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

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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-pricryl-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 ß-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 H_2O_2 (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

examine the α,α-diphenyl-β-pricryl-hydrazyl (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 strained and then concentrated using an 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. The residues were stored at −86°C until use.

α,α-*Diphenyl-β-pricryl-hydrazyl 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 0·04–1·25 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 spectrophometrically at 517 nm (Ultrespec 500/1100 *pro* UV/Vis; BioChrom, Cambridge, UK) and converted into the percentage antioxidant activity using the following equation:

$$\text{Scavenging effect (\%)} = \left(1 - \frac{\mathbf{A}_{Sample\,(517\,nm)}}{\mathbf{A}_{Control\,(517\,nm)}}\right) \times 100\,\%$$

Animals and food intakes

Three-month-old SAMP8 mice were used 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),

Table 1. Compositions of the experimental diets

Ingredient(g/kg diet)	Group			
	A	B	C	D
Casein*	200·0	200·0	200·0	200·0
α-Starch	445·0	438·3	438·3	438·3
Sucrose	222·5	219·2	219·2	219·2
AIN-93G vitamin mix†	10·0	10·0	10·0	10·0
AIN-93G mineral mix†	50·0	50·0	50·0	50·0
Cellulose	20·0	20·0	20·0	20·0
Maize oil	50·0	50·0	50·0	50·0
Choline	2·5	2·5	2·5	2·5
Toona sinensis Roemor extracts	0·0	10·0	10·0	10·0

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.

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.

Measurements of brain amyloid β-protein

After the learning and memory examination, the mice were killed by ether anaesthesia 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 a mouse brain matrix to ensure that the sections analysed were equivalent between the tested mice (coronal slices model, Sunpoint 1 mm; Kent Scientific Corporation, CT, Tortington, USA). It is believed that an increase in β-amyloid deposition

in the hippocampus, an area important for learning and memory, is highly correlated with ageing in SAMP8 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 neo-cortex, corpus callosum, hippocampus and thalamus, the B sections of all the mice were chosen for evaluation.

Brain tissues were processed by histopathological tech-niques, sectioned at 5 μm thickness by microtome (RM 2145; Leica, Nussloch, Germany) and observed under a light microscope (Opticphot-2; Nikon, Tokyo, Japan). Anti-β-amy-loid immunohistochemistry staining in the B section of the brains was performed using the UltraTech H (DAB) Streptavi-din-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 poly-valent-biotinylated antibody (goat anti-rabbit antibodies, 1:1000 v/v) for 45 min, followed by DAB substrate for 30 min. They were then stained with Mayer's haematoxylin (Sigma Chemical Co.) for 3 min. The area of β-amyloid was 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 modifi-cation. Briefly, each mouse's entire brain middle section was measured by an image analyser (Q500MC; Leica). Brain sec-tions 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:

$$\text{Percentage } \beta\text{-amyloid area} = (\text{sum of amyloid-positive area}$$
$$/\text{total area of brain section}) \times 100\%.$$

Analyses of oxidative status

After the mice had been killed, their brain and liver tissues were immediately diluted by 50 mM (pH 7·4) Na_2PO_4 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) concen-tration and the superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) activities of the super-natants 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 centrifu-ging at 1000 *g* for 10 min. The TBARS concentration of the *n*-butanol layer was measured spectrophometrically at 532 nm. Experimental data were expressed as mol equivalent malondialdehyde μM/g 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 hom-ogenates, 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 H_2O_2 (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 cal-culated in terms of mole H_2O_2 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-gluta-thione, 620 μl 0·25 mole phosphate buffer (pH 7·4), 100 μl tissue homogenates, 10 μl 20 mM-ß-NADPH salt and 20 μl 15 mM-cumene hydroperoxide. The production of formazan was deter-mined 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-pricryl-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 buty-lated 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 $2·09 \times 10^{-1}$, $2·85 \times 10^{-1}$
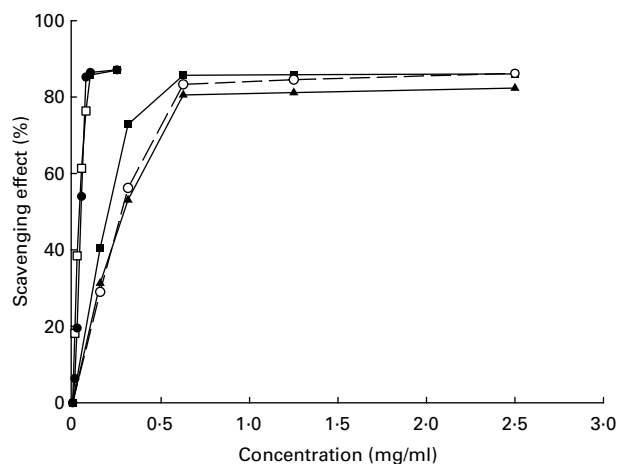
Fig. 1. The α,α-diphenyl-β-pricryl-hydrazyl radical-scavenging activities of water extracts from *Toona sinensis* Roemor leaf extract (TSL, ■), root extract (TSR, ▲) and bark extract (TSB, ○), using commercial antioxidant butylated hydroxytoluene (□) and vitamin C (●) as the controls. For details of diets and procedures, see p. 401.

and 2.77×10^{-1} mg/ml, respectively. These results indicated that all *Toona sinensis* Roemor extracts had DPPH radical-scavenging activity; higher concentrations were, however, needed for the extracts to reach DPPH radical-scavenging activities similar to those of vitamin C and butylated hydroxytoluene.

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

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

(Values are means with their standard errors)

		Body weight				Food intake (g/d)	
		Initial (g)		Final (g)			
Group	n	Mean	SE	Mean	SE	Mean	SE
A	16	27·37	0·78	33·45	0·75	5·73	0·14
B	16	27·94	0·40	34·04	0·58	5·49	0·15
C	16	27·78	0·55	33·96	0·55	5·49	0·18
D	16	27·29	0·85	34·17	0·76	5·52	0·16

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.

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, 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 2.09×10^{-1}, 2.85×10^{-1} and 2.77×10^{-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 2.04×10^{-1} mg/ml and

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)

		Related organ weights (g/100 g body weight)											
		Brain		Heart		Lung		Liver		Spleen		Kidney	
Group	*n*	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
A	10	1·393	0·044	0·527	0·012	0·700	0·027	4·256	0·233	0·308	0·017	1·398	0·059
B	10	1·419	0·028	0·526	0·031	0·713	0·029	4·380	0·305	0·342	0·017	1·321	0·047
C	10	1·392	0·033	0·527	0·019	0·671	0·026	4·047	0·159	0·333	0·019	1·293	0·036
D	10	1·375	0·036	0·521	0·020	0·633	0·034	3·965	0·211	0·338	0·020	1·313	0·071

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.

4.75×10^{-1} mg/ml, respectively. The present results proved that *Toona sinensis* Roemor extracts had antioxidant 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 and *Toona sinensis* Roemor diet groups were decreased with time, 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 test and made better avoidance responses.
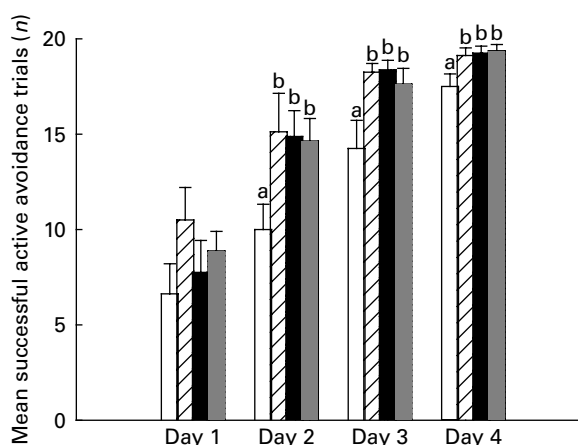

Fig. 2. Mean number of successful active avoidance among twenty trials of shuttle avoidance tests in 3-month-old senescence-accelerated-prone 8 mice fed with different diets for 12 weeks. Values were means with their standard errors. Pair-wise comparisons were conducted employing a least significant difference test. (□) A, control group (*n* 8); () B, 1 % *Toona sinensis Roemor* leaf extract (*n* 8); (■) C, 1 % *Toona sinensis* Roemor root extract; (*n* 8); () D, 1 % *Toona sinensis* Roemor bark extract (*n* 8). [a,b]Values with unlike superscript letters were significantly different at $P<0.05$. For details of diets and procedures, see p. 401.

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 & Nitta, 1994; Nitta *et al.* 1997; Yamada *et al.* 1999; 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 thiobarbituric acid reactivity as well as an age-dependent decrease in glutathione level in the brain. The levels of malordialdehyde 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 malonaldehyde (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
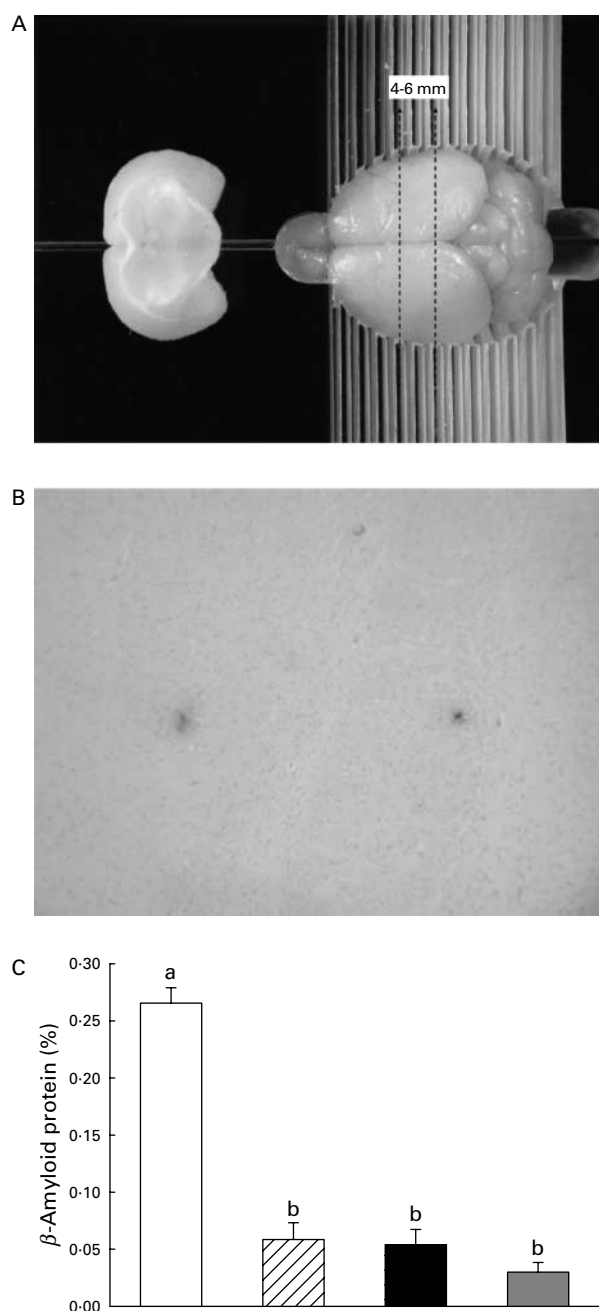
Fig. 3. β-Amyloid protein deposition in the middle brain section in 3-month-old male senescence-accelerated-prone 8 mice fed with different diets for 12 weeks. (A) The brain region stretching from the anterior to the posterior margin at 4–6 mm was chosen and immunostained with a monoclonal antibody to β-amyloid amino acids 17–24 (Biodesign International, Saco, ME, USA), which appeared brownish in colour. (B) The positive area of β-amyloid in each brain section was observed under microscopy with × 40 magnification. (C) More than twenty fields were totally counted in a full-brain section using an image analyser, and percentages were calculated as described on page 3. Values were means with their standard errors. Pair-wise comparisons were conducted employing a least significant difference test. [a,b]Values with unlike superscript letters were significantly different at $P<0.05$. (□)A, control group (*n* 8); (▨) B, 1 % *Toona sinensis* Roemor leaf extract (*n* 8); (■) C, 1 % *Toona sinensis* Roemor root extract (*n* 8); (▦) D, 1 % *Toona sinensis* Roemor bark extract (*n* 8). 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). Ullegaddi *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-glucoside (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 H_2O_2-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.

Table 4. Thiobarbituric acid-reactive substances (TBARS) concentration, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) activities, and total protein content in 3-month-old male senescence-accelerated-prone 8 mice fed with different diets for 12 weeks

(Values are means with their standard errors)

	A (n 8)		B (n 8)		C (n 8)		D (n 8)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
TBARS concentration (μmol/g tissue)								
Hippocampus	3·11[a]	0·10	2·44[b]	0·09	2·31[bc]	0·07	2·07[c]	0·15
Striatum	5·00[a]	0·25	3·25[b]	0·28	3·22[b]	0·18	3·10[b]	0·20
Cortex	2·21[a]	0·11	1·27[b]	0·05	1·11[b]	0·05	1·21[b]	0·10
Liver	1·41[a]	0·26	0·62[b]	0·06	0·60[b]	0·06	0·76[b]	0·03
SOD activity (unit/g protein)								
Hippocampus	36·42[a]	0·88	55·40[b]	1·18	76·58[c]	1·30	53·75[b]	1·41
Striatum	44·25[a]	0·93	80·55[b]	1·40	70·71[c]	2·06	72·89[c]	1·45
Cortex	38·07[a]	0·93	77·92[b]	2·73	65·43[c]	0·80	64·86[c]	1·18
Liver	5·11[a]	0·13	15·97[b]	0·23	25·97[c]	1·19	13·66[d]	0·30
CAT activity (unit/g protein)								
Hippocampus	0·09[a]	0·00	0·13[b]	0·01	0·15[b]	0·02	0·13[b]	0·02
Striatum	0·07[a]	0·00	0·10[b]	0·00	0·11[b]	0·00	0·11[b]	0·01
Cortex	0·09[a]	0·00	0·12[b]	0·00	0·13[c]	0·00	0·14[c]	0·00
Liver	0·71[a]	0·05	1·99[b]	0·13	2·72[c]	0·10	1·49[d]	0·11
GSH-Px activity (unit/g protein)								
Hippocampus	0·13[a]	0·01	0·17[b]	0·01	0·29[c]	0·01	0·43[d]	0·01
Striatum	0·15[a]	0·01	0·23[b]	0·01	0·25[c]	0·01	0·54[d]	0·01
Cortex	0·13[a]	0·01	0·23[b]	0·01	0·31[c]	0·01	0·37[d]	0·02
Liver	0·39[a]	0·03	1·67[b]	0·07	1·45[c]	0·03	1·16[d]	0·04
Total protein content (mg/g tissue)								
Hippocampus	114·83	4·90	112·40	3·14	108·79	2·53	112·01	3·52
Striatum	112·48	6·91	109·16	3·95	111·04	3·42	110·89	4·76
Cortex	113·27	7·83	110·00	4·61	115·91	5·63	113·06	6·02
Liver	158·65	17·03	158·95	15·45	153·85	17·77	148·82	13·42

Pair-wise comparisons were conducted employing a least significant difference test.

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.

[a,b,c,d] Mean values within a row with unlike superscript letters were significantly different at $P < 0.05$.

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