensis*, in different seasons, namely summer (June 2007), autumn (September 2007), winter (December 2007), and spring (March 2008), were sampled from the agriculture experimental station of National Chung Hsing University in Taichung County. The species were identified by Prof. Tzer-Kuan Hu (Department of Agronomy, National Chung Hsing University).

2.3. Extraction and isolation

The samples were cleaned with tap water and air-dried at ambient temperature (25 °C). They were then extracted with methanol by soaking each one for 1 week at ambient temperature twice. The extracts were decanted, filtered under vacuum, concentrated in a rotary evaporator, and then lyophilised. Of these, the resulting crude extract of *V. kelungensis* stem (72.0 g; 1.6%) was further fractionated, successively, with ethyl acetate (EtOAc), *n*-butanol (BuOH) and water, to yield soluble fractions of EtOAc (44.1 g; 61.3%), BuOH (10.0 g; 13.9%), and H_2O (16.8 g; 23.3%). The EtOAc fraction was separated by a silica gel 60 column (Merck, Darmstadt, Germany) eluted with a stepwise gradient of EtOAc/*n*-hexane 2/98 (v/v) to 100/0 (v/v), to give various subfractions (Fig. 1A). Ten major compounds were isolated and purified from the high yields of **A** (compounds **1**–**3**), **B** (compounds **4** and **5**), **C** (compounds **6** and **7**), **D** (compound **8**), **E** (compounds **8** and **9**), **F** (compound **9**), and **G** (compound **10**) subfractions by semipreparative HPLC, with a model PU-2080 pump (Jasco, Japan) equipped with a RI-2031 refractive index detector (Jasco, Japan) and a 250 × 10.0 mm i.d., 5 μm Luna silica column (Phenomenex, Torrance, CA). Separation was achieved using an isocratic system of EtOAc/*n*-hexane at a flow rate of 4 ml/min. NMR spectra were recorded on a Bruker Avance 500 NMR spectrometer (Rheinstetten, Germany) in $CDCl_3$ (**1**–**7**), acetone-d_6 (**8** and **10**) or CD_3OD (**9**). Chemical shifts were determined in δ (ppm) relative to the solvent $CDCl_3$ (δ_H 7.26, δ_C 77.0), acetone-d_6 (δ_H 2.04, δ_C 29.8) or CD_3OD (δ_H 3.31, δ_C 49.1) as internal standard. ESI-MS spectra were measured on a Finnigan LCQ ion-trap mass spectrometer (San Jose, CA). The standard conditions employed included spray voltage 4.0 kV, capillary temperature 280 °C, and sheath gas flow rate 5 arbitrary units. The structures of compounds **1**–**8** and **10** (as shown in Fig. 1B) were identified by ESI-MS and NMR, and all spectral data were consistent with those reported in the literature (Chang, Chang, & Wu, 2000; Hisham, Kumar, Fujimoto, & Hara, 1996; Kuo & Lee, 1997; Kurihara, Kawabata, Ichikawa, Mishima, & Mizutani, 1991; Lin, Chen, & Kuo, 1991; Tanaka, Tabuse, & Matsunega, 1998; Tanaka et al., 2000). Compound **9** was, for the first time, isolated from nature and is reported here as a new natural product.

2-(4-Hydroxyphenyl)-2,3-dihydrobenzo[*b*]furan-3,4,6-triol (**9**): light-yellow amorphous solid; mp: 277–278 °C; $[\alpha]_D^{24}$ −0.456° (MeOH; *c* 1.00); UV (MeOH), λ_{max}, nm (log ε): 285 (3.77); IR (KBr), ν_{max}, cm^{-1}: 3321, 1604, 1512, 835; HREIMS *m*/*z*: 260.0674, $[M]^+$ calcd 260.0685 for $C_{14}H_{12}O_5$; ^1H NMR (500 MHz, CD_3OD): δ 6.93 (d, 2, *J* = 8.5 Hz, H-2, 6), 6.67 (d, 2, *J* = 8.5 Hz, H-3, 5), 6.53 (d, 1, *J* = 1.8 Hz, H-11), 6.11 (d, 1, *J* = 1.8 Hz, H-13), 4.47 (s, 1, H-7), 3.74 (s, 1, H-8); ^{13}C NMR (125 MHz, CD_3OD): δ 159.5 (C-12), 156.4 (C-4), 155.7 (C-14), 151.0 (C-10), 138.6 (C-1), 129.3 (C-2, 6), 124.0 (C-9), 116.1 (C-3, 5), 103.5 (C-11), 102.7 (C-13), 61.1 (C-8), 54.9 (C-7).

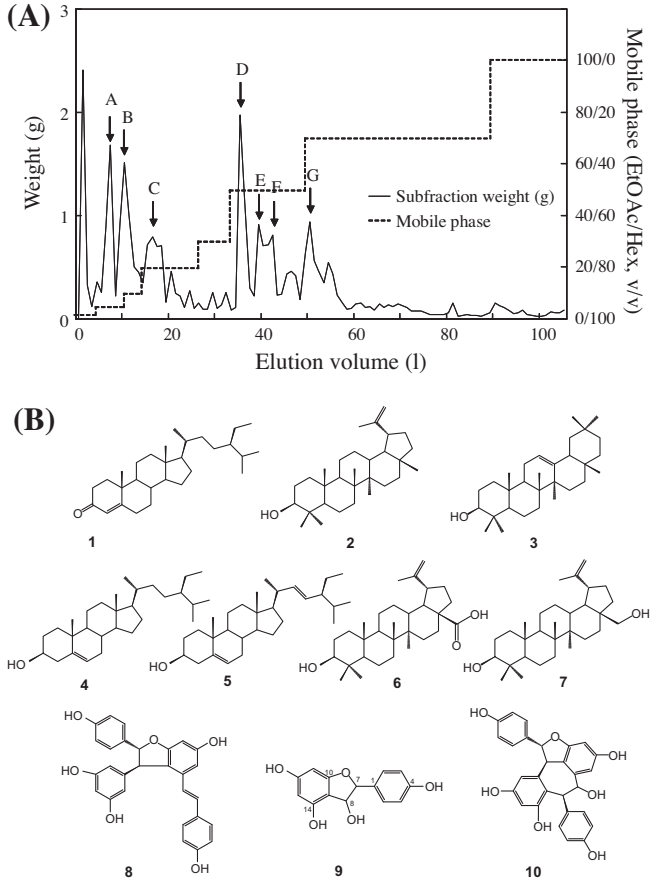

Fig. 1. (A) Subfraction weights of EtOAc fraction from *V. kelungensis* stem. (B) Phytocompounds isolated from EtOAc fraction of *V. kelungensis* stem. (**1**) β-Sitostenone, (**2**) lupeol, (**3**) β-amyrin, (**4**) β-sitosterol, (**5**) stigmasterol, (**6**) betulinic acid, (**7**) betulin, (**8**) (−)-ε-viniferin, (**9**) 2-(4-hydroxyphenyl)-2,3-dihydrobenzo[*b*]furan-3,4,6-triol, and (**10**) (+)-balanocarpol.

2.4. Cell cultures

The macrophage RAW 264.7 cell line, obtained from the ATCC (Manassas, VA), was grown in Dulbecco's modified Eagle's medium (DMEM) (Gibico/BRL, Grand Island, NY) supplemented with 10% heat-inactivated foetal bovine serum, 1 mM sodium pyruvate, 100 units/ml of penicillin, and 100 μg/ml of streptomycin at 37 °C in a humidified 5% CO_2 incubator.

2.5. Measurement of NO production and cell viability

To investigate the anti-inflammatory activity of test samples, NO production in LPS-stimulated RAW 264.7 cells was examined. For NO determination, RAW 246.7 cells were seeded in 96-well plates at a density of 2×10^5 cells/well and grown for 4 h for adherence. The cells were treated with test samples for 1 h and then incubated for 24 h in fresh DMEM, with or without 1 μg/ml of LPS. The nitrite concentration in the culture medium was measured as an indicator of NO production according to the Griess reaction. Briefly, 100 μl of cell culture supernatant were reacted with 100 μl of Griess reagent (1:1 mixture of 0.1% *N*-(1-naphthyl)ethylenediamine dihydrochloride in water and 1% sulfanilamide in 5% phosphoric acid) in a 96-well plate, and absorbance at 540 nm was recorded using an ELISA reader.

The cell viability assay was determined on the basis of MTT assay. After culturing, supernatants were collected for NO measurement, 100 μl of tetrazolium salt solution (1 ml MTT/10 ml DMEM) were added to each well, and then incubated for 1 h at 37 °C in a 5% CO_2 incubator. The medium was then aspirated and the insoluble formazan product was dissolved in 100 μl of DMSO. The extent of MTT reduction was quantified by measuring the absorbance at 570 nm.

2.6. Animals

Six-week-old male ICR mice (National Laboratory Animal Center, Taiwan) were given a standard laboratory diet and distilled water *ad libitum*, and kept on a 12 h light/dark cycle at 22 ± 2 °C.

2.7. Induction of acute liver injury

For the hepatotoxicity study, CCl_4 was used to induce acute liver damage according to Shyur et al. (2008) with slight modifications. Forty-two male ICR mice were randomly divided into seven groups for treatment ($n = 6$ per group): DMSO plus olive oil (vehicle group), DMSO plus CCl_4 (CCl_4 group), silymarin plus CCl_4 (SM group), resveratrol plus CCl_4 (RSV group), crude extract from *V. kelungensis* stem plus CCl_4 (VKS group), (−)-ε-viniferin plus CCl_4 (VNF group), and 2-(4-hydroxyphenyl)-2,3-dihydrobenzo[*b*]-furan-3,4,6-triol plus CCl_4 (HPD group). All test groups (10 mg/kg) were administered via intraperitoneal (*i.p.*) injection for three consecutive days before CCl_4 administration. Mice were challenged with a single *i.p.* administration of 20% CCl_4 solution in olive oil at a dose of 40 μl CCl_4/kg to induce acute liver injury. The mice were sacrificed after collecting blood by retro-orbital bleeding at 6 h after CCl_4 injection.

2.8. Measurement of serum AST and ALT activities

The enzymatic activities of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were used as biochemical markers for acute liver injury. Blood samples were centrifuged at 1400*g* at 4 °C for 15 min and the AST and ALT activities in serum supernatants were determined using a commercial kit from Randox Laboratories (UK).

2.9. Pathological histology

Liver tissue was fixed in 10% buffered formaldehyde and processed for histological examination, according to three kinds of histopathological stain, including haematoxylin and eosin (H&E), Masson, and silver stains.

2.10. Statistical analyses

Statistical significance of differences between treatments was determined by ANOVA. $P < 0.05$ was considered to be statistically significant.

3. Results and discussion

3.1. Anti-inflammatory activity of the stem extracts of V. thunbergii, V. flexuosa, and V. kelungensis

Activation of macrophages is critical to inflammatory processes by their release of a variety of inflammatory mediators, such as NO (Zhuang, Lin, Lin, & Wogan, 1998). NO is important in inflammatory processes of the liver, e.g., septic shock, hepatocarcinoma and autoimmune diseases. iNOS is an important enzyme mediator of inflammatory processes associated with the pathophysiology of many diseases and inflammatory disorders (Surh et al., 2001; Tipoe et al., 2006). In the present study, the LPS-stimulated murine macrophage assay system was employed to evaluate the effects of the stem extracts of *V. thunbergii, V. flexuosa*, and *V. kelungensis* on NO production.

As shown in Fig. 2A, among three crude extracts from the stems of *V. thunbergii, V. flexuosa*, and *V. kelungensis*, the *V. kelungensis* was the best able to inhibit nitric oxide production in LPS-stimulated

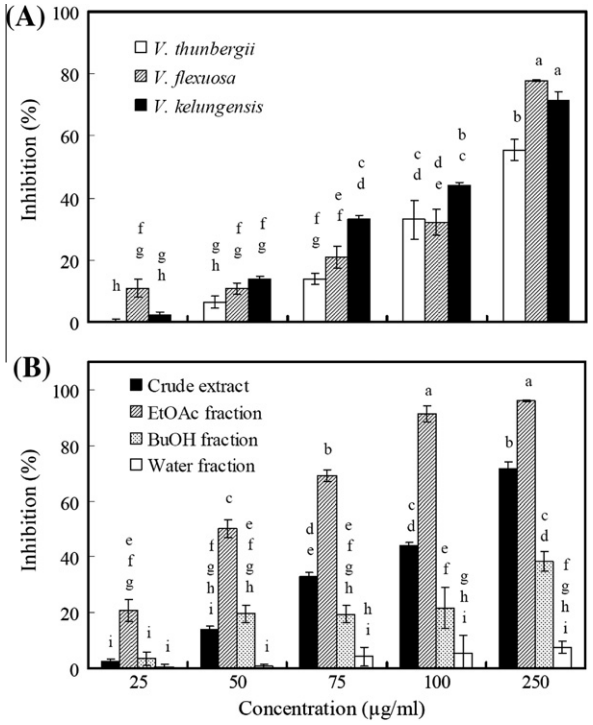

Fig. 2. (A) Effects of the stem extracts of *V. thunbergii*, *V. flexuosa*, and *V. kelungensis* on the nitric oxide production in LPS-stimulated RAW 264.7 macrophage cells. (B) Effects of the stem extract from *V. kelungensis* and its soluble fractions on nitric oxide production in LPS-stimulated RAW 264.7 macrophage cells. Data are presented as means ± SD ($n = 3$). Different letters are significantly different from each other ($P < 0.05$, ANOVA).

RAW 264.7 macrophage cells. The IC_{50} values of the tested extracts were in the following order: *V. kelungensis* (132 μg/ml) > *V. flexuosa* (159 μg/ml) > *V. thunbergii* (214 μg/ml). In addition, MTT assay revealed that concentrations up to 250 μg/ml produced no significant cytotoxic effects on cells treated with the three crude extracts. This result indicated that stem extracts of *V. kelungensis* have an excellent NO-inhibitory activity and merit further investigation.

3.2. Anti-inflammatory activity of the stem extract from V. kelungensis and its derived fractions

As shown in Fig. 2B, anti-inflammatory activity of crude extract from *V. kelungensis* stem and its derived fractions, including the soluble fractions of EtOAc, BuOH and water, revealed a dose-dependent response. Of these, the EtOAc fraction possessed the greatest potential activity. The EtOAc fraction blocked 50% of NO

Table 1
^1H and ^{13}C NMR spectroscopic data for 2-(4-hydroxyphenyl)-2,3-dihydrobenzo[*b*]furan-3,4,6-triol (**9**).

Position	δ_C	δ_H (multiplicity, *J*)	HMBC correlation
1	138.6	–	H-3, H-5, H-8
2	129.3	6.93 (d, *J* = 8.5 Hz)	H-6, H-7
3	116.1	6.67 (d, *J* = 8.5 Hz)	H-5
4	156.4	–	H-2, H-6
5	116.1	6.67 (d, *J* = 8.5 Hz)	H-3
6	129.3	6.93 (d, *J* = 8.5 Hz)	H-2, H-7
7	54.9	4.47 (s)	H-2, H-6
8	61.1	3.74 (s)	–
9	124.0	–	H-7, H-11, H-13
10	151.0	–	H-7, H-8
11	103.5	6.53 (d, *J* = 1.8 Hz)	H-13
12	159.5	–	–
13	102.7	6.11 (d, *J* = 1.8 Hz)	H-11
14	155.7	–	H-8

Table 2
Quantification of resveratrol derivatives from *V. thunbergii*, *V. flexuosa*, and *V. kelungensis* stems in different seasons.

Species	Seasons	Contents (mg/g of air-dried stem)		
		(−)-ε-Viniferin	2-(4-Hydroxyphenyl)-2,3-dihydrobenzo[*b*]furan-3,4,6-triol	(+)-Balanocarpol
V. thunbergii	Spring	2.20 ± 0.08[a]	1.96 ± 0.19[a]	ND
	Summer	0.63 ± 0.01[d]	0.17 ± 0.04[b]	ND
	Autumn	0.75 ± 0.04[c]	Trace	ND
	Winter	0.94 ± 0.00[b]	Trace	ND
V. flexuosa	Spring	0.44 ± 0.03[c]	ND	Trace
	Summer	0.52 ± 0.06[b]	ND	0.17 ± 0.01
	Autumn	1.47 ± 0.06[a]	ND	Trace
	Winter	0.54 ± 0.03[b]	ND	Trace
V. kelungensis	Spring	1.53 ± 0.05[a]	0.89 ± 0.03[a]	Trace
	Summer	0.80 ± 0.04[b]	0.48 ± 0.02[c]	0.11 ± 0.02
	Autumn	0.66 ± 0.06[c]	0.49 ± 0.05[c]	Trace
	Winter	1.54 ± 0.06[a]	0.77 ± 0.03[b]	Trace

Results are means ± SD ($n = 3$). Different letters in superscript indicate significant differences among groups ($P < 0.05$). ND: Not detected.

production in LPS-stimulated cells at approximately 50 μg/ml and more than 90% at 100 μg/ml, with little or no detectable cytotoxicity (treated cells were >90% viable). These results indicate that the anti-inflammatory activity of the methanolic extract of *V. kelungensis* stem can be effectively enriched in the EtOAc fraction. Moreover, the EtOAc fraction had 4.4- and 2.6-fold more mass than had the BuOH and water fractions, respectively. Based on the bioactivity-guided isolation principle, the EtOAc fraction was first subjected to a silica gel column chromatography with a stepwise gradient of *n*-hexane–EtOAc and was subdivided into various subfractions. Among them, subfractions of **A**–**G** possessed high yields, as shown in Fig. 1A. These subfractions were finally subjected to preparative HPLC. Consequently, 10 major constituents, as shown in Fig. 1B, were isolated and identified as β-sitostenone (**1**), lupeol (**2**), β-amyrin (**3**), β-sitosterol (**4**), stigmasterol (**5**), betulinic acid (**6**), betulin (**7**), (−)-ε-viniferin (VNF) (**8**), 2-(4-hydroxyphenyl)-2,3-dihydrobenzo[*b*]furan-3,4,6-triol (HPD) (**9**), and (+)-balanocarpol (**10**). Of these, compound **9** was obtained as a light-yellow amorphous solid. The HREIMS of **9** showed a molecular ion peak at *m/z* 260.0674 [M^+], which corresponded to the molecular formula $C_{14}H_{12}O_5$ (calc. 260.0685), and indicated the presence of nine degrees of unsaturation in the molecule. The IR spectrum suggested that **9** contained hydroxyl groups (3321 cm^{-1}) and benzene rings (1604, 1512 cm^{-1}). The ^1H NMR spectrum (Table 1) showed that **9** had two sets of aromatic proton signals at δ_H 6.53 (1H, d, $J = 1.8$ Hz, H-11), δ_H 6.11 (1H, d, $J = 1.8$ Hz, H-13) and δ_H 6.93 (2H, d, $J = 8.5$ Hz, H-2, 6), δ_H 6.67 (2H, d, $J = 8.5$ Hz, H-3, 5), respectively, as well as two singlet proton signals at δ_H 4.47 (1H, s, H-7) and δ_H 3.74 (1H, s, H-8). The HMBC spectrum of **9** showed long-range correlations from H-7 and H-8 to C-10; from H-7, H-11, and H-13 to C-9. These results suggested that **9** possessed a 2,3-dihydrobenzo[*b*]furan skeleton. On the other hand, in the HMBC spectrum, δ_H 6.93 (H-2, H-6) coupled to δ_C 54.9 (C-7) and δ_H 3.74 (H-8) coupled to δ_C 138.6 (C-1), indicating that the phenolic moiety was attached to C-7. Thus, compound **9** was identified as 2-(4-hydroxyphenyl)-2,3-dihydrobenzo[*b*]furan-3,4,6-triol. This is now, for the first time, isolated from nature and reported here as a new natural product. All ^{13}C signals and heteronuclear multiple bond correlations (HMBC) of this new phytocompound are shown in Table 1.

3.3. Quantification of major resveratrol-derived compounds from different seasons of V. thunbergii, V. flexuosa, and V. kelungensis stems

The quantification of major resveratrol-derived compounds, from different seasons of three wild grape stems, are shown in Table 2. The contents of VNF ranged from 0.63 to 2.20 mg, 0.44 to 1.47 mg, and 0.66 to 1.54 mg per gramme of air-dried stems of *V. thunbergii*, *V. flexuosa*, and *V. kelungensis*, respectively. Accordingly, *V. thunbergii* stem had the highest content of VNF in spring and the lowest content in summer. By contrast, the lowest VNF content was found in spring for *V. flexuosa* stem, while the highest content was found in autumn. As for *V. kelungensis* stem, the highest VNF content was found in winter and spring, and the lowest content was found in autumn. Furthermore, a new compound, HPD, was only present in *V. thunbergii* and *V. kelungensis* stems.

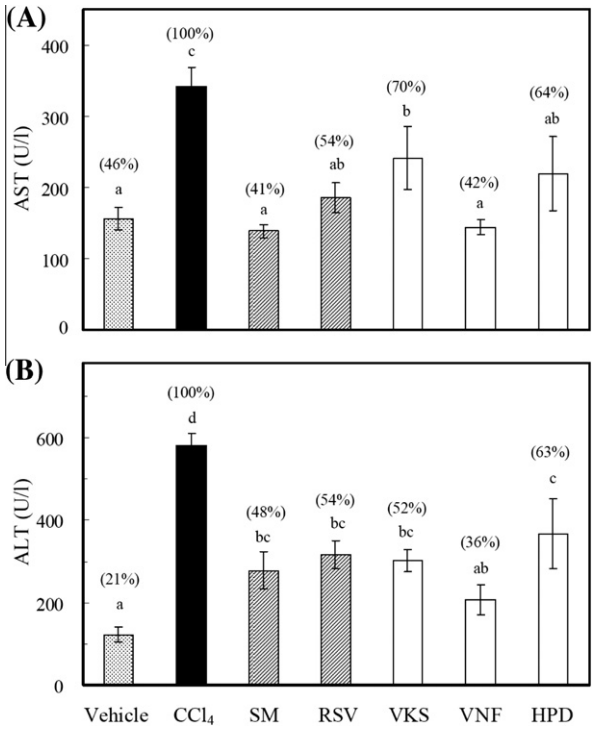

Fig. 3. Effects of the stem extract from *V. kelungensis* and its major resveratrol derivatives on serum AST and ALT activities in CCl_4-induced acute liver injury in mice. Aspartate aminotransferase (AST) (A) and alanine aminotransferase (ALT) (B) activities were determined in the serum from DMSO plus olive oil (vehicle group), DMSO plus CCl_4 (CCl_4 group), silymarin plus CCl_4 (SM group), resveratrol plus CCl_4 (RSV group), crude extract from *V. kelungensis* stem plus CCl_4 (VKS group), (−)-ε-viniferin plus CCl_4 (VNF group), and 2-(4-hydroxyphenyl)-2,3-dihydrobenzo[*b*]-furan-3,4,6-triol plus CCl_4 (HPD group). Data are presented as means ± SEM ($n = 6$). Different letters are significantly different from each other ($P < 0.05$, ANOVA).

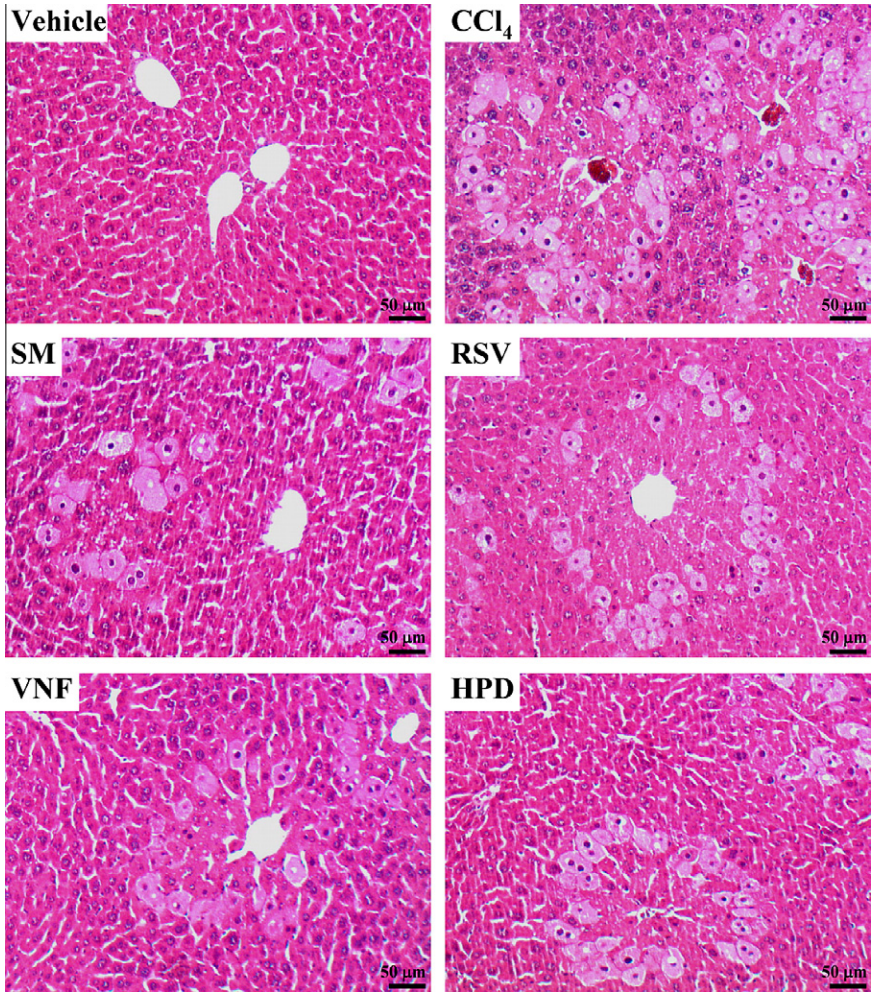

Fig. 4. Histopathological study of liver tissues. Specimens were photographed with a SPOT Idea 3 MP camera on an Olympus CKX41 inverted microscope (H&E stain, magnification: ×100).

Both species showed the highest amount of this new compound in spring with values of 1.96 and 0.89 mg/g, respectively. On the other hand, (+)-balanocarpol was only present in *V. flexuosa* and *V. kelungensis* stems. Among all seasons, these two species showed the highest amount of (+)-balanocarpol in summer, with values of 0.17 and 0.11 mg/g, respectively.

3.4. Hepatoprotective properties of the stem extract from *V. kelungensis* and its major resveratrol derivatives

Exogenous administration of either anti-inflammatory compounds or antioxidants (e.g., resveratrol) is an effective hepatoprotectant through modulation of NO production in rats (Farghali et al., 2009). As the extract of *V. kelungensis* stem (VKS) inhibited NO, we investigated the hepatoprotective activity of VKS and its major resveratrol derivatives, namely VNF and HPD, in CCl_4-induced acute liver injury, a widely used model for testing hepatoprotection in natural compounds. Silymarin (SM) and resveratrol (RSV), the well-known natural remedies for liver disorders, were examined, in parallel, as positive controls (Hoofnagle, 2005; Kawada, Seki, Inoue, & Kuroki, 1998).

Serum aminotransferase activities have long been used, clinically, as indicators of hepatic injury. The protective effects of VKS, VNF and HPD on CCl_4-induced elevation of serum AST and ALT activities are presented in Fig. 3. The serum AST and ALT activities of the CCl_4 group were dramatically elevated to 342 and 581 U/l ($P < 0.0001$), while these values were 156 and 122 U/l, respectively, in the vehicle group. However, the groups treated with 10 mg/kg of SM or RSV showed significant decrease in elevation of serum AST and ALT activities, with the values of 139 and 277 U/l (SM group) or 186 and 316 U/l (RSV group), respectively. Pretreatment of mice with 10 mg/kg of VKS, VNF and HPD, before CCl_4 challenge, significantly attenuated the CCl_4-induced increase in AST and ALT activities. In addition, there were no significant differences among the groups treated with SM, RSV, VKS, VNF and HPD. This hepatoprotection of SM, RSV, VKS, VNF and HPD was also seen in CCl_4-induced histopathological changes of mouse liver (Fig. 4). Panlobular focal hepatocellular necrosis and neutrophil leucocyte infiltration were observed after CCl_4 intoxication for 6 h in the CCl_4 group, whereas SM-, RSV-, VKS-, VNF-, and HPD-pretreated mice had much less severe hepatocellular necrosis. Thus, VKS and its major resveratrol derivatives, VNF and HPD have protective effects similar to that of silymarin or resveratrol against CCl_4-induced acute liver injury.

4. Conclusions

This is the first study to demonstrate that chemical constituents from the stem extract of *V. kelungensis* possess anti-inflammatory activity *in vitro* and *in vivo* that may contribute to the hepatoprotective effect against CCl_4-induced liver injury in mice. The results clearly indicate that the stem extract of *V. kelungensis* and its major

resveratrol derivatives, VNF ((−)-ε-viniferin) and HPD (2-(4-hydroxyphenyl)-2,3-dihydrobenzo[*b*]furan-3,4,6-triol), could reduce CCl_4-induced toxicity, particularly hepatotoxicity, by suppressing ALT and AST activities. It is therefore suggested that *V. kelungensis* stem extract and its major resveratrol derivatives may represent a new type of hepatoprotective agent and they may have a potent hepatoprotective effect in clinical use.

Acknowledgements

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