R. F. Santos[1]
J. C. F. Galduróz[1]
A. Barbieri[2]
M. L. V. Castiglioni[2]
L. Y. Ytaya[3]
O. F. A. Bueno[1]

Cognitive Performance, SPECT, and Blood Viscosity in Elderly Non-demented People Using Ginkgo Biloba

The aging process is associated with several cognitive alterations. This study looks at the effects of taking dried extract of *Ginkgo biloba*, which has been used in several countries in an attempt to minimize these effects. The subjects were 48 men aged 60–70 matched between control and experimental groups for educational level. Evaluation was based on a number of neuropsychological tests in an attempt to cover the largest possible number of functions including Single Photon Emission Computer Tomography (SPECT) and measures of blood viscosity. The study was run on a double-blind basis with placebo and *Ginkgo biloba* groups evaluated over a period of 8 months. After treatment, the experimental group showed a reduction in blood viscosity, improved cerebral perfusion in specific areas and improved global cognitive functioning. The control group showed the opposite – higher blood viscosity, a reduction in cerebral perfusion (in specific areas), and cognitive deterioration in different functions. Although the mechanisms by which *Ginkgo biloba* may contribute to overall enhancement of the parameters evaluated have not been specified, this plant extract certainly appears to be effective in the treatment of cognitive deficits in older people. Further research into its use is called for on the basis of the results obtained here.

Introduction

Many cognitive abilities tend to decline with advancing age, particularly memory, attention and psychomotor functions.

One promising drug treatment to alleviate age-related cognitive decline is the chronic administration of an extract of the dried leaves of *Ginkgo biloba*. Neuroprotective properties of this extract have been found both in rats [4,7,19,40] and humans [30]. These properties are thought to result in beneficial effects in the treatment of a number of illnesses and symptoms, such as dementia [24,28,31,38], cerebral insufficiency [21,25], adverse effects of stress [40] as well as tinnitus and dizziness [20,32,47]. The effects of dried extract of *Ginkgo biloba* on cognitive decline and memory loss in the elderly have also been described [12]. However, these studies are still few in number and have often been based on a limited range of cognitive tests.

Of particular interest to the present study are the vascular effects of dried *Ginkgo biloba* extract, since vascular parameters are known to change with age. Several studies have found that cerebral blood flow (CBF) is reduced in aging subjects [10,16,33]; increment in blood viscosity with age has also been reported [5,15]. Some reports have shown that dried *Ginkgo biloba* extract may have an effect on CBF and BV levels in humans [44,52,55] and in rats [27].

Our aim in the present study was to extend the knowledge about the effects of *Ginkgo biloba* extract to a wide range of neuropsychological functions and, simultaneously, search for possible relationships between its cognitive effects and BV and CBF parameters in elderly non-demented people.

Affiliation
[1] Department of Psychobiology, Universidade Federal de São Paulo-Escola Paulista de Medicina
[2] Imaging Diagnostics/Nuclear Medicine Department, Universidade Federal de São Paulo – Escola Paulista de Medicina
[3] Nuclear Medicine Service, Hospital Israelita Albert Einstein, Brazil

Correspondence
Dr. Ruth Ferreira Santos · R. Napoleão de Barros, 925 · São Paulo - SP - CEP: 04024-002 · Brazil ·
E-Mail: ruthfs@uol.com.br, ruth@psicobio.epm.br

Received 19.12.2001 · **Revised** 2.9.2002 · **Accepted** 16.9.2002

Bibliography
Pharmacopsychiatry 2003; 36: 127–133 · © Georg Thieme Verlag Stuttgart · New York · ISSN ISSN 0176-3679

Materials and Methods

Participants

A total of 48 male volunteers aged 60 – 70 years were recruited through a newspaper advertisement. Health status was assessed by a clinical-psychiatric interview before inclusion in the study and at monthly intervals thereafter. In addition, a battery of laboratory examinations and the Mini-Mental State Examination [6] with a cut-off score of 23 were applied. Except for complaints of mild loss of memory, they presented no gross psychiatric or neurological disorders, or history of drug addiction or heavy alcohol drinking, and were not on medication or drugs that might interfere with neuropsychological testing or with the effects of the *Ginkgo biloba* extract at the time of the study. In addition, IQ and MQ testing confirmed that cognitive abilities of the sample subjects were all within the normal range, although group differences were detected (see Table **1**).

Dried extract of *Ginkgo biloba*

The extract used in this study was produced by Maze Produtos Químicos e Farmacêuticos Ltda., and prepared by Magister Medicamentos Ltda. Composition (per 100 mg) was as follows: 24% flavonoide, 6.1% terpenoide, 2.7% bilobalide, 1.7% ginkgolide A, 0.9% ginkgolide B, and 0.8% ginkgolide C.

Procedures

A double-blind, independent group design was adopted. One group (25 subjects) was treated with placebo and the other group (23 subjects) with dried *Ginkgo biloba* extract for a period of 8 months. Two other participants allocated to the experimental group were excluded from the study for medical reasons not related to the use of the substance. Volunteers were randomly allocated between the groups, except for matching years in formal education (a random draw was carried out by an individual who had no contact with volunteers or examination or test results). Subjects were instructed to take one capsule per day at bedtime. The dose used in this study was 80 mg per day, a clinically used dosage. Placebo capsules did not differ in appearance from the drug capsules and were furnished monthly in dark flasks. Patient compliance was controlled by a number of control procedures. No alterations of the schedule were detected.

Single Photon Emission Computed Tomography (SPECT)

All subjects remained in a supine position with their eyes open in a quiet and dimly lit room for about 30 minutes after insertion of a peripheral venous access. At the end of this period, 1,110 MBq (30 mCi) of ^{99m}Tc-HM-PAO (Ceretec. Amersham International) was injected. SPECT acquisition began 20 minutes later. Special care was taken in positioning and securing patients' heads in the head holder. The canthomeatal line was aligned perpendicularly to the horizontal plane. Images were obtained using a single-head rotating gamma camera (APEX SPX-4HR. ELSCINT), with a low-energy, high resolution, parallel-hole collimator (LEHR) and energy centered at 140 KeV for ^{99m}Tc, and symmetrical 20% window. Sixty projections in a 64 × 64 × 16 matrix with an imaging time of 20 sec per projection were obtained. After attenuation correction, the 9-mm slices were reconstructed by back-projection with a Butterworth filter. A semi-quantitative method was used to evaluate tracer uptake. A set of 10 regions of interest (ROIs) was located in the cortical ribbon and pons over three slices of the axial plane (Fig. **1**). A cerebellar ROI was used as a reference, since there were no observations of perfusion abnormality in the cerebellums of the population studied (Fig. **1A**).

Table **1** MMSE scores and intelligence and memory global quotients

	Placebo (25)	Ginkgo biloba *(23)*	*t*	*p*
MMSE (cuttoff 23)	26.8 ± 1.7	28.7 ± 1.8	–3.1	0.002*
VERBAL IQ	103.9 ± 10.3	101.2 ± 8.4	1.4	0.18
EXECUTION IQ	101.8 ± 16.0	93.0 ± 16.2	1.9	0.06
GLOBAL IQ	102.9 ± 12.8	94.5 ± 11.6	2.4	0.02*
DETERIORATION INDEX	26.2 ± 16.0	29.5 ± 27.8	–0.5	0.62
GLOBAL MQ	87.9 ± 14.4	68.2 ± 11.8	2.6	0.02*

Blood viscosity

10 ml of venous blood was collected and stabilized with EDTA 0.1%. Whole-blood viscosity was obtained within 30 min with a rotational viscosimeter (Wells-Brookfield Cone/Plate Viscometer – Brookfield Engineering Labs. Inc., Soughton, MA. USA) following a technique standardized by Galduróz et al. [15].

Neuropsychological assessment

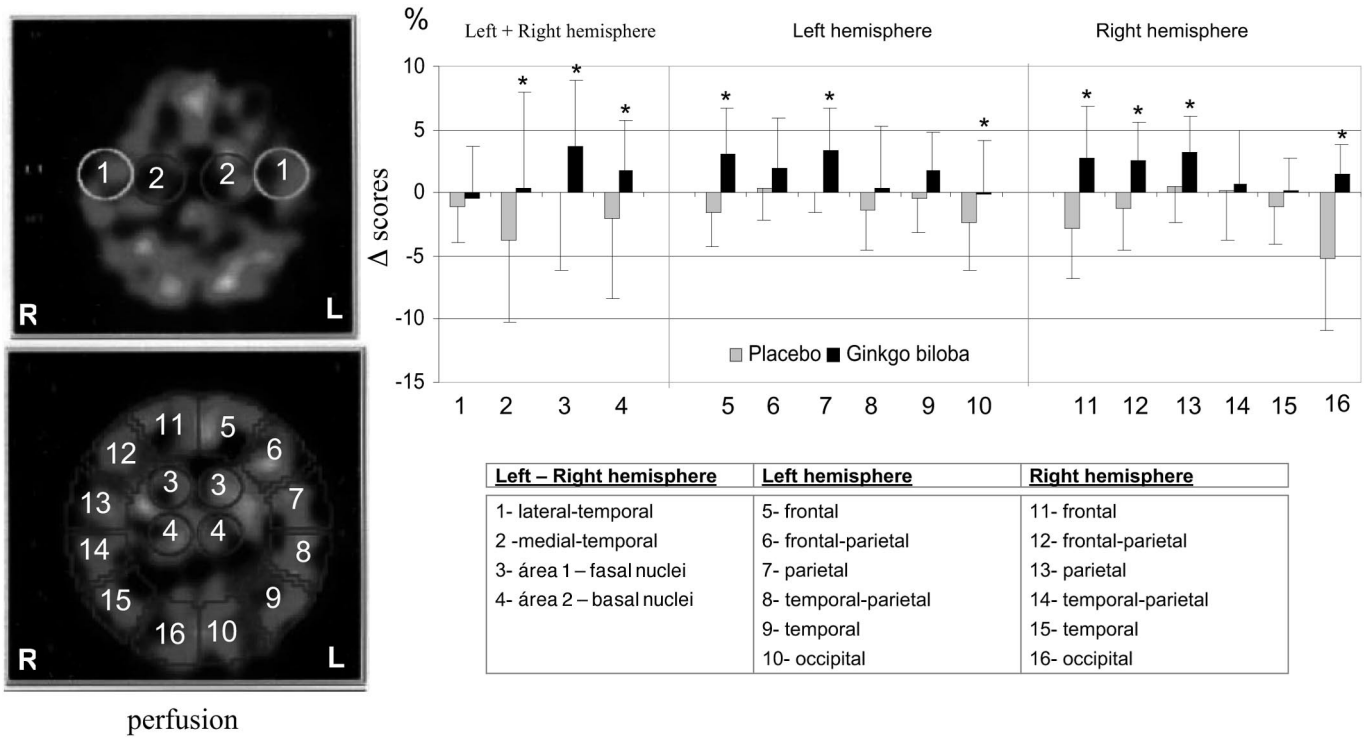
A thorough neuropsychological assessment was carried out on two occasions (before and after an 8-month administration period of dried extract or placebo) using the following tests: a) Wechsler Adult Intelligence Scale-Revised (WAIS-R): Information; Digit Span (forward and backward); Vocabulary; Arithmetic; Comprehension; Similarities; Picture Arrangement; Picture Completion; Block Design; Object Assembly; Digit Symbol; b) Wechsler Memory Scale – Revised (WMS-R): Information; Orientation; Mental Control; Logical Memory; Verbal Paired Associates; c) Corsi Block-Tapping Test; d) Rey-Osterrieth Complex Figure Test; e) Wisconsin Card Sorting Test (WCST); f) Toulouse-Piéron Concentrated Attention [43]; g) Verbal Free Recall [3, 36]. Unless specified, references about the tests can be found in Lezak [29] and in Spreen & Strauss [48]. The testing sequence was planned to avoid interference between tests as far as possible.

Ethical procedures

The research protocol was guided and approved by the Medical Ethics Commission at UNIFESP and all volunteers signed consent forms.

Statistical analyses

Post-treatment minus pre-treatment values (Δ scores) were calculated for each measure. Differences in Δ scores between the two groups were statistically analyzed by Student's *t*-test for independent measures. In addition, intragroup effects (before and after treatment) were compared by Student's *t*-test for dependent measures. Bonferroni corrections were applied [42].



Left – Right hemisphere	Left hemisphere	Right hemisphere
1- lateral-temporal	5- frontal	11- frontal
2 -medial-temporal	6- frontal-parietal	12- frontal-parietal
3- área 1 – fasal nuclei	7- parietal	13- parietal
4- área 2 – basal nuclei	8- temporal-parietal	14- temporal-parietal
	9- temporal	15- temporal
	10- occipital	16- occipital

Fig. **1** Effects of *Ginkgo biloba* on Δ scores (post-minus pre-treatment) cerebral. **A:** ROIs-areas demarcated by the ELSCINT application, in semi quantitative processing of cerebral perfusion, using cerebellum as reference level. **B:** Comparision of different cerebral perfusion in the two groups. * "t" test for independent samples. Bonferroni correction $p < 0.004$.

Results

SPECT

Significant differences were found in the Δ scores of groups in the following areas: medial-temporal ($t = -2.1$, $p = 0.04$), area 1 basal ganglia ($t = -2.1$, $p = 0.04$), and area 2 basal ganglia ($t = -2.5$, $p = 0.02$). Significant differences between the groups in Δ scores of cerebral perfusion (Fig. **1**) were found in areas of the right hemisphere (frontal: $t = -4.8$, $p < 0.0001$; frontoparietal: $t = -3.9$, $p < 0.001$; parietal: $t = -3.3$, $p = 0.001$; and occipital: $t = -5.2$ $p < 0.0001$), of the left hemisphere (frontal: $t = -5.2$, $p < 0.0001$; parietal: $t = -4.5$, $p < 0.0001$; occipital: $t = -2.7$, $p = 0.01$). During the 8-month interval of treatment, a significant reduction of cerebral perfusion was observed in the placebo-treated group in left hemisphere areas (frontal: $t = 305$, $p = 0.001$; occipital: $t = 4.5$, $p = 0.0001$) and in the occipital area of the right hemisphere ($t = 3.3$, $p = 0.003$). In the *Ginkgo biloba*-treated group there was a significant increase of cerebral perfusion in left hemisphere areas (frontal: $t = -3.3$, $p = 0.003$; frontoparietal: $t = -3.9$, $p < 0.0001$; parietal: $t = -5.2$, $p < 0.0001$), and in areas of the right hemisphere (frontal: $t = -4.2$, $p < 0.001$; parietal: $t = 4.8$, $p < 0.0001$).

Blood viscosity

A significant difference between the groups in Δ scores of blood viscosity was found ($t = 6.3$, $p < 0.0001$). Blood viscosity in the placebo group increased significantly between the first and second evaluation (4.1 ± 0.8 and 4.8 ± 0.7; $p < 0.0001$), while there was a significant reduction in the *Ginkgo biloba* group (4.6 ± 0.6 and 3.6 ± 0.6; $p < 0.0001$, Fig. **2**).

Cognitive functions

Corsi Blocks: Analyses of Δ scores revealed significant differences between groups favoring the *Ginkgo biloba*-treated group in both forward ($t = -3.7$, $p < 0.001$) and backward retrieval ($t = -4.7$, $p < 0.0001$). The performance of the plant extract group improved significantly from the first to the second evaluation ($t = -5.7$, $p < 0.0001$ and $t = -6.5$, $p < 0.0001$; forward and backward, respectively) (Fig. **3A**).

Toulouse-Piéron Concentrated Attention: Comparing the effects of treatment on groups' performance, significant differences were found on Δ scores, both in numbers of errors and in number of cancellations ($t = -3.7$, $p < 0.001$, see Fig. **3B**). There was a decrease in the number of cancellations in relation to the number of errors in the placebo-treated group (Δ scores = −24.7 ± 42.8; $t = 2.9$, $p = 0.01$), but there was an increase in the number of

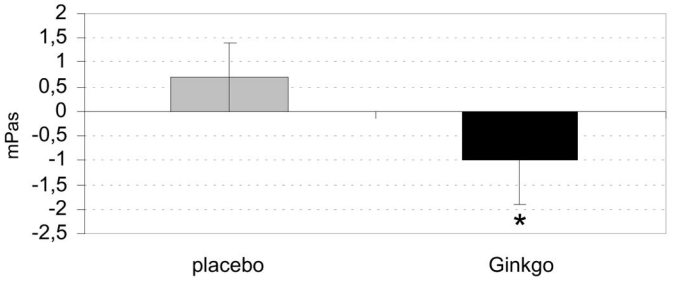


Fig. **2** Effects of *Ginkgo biloba* on Blood viscosity (post-minus pre-treatment). * Test "t" for independent samples.

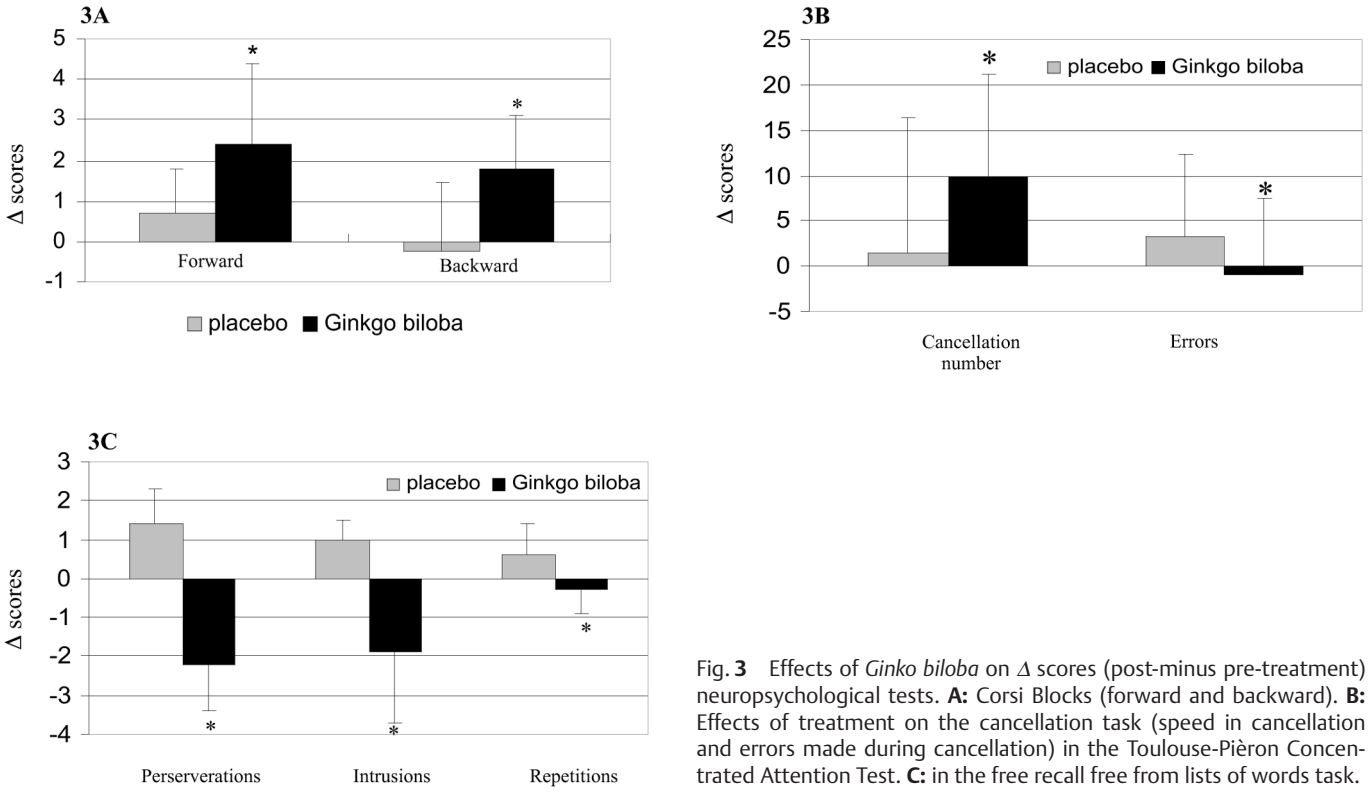


Fig. **3** Effects of *Ginko biloba* on Δ scores (post-minus pre-treatment) neuropsychological tests. **A:** Corsi Blocks (forward and backward). **B:** Effects of treatment on the cancellation task (speed in cancellation and errors made during cancellation) in the Toulouse-Pièron Concentrated Attention Test. **C:** in the free recall free from lists of words task.

cancellations in relation to the number of errors in the *Ginkgo biloba*-treated group (Δ scores = –26.5 ± 82.8; t = –2.4, p = 0.02).

Verbal Free Recall: No effect of treatment with *Ginkgo biloba* was detected on the Δ scores of total number of words recalled. Differences between the groups were seen in the number of errors made by the subjects: intrusions of words (t = 11.3, p < 0.001), perseverations (t = 8.0, p < 0.001) and repetitions (t = 4.3; p = 0.001). The placebo group made significantly more errors after treatment in comparison to pre-treatment phase: intrusions (t = –7.3; p < 0.0001); perseverations (t = –11.4; p < 0.0001); and repetitions (t = –3.7; p = 0.001). The experimental group made significantly fewer errors: intrusions (t = 8.5; p < 0.0001); perseverations (t = 5.1; p = 0.001) and repetitions (t = 2.3; p < 0.03).

WAIS-R: Comparing the Δ scores of the two treatments, significant differences were detected in the following sub-tests: Vocabulary (t = –4.5, p < 0.001); Block Design (t = –3.1, p = 0.002); Arithmetic (t = –4.5, p < 0.001); Object Assembly (t = –4.9, p < 0.001); Comprehension (t = –6.3, p < 0.001); Digit Symbol (t = –4.5, p < 0.001) and Similarities (t = –3.8, p < 0.001); in all cases, the *Ginkgo biloba* treated group performed better than the placebo group. Besides, a significant deterioration in performance was detected between the first and the second evaluation in the placebo-treated group: Digit Span – backward (t = 3.6, p = 0.001); Object Assembly (t = 4.2, p < 0.001) and Comprehension (t = 4.6, p = 0.001). On the contrary, an improvement in performance was observed in the group treated with *Ginkgo biloba*: Information (t = –3.6, p = 0.001); Vocabulary (t = –4.4, p < 0.001); Block Design (t = –3.3, p = 0.003); Arithmetic (t = –4.2, p < 0.001); Object Assembly (t = –3.3, p = 0.003); Comprehension (t = –4.9, p < 0.001); Digit Symbol (t = –6.5, p < 0.001) and Similarities (t = –5.2, p < 0.0001), but also a significant deterioration in the Digit Span – forward subtest (t = 3.6, p = 0.001) (Table **2**).

WMS-R: The groups' Δ scores were significantly different in: Mental Control (t = 7.2, p < 0.001 and t = 4.5, p < 0.001, time and errors, respectively) and Verbal Paired Associates – no semantic relation (t = –4.2, p = 0.001 on 1st trial; t = –3.8, p < 0.001 on 2nd trial and t = –4.1, p < 0.001 on 3rd trial). In the placebo-treated group, there was a significant improvement on the Verbal Paired Associates test, but only for closely related semantically pairs (t = –3.5, p = 0.002 on 1st trial; t = –4.0, p < 0.001 on 2nd trial and t = –4.0, p < 0.001). In the *Ginkgo biloba*-treated group, performance was significantly higher on: Mental Control (t = 7.4, p < 0.001 and t = 4.2, p < 0.001 – time and errors, respectively, and Verbal Paired Associates – semantically related (t = –3.5, p = 0.002 on 2nd trial and t = –4.1, p < 0.001 on 3rd trial) and non-semantically related pairs (t = –3.6, p = 0.001 on the 1st trial; t = –6.1, p < 0.001 on the 2nd trial; t = –5.9, p < 0.001 on the 3rd trial and t = –5.2, p < 0.001 on the 4th trial, see Table **2**).

Rey-Osterrieth Complex Figure: Significant differences between the groups were found between the Δ scores only on delayed retrieval (t = –3.1, p = 0.003). A significant improvement in immediate retrieval was detected in the placebo-treated group (t = –3.3, p = 0.003); but there was a significant improvement in the three tasks in the *Ginkgo biloba*-treated group (copy: t = –3.6, p = 0.001; immediate retrieval: t = –3.9, p < 0.001; and late retrieval: t = –4.9, p < 0.001, see Table **2**).

Wisconsin Card Sorting Test: Comparing the effects of treatments on the Δ scores of the groups, statistically significant differences

Table **2** Performance on neuropsychological tests: pre-treatment, post-treatment and Δ scores (pre- minus post treatment)

			Placebo		Ginko biloba		*Placebo*	Ginko biloba
			Pre-treatment	*Post-treatment*	*Pre-treatment*	*Post-treatment*		
WAIS-R								
Information			20.2 ± 4.8	21.1 ± 4.0	**19.7 ± 4.4**	**21.0 ± 3.6**	1.0 ± 3.7	1.3 ± 1.9
Picture completion			13.5 ± 2.3	13.2 ± 3.1	11.9 ± 4.6	12.9 ± 5.1	−0.4 ± 1.9	1.0 ± 4.2
Digit span – forward			5.8 ± 1.5	5.3 ± 1.7	5.3 ± 1.0	4.3 ± 1.2	−0.6 ± 1.2	−1.0 ± 1.4
Digit span – backward			**5.3 ± 1.7**	**4.0 ± 1.4**	4.0 ± 0.8	4.2 ± 1.1	−1.3 ± 1.8	0.2 ± 1.1
Picture arrangement			8.6 ± 4.5	4.1 ± 4.2	6.9 ± 3.9	8.2 ± 3.2	−1.5 ± 2.8	1.3 ± 4.6
Vocabulary			45.5 ± 12.5	44.8 ± 12.1	**42.7 ± 10.9**	**47.3 ± 9.8**	**−0.7 ± 2.9**	**4.6 ± 5.0**
Block design			21.8 ± 9.7	20.9 ± 12.8	**15.9 ± 13.7**	**22.5 ± 11.3**	**−0.9 ± 6.8**	**6.6 ± 9.5**
Arithmetic			18.2 ± 5.4	17.6 ± 6.1	**15.0 ± 6.1**	**17.7 ± 5.8**	**−0.6 ± 1.8**	**2.7 ± 3.1**
Object Assembly			**25.0 ± 9.1**	**20.9 ± 10.9**	**17.7 ± 11.4**	**24.2 ± 11.0**	**−4.1 ± 4.9**	**6.5 ± 9.6**
Comprehension			**24.1 ± 5.3**	**18.2 ± 3.8**	**20.8 ± 2.9**	**24.7 ± 4.1**	**−5.9 ± 6.4**	**3.8 ± 3.8**
Digit symbol			38.7 ± 19.7	32.6 ± 16.2	**30.3 ± 6.9**	**39.8 ± 7.8**	**−6.2 ± 15.2**	**9.4 ± 6.9**
Similarities			16.0 ± 6.8	15.5 ± 6.8	**13.0 ± 4.5**	**16.3 ± 5.1**	**−0.5 ± 3.8**	**3.3 ± 3.1**
WMS-R								
Information			5.7 ± 0.5	5.8 ± 0.4	5.6 ± 0.5	5.9 ± 0.3	0.3 ± 0.6	0.3 ± 0.6
Orientation			5.0 ± 0.2	4.9 ± 0.3	4.9 ± 0.3	4.9 ± 0.3	0.0 ± 0.4	0.0 ± 0.4
Mental Control – time			40.6 ± 25.1	44.4 ± 29.5	**43.7 ± 13.6**	**35.3 ± 9.8**	**3.7 ± 6.0**	**−8.3 ± 5.4**
Mental Control – errors			4.2 ± 3.8	4.9 ± 4.6	**3.6 ± 3.3**	**2.0 ± 2.4**	**0.6 ± 1.6**	**−1.5 ± 1.7**
Logical memory	immediate retrieval		8.8 ± 3.4	7.9 ± 3.5	8.0 ± 2.7	9.1 ± 2.3	**−0.8 ± 2.2**	**1.2 ± 2.9**
	delayed retrieval		7.3 ± 3.2	7.6 ± 2.7	6.0 ± 3.3	7.6 ± 2.2	0.3 ± 2.1	1.5 ± 2.5
Associated Pairs	Semantically related	1st trial	**2.7 ± 1.1**	**3.6 ± 0.7**	2.1 ± 1.4	2.9 ± 1.4	0.9 ± 1.1	0.8 ± 1.7
		2nd trial	**2.6 ± 0.9**	**3.6 ± 0.9**	**2.9 ± 1.3**	**3.9 ± 0.8**	1.0 ± 1.1	1.0 ± 1.2
		3rd trial	**3.0 ± 1.0**	**4.0 ± 0.5**	**3.0 ± 0.9**	**4.1 ± 0.8**	1.0 ± 1.1	1.1 ± 1.1
		4th trial	2.8 ± 1.4	3.6 ± 0.7	2.8 ± 1.5	3.6 ± 0.5	0.8 ± 1.7	0.8 ± 1.9
	Non-semantically related	1st trial	1.1 ± 1.4	0.5 ± 0.5	**0.1 ± 0.3**	**1.4 ± 1.5**	**−0.7 ± 1.3**	**1.3 ± 1.6**
		2nd trial	1.2 ± 0.9	1.3 ± 1.3	**0.5 ± 0.5**	**2.2 ± 1.1**	**0.1 ± 1.5**	**1.7 ± 1.2**
		3rd trial	1.4 ± 1.1	1.5 ± 1.0	**0.9 ± 0.8**	**2.8 ± 1.0**	**0.1 ± 1.4**	**1.9 ± 1.4**
		4th trial	1.1 ± 1.2	1.5 ± 0.8	**0.7 ± 0.9**	**2.4 ± 1.3**	0.3 ± 1.7	1.7 ± 1.4
Rey-Osterrieth Complex Figure								
Copying			24.1 ± 8.4	25.5 ± 7.9	**24.7 ± 4.4**	**28.6 ± 3.8**	1.4 ± 5.1	4.0 ± 5.0
Immediate retrieval			10.6 ± 6.2	14.2 ± 7.4	**9.5 ± 5.7**	**16.2 ± 5.4**	3.6 ± 8.0	6.8 ± 8.0
Delayed retrieval			8.8 ± 5.5	11.1 ± 6.6	**5.9 ± 5.2**	**13.8 ± 6.2**	**2.3 ± 4.8**	**7.9 ± 7.4**
Wisconsin Card Sorting Test								
Number of trials/categories			55.2 ± 42.1	41.4 ± 19.2	**31.4 ± 15.5**	**20.0 ± 8.2**	−13.8 ± 47.2	−11.4 ± 14.3
Non-perseverative errors/categories			8.5 ± 12.0	12.0 ± 16.6	**4.6 ± 4.8**	**1.1 ± 1.0**	**3.5 ± 7.9**	**−3.5 ± 4.2**
Perserverative errors /categories			15.1 ± 13.7	9.5 ± 4.9	7.0 ± 4.5	4.4 ± 2.9	−5.6 ± 15.9	−2.6 ± 5.1

Bold: Significant differences between groups or intra-group. $p < 0,01$ at least.

were detected only in relation to the mean number of non-perseverative errors made within one category ($t = 3.8$, $p < 0.001$). Intra-group effects were found to be statistically different only in the *Ginkgo biloba* treated group (on the number of trials to close a category; $t = 3.8$, $p = 0.001$; and on the number of non-perseverative errors within the same category $t = 4.0$, $p < 0.001$, Table **2**).

Discussion

Improvement in several cognitive aspects were observed after treatment with a *Ginkgo biloba* extract over a period of 8 months. These cognitive aspects benefited by the extract may be summarized as general intelligence (exemplified by tests such as Vocabulary, Comprehension, Similarities), visuospatial abilities (Block Design, Object Assembly, Corsi's Blocks), attentional processes (Digit Symbol, Toulouse), and information processing speed (assessed by timed tests).

Improvements in short-term working memory speed by *Ginkgo biloba* has already been indicated in normal adults [41].

Beneficial effects of the extract have also been reported by several authors in demented patients as well [17,18,24,28,31].

Other studies, however, have not found any beneficial effects. Nathan et al. [37] aimed to study 1-day administration effects of the extract in healthy old volunteers, and Moulton et al. [35] have investigated healthy male subjects after 5 days of treatment. The acute administration can explain the failure to get beneficial studies in these latter studies. However, the study by van Dongen et al. [53] was intended to evaluate the efficacy of prolonged Ginkgo treatment in demented and age-associated memory impaired (AAMI) patients. Their results contrast with those of previously trials cited above, in that efficacy of the extract was not observed.

The difference in outcome between our results and those from the van Dongen et al. [53] study may stem from the difference in diagnosis between our samples, that is, our subjects were not demented, nor could they be diagnosed with AAMI. They used to complain of memory deficits, but as noted in several studies [2,8], subjective memory complaints have a low predictive value for dementia. Certainly, the patients of van Dongen at al. suffered from more intense cognitive deficits that were harder to overcome by the treatment than in our volunteers. Or, alternatively, intrinsic differences in the cognitive profiles of demented/AAMI patients subjective memory complainers should also be considered.

Chronic treatment with dried *Ginkgo biloba* extract led to a significant reduction in blood viscosity and increased cerebral perfusion in several ROIs corresponding to bilateral frontal, bilateral parietal, right frontal-parietal, left temporal and right occipital areas. Pre-frontal areas are known to be involved in central executive functions that play a key role in mental organization, problem solving strategies, improved attention and concentration, and in minimizing losses in other cognitive functions in the elderly [45,50]. Areas involving the parietal lobe and right frontoparietal areas contribute to visuospatial functions, including visuospatial short-term memory. *Ginkgo biloba* treatment was also effective in boosting performance in another non-verbal memory task (Rey-Osterrieth figure).

Typical verbal memory tasks such as free recall of word lists were not affected, except for a reduction in some kinds of errors more likely to be related to frontal functions (number of perseverations, intrusions and repetitions). This result does not fit well with blood perfusion alterations on left temporal regions known to be associated to episodic encoding of words [39]. However, the extract treated group performed much better than the placebo treated group on previously unrelated Verbal Paired Associates, another verbal memory task, perhaps more difficult than verbal free recall. When both words were semantically related, the task was performed easily by most participants and no beneficial effect of *Ginkgo biloba* was detected. An increment in its performance would also be expected to be related to an increase in left temporal lobe perfusion, which did occur. Alternatively, the ability in establishing semantic elaboration, impaired in older people [22], could be ameliorated by the extract. Moreover, the use of visual imagery mediated by primary visual cortex [26] is a strategy that the subjects may use to improve association learning of unrelated words [14]. Therefore, it is possible that a perfusion increase in the occipital area facilitates performance of this task.

Summing up the neuropsychological results, it seems that the beneficial effects of *Ginkgo biloba* are exerted on those cognitive domains that are more likely to be affected by aging [23].

A plethora of effects that *Ginkgo biloba* shows have been reported in recent years. This may help explain the cognitive improvement brought about by its extract. *Ginkgo biloba* has an effect on the platelet aggregation factor [1,46], and this may explain why it has the effect of lowering blood viscosity. Thus, the cognitive improvement may be related to the improvement in blood flow brought about by chronic use of extract of *Ginkgo biloba,* which may in turn decrease blood viscosity [11,27,51] and increase the supply of oxygen and of glucose to neurons. Improvement in attentional functions, at least such as those seen in the speedy execution of tasks, may be attributed to the power of *Ginkgo biloba* to inhibit MAO (monoamine oxidase) [54], thus raising the levels of neurotransmitters such as dopamine by reducing their degradation. A similar effect may take place regarding serotonin levels, thus contributing to cerebral vasodilatation (5HT2 receptors), particularly on higher-caliber blood vessels [34].

Other possibility to explain the cognitive effects is based on recent studies that have shown its antioxidant action on the hippocampus [9]. The organism seems to possess an endogenous antioxidant system to reduce the oxidation effect of free radicals. This system declines with age, and may be given a boost through taking *Ginkgo biloba* to curb the pace of this process. The antioxidation effect itself [13,30,40,49] may contribute to vascular improvement, since it may reduce the rate of degeneration from free radical aggressions.

Although it is not currently possible to specify more precisely the mechanism through which *Ginkgo biloba* acts, it does appear to be useful in the treatment of cognitive deficits in older people and further research is called for in the light of the results obtained in studies of this plant.

Acknowledgements

We are grateful to AFIP and FAPESP for financial support and to Maria Laura Nogueira Pires, Ana Amélia Benedito Silva, Paulo Henrique F. Bertolucci and two anonimous reviewers for helping in statistical analyses and suggestions to improve the manuscript.

References

[1] Akiba S, Kawauchi T, Oka T, Hashizume T, Sato T. Inhibitory effect of the leaf extract of Ginkgo biloba L. on oxidative stress-induced platelet aggregation. Biochem Mol Biol Int 1998; 46(6): 243 – 8
[2] Almeida OP. Memory complaints and the diagnosis of dementia. Arq Neuropsiquiatr 1998; 56: 412 – 418
[3] Andrade VM, Oliveira MGM, Miranda MC, Oliveira ASB, Oliveira EML, Bueno OFA. Semantic relations and repetition of items enhance the free recall of words by multiple sclerosis patients. (submitted)
[4] Attella MJ, Hoffman SW, Stasio MJ, Stein DG. Ginkgo biloba extract facilitates recovery from penetrating brain injury in adult male rats. Exp Neurol 1989; 105: 62 – 71
[5] Bernasconi C, Del Santo A, Marrali F, Laffranchi M, Milani S, Agostoni A. Reference limits for blood viscosity: Influence of sex and age. Clin Hemorheol 1991; 11: 447 – 457

[6] Bertolucci PHF, Brucki SMD, Campacci SR, Juliano Y. The Mini-Mental State Examination in ann outpatient population: impact of literacy. Arq Neuropsiquiatr 1994; 52: 1 – 7
[7] Bolanos-Jimenez F, DeCastro RM, Sarhan H, Prudhome N, Drieu K, Fillion G. Stress-induced 5-HT1A receptor desensitization: Protective effects of Ginkgo biloba extract (Dried extract 761). Fundamental Clin Pharmacol 1995; 9: 169 – 174
[8] Bolla KI, Lindgren KN, Bonaccorsy C, Bleecker ML. Memory complaints in older adults: Fact or Fiction. Archives of Neurology 1991; 48: 61 – 64
[9] Bridi R, Crossetti F, Steffen V, Henriques AT. Estudo da atividade antioxidante do extrato padronizado de Ginkgo biloba (EGB 761) em hipocampo de ratos. XV Simpósio de Plantas Medicinais do Brasil, Águas de Lindóia – SP. October, 1998
[10] Celsis P, Agniel A, Cardebar D, Démonet JF, Ousset PJ, Puel M. Age related cognitive decline: a clinical entity? A longitudinal study of cerebral blood flow and memory performance. J Neurol Neurosurg Psychiatr 1997; 6: 601 – 608
[11] Chung TW, Ho CO. Changes in viscosity of low shear rates and viscoelastic properties of oxidative erythrocyte suspensions. Clinical Hemorheology & Microcirculation 1999; 21(2): 99 – 103
[12] Curtis-Prior P, Vere D, Fray P. Therapeutic value of Ginkgo biloba in reducing symptoms of decline in mental function. J Pharmacy & Pharmacol 1999; 51: 535 – 41
[13] Dumont E, Arbigny PD, Nouvelot A. Protection of polyunsaturated fatty acids against iron-dependent lipid peroxidation by a Ginkgo biloba extrat (Egb 761). Methods Find. Exp. Clin. Pharmacol. 1995; 17: 83 – 88
[14] Dunlosky J, Hertzog C. Updating knowledge about encoding strategies. A componential analysis of learning about strategy effectiveness from task experience. Psychology and Aging 2000; 15: 462 – 474
[15] Galduróz JCF, Santos RF, Carlini EA. Os efeitos da idade e sexo sobre a viscosidade sangüínea em voluntários normais. Temas 1995; 50: 159 – 166
[16] Goldstein S, Reivich M. Cerebral blood flow and metabolism in aging and dementia. Clin Neuropharmacol 1991; 14 (Suppl.): S34 – 44
[17] Haase J, Halama P, Hörr R. Efficacy of short-term treatment with intravenously administered Ginkgo biloba special extract EGb 761 in Alzheimer type and vascular dementia. Zeitschrift für Gerontologie und Geriatrie 1996; 29: 302 – 309
[18] Hofferberth B. The efficacy of EGB-761 in patients with senile dementia of the Alzheimer-type. A double-blind, placebo-controlled study on different levels of investigation. Human Psychopharmacology: Clinical and Experimental 1994; 9: 215 – 222
[19] Hoffman SW, Stein DG. Extract of Ginkgo biloba (dried extract 761) improves behavioral performance and reduces histopathology after cortical contusion in the rat. Restorative Neurol Neurosci 1997; 11: 1 – 12
[20] Holgers KM, Axelsson A, Pringle I. Ginkgo biloba extract for the treatment of tinnitus. Audiology 1994; 33: 85 – 92
[21] Hopfenmuller W. Evidence for a therapeutic effect of Ginkgo biloba special extract. Meta-analysis of 11 clinical studies in patientswith cerebrovascular insufficiency in old age. Arzneimittel-Forsch/Drug Res 1994; 44: 1005 – 1013
[22] Howard DV, Fry AF, Brune CM. Aging and memory for new associations: Direct versus indirect measures. Journal of Experimental Psychology: Learning, Memory, and Cognition 1991; 17: 779 – 792
[23] Jenkins L, Myerson J, Joerding JA, Hale S. Converging evidence that visuospatial cognition is more age-sensitive than verbal cognition. Psychology and Aging 2000; 15: 157 – 175
[24] Kanowski S, Herrmann WM, Stephan K, Wierich W, Hörr R. Proof of efficacy of the Ginkgo biloba special extract EGb 761 in outpatients suffering from mild to moderate primary degenerative dementia of the Alzheimer type or multi-infarct dementia. Pharmacopsychiatry 1996; 29: 47 – 56
[25] Kleijnen J, Knipschild P. Ginkgo biloba for cerebral insufficiency. Brit J Clin Pharmacol 1992; 34: 352 – 358
[26] Klein I, Paradis A-L, Poline JB, Kosslyn SM, Bihan DL. Transient activity in the human calcarine cortex during visual-mental imagery: an event-related fMRI study. Journal of Cognitive Neuroscience 2000; 12: S15 – 23
[27] Krieglstein J, Beck T, Seibert A. Influence of an extract of Ginkgo biloba on cerebral blood flow and metabolism. Life Sci 1986; 39: 2327 – 2334
[28] LeBars PL, Katz MM, Berman N, Itil TM, Freedman AM, Schatzberg AF. A placebo-controlled, double-blind, randomized trial of an extract of Ginkgo biloba for dementia. JAMA 1997; 278: 1327 – 1332
[29] Lezak MD. Neuropsychological Assessment.; 3rd Edition New York: Oxford University Press, 1995
[30] Maitra I, Marcocci L, Droy-Lefaix MT, Packer L. Peroxyl radical scavenging activity of Ginkgo biloba dried extract EGb 761. Biochem Pharmacol 1995; 49: 1649 – 1655
[31] Maurer K, Ihl R, Dierks T, Frolich L. Clinical efficacy of Ginkgo biloba special extract (dried extract 761) in dementia of the Alzheimer type. Phytomedicine 1998; 5: 417 – 424
[32] Meyer B. A multicenter. randomized. double-blind drug versus placebo study of Ginkgo biloba extract in the treatment of tinnitus. Presse Medicale 1986; 15: 1562 – 1564
[33] Meyer JS, Terayama Y, Takashima S. Cerebral circulation in the elderly. Cerebral Vascular and Brain Metabolism Reviews 1993; 5: 122 – 146
[34] Mizumoto N. Regulação do fluxo sangüíneo cerebral. In: Stávale, M.C. Bases da Terapia Intensiva Neurológica Livraria Editora Santos, 1996
[35] Moulton PL, Boyko LN, Fitzpatrick JL, Petros TV. The effect of Ginkgo biloba on memory in healthy male volunteers. Physiology & Behavior 2001; 73: 659 – 665
[36] Murdock BB Jr. The serial position effect of free recall. Journal of Experimental Psychology 1962; 64: 482 – 488
[37] Nathan PJ, Ricketts E, Wesnes K, Mrazek L, Greville W, Stough C. The acute nootropic effects of Ginkgo biloba in healthy older human subjects: A preliminary investigation. Human Psychopharmacology: Clinical and Experimental 2002; 17: 45 – 49
[38] Oken BS, Storzbach DM, Kaye JA. The efficacy of Ginkgo biloba on cognitive function in Alzheimer disease. Arch Neurol 1998; 55: 1409 – 1415
[39] Otten LJ, Henson RNA, Rugg MD. Depth of processing effects on neural correlates of memory encoding. Brain 2001; 124: 399 – 412
[40] Oyama Y, Chikahisa L, Ueha T, Kanemaru K, Noda K. Ginkgo biloba extract protects brain neurons against oxidative stress induced by hydrogen peroxide. Brain Res 1996; 712: 349 – 352
[41] Polich J, Gloria R. Cognitive effects of a Ginkgo biloba/vinpocetine compound in normal adults: Systematic assessment of perception, attention and memory. Human Psychopharmacology: Clinical and Experimental 2001; 16: 409 – 416
[42] Portney LG. Watkins, Foundations of Clinical Research: Applications to Practice. Norwik: Appleton and Lange, 1993
[43] Rainho O (Ed.). Manual da Bateria CEPA de Aptidões Específicas. Rio de Janeiro: CEPA – Centro Editor de Psicologia Aplicada, 1992
[44] Ritch R. Potential role for Ginkgo biloba extract in the treatment of glaucoma. Med Hypotheses 2000; 54: 221 – 35
[45] Smith EE, Jonides J. Storage and executive processes in the frontal lobes. Science 1999; 283: 1657 – 1661
[46] Smith PF, Maclennan K, Darlington CL. The neuroprotective properties of the Ginkgo biloba leaf: a review of the possible relationship to platelet-activating factor (PAF). J Ethnopharmacol 1996; 50: 131 – 9
[47] Soholm B. Clinical improvement of memory and other cognitive functions by Ginkgo biloba: Review of relevant literature. Advances in Therapy 1998; 15: 54 – 65
[48] Spreen O, Strauss EA. Compendium of Neuropsychological Tests. New York: Oxford University Press, 1991
[49] Stein DG. Brain damage and recovery. Progr Brain Res 1994; 100: 203 – 11
[50] Stuss DT, Benson DF. The frontal lobes and control of cognition and memory. In: Perecman, E. (ed.). The Frontal Lobe Revisited, New York: The IRBN Press, 1987
[51] Tadano T, Nakagawasai O, Tan-no K, Morikawa Y, Takahashi N, Kisara K. Effects of Ginkgo biloba extract on impairment of learning induced by cerebral ischemia in mice. American Journal of Chinese Medicine 1998; 26: 127 – 32
[52] Timerbaeva SL, Suslina ZA, Bodareva EA, Fedin PA, Korepina OS, Pervosvanskii BE. Tanakan in the treatment of primary manifestations of insufficiency of brain blood supply. Zh Nevrol Psikhiatr Im S S Korsakova; 2000; 100(8): 24 – 8
[53] van Dongen MCJM, van Rossum E, Kessels AGH, Sielhorst HJG, Knipschild PG. The efficacy of ginkgo for elderly people with dementia and age-associated memory impairment: New results of a randomized clinical trial. Journal of the American Geriatrics Society 2000; 48: 1183 – 1194
[54] White HL, Scates PW, Cooper BR. Extracts of Ginkgo biloba leaves inhibit monoamine oxidase. Life Sciences 1996; 58: 1315 – 1321
[55] Zhang WR, Hayashi H Sasaki C, Sakai K, Warita H, Wang JM, Shiro Y, Uchida M, Abe K. protective effect of Ginkgo extract on rat brain with transient middle cerebral artery occlusion. Neurol Res 2000; 22: 517 – 21