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Bioactive Phytochemicals of Leaf Essential Oils of *Cinnamomum osmophloeum* Prevent Lipopolysaccharide/D-Galactosamine (LPS/D-GalN)-Induced Acute Hepatitis in Mice

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ABSTRACT: The purpose of this study was to investigate the bioactive phytochemicals of leaf essential oils of *Cinnamomum osmophloeum* on lipopolysaccharide/D-galactosamine (LPS/D-GalN)-induced acute hepatitis. The results revealed that post-treatment with 100 μmol/kg *trans*-cinnamaldehyde, (−)-aromadendrene, T-cadinol, or α-cadinol significantly decreased the aspartate aminotransferase (AST), alanine aminotransferase (ALT), tumor necrosis factor-α (TNF-α), and interleukin 6 (IL-6) levels in serum. Moreover, both T-cadinol and α-cadinol treatments decreased the expressions of cleaved caspase-3 and cleaved poly-ADP ribose polymerase (PARP) in the liver tissues when compared with the LPS/D-GalN group. Liver histopathology also showed that silymarin, *trans*-cinnamaldehyde, (−)-aromadendrene, T-cadinol, or α-cadinol significantly reduced the incidence of liver lesions induced by LPS/D-GalN. These results suggest that the above phytochemicals exhibit potent hepatoprotection against LPS/D-GalN-induced liver damage in mice, and their hepatoprotective effects may be due to the modulation of anti-inflammatory activities.

KEYWORDS: *Cinnamomum osmophloeum*, essential oil, hepatoprotection, lipopolysaccharide/D-galactosamine (LPS/D-GalN), acute liver failure (ALF)

INTRODUCTION

Accumulating evidence indicates that endotoxemia and sepsis occur frequently in patients as a result of acute liver failure (ALF). This type of disorder still has extremely poor prognosis and high mortality because of the lack of effective therapy.[1,2] At present, no specific therapy is available except for liver transplantation.[3] Therefore, the need is urgent for effective therapy for ALF. Rodents treated with lipopolysaccharide/D-galactosamine (LPS/D-GalN) showed marked sensitization to LPS response and potentiation of tumor necrosis factor-α (TNF-α) induced hepatocyte apoptosis.[4] Thus, this experimental acute hepatitis model is widely used to investigate the underlying mechanisms of clinical ALF and develop effective therapeutic strategies for endotoxin challenge.[5]

Cinnamomum osmophloeum Kaneh. (Lauraceae) is a tree indigenous to Taiwan at elevations between 400 and 1500 m and is traditionally used as a medicinal plant.[6] An aqueous extract of *C. osmophloeum* leaves is used in Taiwan for healing inflammation, diabetes, and enteric infection. Tung et al.[6] reported that the leaf essential oils from cinnamaldehyde and mixed types of *C. osmophloeum* strongly inhibited nitric oxide (NO) production, with IC_{50} values ranging from 9.7 to 15.5 μg/mL. In addition, *trans*-cinnamaldehyde, (−)-aromadendrene, T-cadinol, and α-cadinol were confirmed as the major bioactive phytochemicals in these essential oils. These results imply that the essential oils or the derived phytochemicals from *C. osmophloeum* leaves have great potential to prevent diseases caused by the overproduction of reactive nitrogen species and inflammatory disorders such as hepatitis.

It is well-known that activation of macrophages is important to inflammatory response because they release inflammatory mediators.[7,8] NO is important in the inflammatory response of the liver. Inducible nitric oxide synthase (iNOS) is an important enzyme mediator of inflammatory response associated with inflammatory disorders.[9] In the present study, the LPS-stimulated macrophage system was employed to evaluate the effect and mechanism of bioactive compounds on anti-inflammatory activity. In addition, to the best of our knowledge, no prior studies have investigated the hepatoprotective effects of the bioactive phytochemicals of leaf essential oils of *C. osmophloeum*. Accordingly, in this study, we also aimed to provide scientific evidence for the hepatoprotective efficacy of *trans*-cinnamaldehyde, (−)-aromadendrene, T-cadinol, and α-cadinol against LPS/D-GalN-induced acute hepatitis in mice.

MATERIALS AND METHODS

Chemicals. LPS, Greiss reagent, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), curcumin, silymarin, *trans*-cinnamaldehyde, and (−)-aromadendrene were purchased from Sigma

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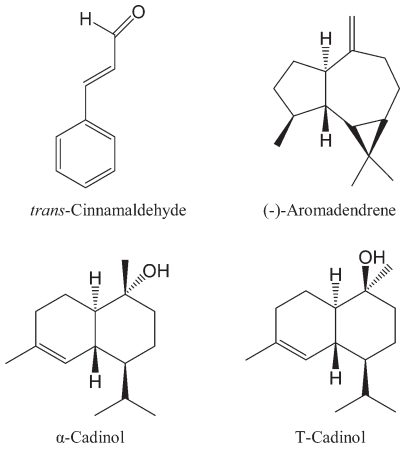
Chemical Co. (St. Louis, MO). Dimethyl sulfoxide (DMSO) was purchased from Acros (Belgium). T-Cadinol and α-cadinol were provided by Prof. Yueh-Hsiung Kuo (Tsuzuki Institute for Traditional Medicine, College of Pharmacy, China Medical University, Taiwan). The other chemicals and solvents used in this experiment were of the highest quality available.



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

Measurement of NO Production and Cell Viability. To investigate the anti-inflammatory activity of the test samples, NO production in the LPS-stimulated RAW 264.7 cells was carried out according to the method followed by Wu et al.[10] 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 was 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 was recorded at 540 nm using an ELISA reader (Labsystems multiskan MS, Helsinki, Finland).

The cell viability assay was determined on the basis of the MTT assay. After culturing, supernatants were collected for NO measurement; 100 μL of tetrazolium salt solution (1 mL MTT/10 mL DMEM) was 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.

Western Blot Analysis of α-Cadinol in LPS-Stimulated RAW 264.7 Cells. Western blot analysis was performed according to the method reported by Pan et al.[11] Briefly, RAW 264.7 cells (5×10^6 cells) were seeded on a 6 cm dish for 2 h; afterward, the cells were pretreated with α-cadinol (0, 50, 75, and 100 μM) for 1 h and then stimulated with or without 1 μg/mL LPS for different times. The expressions of iNOS, inhibitor κBα (IκBα), phospho-IκB kinase α/β (p-IKKα/β), and the nuclear factor-κB subunit p65 (p65) proteins were determined. In the iNOS, IκBα, p-IKKα/β, and p65 protein expressions, cells were stimulated with LPS for 24 h, 30 min, 30 min, and 45 min, respectively. The protein samples (50 μg) were resolved by a 10% SDS-PAGE and electrophoretically transferred to a PVDF membrane. Thus, the membrane was incubated with primary antibody (4 °C, overnight) and further incubated for 1−2 h with anti-mouse or anti-rabbit IgG antibody conjugated to HRP. The protein expressions were detected by the enhanced chemiluminescence reagents (ECL, Pierce). β-Actin and lamin A/C levels were measured as internal controls.

Animals. Thirty-five pathogen-free, male ICR mice, 6 weeks old, 18−20 g body weight, were purchased from the National Laboratory Animal Center, Taiwan. Upon delivery, the mice were kept in a pathogen-free rodent facility, were given a standard laboratory diet and distilled water ad libitum, and kept on a 12 h light/dark cycle at 22 ± 2 °C. This study was conducted according to institutional guidelines and approved by the Institutional Animal Care and Utilization Committee of National Chung Hsing University, Taiwan.

Effects of *trans*-Cinnamaldehyde, (−)-Aromadendrene, T-Cadinol, and α-Cadinol on Acute Hepatitis in Mice. The in vivo therapeutic potential of the bioactive phytochemicals on LPS/D-GalN-induced acute hepatitis was investigated and compared with the hepatoprotective agent, silymarin. Thirty-five mice were randomly assigned to seven groups (n = 5 per group) for treatment: vehicle, LPS/D-GalN, silymarin, *trans*-cinnamaldehyde, (−)-aromadendrene, T-cadinol, and α-cadinol. Silymarin (20 mg/kg), *trans*-cinnamaldehyde (100 μmol/kg), (−)-aromadendrene (100 μmol/kg), T-cadinol (100 μmol/kg), and α-cadinol (100 μmol/kg) were administered intraperitoneally (ip) after 1 h of reatment with 500 ng of LPS and 25 mg of D-GalN in 250 μL of saline as described previously.[12] Blood samples were collected by retro-orbital bleeding 8 h after LPS/D-GalN injection. Then, all mice were sacrificed and liver tissues collected.

Measurement of the Levels of AST, ALT, TNF-α, and IL-6 in Serum. The serum levels of AST, ALT, TNF-α, and IL-6 were used as biochemical markers for acute hepatitis. Blood samples were centrifuged at 1400*g* at 4 °C for 15 min. Serum AST and ALT activities were determined by use of commercial kits from Randox Laboratories (U.K.). Serum levels of TNF-α and IL-6 were measured using an enzyme immunoassay (R&D Systems, Inc., Minneapolis, MN).

Pathological Histology. Liver tissues were fixed in 10% buffered formaldehyde and processed for histological examination according to hematoxylin and eosin (H&E) stain.

Western Blot Analysis. The total protein of the liver tissues (0.1 g) from each mouse was homogenized in a mixer ball mill (MM301, Retsch, Haan, Germany) for 2 min, extracted by adding 0.4 mL of lysis buffer, and centrifuged at 15000*g* for 30 min at 4 °C. Protein determination and Western blotting were carried out according to the method proposed by Huang et al.[13]

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

RESULTS

***trans*-Cinnamaldehyde, (−)-Aromadendrene, T-Cadinol, and α-Cadinol Suppress NO Production Induced by LPS in Vitro.** As shown in Figure 1, all phytochemicals were found to dose-dependently inhibit NO production in LPS-activated macrophages. The NO inhibitory effect of the phytochemicals was in the following order: α-cadinol (IC_{50} = 58 μM) > T-cadinol (IC_{50} = 89 μM) > (−)-aromadendrene (IC_{50} = 94 μM) > *trans*-cinnamaldehyde (IC_{50} = 107 μM). Accordingly, α-cadinol was the most effective in inhibiting NO production in LPS-stimulated RAW 264.7 macrophage cells. The addition of 10, 25, 50, 100, and 200 μM α-cadinol to LPS-stimulated cells resulted in decreased NO production at levels of 17, 20, 35, 95, and 100%, respectively, as compared to that of the cells treated with LPS alone. In addition, except for curcumin

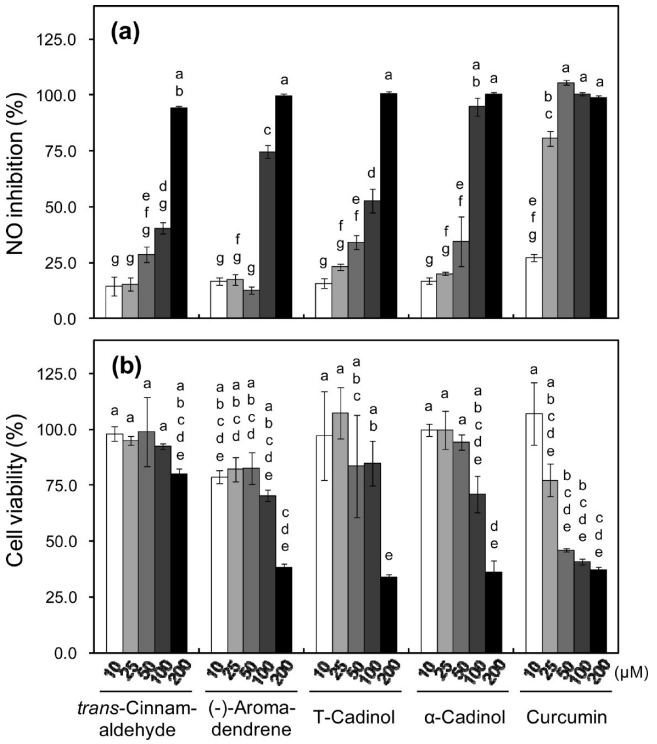


Figure 1. Effects of the bioactive phytochemicals of *C. osmophloeum* leaves and curcumin on nitric oxide production of LPS-stimulated RAW 264.7 macrophage cells: (a) concentration-dependent inhibition of nitric oxide production; (b) cytotoxicty of the bioactive phytochemicals measured by the MTT assay. Results are the mean ± SD (n = 3). Different letters indicate significant difference within treatments (P < 0.05, ANOVA).

(positive control), the MTT assay revealed that concentrations of up to 100 μM produced no significant cytotoxic effects on cells treated with all phytochemicals (Figure 1b).

α-Cadinol Inhibits iNOS Expression in LPS-Induced RAW 264.7 Murine Macrophage Cells via NF-κB Signaling Pathway. Among the four phytochemicals, α-cadinol showed a remarkable NO inhibitory effect. We further examined the anti-inflammatory mechanism of α-cadinol. In RAW 264.7 macrophages, LPS resulted in iNOS protein after 24 h of LPS-activated macrophages (Figure 2), whereas NO production was detectable (Figure 1). Treatment with α-cadinol significantly reduced iNOS induction after LPS-stimulated cells; thus, iNOS was paralleled by the reduction of NO production. The result implies that α-cadinol may serve as an anti-inflammatory agent through down-regulation in iNOS and NO production. We assume that the reduction of iNOS expression by α-cadinol could be the result of poor nuclear translocation of NF-κB. IκBα degradation is an important step for NF-κB activation and expression of its target iNOS induced by LPS, and α-cadinol showed significant inhibition of IκBα degradation. IκB is a NF-κB inhibitor that can be degraded by IKK after LPS stimulation. Figure 2 shows that the expression of IκBα increased with α-cadinol in parallel with a p-IKKα/β decrease. According to these results, we found that α-cadinol's preferential targeting of the binding of NF-κB to its consensus DNA sequence could be due to NF-κB inactivation resulting from stabilization of IκB. Furthermore, IKKα/β phosphorylation was involved in this down-regulation of the anti-inflammatory response.

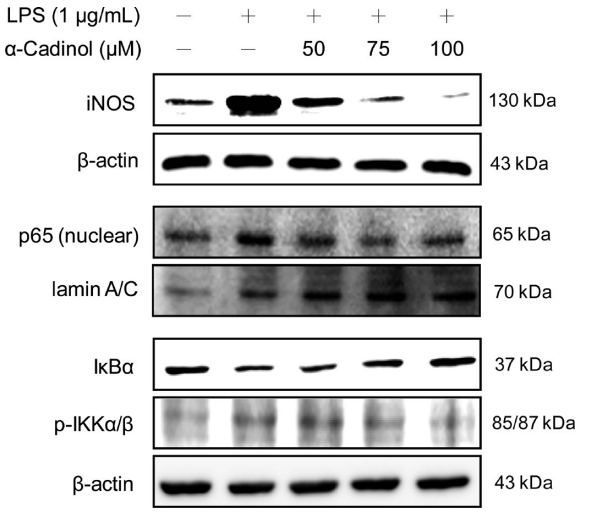


Figure 2. Effects of α-cadinol on iNOS, nuclear p65, p-IKKα/β, and IκBα of LPS-stimulated RAW 264.7 macrophage cells. All proteins were determined by Western blot analysis. The indicated apparent molecular masses of iNOS, nuclear p65, p-IKKα/β, IκBα, lamin A/C (internal control), and β-actin (internal control) were 130, 65, 85/87, 37, 70, and 43 kDa, respectively.

Effects of *trans*-Cinnamaldehyde, (−)-Aromadendrene, T-Cadinol, and α-Cadinol on Serum Biochemical Parameters of Liver Function. Serum AST and ALT activities have long been used clinically as indicators of hepatic injury.[14] At 8 h after the injection of LPS/D-GalN, the serum levels of AST and ALT activities were significantly higher than those of the vehicle group. As shown in Figure 3, the serum AST and ALT activities of the LPS/D-GalN group were dramatically elevated to 2618 and 3200 U/L, whereas the values were 244 and 163 U/L, respectively, in the vehicle group. However, after LPS/D-GalN challenge, the mice administered 20 mg/kg silymarin or 100 μmol/kg *trans*-cinnamaldehyde, (−)-aromadendrene, T-cadinol, or α-cadinol showed significant decreases in the elevation of serum AST and ALT activities, with values of 1108 and 1312 U/L (silymarin group), 1164 and 1270 U/L (*trans*-cinnamaldehyde group), 1234 and 1944 U/L ((−)-aromadendrene), 1298 and 1769 U/L (T-cadinol), or 616 and 731 U/L (α-cadinol), respectively. These results demonstrate that treatment with these compounds, especially α-cadinol, significantly attenuates the LPS/D-GalN-induced elevation of serum ALT and AST levels.

Effects of *trans*-Cinnamaldehyde, (−)-Aromadendrene, T-Cadinol, and α-Cadinol on Serum Cytokines. It is becoming increasingly clear that the link between LPS-induced macrophage activation and endotoxin-induced hepatitis is strong and that the interplay between the inflammatory mediators (i.e., NO and TNF-α) and the development and remission of hepatitis is critical. Therefore, we used the in vitro RAW 264.7 macrophage cell assay with LPS stimulation to screen the anti-inflammatory activities of phytocompounds that may have great potential as effective agents against endotoxin-induced hepatitis. In addition, the determination of chemical reaction-based assay of NO production is more cost-effective than using an ELISA-based test of TNF-α secretion in LPS-stimulated RAW 264.7 cells. On the other hand, endotoxin-induced fatal hepatitis is mediated by TNF-α. Therefore, measurement of TNF-α levels in mouse sera is more important than NO production. IL-6, like TNF-α, is a cytokine with both pro- and anti-inflammatory properties.

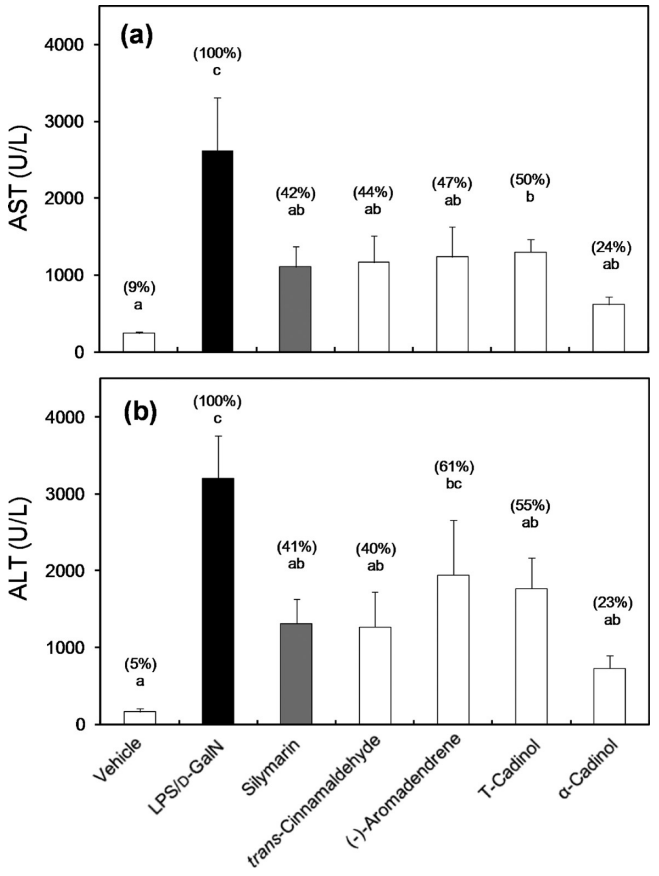


Figure 3. Hepatoprotective effects of the bioactive phytochemicals of *C. osmophloeum* leaves and silymarin on LPS/D-GalN-induced acute hepatitis: serum levels of AST activity (a) and ALT activity (b) in mice treated with silymarin (20 mg/kg) or the bioactive phytochemicals (100 μmol/kg) followed by LPS/D-GalN challenge. Data are presented as the mean ± SEM (n = 5). Different letters indicate significant difference within treatments (P < 0.05, ANOVA).

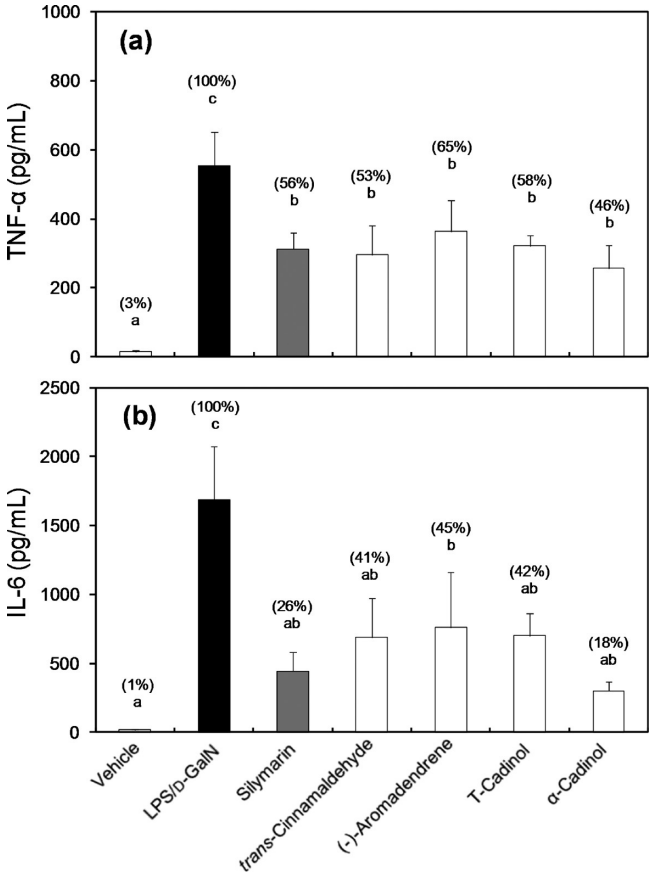


Figure 4. Hepatoprotective effects of the bioactive phytochemicals of *C. osmophloeum* leaves and silymarin on LPS/D-GalN-induced acute hepatitis: serum levels of TNF-α (a) and IL-6 (b) in mice treated with silymarin (20 mg/kg) or the bioactive phytochemicals (100 μmol/kg) followed by LPS/D-GalN challenge. Data are presented as the mean ± SEM (n = 5). Different letters indicate significant difference within treatments (P < 0.05, ANOVA).

Figure 4 shows the serum levels of TNF-α and IL-6 at 8 h post-LPS/D-GalN injection. Accordingly, LPS/D-GalN treatment increased the serum levels of TNF-α (555 pg/mL) and IL-6 (1685 pg/mL) when compared with those in the vehicle control group, showing values of 17 and 15 pg/mL, respectively. However, the serum levels of TNF-α were significantly diminished in the groups treated with 20 mg/kg silymarin (312 pg/mL), 100 μmol/kg *trans*-cinnamaldehyde (295 pg/mL), 100 μmol/kg (−)-aromadendrene (363 pg/mL), 100 μmol/kg T-cadinol (322 pg/mL), and 100 μmol/kg α-cadinol (256 pg/mL) as compared to those administered LPS/D-GalN alone (555 pg/mL). Additionally, the groups treated with 20 mg/kg silymarin (445 pg/mL), 100 μmol/kg *trans*-cinnamaldehyde (689 pg/mL), 100 μmol/kg (−)-aromadendrene (759 pg/mL), 100 μmol/kg T-cadinol (701 pg/mL), and 100 μmol/kg α-cadinol (300 pg/mL) were found to have significant decreases in serum levels of IL-6 when compared with the LPS/D-GalN group (1685 pg/mL). Of these, α-cadinol possessed the most potential protection. By comparison of the configurations of α-cadinol and T-cadinol, the result also revealed that cadinane-type sesquiterpenoids with an equatorial hydroxyl group at C-9 exhibited better protection against LPS/ D-GalN-induced acute hepatitis. These results demonstrate that normal uptake in the bioactive phytochemicals might be due to a summation of effects by direct modulation of pro-inflammatory TNF-α and IL-6 production and their important downstream molecules in LPS/D-GalN-induced acute hepatitis in mice.

Pathological Histology. The livers of LPS/D-GalN-challenged mice showed an infiltration of numerous inflammatory cells into liver lobules, loss of sinusoidal cells, tissue destruction, and erythrocyte influx (hemorrhage) in liver sections (Figure 5). These LPS/D-GalN-induced histopathological changes were significantly attenuated in the treatments, which indicated that phytochemicals, that is, silymarin, *trans*-cinnamaldehyde, (−)-aromadendrene, T-cadinol, and α-cadinol, effectively protected the liver against dysfunction caused by LPS/D-GalN. Of these, α-cadinol possessed the most potential protection. In addition, there were no significant differences among the groups treated with silymarin, *trans*-cinnamaldehyde, (−)-aromadendrene, and T-cadinol. Thus, the bioactive phytochemicals of the leaf essential oil of *C. osmophloeum* possess a protective effect similar to that of silymarin against LPS/D-GalN-induced acute hepatitis.

Effects of α-Cadinol and Its Isomer, T-Cadinol, on Hepatic Cleaved Caspase-3 and Cleaved PARP Protein Expressions. Among the four phytochemicals, α-cadinol showed a remarkable hepatoprotective effect similar to that of silymarin against

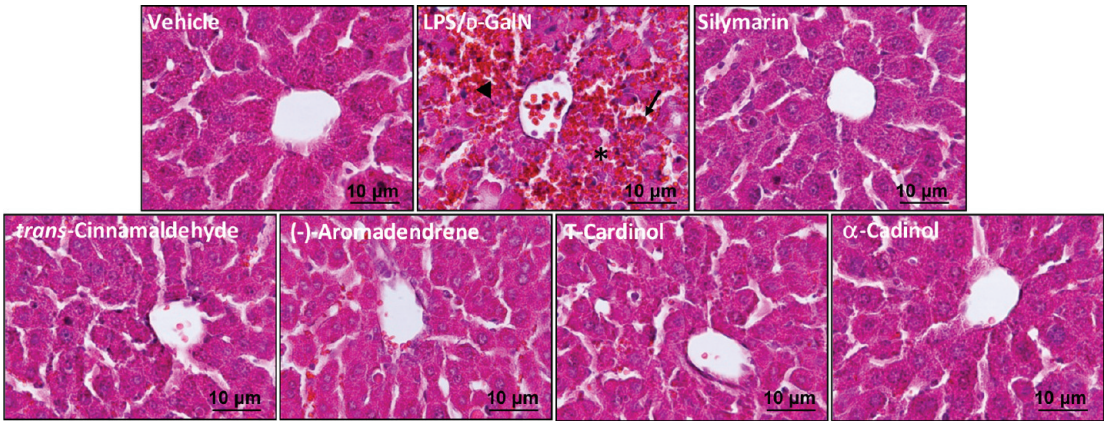


Figure 5. Pathological examination by H&E stain of livers from mice post-treated with the vehicle, silymarin (20 mg/kg) or the bioactive phytochemicals (100 μmol/kg) of *C. osmophloeum* leaves in LPS/D-GalN-treated mice. Liver sections from LPS/D-GalN-treated mice showed acute hepatitis with an infiltration of numerous inflammatory cells into liver lobules (arrowhead, ▼), loss of sinusoidal cells, tissue destruction (star, ∗) and erythrocyte influx (arrow, ←).

LPS/D-GalN-induced acute hepatitis. We further examined the antiapoptotic effect of α-cadinol and its isomer, T-cadinol, on LPS/D-GalN-induced liver cell apoptosis. Figure 6 shows a representative Western blot of cleaved caspase-3 (19 and 17 kDa) and cleaved PARP (89 kDa) levels in liver tissues. Mice in the LPS/D-GalN group showed significantly increased hepatic cleaved caspase-3 and cleaved PARP levels as compared to those observed in the vehicle control group. However, the groups treated with T-cadinol or α-cadinol showed decreased expressions of cleaved caspase-3 by 32 or 29% and of cleaved PARP by 75 or 93%, respectively, as compared to the LPS/D-GalN group. The levels of proteolytic cleavage of caspase-3 and its substrate PARP, the hallmarks of apoptosis, were prominently increased in the LPS/D-GalN-treated mice; post-treatment with silymarin, T-cadinol or α-cadinol significantly suppressed the cleavage forms of these proapoptotic proteins. These results suggest that T-cadinol or α-cadinol possesses a protective effect similar to that of silymarin against LPS/D-GalN-induced acute hepatitis.

DISCUSSION

Earlier studies have shown that the leaf essential oils of *C. osmophloeum* possess potent anti-inflammatory properties.[6,15] Tung et al.[6] reported that the leaf essential oils of *C. osmophloeum* strongly inhibited NO production. In addition, we found that *trans*-cinnamaldehyde, (−)-aromadendrene, T-cadinol, and α-cadinol of these essential oils significantly inhibit NO production in LPS-activated macrophages. Among the four phytochemicals, α-cadinol showed a remarkable NO inhibitory effect. α-Cadinol inhibited LPS-induced NO production in macrophages and showed dose-dependent inhibition of iNOS expression. The induction of NF-κB by LPS was markedly inhibited with different doses of α-cadinol. α-Cadinol inhibited the LPS-induced IKK and NF-κB associated with the inhibition of iNOS expression. These results imply that the α-cadinol inhibits iNOS expression in LPS-induced RAW 264.7 cells via NF-κB signaling pathway.

Anti-inflammatory activity makes the medicinal plants an ideal candidate for a triad of ailments, including acute liver failure.[1,2] We have previously shown that the bioactive phytochemicals, that is, *trans*-cinnamaldehyde, (−)-aromadendrene, T-cadinol, and α-cadinol, of leaf essential oils of *C. osmophloeum* significantly decrease the serum levels of AST, ALT, TNF-α, and IL-6 in ALF mice at a dosage of 100 μmol/kg. At the same dosage (100 μmol/kg), animals treated with *trans*-cinnamaldehyde, (−)-aromadendrene, T-cadinol, and α-cadinol all showed significant reductions of AST and ALT activities by 56/60, 53/39, 50/45, and 76/76%, respectively. Wan et al.[16] reported that baicalin, a traditional anti-inflammatory drug, at 50 mg/kg of body weight can decrease the elevation of plasma AST activity (20%) and that of ALT activity by 30% in LPS/D-GalN-induced acute hepatitis. Zhang et al.[17] also found that administration of asiaticoside, a triterpenoid product isolated from *Centella asiatica*, significantly reduced serum AST and ALT activities by 50 and 40%, respectively, as compared to the LPS/D-GalN group at a dosage of 20 mg/kg. Comparisons of these data revealed that the bioactive phytochemicals (*trans*-cinnamaldehyde, (−)-aromadendrene, T-cadinol, and α-cadinol) of the leaf essential oils of *C. osmophloeum* examined in this study exhibited great hepatoprotective effects. Among the four compounds, α-cadinol showed a remarkable hepatoprotective effect similar to that of silymarin against LPS/D-GalN-induced acute hepatitis.

In this animal model, hepatitis depends on pro-inflammatory cytokines, including IL-6 and TNF-α. TNF-α plays a vital role in the pathogenesis of LPS/D-GalN-induced acute hepatitis.[18,19] LPS activates macrophages and kupffer cells to produce TNF-α, which induces hepatocyte apoptosis in the early stage of LPS/D-GalN-induced hepatitis in mice. Thus, TNF-α-induced neutrophil transmigration occurs in the later stage of hepatitis and causes serious hepatocyte necrosis and organ failure.[20−23] Furthermore, at a dosage of 100 μmol/kg, administration of the bioactive phytochemicals (*trans*-cinnamaldehyde, (−)-aromadendrene, T-cadinol, and α-cadinol) of the leaf essential oils of *C. osmophloeum* significantly reduced the serum TNF-α and IL-6 levels by 47/59, 35/55, 42/58, and 54/82%, respectively, as compared to the LPS/D-GalN group. Zhang et al.[17] found that administration of asiaticoside significantly reduced the serum TNF-α level by 40% at a dosage of 10 mg/kg. Comparisons of the aforesaid results indicate that α-cadinol has an excellent hepatoprotective effect.

In regard to apoptosis, TNF-α combines with TNF-α receptor on the hepatocyte membrane to activate caspase-3, a cysteinprotease of the CED-3/ICE family, and eventually induces apoptosis at the early stages through signal transmission. In this

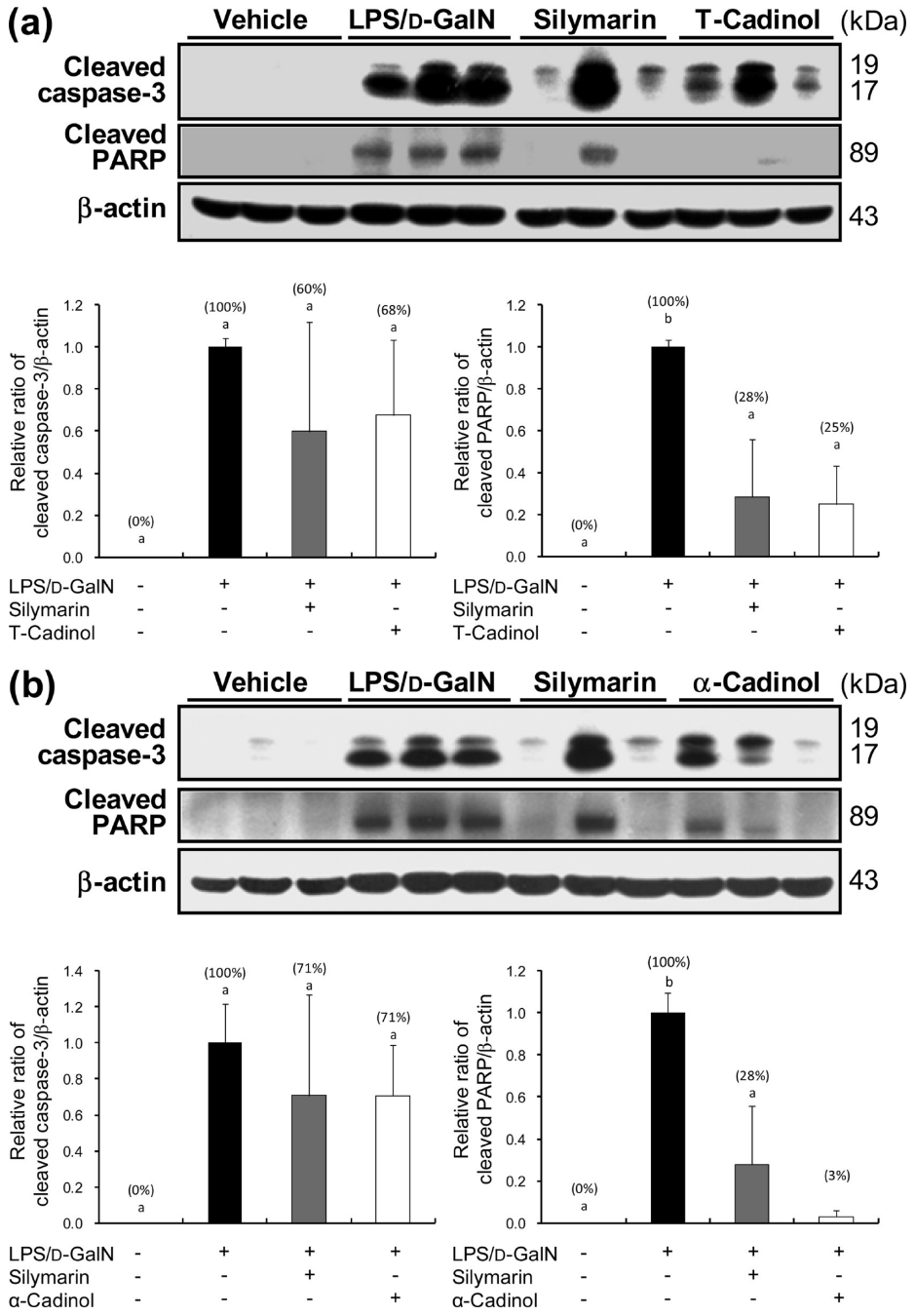


Figure 6. Hepatoprotective effects of T-cadinol (a) and α-cadinol (b) on LPS/D-GalN-treated mice. Liver tissues were obtained from mice after 8 h of LPS/D-GalN administration. Cleaved forms of caspase-3 and PARP were determined by Western blot analysis. The indicated apparent molecular masses of cleaved caspase-3, cleaved PARP, and β-actin (internal control) were 19, 17, 89, and 43 kDa, respectively. Data are the mean ± SEM ($n = 3$). Different letters indicate significant difference within treatments ($P < 0.05$, ANOVA).

study, LPS/D-GalN significantly up-regulated the serum level of TNF-α. In contrast, administration of α-cadinol reduced the LPS/D-GalN-induced serum TNF-α level and diminished the increase of caspase-3 induced by TNF-α. Accordingly, the present study shows that T-cadinol and α-cadinol might attenuate hepatocyte apoptosis by inhibiting the serum of TNF-α and caspase-3 in liver tissues.

In conclusion, LPS/D-GalN-induced acute hepatitis in mice has been widely used as a model system that closely mimics the cascade of events leading to clinical hepatitis caused by endotoxemia and sepsis. The advantage of this model is that D-GalN can potentiate the acute toxicity of LPS within a few hours and produce fatal acute hepatitis within a few days.[24] Herein, the results demonstrate that treatment with the *trans*-cinnamaldehyde, (−)-aromadendrene, T-cadinol, and α-cadinol attenuated LPS/D-GalN-induced liver injury, as indicated by a reduction in serum levels of AST, ALT, TNF-α, and IL-6, as well as hepatic inflammation and necrotic and apoptotic tissue injury. It is therefore suggested that the bioactive phytochemicals of the leaf essential oils of

C. osmophloeum may represent a new type of hepatoprotective agent and may provide a potent hepatoprotective effect for clinical use.

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Author Contributions

‖Equal contribution to this paper.

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