Hepatoprotective Effect of *Combretum quadrangulare* and Its Constituents

Arjun Hari Banskota, Yasuhiro Tezuka, I Ketut Adnyana, Quanbo Xiong, Koji Hase, Kim Qui Tran, Ken Tanaka, Ikuo Saiki, and Shigetoshi Kadota

Institute of Natural Medicine, Toyama Medical and Pharmaceutical University, 2630 Sagitaiga, Toyama 930-0194, Japan, National University Ho Chi Minh City, Ho Chi Minh City, Vietnam, and National Research Institute of Police Science, Sanban-cho, Chiyoda-ku, Tokyo 102-0075, Japan. Received November 12, 1999; accepted December 27, 1999

The MeOH extract of leaves of *Combretum quadrangulare* showed significant hepatoprotective effect on *p*-galactosamine (*p*-GalN)/lipopolysaccharide (LPS)-induced experimental liver injury in mice and on *p*-GalN/tumor necrosis factor-α (TNF-α)-induced cell death in primary cultured mouse hepatocytes. Phytochemical investigation led to the isolation of thirty cycloartane-type triterpenes together with betulinic acid, β-sitosterol, β-sitosterol glucoside, 4 flavones (34–37), and 3 flavone C-glucosides (38–40). These compounds showed various potencies of hepatoprotective effect on *p*-GalN/TNF-α-induced cell death in primary cultured mouse hepatocytes. Quadrangulararol B (29), methyl quadrangulararate I (33), kamatakein (34), 5,7,4′-trihydroxy-3,3′-dimethoxyflavone (35), 5,4′-dihydroxy-3,7,3′-trimethoxyflavone (36) and isokaempferide (37) showed strong inhibitory effect on TNF-α-induced cell death with IC₅₀ values of 34.3, 33.7, 13.3, 22.4, 13.4 and 22.8 μM, respectively, whereas clinically-used sildalin had an IC₅₀ value of 39.6 μM and glycyrhrizin showed very weak inhibitory effect. Methyl quadrangularates A (30) and N (32), norquadrangularic acid B (31) and vitexin (40) also showed potent inhibition on TNF-α-induced cell death with IC₅₀ values of 45.7, 89.3, 67.6 and 40.1 μM, respectively. The flavonoids and some of the cycloartane-type triterpenes appeared to be the hepatoprotective principles of the leaves of *C. quadrangulare*.

Key words  *Combretum quadrangulare*; hepatoprotective effect; flavone; cycloartane-type triterpene; flavone C-glycoside

*Combretum* species (Combretaceae) are widely distributed in Asia and Africa. These plants are used as folk medicine for the treatment of hepatitis, malaria, respiratory infection and even cancer by rural people of different parts of Vietnam, India and Somalia. *C. quadrangulare* is an evergreen tree indigenous to eastern Asia commonly known as “Tram bar” in Vietnam. The leaves and seeds as well as stem bark of the plant are used in Vietnamese traditional medicine as antihepatitis, antipyretic, antidyserteric, and anthelminthic agents. As a part of our continuing studies on Vietnamese medicinal plants, we found that the MeOH extract of the leaves of *C. quadrangulare* showed promising hepatoprotective effect on *p*-galactosamine (*p*-GalN)/lipopolysaccharide (LPS)-induced experimental liver injury in mice. Further chemical examination of the MeOH extract afforded 30 cycloartane-type triterpenes together with betulinic acid (28), β-sitosterol (18), β-sitosterol glucoside (19), 4 flavones, and 3 flavone C-glucosides. Among them some of the cycloartane-type triterpenes and flavones possessed cytotoxic activity towards liver-metastatic murine colon 26-L5 carcinoma cells. We further examined the hepatoprotective effect of the isolated compounds on *p*-GalN/TNF-α-induced cell death in primary cultured mouse hepatocytes. In this paper, we wish to report the hepatoprotective activity of the MeOH extract of the leaves of *C. quadrangulare* and its constituents.

MATERIALS AND METHODS

Plant Material  Leaves of *C. quadrangulare* Kurz were purchased at a local market at Ho Chi Minh City, Vietnam in 1995. A voucher sample (TMPW 18999) is preserved in the Museum for Materia Medica, Toyama Medical and Pharmaceutical University, Toyama, Japan, as a reference.

Extraction and Isolation  Air dried leaves (2.65 kg) were extracted by refluxing with MeOH (161, 3 h×3). The filtrate was evaporated under reduced pressure to yield a dark green MeOH extract (610 g). A part of the MeOH extract (400 g) was chromatographed on silica gel with a CHCl₃/MeOH gradient system to give eleven fractions. The isolation procedures of compounds 1—17, 19—37 from fractions 3—9 were reported in the previous papers. Fractions 10 and 11 were combined and the combined fraction (60.2 g) was chromatographed on Diaion HP-20 and eluted with water and then acetone to give water eluate (40.0 g) and acetone eluate (15.1 g). The water eluate was further chromatographed on Cosmosil 75C₂₅-OPN with H₂O–MeOH–CH₃CN (1:1:1) to give twenty subfractions. Reversed-phase preparative TLC (MeOH–H₂O, 1:1) of the subfractions 12, 15 and 17 gave vitexin (40, 6.7 mg), isovitexin 4′-methyl ether (39, 62.4 mg), and isoorientin (38, 49.9 mg), respectively. Their structures were confirmed by comparing the spectral data with those in the literature. The acetone-soluble part gave β-sitosterol glucoside (33, 15.1 mg) as a precipitate and confirmed by comparing the 'H- and 13C-NMR data with those of an authentic sample.

Chemicals  *p*-GalN and collagenase were from Wako Pure Chemicals Industry (Osaka, Japan). LPS (Escherichia coli 055: B5) was from Difco Laboratories (Detroit, MI, U.S.A.). Mouse recombinant TNF-α, sildalin, William’s E medium, bovine serum albumin (BSA), insulin, dexamethasone, penicillin G, streptomycin and 3-(4,5-dimethylthiazol-2-yl)-2,5-dime-thyltetrazolium bromide (MTT) were purchased from Sigma Chemicals (St. Louis, MO, U.S.A.). Ethyleneglycol-0,0-bis(2-aminoethyl)-N,N,N′-tetraacetic acid (EGTA) was purchased from Fluka Chemie (Switzerland). Heat-inactivated calf serum and Hanks’ balanced salt solution (HBSS) were from Gibco BRL Products (Gaithersburg, MD, U.S.A.). Falcon primary surface-modified polystyrene culture plates with 96 wells were from Becton Dick-
Animals Male ddY mice, 6-weeks-old (30—32 g) were used for the d-GalN/LPS-induced liver model. All animals were purchased from Shizuoka Laboratory Animal Center (Shizuoka, Japan) and were maintained on a 12 h light/dark cycle in a temperature and humidity controlled room. The animals were fed with a laboratory pelleted chow (CE-2; CLEA Japan Inc., Tokyo, Japan) and water ad libitum before the experiment.

d-GalN/LPS-Induced Liver Injury in Mice Liver injury was introduced by d-GalN/LPS in mice according to the method of Tieg et al. In each group 7 or 10 mice were used. After 12 h fasting, mice were given an i.p. injection of d-GalN (700 mg/kg) and LPS (20 μg/kg). The MeOH and water extracts of *C. quadrangularare* leaves were given s.c. injection of 50 mg/kg, twice at 18 and 2 h before d-GalN/LPS challenge. Blood alanin aminotransferase (ALT) levels were examined 8 h postinjection of d-GalN/LPS to evaluate the extent of liver damage.

TNF-α-Induced Cell Death in Primary Cultured Mouse Hepatocytes Mouse liver parenchymal cells were isolated according to the procedure described previously by Seglen et al. In brief, the liver was perfused with Ca²⁺-free HBSS containing 0.5% BSA and 5 mM EGTA, then recirculated with collagenase solution composed of Ca²⁺-free HBSS, 0.075% collagenase, 4 mM CaCl₂, and 0.005% trypsin inhibitor. Isolated hepatocytes were cultured in William's E medium supplemented with 10% calf serum, 100 IU/ml peni-
RESULTS AND DISCUSSION

The d-GalN/LPS-induced liver failure model in mice is now recognized as a promising experimental basis for the understanding of the mechanism of clinical liver complaints and for the evaluation of the efficiency of hepatoprotective activity. Using this model, we have evaluated many traditional medicines for their hepatoprotective effect. The MeOH and water extracts of leaves of C. quadrangularis were also tested for their hepatoprotective effect on d-GalN/LPS-induced liver injury in mice. At 8 h after d-GalN/LPS challenge, the blood ALT level was elevated to 1888±554 U/L in the d-GalN/LPS-treated group, while in the normal group the blood ALT level was 66±17 U/L at the same time interval. The blood ALT level of the group pretreated by the MeOH extract significantly decreased to 493±106 U/L whereas the group pretreated by the water extract showed a blood ALT level of 1701±301 U/L (Table 1). This result suggested that the MeOH extract of leaves of C. quadrangularis possessed potent hepatoprotective effect.

During d-GalN/LPS-induced liver injury, LPS stimulates macrophages to secret various pro-inflammatory cytokines, including interleukin-1 (IL-1), IL-6 and considerable amounts of TNF-α. TNF-α induces hepatocyte apoptosis and neutrophil transmigration that works as a critical step for the hepatocyte necrosis occurring at the later stage of this liver injury. Thus, TNF-α plays a central role in the pathogenesis of this liver injury. We further tested the hepatoprotective activity of both MeOH and water extracts of leaves of C. quadrangularis on d-GalN/TNF-α-induced cell death in primary cultured mouse hepatocytes. In this system, hepatocytes apoptosis was reported to occur beginning from 8 h and lasting until 20 h and necrosis was later at 16 h after d-GalN/TNF-α-challenge, findings similar to those in vivo but with a time lag. Both MeOH and water extracts of the leaves showed hepatoprotective effect dose-dependently with cell survival rates of 88.2±8.8% and 60.0±6.7%, respectively, at 100 µg/ml concentration as compared to control which have cell survival rate 43.7±5.7% (Table 2). This result indicated that the MeOH extract had stronger hepatoprotective effect than the water extract, which is parallel to that obtained from the in vivo experiment.

Considering the above results, we further subjected the MeOH extract to chemical investigation, which afforded forty compounds including thirty cycloartane-type triterpenes (1—17, 20—27 and 29—33, all are new except 10 and 11), betulinic acid (28), β-sitosterol (18), β-sitosterol glucoside (19), 4 flavones [kanakenin (34), 5,7,4′-trihydroxy-3,3′-dimethoxyflavone (35), 5,4′-dihydroxy-3,7,3′-trimethoxyflavone (36), isokaempferide (37)] and three flavones C-glucosides [sooorientin (38), isovitexin 4′-methyl ether (39), vitexin (40)].

### Table 1. Hepatoprotective Effects of C. quadrangularis on LPS-Induced Liver Injury in d-GalN-Sensitized Mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>n</th>
<th>ALT level (U/L)</th>
<th>HP effect (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>d-GalN/LPS treated control</td>
<td>7</td>
<td>66±17</td>
<td>10</td>
<td>1888±554</td>
</tr>
<tr>
<td>C. quadrangularis</td>
<td>10</td>
<td>493±106</td>
<td>76.5</td>
<td></td>
</tr>
<tr>
<td>MeOH extract</td>
<td>50</td>
<td>1701±301</td>
<td>10.0</td>
<td></td>
</tr>
<tr>
<td>Water extract</td>
<td>100</td>
<td>60.0±6.7</td>
<td>37.3</td>
<td></td>
</tr>
</tbody>
</table>

Results are the mean±S.E., *p<0.05. Student's t-test was used for statistical evaluation of ALT level. a) Each extract was administered at 50 mg/kg through subcutaneous injection, 2 times at 18 and 2 h before d-GalN/LPS challenge. b) Blood ALT level was measured at 8 h after d-GalN/LPS injection. c) Hepatoprotective effect (HP effect, %) was determined by evaluation of serum enzyme level in comparison with the d-GalN/LPS-treated control.

### Table 2. Hepatoprotective Effects of Different Extracts of C. quadrangularis Leaves on d-GalN/TNF-α-Induced Cell Death in Primary Cultured Mouse Hepatocytes

<table>
<thead>
<tr>
<th>Extract and fractions</th>
<th>Concentration (µg/ml)</th>
<th>Cell survival rate±S.D. (% of normal)</th>
<th>Inhibition (% of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>100.0±7.6</td>
<td>43.7±5.7</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>113.2±7.9</td>
<td>88.2±8.8</td>
<td>53.2±6.2</td>
</tr>
<tr>
<td>MeOH extract</td>
<td>200</td>
<td>61.5±5.4</td>
<td>60.0±6.7</td>
</tr>
<tr>
<td>Water extract</td>
<td>100</td>
<td>60.0±6.7</td>
<td>37.3</td>
</tr>
</tbody>
</table>

Results are expressed as mean±S.D. (n=4; for normal and control, n=8). *p<0.05, **p<0.01. Significantly different from control.

The isolated compounds were tested for their hepatoprotective effect at 200, 100 and 50 µM concentration and showed various potencies of hepatoprotective effect on d-GalN/TNF-α-induced cell death in primary cultured mouse hepatocytes (Table 3). These compounds which have interesting hepatoprotective effect were tested at lower concentration and the results are summarized in Table 4. Among the cycloartane-type triterpenes, quadrangularol B (29, IC50 34.3 µM) and methyl quadrangularate 1 (33, IC50 33.7 µM) possessed the strongest inhibitory effect on TNF-α-induced cell death in primary cultured mouse hepatocytes. Methyl quadrangularates A (30) and N (32) and norquadrangularic acid B (31) showed an IC50 value of 45.7, 89.3 and 67.6 µM, respectively. Among the rest 11, 12, 13, 16, 17, 27 and epimeric mixture of 6 and 7 had weak hepatoprotective activity against d-GalN/TNF-α-induced cell death in primary cultured mouse hepatocytes. All these compounds possessed stronger hepatoprotective effect than glycyrrhizin and the first 2 (29 and 33) had stronger inhibitory activity than clinically used positive control silibinin (IC50 39.6 µM). Both of the 29-norcycloartanes (27 and 31) and all the triterpenes with an α,β-unsaturated ketone (16, 29 and 32) possessed hepatoprotective activity. Thus, it seems that 29-norskeleton and the α,β-unsaturated ketone play an important role for the hepatoprotective activity of cycloartane-type triterpenes. Regarding the previous literature, triterpenes belonging to urane, oleane and dammarane were reported to have hepatoprotective activity. Several studies were done on glycyrrhizin, an oleane saponin, used as a hepatoprotective
Table 3. Hepatoprotective Effects of Compounds Isolated from the MeOH Extract of \textit{C. quadrantrangle} Leaves on \(\beta\)-GalN/TNF-\(\alpha\)-Induced Cell Death in Primary Cultured Mouse Hepatocytes

<table>
<thead>
<tr>
<th>Compds.</th>
<th>Conc. ((\mu)M)</th>
<th>Cell survival rate±S.D. (% of normal)</th>
<th>% Inh(^a)</th>
<th>Compds.</th>
<th>Conc. ((\mu)M)</th>
<th>Cell survival rate±S.D. (% of normal)</th>
<th>% Inh(^a)</th>
<th>Compds.</th>
<th>Conc. ((\mu)M)</th>
<th>Cell survival rate±S.D. (% of normal)</th>
<th>% Inh(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal-1</td>
<td>100.0±4.8</td>
<td>53.9±9.1</td>
<td>6.7</td>
<td>200.7±7.1</td>
<td>47.3±3.5</td>
<td>20.0±7.1</td>
<td>33.3</td>
<td>Glycyrhrizin</td>
<td>200</td>
<td>66.4±2.9</td>
<td>16.7</td>
</tr>
<tr>
<td>Control-1</td>
<td>10.5±6.9</td>
<td>67.4±2.3</td>
<td>25.2</td>
<td>200</td>
<td>37.7±7.4</td>
<td>20.0</td>
<td>9.8±0.7</td>
<td>100</td>
<td>19.9±4.8</td>
<td>8.0</td>
<td>19.9±4.8</td>
</tr>
<tr>
<td>Silibin</td>
<td>100</td>
<td>86.7±1.4</td>
<td>60.9</td>
<td>50</td>
<td>41.9±2.2</td>
<td>10.9</td>
<td>100</td>
<td>58.0±5.3</td>
<td>1.9</td>
<td>50</td>
<td>46.0±5.3</td>
</tr>
<tr>
<td>200</td>
<td>50</td>
<td>25</td>
<td>37.8</td>
<td>50</td>
<td>47.1±7.0</td>
<td>50</td>
<td>47.1±7.0</td>
<td>50</td>
<td>48.3±2.3</td>
<td>50</td>
<td>48.3±2.3</td>
</tr>
<tr>
<td>10</td>
<td>100</td>
<td>47.2±5.0</td>
<td>0.6</td>
<td>200</td>
<td>24.0±2.4</td>
<td>22</td>
<td>24.0±2.4</td>
<td>200</td>
<td>49.3±1.7</td>
<td>100</td>
<td>49.3±1.7</td>
</tr>
<tr>
<td>50</td>
<td>100</td>
<td>35.2±2.5</td>
<td>1.3</td>
<td>50</td>
<td>44.1±4.0</td>
<td>100</td>
<td>44.1±4.0</td>
<td>50</td>
<td>50.7±5.7</td>
<td>100</td>
<td>50.7±5.7</td>
</tr>
<tr>
<td>200</td>
<td>50</td>
<td>20.0</td>
<td>1.3</td>
<td>50</td>
<td>43.2±6.0</td>
<td>50</td>
<td>43.2±6.0</td>
<td>50</td>
<td>54.6±3.6</td>
<td>50</td>
<td>54.6±3.6</td>
</tr>
</tbody>
</table>

Results are expressed as mean±S.D. (\(n=4\) for normal and control, \(n=8\), \(* p<0.05, ** p<0.01\). Significantly different from control. \(a\) Inhibition (% of control).

agent with membrane-stabilizing activity. But in the present study glycyrhrizin exhibited very weak hepatoprotective activity on \(\beta\)-GalN/TNF-\(\alpha\)-induced cell death in primary cultured mouse hepatocytes (Table 3). The mechanism of the hepatoprotection by cycloartenyl-type triterpenes is not clear by this study, but these cycloartanes may have a different protection mechanism than that of glycyrhrizin. Furthermore, it is worthy to note here that this is the first report of a hepatoprotective effect for cycloartenyl-type triterpenes.

All the flavonoids, with (38—40) or without C-glucoside (34—37), possessed an inhibitory effect on TNF-\(\alpha\)-induced cell death in a concentration-dependent manner. In comparison to flavone C-glucosides, flavones without C-glucoside possessed stronger inhibitory effects. Kamatakenin (34), 5,7,4'-trihydroxy-3,3'-dimethoxyflavone (35), 5,4'-dihydroxy-3,3',3-trimethoxyflavone (36) and isokaempferide (37) have IC\(_{50}\) values of 13.3, 22.4, 13.4 and 22.8 \(\mu\)M, respectively, whereas the clinically-used silibinin had an IC\(_{50}\) value of 39.6 \(\mu\)M. The flavone C-glucoside, vitexin (40) also has a similar extent of inhibitory effect (IC\(_{50}\), 40.1 \(\mu\)M) to that of silibinin and the remaining two C-glucosides, 38 and 39, had weaker inhibitory activity. \(\beta\)-Sitosterol glucoside also possesses weak hepatoprotective activity at 200 \(\mu\)M (56.0±5.9% cell survival rate as compared to 47.7±3.5% of control).

Increasing evidence indicates that the total balance between reactive oxygen species (ROS) and antioxidants possibly affects the signalling mechanisms of various responses to TNF-\(\alpha\). All the above flavonoids are known to be radical scavengers. Therefore, though other mechanisms such as attenuation of the inhibition of protein synthesis induced by \(\beta\)-GalN(22) and enzyme (i.e., lipooxygenase) inhibition(23) may be implicated in their actions against cell death, the radical scavenging activity may play the most important role. That is, by scavenging the ROS induced by TNF-\(\alpha\), they could interfere with the signal transduction triggered by TNF-\(\alpha\), and thus could protect from the subsequent cell injury.
Table 4. Hepatoprotective Effects of Compounds Isolated from the MeOH Extract of C. quadangularis Leaves on α-GalN/TNF-α-Induced Cell Death in Primary Cultured Mouse Hepatocytes

<table>
<thead>
<tr>
<th>Compds.</th>
<th>Conc. (μM)</th>
<th>Cell survival rate ± S.D. (as % of normal)</th>
<th>Inhibition (% of control)</th>
<th>IC₅₀ (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silybin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal-1</td>
<td>40</td>
<td>99.9 ± 5.9</td>
<td>39.6°</td>
<td></td>
</tr>
<tr>
<td>Control-1</td>
<td></td>
<td>42.4 ± 3.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal-2</td>
<td>32</td>
<td>59.8 ± 9.5**</td>
<td>60.3</td>
<td>89.3</td>
</tr>
<tr>
<td>Control-2</td>
<td></td>
<td>46.2 ± 3.2**</td>
<td>23.9</td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>50</td>
<td>73.4 ± 3.1**</td>
<td>37.3</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>50</td>
<td>71.2 ± 2.3**</td>
<td>68.9</td>
<td>45.7</td>
</tr>
<tr>
<td>31</td>
<td>50</td>
<td>69.5 ± 2.6**</td>
<td>64.0</td>
<td>45.7</td>
</tr>
<tr>
<td>32</td>
<td>50</td>
<td>63.3 ± 4.1**</td>
<td>49.4</td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>50</td>
<td>58.2 ± 5.2**</td>
<td>37.3</td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>50</td>
<td>59.9 ± 4.5**</td>
<td>34.4</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>50</td>
<td>52.9 ± 1.0**</td>
<td>42.6</td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>50</td>
<td>61.1 ± 4.4**</td>
<td>42.6</td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>50</td>
<td>53.6 ± 1.4**</td>
<td>42.6</td>
<td></td>
</tr>
<tr>
<td>38</td>
<td>50</td>
<td>61.0 ± 4.2**</td>
<td>37.3</td>
<td></td>
</tr>
<tr>
<td>39</td>
<td>50</td>
<td>52.6 ± 2.6**</td>
<td>44.7</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>50</td>
<td>65.1 ± 1.7**</td>
<td>22.4</td>
<td></td>
</tr>
<tr>
<td>41</td>
<td>50</td>
<td>54.3 ± 2.1**</td>
<td>45.5</td>
<td></td>
</tr>
<tr>
<td>42</td>
<td>50</td>
<td>43.0 ± 2.4**</td>
<td>14.3</td>
<td></td>
</tr>
<tr>
<td>43</td>
<td>50</td>
<td>73.1 ± 1.4**</td>
<td>97.6</td>
<td>13.4</td>
</tr>
<tr>
<td>44</td>
<td>50</td>
<td>60.4 ± 2.0**</td>
<td>61.8</td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>50</td>
<td>53.6 ± 5.2**</td>
<td>61.8</td>
<td></td>
</tr>
<tr>
<td>46</td>
<td>50</td>
<td>53.8 ± 0.7**</td>
<td>61.8</td>
<td>22.8</td>
</tr>
<tr>
<td>47</td>
<td>50</td>
<td>52.3 ± 1.4**</td>
<td>40.3</td>
<td>15.6</td>
</tr>
<tr>
<td>48</td>
<td>50</td>
<td>43.1 ± 4.8**</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Results are expressed as mean ± S.D. (n=4; for normal and control, n=8). *p < 0.05, **p < 0.01. Significantly different from control. a) IC₅₀ value was calculated from the data shown in Table 3.

In conclusion, the present report shows that the MeOH extract of the leaves of C. quadangularis showed significant hepatoprotective activity both in vitro and in vivo experiments. Flavonoids and a flavone C-glucoside, vitexin, were isolated as active components of the MeOH extract and some of the cycloartenyl-type triterpenes also possessed potent hepatoprotective activity. Though the hepatoprotective effect of these compounds, with consideration of their yields, were weaker than the MeOH extract, these compounds may have contributed to the hepatoprotective activity of the MeOH extract of the leaves of C. quadangularis.

Acknowledgment This work was supported in part by a Grant-in-Aid for International Scientific Research (No. 09041177) from the Ministry of Education, Science, Sports, and Culture, Japan.

REFERENCES AND NOTES