Beneficial effect of Toona sinensis Roemor on improving cognitive performance and brain degeneration in senescence-accelerated mice

Jiunn-Wang Liao¹, Cheng-Kuang Hsu², Ming-Fu Wang³, Wen-Min Hsu³ and Yin-Ching Chan³*

¹Graduate Institute of Veterinary Pathology, National Chung Hsing University, Taichung, Taiwan, Republic of China
²Department of Biotechnology and Bioinformatics, Asia University, Wufung, Taichung County, Taiwan, Republic of China
³Department of Food and Nutrition, Providence University, Taiwan, Republic of China

(Received 30 September 2005 – Revised 29 March 2006 – Accepted 30 March 2006)

The purpose of the present study was to examine the effects of Toona sinensis Roemor extracts on antioxidative activities, brain morphological changes and cognitive ability. In an in vitro study, the antioxidant capacities of water extracts from Toona sinensis Roemor leaf (TSL), root (TSR) and bark (TSB) were evaluated by an α,α-diphenyl-β-primryl-hydrazyl radical-scavenging test. The results showed that the scavenging activities of all Toona sinensis Roemor extracts were over 80% at a concentration of 0.625 mg/ml. In an in vivo study, 3-month-old male senescence-accelerated-prone 8 mice were used as the tested subjects and fed four different diets: casein diet or casein diet supplemented with 1% TSL, TSR or TSB extract for 12 weeks. The results showed that the mice supplemented with Toona sinensis Roemor extracts demonstrated significantly less amyloid β-protein deposition and lower levels of thiobarbituric acid-reactive substances than the control group. All Toona sinensis Roemor diet groups also showed better active shuttle avoidance responses, and higher superoxide dismutase, catalase and glutathione peroxidase activities, than the control group. It can thus be concluded that supplementation with either TSL, TSR or TSB extract could not only reduce the incidence of β-amyloid plaques, but also improve learning and memory ability in senescence-accelerated-prone 8 mice. This might be due to the beneficial effects of Toona sinensis Roemor extracts on promoting the antioxidative defence system.

Toona sinensis Roemor: Cognitive ability: Amyloid β-protein deposition: Antioxidative system

Free radical-mediated reactions and reactive oxygen species have been proposed to be the major contributors to the pathological processes of neurodegenerative diseases such as Alzheimer’s and Parkinson’s disease. Alzheimer’s disease is accompanied by cognitive impairment as well as involving the pathological build-up of extracellular, vascular and parenchymal deposits of a 40–42-amino peptide, amyloid β-protein. It has been demonstrated that oxidative damage increased both in the brains of individuals with Alzheimer’s disease (Friedlich & Butcher, 1994; Smith et al. 1997; Montine et al. 1999) and in β-amyloid plaque-forming transgenic mouse models of Alzheimer’s disease (Perry & Smith, 1997; Pappolla et al. 1998; Smith et al. 1998), suggesting that an elevated level of amyloid β-protein is sufficient to stimulate oxidative damage. In an in vitro study, a direct application amyloid β-protein to neuronal cells increased the production of H₂O₂ (Behl et al. 1994), leading to amyloid β-protein neurotoxicity, which could be prevented by the use of antioxidants (Behl et al. 1992; Goodman et al. 1994).

The development of rodent models of Alzheimer’s disease will elucidate some important aspects of the aetiology of the disease and the development of therapeutic methods. In a murine model of accelerated senescence (senescence-accelerated mouse), severe senile amyloidosis is one of the most characteristic age-associated disorders (Higuchi et al. 2004). Ohta et al. (1989) and Flood & Morley (1993) have reported that the senescence-accelerated-prone 8 (SAMP8) mouse exhibits a remarkable age-related deterioration in its learning ability and memory. It is therefore an excellent Alzheimer’s disease model for studying the pathological changes in the brain and deficits in cognitive ability.

Toona sinensis Roemor is an upland tree that is widely distributed in southern Taiwan as well as in China. The leaves of Toona sinensis Roemor are a vegetable that has been very popular with vegetarians in Taiwan. The tender leaves and stem have been used as a carminative and to treat enteritis, dysentery and itch in oriental medicine (Edmonds & Staniforth, 1998). The crude extract of Toona sinensis Roemor leaf (TSL) has been proved to possess antiproliferative effects and also to promote the apoptosis of human lung cancer cells (Chang et al. 2001). Hsu et al. (2003) reported that TSL Roemor leaf extract could increase lipolytic activity in 3T3-L1 adipocytes by mediating the protein kinase C pathway. In addition, studies have pointed out that a water extract of Toona sinensis Roemor is able to enhance glucose uptake in 3T3-L1 adipocytes (Yang et al. 2003), increase the level of GLUT 4 protein and mRNA expression, and reduce blood sugar level in Alloxan-induced diabetes mellitus mice (Wang, 2000). The purposes of the present study were to

Abbreviations: CAT, catalase; DPPH, α,α-diphenyl-β-primryl-hydrazyl; GSH-Px, glutathione peroxidase; SAMP8, senescence-accelerated-prone 8; SOD, superoxide dismutase; TBARS, thiobarbituric acid-reactive substances; TSB, Toona sinensis Roemor bark; TSL, Toona sinensis Roemor leaf; TSR, Toona sinensis Roemor root.

* Corresponding author: Dr Y.-C. Chan, fax +86 4 26530027, email ycchan@pu.edu.tw
examine the α,α-diphenyl-β-ricryl-hydrayl (DPPH) radical-scavenging activities of water extracts from TSL, root (TSR) and bark (TSB), as well as to evaluate the effects of the antioxidant activities of these extracts on brain morphological changes, antioxidant status and cognitive ability in memory-deficient SAMP8 mice.

Methods

Procedures for Toona sinensis Roemor extraction

TSL, TSR and TSB were cut into small pieces, weighed and extracted with water (1:4 w/v) at 100°C for 30 min, 4 h and 4 h, respectively. The supernatants were then obtained using a vacuum evaporator (rotary vacuum evaporator, type N-1; Eyela, Tokyo, Japan) under reduced pressure. The extracts of TSL, TSR and TSB were obtained at yields of 6 %, 9 % and 10 %, respectively. These residues were stored at −86°C until use.

α,α-Diphenyl-β-ricryl-hydrayl radical-scavenging ability of Toona sinensis Roemor

The DPPH radical-scavenging activities of the water extracts of TSL, TSR and TSB were determined according to the method of Yamaguchi et al. (1990). DPPH, ascorbic acid (vitamin C) and butylated hydroxytoluene were purchased from Sigma Chemical Co. (St Louis, MO, USA). All other chemicals were reagent grade or purer. An aliquot of the extract (0.1 ml, range approximately 0.156–15 mg/ml), vitamin C (0.1 ml, approximately 0.156–15 mg/ml), butylated hydroxytoluene were purchased from Sigma Chemical Co. (St Louis, MO, USA). All other chemicals were reagent grade or purer. An aliquot of the extract (0.1 ml, range approximately 0.156–15 mg/ml), vitamin C (0.1 ml, approximately 0.156–15 mg/ml), butylated hydroxytoluene (0.1 ml, approximately 0.04–1.25 mg/ml) or butylated hydroxytoluene (0.1 ml, approximately 0.04–1.25 mg/ml) was mixed with 0.4 ml Tris-HCl buffer (100 mm, pH 7.4). This was then added to 0.5 ml 250 μm-DPPH in ethanol. The mixture was allowed to react in the dark at room temperature. After 20 min, the absorbance of the resulting solution was measured spectrophotometrically at 517 nm (Ultrospec 500/1100 pro UV/Vis; BioChrom, Cambridge, UK) and converted into the percentage antioxidant activity using the following equation:

\[
\text{Scavenging effect (} \% \text{)} = \left( 1 - \frac{A_{\text{Sample}} \text{(517 nm)}}{A_{\text{Control}} \text{(517 nm)}} \right) \times 100 \%
\]

Animals and food intakes

Three-month-old SAMP8 mice were utilized in this experiment. About five mice were housed per cage under controlled environmental conditions (25 ± 2°C, 65 ± 5 % relative humidity, 07.00–19.00 hours lighting period). The mice were divided into control, 1 % TSL, 1 % TSR and 1 % TSB diet groups (sixteen per group; Table 1) and allowed free access to the diets for 12 weeks. The food intake of the mice in each cage was recorded every day and the value divided by the number of the mice in that cage to represent the mean food intake for those mice. The mice were weighed weekly. The study protocol was approved by the animal research ethics committee at Providence University, Taichung, Taiwan.

Memory evaluation

At 1 week before the learning and memory examination, the mice were placed individually in cubic boxes (sides 25 cm), and the ambulatory activity of all the mice was then measured for 10 min with a video-activity monitor (E61-21; Coulbourn Instruments, Philadelphia, PA, USA). The mice that failed to pass this test were excluded from the subsequent tests; about two or three mice were removed from each group.

An active avoidance test was performed to evaluate the learning and memory ability of the tested mice after feeding them the experimental diets for 12 weeks. Before receiving subsequent stimuli, each mouse exposed to this test was placed for 10 s in a shuttle box (35 cm × 17 cm × 20 cm, width × length × height; model E10-15; Coulbourn Instruments), which was divided into two equal compartments connected by a small opening (7.5 cm × 6.5 cm). This allowed the mice to adapt to the box.

A successful avoidance response was logged if the mouse tested moved itself from one compartment to the other in the shuttle box after receiving a 10 s conditioned stimulus – a tone and a red, yellow and green light. If the mouse did not perform a successful avoidance response, an unconditioned stimulus of a 0.3 mA, 0.5 s scrambled foot shock was given immediately after the conditioned stimulus had been presented. Each mouse received four daily sessions of a combination of five conditioned/unconditioned conditioned stimulus trials, giving a total of twenty trials each day, for 4 consecutive days. Between the sessions, the mice were allowed to rest for 15–20 min. The avoidance responses of the mice were recorded automatically.

Table 1. Compositions of the experimental diets

<table>
<thead>
<tr>
<th>Ingredient (g/kg diet)</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Casein*</td>
<td>200.0</td>
</tr>
<tr>
<td>α-Starch</td>
<td>448.5</td>
</tr>
<tr>
<td>Sucrose</td>
<td>222.6</td>
</tr>
<tr>
<td>AIN-93G vitamin mix†</td>
<td>10.0</td>
</tr>
<tr>
<td>AIN-93G mineral mix†</td>
<td>50.0</td>
</tr>
<tr>
<td>Cellulose</td>
<td>20.0</td>
</tr>
<tr>
<td>Maize oil</td>
<td>50.0</td>
</tr>
<tr>
<td>Choline</td>
<td>2.5</td>
</tr>
<tr>
<td>Toona sinensis Roemor extracts</td>
<td>0.0</td>
</tr>
</tbody>
</table>

A, control group; B, 1 % Toona sinensis Roemor leaf extract; C, 1 % Toona sinensis Roemor root extract; D, 1 % Toona sinensis Roemor bark extract.

* Casein (92 % crude protein) was purchased from Oriental Yeast Co. (Tokyo, Japan).
† Obtained from ICN Pharmaceuticals Inc., Costa Mesa, CA, USA. Compositions of AIN-93G vitamin and mineral mixtures are given in Reeves (1997). For details of diets and procedures, see this page.

Measurements of brain amyloid β-protein

After the learning and memory examination, the mice were killed by ether anesthesia for the subsequent investigations. In order to examine the changes in brain morphology, the whole brains of the mice were quickly dissected and individually fixed in a 10 % buffered neutral formalin solution for 2 weeks. Brain regions at the A, B, C and D sections (as defined based on Popesko et al. (1992)) were dissected at intervals by quantitative morphometry.
in the hippocampus, an area important for learning and memory, is highly correlated with ageing in SAMPS8 mice (Farr et al. 2003). Because the B (middle) section of the brain stretching from the anterior to the posterior margin with a width of 4–6 mm (see Fig. 3(A) later) consists of neocortex, corpus callosum, hippocampus and thalamus, the B sections of all the mice were chosen for evaluation.

Brain tissues were processed by histopathological techniques, sectioned at 5 μm thickness by microtome (RM 2145; Leica, Nussloch, Germany) and observed under a light microscope (Optiphot-2; Nikon, Tokyo, Japan). Anti-β-amyloid immunohistochemistry staining in the B section of the brains was performed using the UltraTech H (DAB) Streptavidin-Biotin Universal Detection System (Immunotech, Cedex, France). To identify β-amyloid plaque, sections were immersed into 10 mmol citrate buffer and microwaved for three periods of 2 min each. The sections were then incubated with 1:300 v/v diluted primary anti-β amyloid antibody (monoclonal antibody to β-amyloid amino acids 17–24; Biodesign International, Saco, ME, USA) for 2 h at room temperature. Slides of the brains were incubated in polyvalent-biotinylated antibody (goat anti-rabbit antibodies, positively immunostained with a monoclonal antibody with monoclonal antibody to β-amyloid deposition in the hippocampus, an area important for learning and memory, is highly correlated with ageing in SAMPS8 mice (Farr et al. 2003). Because the B (middle) section of the brain stretching from the anterior to the posterior margin with a width of 4–6 mm (see Fig. 3(A) later) consists of neocortex, corpus callosum, hippocampus and thalamus, the B sections of all the mice were chosen for evaluation.

Brain tissues were processed by histopathological techniques, sectioned at 5 μm thickness by microtome (RM 2145; Leica, Nussloch, Germany) and observed under a light microscope (Optiphot-2; Nikon, Tokyo, Japan). Anti-β-amyloid immunohistochemistry staining in the B section of the brains was performed using the UltraTech H (DAB) Streptavidin-Biotin Universal Detection System (Immunotech, Cedex, France). To identify β-amyloid plaque, sections were immersed into 10 mmol citrate buffer and microwaved for three periods of 2 min each. The sections were then incubated with 1:300 v/v diluted primary anti-β amyloid antibody (monoclonal antibody to β-amyloid amino acids 17–24; Biodesign International, Saco, ME, USA) for 2 h at room temperature. Slides of the brains were incubated in polyvalent-biotinylated antibody (goat anti-rabbit antibodies, positively immunostained with a monoclonal antibody with which it appeared brownish in colour (see Fig. 3(B) later).

The β-amyloid-positive area of each brain section was measured according to Lim et al. (2000), with slight modification. Briefly, each mouse’s entire brain middle section was measured by an image analyser (Q500MC; Leica). Brain sections from eight mice in each group were measured under 40-fold magnifications, and more than twenty fields were totally counted in each entire brain section. We believed that counts from twenty different observation fields in a mouse brain and eight mice per group would be sufficient for the quantitation and statistical analysis of the difference in β-amyloid deposition between the groups. The percentage of β-amyloid-positive area was calculated using the following equation:

\[
\text{Percentage } \beta\text{-amyloid area} = \frac{\text{sum of amyloid-positive area}}{\text{total area of brain section}} \times 100\%.
\]

### Analyses of oxidative status

After the mice had been killed, their brain and liver tissues were immediately diluted by 50 mmol (pH 7.4) Na2PO4 buffer, respectively, homogenised and centrifuged at 3000 g for 10 min in a refrigerated centrifuge (Hettich Universal 16 R; Tuttlingen, Germany). The supernatants were then collected. The thiobarbituric acid-reactive substances (TBARS) concentration and the superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) activities of the supernatants were determined to evaluate the oxidative status of the brain and liver tissues.

The concentrations of TBARS in the tissue homogenates were measured according to the method described by Ohkawa et al. (1979). A mixture was obtained by mixing the supernatants with 2-thiobarbituric acid (4 g/kg in 0.2 mol HCl) and butylated hydroxytoluene (2 g/kg in 95 % ethanol) in a ratio of 1:2:0.3 v/v, and the mixture was then heated at 90°C for 45 min. After cooling the mixture, 5 ml n-butanol was added, and the n-butanol layer was separated by centrifuging at 1000 g for 10 min. The production of formazan was measured spectrophotometrically at 532 nm. Experimental data were expressed as μmol malondialdehyde μg tissue, using tetramethoxypropane as an external standard and double-distilled water as the control.

The method of Marklund & Marklund (1974) was applied to the measurement of SOD activity. A final 3.017 ml volume of the reaction systems contains 10 μl tissue homogenates, 50 mm-Tris-HCl (pH 8.2) and 50 mm-pyrogallol. The production of formazan was determined at 325 nm in a spectrophotometer at 25°C. One unit of SOD was defined as the amount of protein that inhibited the rate of pyrogallol reduction by 50%. The measurement was total SOD activity without discriminating between the isoforms of SOD.

CAT activity was determined using the method of Aebi (1983). An assay mixture consisted of 1.0 ml H2O2 (30 mm) and 2 ml of the supernatants of the tissue homogenates in a final volume of 3.0 ml. Changes in absorbance were recorded at 240 nm in a spectrophotometer at 25°C. CAT activity was calculated in terms of mole H2O2 consumed/min per g tissue protein.

GSH-Px activity was measured according to the method of Paglia & Valentine (1967). Briefly, the assay mixture consisted of 200 μl 5 (units/ml) glutathione reductase, 50 μl 40 mm-glutathione, 620 μl 0.25 mole phosphate buffer (pH 7.4), 100 μl tissue homogenates, 10 μl 20 mm-β-NADPH salt and 20 μl 15 mm-cumene hydroperoxide. The production of formazan was determined at 340 nm in a spectrophotometer at 25°C. One unit of GSH-Px was defined as the amount of μm-oxidative NADPH. Data for SOD, CAT and GSH-Px activities were expressed as unit/g protein. The Pierce Micro BCA protein assay was used to determine the soluble protein in the samples.

### Statistical analysis

All data were expressed as means and their standard errors and analysed using SPSS 8.0 software (SPSS Inc., Chicago, IL, USA). Data were evaluated by one-way ANOVA. The least significant difference test was used for pair-wise comparisons when the F test was significant. Differences were considered to be significant at the 0.05 level.

### Results

**α,α-Diphenyl-β-priseyl-hydrazyl radical-scavenging activity of Toona sinensis Roemor**

The dose–response curves for the DPPH radical-scavenging activities of Toona sinensis Roemor extracts, vitamin C and butylated hydroxytoluene are shown in Fig. 1. The results showed that 0.625 mg/ml concentrations of different Toona sinensis Roemor extracts were needed to obtain 80 % DPPH radical-scavenging activity; however, to achieve the same goal, 0.1 mg/ml vitamin C or 0.075 mg/ml butylated hydroxytoluene was required. The inhibitory concentration 50 % values of the DPPH radical-scavenging activities of TSL, TSR and TSB extracts were 2.09 × 10⁻¹, 2.85 × 10⁻¹, and 2.00 × 10⁻¹, respectively.
**Toona sinensis, memory and brain morphology**

403

**Effect of Toona sinensis Roemor on body weight, food intake and locomotory activity**

No significant differences ($P>0.05$) were shown in terms of accumulated body weights, organ weights and food intakes in 3-month-old SAMP8 mice from different diet groups: control, TSL, TSR and TSB (Tables 2 and 3). A locomotion activity test revealed that the ambulatory activities of the mice did not differ between the diet groups ($P>0.05$; data not shown).

**Effect of Toona sinensis Roemor on cognitive ability**

The successful avoidance times of SAMP8 mice from the TSL, TSR and TSB diet groups were greater than those from the control group on days 2, 3 and 4 (Fig. 2). This indicated that the addition of all *Toona sinensis* Roemor extracts to the diet could improve the cognitive ability of the SAMP8 mice. There was, however, no difference between the TSL, TSR and TSB groups. In all the *Toona sinensis* Roemor diet groups and the control group, the successful avoidance times on days 2, 3 and 4 were significantly higher than those on day 1, indicating that the mice were more familiar with the test and made better avoidance responses after day 1.

**Effect of Toona sinensis Roemor on brain morphology and oxidative status**

Fig. 3 shows the effect of *Toona sinensis* Roemor extracts on the morphological changes in the brain of SAMP8 mice. In comparison with the control group, lower amyloid β-protein accumulation was found in the brain tissues of the TSL, TSR and TSB diet groups. The TBARS concentrations and the activities of SOD, CAT and GSH-Px in the brain and liver tissues are shown in Table 4. Lower TBARS concentrations were observed in both the brain and liver tissues of the mice fed with the TSL, TSR and TSB diets than in those of the control group. No significant difference was found in TBARS level between the *Toona sinensis* Roemor diet groups except that the TSB group showed a lower TBARS concentration in the hippocampus than did the TSL group. SOD, CAT and GSH-Px activities in the hippocampus, striatum, cortex and liver were higher in the TSL, TSR and TSB diet groups than in the control group. Moreover, significantly high GSH-Px activity in the brain was noted in the TSB group. However, the total protein content of the tissues did not differ between the four groups. These results indicated that the diet supplemented with all *Toona sinensis* Roemor extracts could reduce brain degeneration and lipid peroxidation, and promote the antioxidative defence system.

**Discussion**

In the present study, the DPPH radical-scavenging activities of water extracts from TSL, TSR and TSB at various concentrations were measured. The results showed that the scavenging activities increased with the concentrations of *Toona sinensis* Roemor extracts up to a certain point (0.625 mg/ml), after which they levelled off.

The DPPH radical is one of the compounds that possesses a proton free radical with a characteristic absorption, which decreases significantly on exposure to proton-radical scavengers (Yamauchi et al. 1990). Chen & Ho (1995) demonstrated that the DPPH radical could be scavenged by antioxidants as a result of their hydrogen-donating abilities. Therefore, in the present study, the *Toona sinensis* Roemor extracts showed a concentration-dependent scavenging activity towards the DPPH radical, which may also be attributed to their hydrogen-donating abilities. The inhibitory concentration 50% values of the DPPH radical-scavenging activity of TSL, TSR and TSB were 2.09 x 10^{-1}, 2.85 x 10^{-1} and 2.77 x 10^{-1} mg/ml, respectively. Fang (2000) also found that *Toona sinensis* Roemor extract showed significant anti-superoxide formation activity and an inhibitory effect on xanthine oxidase, the inhibitory concentration 50% values being 2.04 x 10^{-1} mg/ml and

---

**Fig. 1.** The a,-diphenyl-b-2,4-6-trinitro-2,4-6-trinitrobenzene radical-scavenging activities of water extracts from *Toona sinensis* Roemor leaf extract (TSL, ▲), root extract (TSR, ▼) and bark extract (TSB, ◆), using commercial antioxidant butylated hydroxytoluene (□) and vitamin C (●) as the controls. For details of diets and procedures, see p. 401.

**Fig. 2.** The DPPH radical-scavenging activities of the water extracts from *Toona sinensis* Roemor on cognitive ability.

**Fig. 3.** The DPPH radical-scavenging activities of the water extracts from *Toona sinensis* Roemor on brain morphology.

---

**Table 2.** Body weights and food intakes in 3-month-old male senescence-accelerated-prone 8 mice fed with different diets for 12 weeks.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Initial (g) Mean ± SE</th>
<th>Final (g) Mean ± SE</th>
<th>Food intake (g/d) Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>16</td>
<td>27.37 ± 0.78</td>
<td>33.45 ± 0.75</td>
<td>5.73 ± 0.14</td>
</tr>
<tr>
<td>B</td>
<td>16</td>
<td>27.94 ± 0.40</td>
<td>34.04 ± 0.58</td>
<td>5.49 ± 0.15</td>
</tr>
<tr>
<td>C</td>
<td>16</td>
<td>27.78 ± 0.55</td>
<td>33.96 ± 0.55</td>
<td>5.49 ± 0.18</td>
</tr>
<tr>
<td>D</td>
<td>16</td>
<td>27.29 ± 0.85</td>
<td>34.17 ± 0.76</td>
<td>5.52 ± 0.16</td>
</tr>
</tbody>
</table>

Pair-wise comparisons were conducted employing a least significant difference test. There were no significant differences at $P<0.05$.

A, control group; B, 1% *Toona sinensis* Roemor leaf extract; C, 1% *Toona sinensis* Roemor root extract; D, 1% *Toona sinensis* Roemor bark extract.

For details of diets and procedures, see p. 401.

---

**Table 3.** The antioxidative defence system.

<table>
<thead>
<tr>
<th>Group</th>
<th>Concentration (mg/ml)</th>
<th>TBARS (nmol/ml)</th>
<th>SOD (U/mg protein)</th>
<th>CAT (U/mg protein)</th>
<th>GSH-Px (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.00</td>
<td>0.23</td>
<td>6.42</td>
<td>6.78</td>
<td>6.67</td>
</tr>
<tr>
<td>B</td>
<td>0.25</td>
<td>0.30</td>
<td>5.97</td>
<td>6.12</td>
<td>6.43</td>
</tr>
<tr>
<td>C</td>
<td>0.50</td>
<td>0.35</td>
<td>5.87</td>
<td>6.34</td>
<td>6.57</td>
</tr>
<tr>
<td>D</td>
<td>1.00</td>
<td>0.40</td>
<td>5.64</td>
<td>6.43</td>
<td>6.57</td>
</tr>
</tbody>
</table>

---

**Table 4.** The antioxidative defence system.

<table>
<thead>
<tr>
<th>Group</th>
<th>Concentration (mg/ml)</th>
<th>TBARS (nmol/ml)</th>
<th>SOD (U/mg protein)</th>
<th>CAT (U/mg protein)</th>
<th>GSH-Px (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.00</td>
<td>0.23</td>
<td>6.42</td>
<td>6.78</td>
<td>6.67</td>
</tr>
<tr>
<td>B</td>
<td>0.25</td>
<td>0.30</td>
<td>5.97</td>
<td>6.12</td>
<td>6.43</td>
</tr>
<tr>
<td>C</td>
<td>0.50</td>
<td>0.35</td>
<td>5.87</td>
<td>6.34</td>
<td>6.57</td>
</tr>
<tr>
<td>D</td>
<td>1.00</td>
<td>0.40</td>
<td>5.64</td>
<td>6.43</td>
<td>6.57</td>
</tr>
</tbody>
</table>
4.75 × 10⁻³ mg/ml, respectively. The present results proved that *Toona sinensis* Roemor extracts had antioxidative capacity in an *in vitro* situation.

In the present study, the learning and memory function of male SAMP8 mice was evaluated by the active avoidance test, and the results illustrated that the TSL, TSR and TSB diet groups all had better learning and memory ability than the control group on days 2, 3 and 4, indicating that the age-related memory deterioration in SAMP8 mice might be improved by administrating *Toona sinensis* Roemor extracts. Furthermore, the main difference in the learning between the groups was their speed of learning. The differences in the mean successful avoidance test trials between the control group on days 2, 3 and 4, indicating that the effects of *Toona sinensis* Roemor extracts declined with successive test days. Moreover, the mean successful avoidance test trials of the control and all *Toona sinensis* Roemor diet groups increased with time because the mice became more familiar with the task and made better avoidance responses.

The present results showed that *Toona sinensis* Roemor diet groups had lower amyloid β-protein levels than the control group, and that amyloid β-protein accumulation was negatively correlated to memory ability and oxidative status. It has been reported that amyloid β-protein significantly impairs spatial learning and working memory; it thus plays a critical role in the development of Alzheimer’s disease (Nabeshima et al. 1994; Nitta et al. 1997; Yamada et al. 1997; Yamada & Nabeshima, 2000). Morley et al. (2000) administered antibody to amyloid β-protein into the cerebral ventricles of SAMP8 mice and found that such treatment could alleviate the deficits in the acquisition and retention test performances. Yamada et al. (1999) suggested that antioxidants such as α-tocopherol could prevent the learning and memory deficits caused by amyloid β-protein accumulation. The present results suggested that the administration of *Toona sinensis* Roemor extracts could lessen the memory deterioration and brain degeneration of SAMP8 mice, possibly through a powerful antioxidative capacity.

According to Liu & Mori (1993), SAM mice showed an age-dependent increase in thioibarbituric acid reactivity as well as an age-dependent decrease in glutathione level in the brain. The levels of malondialdehyde and carbonyl in the hippocampus of SAMP8 mice were consistently higher than those seen in SAMR1 mice (Kim et al. 2002). Thus, the acceleration of senescence in SAM mice might correlate to the damage caused by free radicals. The age-dependent oxidative processes could be moderated by applying various antioxidants to improve cerebellar physiology and motor learning in aged rats (Bickford et al. 2000). The present study showed that a diet supplemented with TSL, TSR, and TSB extracts could result in a fall in TBARS concentration. Concerns have, however, been raised regarding the validity of the TBARS assay, including possible interference with other compounds in the sample, the heating conditions during the assay, the presence of Fe in the assay reagents and the rapid metabolism of malondialdehyde (Halliwell & Grootveld, 1987; Pincemail et al. 1996). It is therefore not recommended that this be used as the sole index of lipid peroxidation (Brown & Kelly, 1994).

Supplementation with *Toona sinensis* Roemor extracts also increased SOD, CAT and GSH-Px activities in the brain and liver tissue of SAMP8 mice. The present results thus indicated that the administration of *Toona sinensis* Roemor extracts could promote the antioxidative capacity of SAMP8 mice. It has been suggested that the oxidative imbalance observed in

### Table 3. Related organ weights in 3-month-old male senescence-accelerated-prone 8 mice fed with different diets for 12 weeks (Values are means with their standard errors)

<table>
<thead>
<tr>
<th>Group</th>
<th>Brain Mean</th>
<th>Brain SE</th>
<th>Heart Mean</th>
<th>Heart SE</th>
<th>Lung Mean</th>
<th>Lung SE</th>
<th>Liver Mean</th>
<th>Liver SE</th>
<th>Spleen Mean</th>
<th>Spleen SE</th>
<th>Kidney Mean</th>
<th>Kidney SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1.389</td>
<td>0.044</td>
<td>0.527</td>
<td>0.012</td>
<td>0.700</td>
<td>0.027</td>
<td>4.256</td>
<td>0.233</td>
<td>0.308</td>
<td>0.017</td>
<td>1.398</td>
<td>0.059</td>
</tr>
<tr>
<td>B</td>
<td>1.419</td>
<td>0.028</td>
<td>0.526</td>
<td>0.031</td>
<td>0.713</td>
<td>0.029</td>
<td>4.380</td>
<td>0.305</td>
<td>0.342</td>
<td>0.017</td>
<td>1.321</td>
<td>0.047</td>
</tr>
<tr>
<td>C</td>
<td>1.392</td>
<td>0.033</td>
<td>0.527</td>
<td>0.019</td>
<td>0.671</td>
<td>0.026</td>
<td>4.047</td>
<td>0.159</td>
<td>0.333</td>
<td>0.019</td>
<td>1.293</td>
<td>0.036</td>
</tr>
<tr>
<td>D</td>
<td>1.375</td>
<td>0.036</td>
<td>0.521</td>
<td>0.020</td>
<td>0.633</td>
<td>0.034</td>
<td>3.965</td>
<td>0.211</td>
<td>0.338</td>
<td>0.020</td>
<td>1.313</td>
<td>0.071</td>
</tr>
</tbody>
</table>

Pair-wise comparisons were conducted employing a least significant difference test. Values were not significant at P<0.05. A, control group; B, 1% *Toona sinensis* Roemor leaf extract; C, 1% *Toona sinensis* Roemor root extract; D, 1% *Toona sinensis* Roemor bark extract.

For details of diets and procedures, see p. 401.
Alzheimer’s disease could be due to a decrease in total antioxidant capacity (Guidi et al. 2006). Ulagadgi et al. (2005) found that supplementation with antioxidant vitamins could increase antioxidant capacity and reduce lipid peroxidation products. Thus, antioxidant supplementation may have a potential therapeutic effect in the area of Alzheimer’s disease.

It has been noted that antioxidants might function as free radical scavengers, stimulators of antioxidative enzymes and regulators of genetic expression. For example, melatonin, a hormone from the pineal gland of vertebrates, was found to be not only a strong free radical scavenger, but also a stimulator of antioxidative enzymes (Klepac et al. 2006). Tutunculer et al. (2005) found that melatonin could protect against brain injury by oxidative stress, and this protective effect may be due to its direct scavenging activity and activation of CAT. Feng et al. (2006) demonstrated that melatonin could reinstate the increase in TBARS levels as well as the reduction in SOD activity and glutathione content in a transgenic mouse model of Alzheimer’s disease. In addition, melatonin caused a significant reduction in the upregulated expression of Bax, caspase-3 and Par-4, thus inhibiting the triggering process of neuronal apoptosis in transgenic mice (Feng et al. 2006).

Cheng et al. (2006) investigated the effect of melatonin on symptoms of Alzheimer’s disease and found that melatonin improved cognitive function, acted against oxidative injury and apoptosis, and inhibited the formation of β-amyloid deposition and β-amyloid fibre. The present results also indicated that the Toona sinensis Roemor extracts showed radical-scavenging activity and could increase the activities of antioxidative enzymes. Further investigation will, however, be needed to determine whether the increases in the activities of SOD, CAT and GSH-Px resulted from the effects of Toona sinensis Roemor extracts on gene expression, and whether the Toona sinensis Roemor could cross into the circulation and perform direct scavenging.

The phytochemical components of Toona sinensis Roemor have been investigated; fifteen compounds have been isolated and identified, including methyl gallate, gallic acid, kaempferol, quercitin, quercitrin, rutin, catechin, epicatechin, oleic acid, palmitic acid, linoleic acid, linolenic acid, a mixture of β-sitosterol and stigmasterol, and β-sitosterol-glucose (Park et al. 1996; Hsieh et al. 1999; Tsai et al. 2001). Of these, methyl gallate has been demonstrated to possess antioxidant activity and to inhibit lipid peroxidation (Wu et al. 1998; Mei et al. 1999; Westenburg et al. 2000; Galato et al. 2001). Hsieh et al. (2004) demonstrated that methyl gallate extracted from TSR could suppress H2O2-mediated oxidative stress and DNA damage via scavenging of intracellular reactive oxygen species, inhibiting lipid peroxidation and preventing the depletion of intracellular glutathione level in Madin–Darby canine kidney cells (Hsieh et al. 2004).

In conclusion, the present results illustrated that supplementation with water extracts from TSL, TSR and TSB could reduce amyloid β-protein accumulation, TBARS and cognitive deterioration in mice. We also observed that all Toona sinensis Roemor extracts were effective in promoting the antioxidant system. The protective components of the Toona sinensis Roemor extracts that could slow the progression of ageing in mice are of interest and worth further study.
Acknowledgements

We are grateful to Dr Hseng-Kuang Hsu (Department of Physiology, School of Medical Sciences and Department of Internal Medicine, Kaohsiung Medical University, Taiwan) for providing the Toona sinensis Roemor extract samples. This study was supported by the National Science Council of Taiwan (NSC-93-2316-B-126-006), Republic of China.

References


Fang BJ (2000) The developmental of natural medicines from Taiwan on anti-oxidant and anti-herpes viruses activity. Masters Thesis, Kaohsiung Graduate Institute of Natural Products, Kaohsiung Medical University, Taiwan.


compounds: correlation among electrochemical, visible spectros
tscopy methods and structure-antioxidant activity. Redox Rep 6, 243–250.

Goodman G, Steiner MR, Steiner SM & Matsson MP (1994) Nordi-
hydroguaiaretic acid protects hippocampal neurons against amyloid 
β-peptide toxicity, and attenuates free radical and calcium accumu-

patients with mild cognitive impairment and Alzheimer’s disease.
Neurobiol Aging 27, 262–269.

Halliwell B & Grootveld M (1987) The measurement of free radical

Higuchi K, Xing Y, Fu X, Korenaga T, Guo Z, Nakanura A & Mori M
(2004) Mouse senile amyloidosis in the SAM model. In The Senes-
cence-accelerated Mouse (SAM): An Animal Model of Senescence,
pp. 35–40 [Y Nomura, T Takeda and Y Okuma, editors]. Inter-
national Congress Series 1260. Amsterdam: Elsevier BV.


Hsieh TJ, Liu TZ, Chia YC, Chen CL, Lu FJ, Chuang MC, Mau SY, 
Chen SH & Syu CH (2004) Protective effect of methyl gallate from
Toona sinensis (Meliaceae) against hydrogen peroxide-induced oxidative stress and DNA damage in MDCK cells. Food Chem Toxicol 42, 843–850.

Hsu HK, Yang YC, Hwang JH & Hong SJ (2003) Effects of Toona
sinensis leaf extract on lipolysis in differentiated 3T3-L1 adipocytes.

Kim HC, Bing G, Jhoo GW, Jhoo WK, Klepac N, Rudes Z & Klepac R

Biomed Pharmacother 60, 32–35.


Liu J & Mori A (1993) Age-associated changes in superoxide dismu-
tase activity, thiobarbituric acid reactivity and reduced glutathione 
level in the brain and liver in senescence accelerated mice (SAM): a

Marklund S & Marklund G (1974) Involvement of the superoxide
anion radical in the autooxidation of pyrogallol and a convenient

Mei L, McClements DJ & Decker EA (1999) Lipid oxidation in 
emulsions as affected by charge status of antioxidants and emul-

Montine TJ, Markesbery WR, Zackert W, Sanchez SC, Roberts LJ &
Morrow JD (1999) The magnitude of brain lipid peroxidation

Nabeshima T & Nitta A (1994) Memory impairment and neuronal

Merley JE, Kumar VB, Bernardo AE, Fary SA, Uezu K, Tumosa N

Nabeshima T & Nitta A (1994) Memory impairment and neuronal 
dysfunction induced by beta-amyloid protein in rats. Tohoku J 

infusion of beta-amyloid protein into cerebral ventricle induces 
learning impairment and neuronal and morphological degeneration. 

Ohkawa H, Ohishi N & Yagi K (1979) Assay for lipid peroxides
in animal tissues by thiobarbituric acid reaction. Anal Biochem 95, 
351–358.

Ohta A, Hirano T, Yagi H, Tanaka S, Hosokawa M & Takeda T

Paglia DE & Valentine WN (1967) Studies on the qualitative charac-

Pappolla MA, Chyan YJ, Omar RA, Hsiao K, Perry G, Smith MA &
Bozner P (1998) Evidence of oxidative stress and in vivo neuro-

Park JC, Yu YB, Lee JH, Choi JS & OK KD (1996) Phenolic com-
ounds from the rachis of Cedrela sinensis. Korea J Pharmacog-
ousy 27, 219–223.

Perry G & Smith MA (1997) A central role for oxidative damage in 
the pathogenesis and therapeutics of Alzheimer’s disease. 
Alzheimers Res 2, 319–324.

Pincencail J, Defraigne JO & Limet R (1996) Oxidative stress in 

The Anatomy of Laboratory Animals, vol. 2. London: Wolfe Publish-
ing.

Reeves PG (1997) Components of the AIN-93 diets as improvements in 
the AIN-76A diet. J Nutr 127, Suppl. 5, 8385–8415.

Smith MA, Hirai K, Hsiao K, Pappolla MA, Harris PLR, Siedlank SL, 

Smith MA, Richey Harris PL, Sayre LM, Beckman JS & Perry G

Tsal IL, Jeng YF, Duh CY & Chen IS (2001) Cytotoxic constituents 

Tutunculer F, Eskiçok S, Basaran UN, Ekekli G, Ayvaz S & 
Vatansever U (2005) The protective role of melatonin in experi-

Ullegaddi R, Powers HJ & Gariballa SE (2005) Antioxidant supple-
mentation enhances antioxidant capacity and mitigates oxi-

Wang PH (2000) The study of hypoglycemic effects of Toona sinen-
is aqueous leaf extracts on Alloxan-induced diabetic rats. Masters
Thesis, Graduate Institute of Medicine, Kaohsiung Medical Uni-
versity, Taiwan.

Westenburg HE, Lee KJ, Lee SK, Fong HH, van Bremen RB, Pezzuto 
JM & Kinghorn AD (2000) Activity-guided isolation of antioxidative 

Wu J, Sugiyama H, Zeng LH, Mickle D & Wu TW (1998) Evidence of 
Troxol and some gallates as synergistic protectors of erythro-


Yamada K, Tanaka T, Han D, Szenaki K, Kameyama T & 
Nabeshima T (1999) Protective effects of idebenone and 
alpha-tocopherol on beta-amyloid1-42 induced learning and
memory deficits in rats: implication of oxidative stress in beta-

Yamauchi N, Watanabe N & Kuriyama H (1990) Suppressive 
effects of intracellular glutathione on hydroxyl radical production 

Yang YC, Hsu HK, Hwang JH & Hong SJ (2003) Enhancement of 
glucose uptake in 3T3-L1 adipocytes by Toona sinensis leaf 