Available online at www.sciencedirect.com
ScienceDirect
Food and Chemical Toxicology 45 (2007) 1882–1890
www.elsevier.com/locate/foodchemtox

Aqueous and hydroalcoholic extracts of Black Maca (*Lepidium meyenii*) improve scopolamine-induced memory impairment in mice

Julio Rubio [a,*], Haixia Dang [b], Mengjuan Gong [b], Xinmin Liu [b], Shi-lin Chen [b], Gustavo F. Gonzales [a]

[a] *Department of Biological and Physiological Sciences, Faculty of Sciences and Philosophy and Instituto de Investigaciones de la Altura, Universidad Peruana Cayetano Heredia, P.O. Box 1843, Lima, Peru*
[b] *Research Center of Pharmacology and Toxicology, Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Pekin Union Medical College, Beijing 100094, China*

Received 12 August 2006; accepted 10 April 2007

Abstract

Lepidium meyenii Walp. (Brassicaceae), known as Maca, is a Peruvian hypocotyl growing exclusively between 4000 and 4500 m altitude in the central Peruvian Andes, particularly in Junin plateau. Previously, Black variety of Maca showed to be more beneficial than other varieties of Maca on learning and memory in ovariectomized mice on the water finding test. The present study aimed to test two different doses of aqueous (0.50 and 2.00 g/kg) and hydroalcoholic (0.25 and 1.00 g/kg) extracts of Black Maca administered for 35 days on memory impairment induced by scopolamine (1 mg/kg body weight i.p.) in male mice. Memory and learning were evaluated using the water Morris maze and the step-down avoidance test. Brain acetylcholinesterase (AChE) and monoamine oxidase (MAO) activities in brain were also determined. Both extracts of Black Maca significantly ameliorated the scopolamine-induced memory impairment as measured in both the water Morris maze and the step-down avoidance tests. Black Maca extracts inhibited AChE activity, whereas MAO activity was not affected. These results indicate that Black Maca improves scopolamine-induced memory deficits.

Keywords: Black Maca; Scopolamine; Memory; Water Morris maze; Step-down avoidance test; AChE activity; MAO activity

1. Introduction

The increase in life expectancy during the 20th century had concomitantly increased the number of people suffering of age related diseases. Particularly, the older people show a greater deficit in cognitive function (Halbreich et al., 1995) being the Alzheimer's disease (AD) the most common cause of progressive loss of memory (Lee et al., 2006) and dementia in the elderly (Heikkinen et al., 2004). Similarities in the memory impairments between Alzheimer patients and scopolamine-treated animals have been reported, and it has been proposed that scopolamine, a muscarinic cholinergic receptor antagonist, could serve as a useful pharmacological tool to produce a partial model of the disorder (Bartus, 2000).

AD is clinically characterized by a progressive loss of cognitive abilities. The pathophysiology of AD is complex and involves several different biochemical pathways (Kang et al., 2005). The key symptoms of AD are primarily caused by cholinergic dysfunction (Wang et al., 2006). It is known that acetylcholine (ACh) is an important neurotransmitter related to memory and learning.

Abbreviations: AChE, acetylcholinesterase; AchEI, acetylcholinesterase inhibitors; AD, Alzheimer's disease; MAO, monoamine oxidase.

* Corresponding author. Fax: +51 1 4821195.
E-mail address: 09008@upch.edu.pe (J. Rubio).

0278-6915/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.fct.2007.04.002

Acetylcholinesterase (AChE) modulates ACh to proper levels by degradation; accordingly, excessive AChE activity leads to constant ACh deficiency, memory, and cognitive impairments (Yamada et al., 2004). In fact, it has been demonstrated that there is a specific deficiency in Ach, cholinacetyltransferase, and an increase in AChE in autopsy material from patients with Alzheimer's disease (Davies and Maloney, 1976). Currently, AchE inhibitors (AChEI) are the first group of compounds for AD treatment. Until now, four AChEI have been approved by the FDA for the treatment of AD: tacrine (Cognex®), donepezil (Aricept®), rivastigmine (Exelon®), and galantamine (Reminyl®) (Lahiri et al., 2002). However, AChEI presents some limitations as their short half-lives and excessive side effects caused by activation of peripheral cholinergic systems, as well as hepatotoxicity, which is the most frequent and important side effect of tacrine therapy (Farlow et al., 1992; Knapp et al., 1994; Rogers et al., 1998). For this reason, alternative and complementary therapies are needed. Several studies have shown the neuroprotective and/or cognition-enhancing properties of natural products and their components using different animal models (Lee et al., 2003; Yan et al., 2004; Houghton et al., 2004; Yamada et al., 2004; Kang et al., 2003, 2005; Yu et al., 2005; Wang et al., 2006).

Lepidium meyenii Walp. (Brassicaceae), known as Maca, is a Peruvian plant growing exclusively between 4000 and 4500 m altitude in the central Peruvian Andes, particularly in Junin plateau. Previous studies were focused to demonstrated the traditional fertility-enhancing properties of the hypocotyls of Maca (Cobo, 1956) in animal models (Zheng et al., 2000; Gonzales et al., 2001a, 2004; Cicero et al., 2001; Ruiz-Luna et al., 2005; Chung et al., 2005; Rubio et al., 2006a) and humans (Gonzales et al., 2001b).

Different biological properties have been observed among different varieties of Maca. In comparison to Yellow and Red Maca, the Black Maca presented the greatest effect on sperm production in male rats (Gonzales et al., 2006) and on latent learning in ovariectomized female mice (Rubio et al., 2006b). Red Maca significantly reduced prostate size in rats (Gonzales et al., 2005).

To our knowledge, nothing is known about the effect of Maca on memory using male animal models. For such reason, we evaluated the effect of two different doses of aqueous and hydroalcoholic extracts of Black Maca on male mice with memory impairment induced by scopolamine using the water Morris maze (Morris, 1984) and the step-down avoidance test (Luo et al., 2003a). In addition, the brain activities of AChE and MAO were assessed.

2. Materials and methods

2.1. Animals

Three-month-old male mice from the Kunming strain (25.68 ± 0.15 g) obtained from the Laboratory Animal Center of the Chinese Academy of Medical Science (Beijing, China) were used for the study. Mice were maintained at ambient temperature (23.0 ± 2.0 °C) with a 12:12 h light/dark cycle in the animal house of the Institute of Medicinal Plant Development (IMPLAD). Mice were provided with laboratory chow and tap water *ad libitum*. All animal experiments were conducted in compliance with "Guide of the care and use of laboratory animals" (National Research Council, 1996).

2.2. Preparation of aqueous and hydroalcoholic extracts of Black Maca (*L. meyenii*)

The dried hypocotyls of Black Maca were obtained from Carhuamayo, Junin at 4000 m altitude. Biological activity of the plant is located in the hypocotyls that are consumed by natives after being naturally dried (Gonzales, 2006). Irma Fernandez, who is a Botanist of the Department of Pharmaceutical Sciences, Universidad Peruana Cayetano Heredia, authenticated the identity of the plant. The voucher number IFV 1885 was deposited at the Department.

The aqueous extract of the hypocotyls were boiled according to the traditional method. After boiling, the aqueous preparation was left standing to cool, filtered, freezed at −70 °C and lyophilized (Lyophilizer freeze Mobile12). One gram of dried Black Maca hypocotyls produced 0.46 g of lyophilized Black Maca. The lyophilized aqueous extract of Black Maca was further diluted in distilled water to obtain different concentrations in 1 ml. Solutions were placed in vials and kept in a refrigerator at 4 °C until use.

Hydroalcoholic extract of Black Maca were prepared with aqueous ethanol (60%, v/v) by percolation at room temperature for 24 h and concentrate at low pressure to constant weight. The extract was prepared by Eng. Alfonso Higa from Agroindustrial Chanchamayo (Lima, Peru). One gram of dried Black Maca hypocotyls produced 0.22 g of hydroalcoholic Black Maca. This extract was further diluted in distilled water to obtain different concentrations in 1 ml. Solutions were placed in vials and kept in a refrigerator at 4 °C until use.

2.3. Treatments

In all experiments a feeding needle no. 18 (Fisher Scientific, Pittsburgh, PA) for oral administration was used to administer aqueous (0.5 and 2.0 g/kg) or hydroalcoholic (0.25 and 1.0 g/kg) Black Maca or vehicle by oral route for 35 days. Mice were weighed twice week. No differences between groups regarding to body weight were observed at the beginning of the experiment (*data not shown*).

Animals were randomly divided in the following groups (10 animals each): control (distilled water), scopolamine (1 mg/kg), two doses of hydroalcoholic extract of Black Maca (0.25 and 1.00 g/kg) and two doses of aqueous extract of Black Maca (0.50 and 2.00 g/kg). Scopolamine (scopolamine hydrobromide, Merck KGaA, Germany) and Maca groups received the drug (1 mg/kg i.p.) 30 min before each session of the Water Morris maze and 10 min before training session in step-down avoidance task. Control group received vehicle (i.p.) in the same way as mentioned above. The purpose of the present study was to evaluate the effect of Black Maca under experimental pathological conditions such as memory impairment induced by scopolamine using experimental models previously described (Yamada et al., 2004).

2.4. Water Morris maze

This task was adapted for mice from the paradigm originally described by Morris (Morris, 1984). The water maze was a circular pool (65 cm in diameter, 25 cm high), filled with water (26 ± 1 °C) and made opaque with black ink, to the depth of 20 cm. The pool was divided into four quadrants. An escape platform was placed in the middle of one quadrant, 1.0 cm below the water surface, equidistant from the sidewall and middle of the pool. The platform providing the only escape from the water was located in the same quadrant on every trial. Three different starting points for mice were placed around the perimeter of the pool. On each of the four training days, all three start points were used once each in a pseudo-random sequence. All assessments were performed using a CRE camera

that was suspended over the center of the pool. The swimming behavior for each mice was recorded using an automated tracking system (developed by China's Cosmonaut training Center and Institute of Medicinal Plant Development, Liu et al., 1998) coupled to a personal computer. The water maze was always located in a large room with a number of extra-maze visual cues including (lights, desks, personal computer and video equipment, etc.). The experimenter was always sat at the same position. All experiments (from day 23 to day 26) were carried out between 10:00 and 16:00.

A trial began by placing the animal in the water facing the wall of the pool at one of the starting points. If the animal failed to escape on the platform within 120 s, it was gently placed there by the researcher and allowed to stay for 15 s. The inter-trial interval was 5–10 min. Three escape trails were given to all mice per day for four consecutive days. The escape latency (s), swimming distance (cm) and average speed (cm/s) to reach the platform were recorded.

2.5. Step-down avoidance test

The apparatus consisted of a $27 \times 15 \times 12$ cm^3 plastic box whose floor was made of parallel bronze bars. The left end of the grid was occupied by a 4 cm diameter, 5 cm high wooden platform. The behavior of each mice were recorded in a personal computer using an automated tracking system (developed by China's Cosmonaut training Center and Institute of Medicinal Plant Development, Liu et al., 1994) coupled to a infrared sensor located in the apparatus. The experiments were carried out between 10:00 and 14:00.

The step-down avoidance test was performed 7 days after the Morris Water maze as previously described (Luo et al., 2003a). Before the beginning of the training session, mice were placed on the apparatus to adapt for 3 min. In the training session (day 34), mice were put on the grid floor and then a continuous electric shock was delivered to the grid floor by an isolated stimulator. When the electric shock was delivered, mice escaped from the grid floor back up onto the platform. The duration of training test was for 5 min and the shock was maintained for this period. In this session, the time that animals used to reach the shock-free zone was recorded for 5 min. Twenty-four hours after training, mice were placed on the platform for the retention test (day 35). The electric shocks were still delivered for 5 min. Step-down latency (time to find the platform) and the number of errors were recorded with improved retention reflected by increased latency and a reduction in errors.

2.6. Biochemical determination of AChE and MAO activities

At the end of the experiments, mice were sacrificed by decapitation and the brains were dissected out. The brains were homogenized in 10.0 ml of Saline (NaCl 0.9%), centrifuged at $3500 \times g$ for 15 min and the supernatant obtained for biochemical determinations (AChE and MAO activities).

The determination of the activity of AChE and MAO in brain was performed using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing City, PR China). All samples were run in a same assay to avoid between-assay variation. Data were referred by mg of protein. Protein concentrations were determined by the Lowry method (Lowry et al., 1951) using bovine serum albumin as a standard.

2.7. Statistical analysis

Data were analyzed using the statistical package STATA (version 8.0) for personal computers (Stata Corporation, 702 University Drive East, College Station, TX, USA).

Data are presented as mean ± standard error of the mean (SEM) or median and interquartile range. Homogeneity of variances was assessed using a Bartlett test. If variances were homogeneous, differences between groups and treatment were assessed by one-way or two-way analysis of variance (ANOVA). If the P value in the ANOVA test was significant, the differences between pair of means were assessed by the Scheffé test. One-way ANOVA was used to analyzed data from AChE and MAO activities; meanwhile, data obtained from the water Morris maze (swimming distance, escape latency and average speed) were analyzed using two-way ANOVA.

When variances were not homogeneous, the Kruskal–Wallis test was used to assess differences between groups. If the result was statistically significant differences between groups were assessed using the methodology described by Siegel and Castellan (1988) for nonparametric analyzes. Data from the Step-down avoidance test (latency and number of errors) were analyzed using the nonparametric tests mentioned above.

A value of $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Effect of aqueous and hydroalcoholic extracts of Black Maca on spatial memory in the water Morris maze

Fig. 1a and b shows the swimming distance to reach the platform of mice with memory impairment induced by scopolamine (1 mg/kg) and treated with 0.25 and 1.00 g/kg of hydroalcoholic extract of Black Maca (Fig. 1a) and 0.50 and 2.00 g/kg of aqueous extract of Black Maca (Fig. 1b).

Two-way ANOVA revealed an effect of the number of days ($F_{3,57} = 38.53$, $P < 0.05$) and groups ($F_{5,57} = 15.52$, $P < 0.05$). Swimming distance was increased by scopolamine administration with respect to the control group in all days of evaluation ($F_{1,78} = 42.17$, $P < 0.05$). In fact, scopolamine-treated mice did not learn the fixed position of the platform during all days of evaluation (P:NS); meanwhile, mice from control group showed shorter swimming distance during training days ($F_{3,36} = 4.80$, $P < 0.05$). The effect of scopolamine on swimming distance was observed in mice treated with the two extracts of Black Maca (both doses) during the first day by increasing significantly this parameter with respect to the control group ($F_{5,53} = 4.16$, $P < 0.05$). From day 2 to day 4, mice treated with aqueous and hydroalcoholic extracts of Black Maca showed a reduction in the swimming distance to reach the platform than scopolamine-treated mice ($F_{4,142} = 19.25$, $P < 0.05$). In addition, swimming distance was shorter in mice treated with 0.25 and 1.00 g/kg of hydroalcoholic extract of Black Maca and 0.50 and 2.00 g/kg of aqueous extract of Black Maca from day 1 to day 4 ($F_{3,36} = 13.97$, $P < 0.05$; $F_{3,36} = 11.79$, $P < 0.05$; $F_{3,36} = 30.18$, $P < 0.05$; $F_{3,36} = 13.31$, $P < 0.05$; respectively). No differences in swimming distance were observed between Black Maca-treated groups (Hydroalcoholic vs. aqueous extracts, P:NS).

Fig. 1c and d shows the effect of hydroalcoholic (0.25 and 1.00 g/kg) and aqueous (0.50 and 2.00 g/kg) extracts of Black Maca on escape latency of mice with scopolamine-induced memory impairment, respectively.

Two-way ANOVA revealed an effect of the number of days ($F_{3,57} = 26.20$, $P < 0.05$) and groups ($F_{5,57} = 6.94$, $P < 0.05$). During all sessions, scopolamine increased the escape latency with respect to control group ($F_{1,78} = 16.97$, $P < 0.05$). Mice treated with scopolamine did not show differences in escape latency from day 1 to day 4

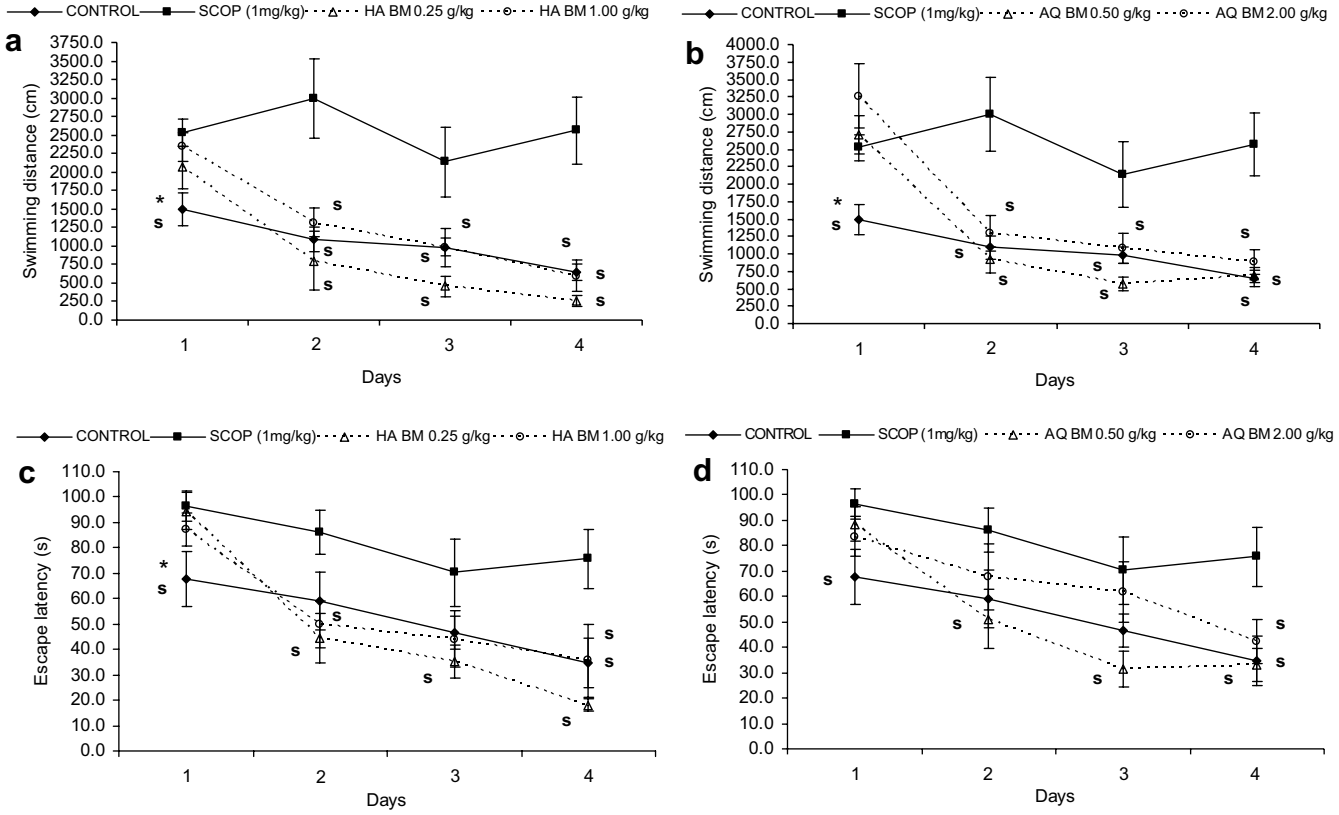

Fig. 1. The effect of aqueous and hydroalcoholic extracts of Black Maca on scopolamine-induced memory impairment on swimming distance (in cm) (a, b) and escape latency (in s) (c, d) in the Morris Water maze. Each day mice received by gavage distilled water (Control and SCOP groups), aqueous (0.50 and 2.00 g/kg) or hydroalcoholic (0.25 and 1.00 g/kg) extracts of Black Maca. All animals were tested for spatial memory 30 min after the injection of vehicle (control group) or scopolamine (1.00 mg/kg, in SCOP and Black Maca-treated groups). Values show are the mean total distance (cm) or escape latency (s) ± SEM. $^{*}P < 0.05$ respect to Black Maca-treated groups and $^{s}P < 0.05$ respect to SCOP group.

(*P*:NS). The lower doses of aqueous and hydroalcoholic extract of Black Maca (0.50 and 0.25 g/kg, respectively) reversed the negative effect of scopolamine on escape latency from day 2 to day 4 reaching similar values than control group ($F_{3,113} = 12.92$, $P < 0.05$). Animals treated with 1.00 g/kg of hydroalcoholic extract of Black Maca showed a lower escape latency on days 2 and 4 when compare to scopolamine group ($F_{2,27} = 3.62$, $P < 0.05$; $F_{2,27} = 3.75$, $P < 0.05$; respectively); meanwhile, the higher dose of aqueous extract of Black Maca (2.00 g/kg) reduced the escape latency only on day 4 ($F_{2,27} = 4.72$, $P < 0.05$). No differences were observed in escape latency when Black Maca-treated groups were compared (*P*:NS).

Average speed was no affected by any treatment during all days of evaluation (*data not shown*).

Fig. 2a–f shows the schematic representation of swimming paths at day 4 in mice treated with vehicle (a), scopolamine (b), 0.25 and 1 g/kg of hydroalcoholic extract of Black Maca (c and d, respectively) and 0.50 and 2.00 g/kg of aqueous extract of Black Maca (e and f, respectively).

As mentioned previously, higher swimming distance and escape latency in mice treated with scopolamine correlates with the higher rate of time the mice spent near the wall of the pool. At day 4, control mice and Black Maca-treated mice showed similar patterns of swimming paths.

3.2. Effect of aqueous and hydroalcoholic extracts of Black Maca on step-down latency

Table 1 shows the effect of aqueous (0.50 and 2.00 g/kg) and hydroalcoholic (0.25 and 1.00 g/kg) extracts of Black Maca on number of errors and latency in the step-down avoidance task in the test session. Data obtained from the test session was evaluated using Kruskal–Wallis and Mann–Whitney *U*-nonparametric tests.

Scopolamine (1 mg/kg) increased the number of errors with respect to control group ($P < 0.05$). Treatment with 0.50 and 2.00 g/kg of aqueous extract of Black Maca reduced the number of errors in the test day with respect to mice from the scopolamine group ($P < 0.05$). Regarding to mice treated with hydroalcoholic extract of Black Maca, only mice treated with 0.25 g/kg of this extract showed a reduction in the number of errors with respect to scopolamine group ($P < 0.05$). Mice treated with 1.00 g/kg of hydroalcoholic extract of Black Maca presented similar values in the number of errors when compared to scopolamine group (*P*:NS).

Step-down latency was reduced in mice from scopolamine group ($P < 0.05$). The treatment with both extracts (both doses) of Black Maca reversed the effect of scopolamine in step-down latency ($P < 0.05$). No differences were

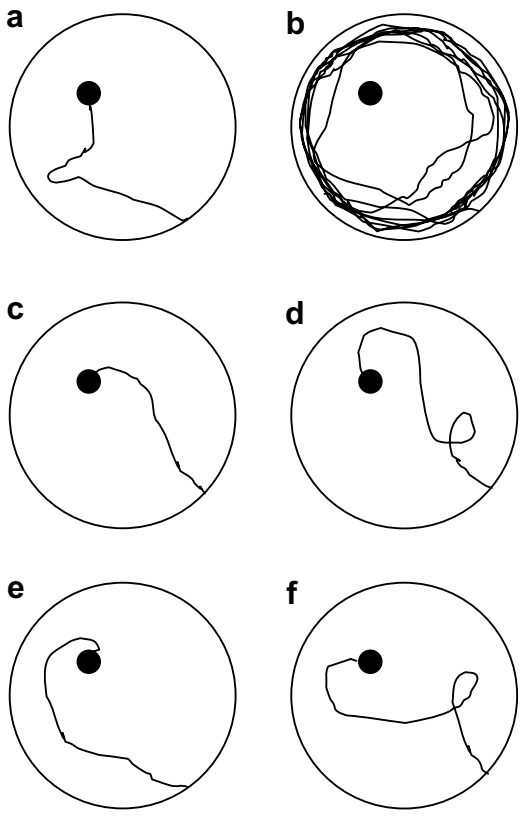

Fig. 2. Representation of swimming paths during the water Morris maze in the last day of evaluation (day 4): (a) Control (distilled water); (b) scopolamine (1 mg/kg); (c) 0.50 g/kg aqueous extract of Black Maca; (d) 2.00 g/kg aqueous extract of Black Maca; (e) 0.25 g/kg hydroalcoholic extract of Black Maca; (f) 1.00 g/kg hydroalcoholic extract of Black Maca.

observed when Black Maca-treated groups were compared (*P*:NS).

3.3. Effect of aqueous and hydroalcoholic extracts of Black Maca on brain AChE and MAO activities

The effect of aqueous and hydroalcoholic extracts of Black Maca in brain AChE and MAO activities are shown in Table 2. Differences between groups regarding to AChE and MAO activities were analyzed using one-way ANOVA.

Table 2
The effect of aqueous and hydroalcoholic extracts of Black Maca on AChE and MAO activity on mice brain treated with scopolamine

Treatment	Dose	AChE activity (U/mgprot)	MAO activity (U/h/mgprot)
Control	–	7 ± 1	15 ± 0.6
Scopolamine	1.00 mg/kg	11 ± 2*	15 ± 1
Hydroalcoholic extract of Black Maca	0.25 g/kg	5 ± 0.4[a]	17 ± 1
	1.00 g/kg	5 ± 0.3[a]	15 ± 1
Aqueous extract of Black Maca	0.50 g/kg	5 ± 0.4[a]	17 ± 1
	2.00 g/kg	5 ± 0.8[a]	18 ± 0.5

Values are presented as mean ± SEM.
* $P < 0.05$ respect to control group.
[a] $P < 0.05$ respect to scopolamine group.

Scopolamine administration resulted in significant increase in the AChE activity (1.50-fold with respect to control group). Meanwhile, aqueous and hydroalcoholic extracts of Black Maca reduced AChE activity reaching similar values to control group. Black Maca had values of AChE representing 46.01% and 48.23% (0.50 and 2.00 g/kg of aqueous extract, respectively) and 47.61% and 46.10% (0.25 and 1.00 g/kg of hydroalcoholic extract, respectively) than that observed in the scopolamine-treated group (Table 2). These results show that administration of Black Maca suppressed the increase of AChE activity by scopolamine administration.

Finally, MAO activity was not affected by any treatment (Table 2).

4. Discussion

Maca is naturally present in different varieties which are characterized by their external color (Tello et al., 1992; Yllescas, 1994). Recently, different biological effects were

Table 1
The effect of aqueous and hydroalcoholic extracts of Black Maca on scopolamine-induced memory impairment on the number of errors and step-down latency (s) in the step-down avoidance test

	Control	Scopolamine	Hydroalcoholic extract		Aqueous extract	
			0.25 g/kg	1.00 g/kg	0.50 g/kg	2.00 g/kg
Step-down latency	217† (152–256)	36* (26–168)	210† (153–254)	243† (151–284)	201† (74–251)	263† (149–291)
Gamma value	−0.67		0.68	0.80	0.62	0.76
P	0.001		0.001	0.000	0.002	0.000
Number of errors	3 (1–5)	5* (4–8)	3†,a (0–4)	5 (3–8)	3†,a (1–4)	2†,a (0.8–4)
Gamma value		0.71	−0.79; 0.62		−0.90; −0.68	−0.83; −0.63
P		0.000	0.000; 0.004		0.000; 0.003	0.000; 0.003

After daily oral treatment with distilled water (Control and SCOP groups) and aqueous (0.50 and 2.00 g/kg) and hydroalcoholic (0.25 and 1.00 g/kg) extracts of Black Maca, animals were tested in the step-down avoidance task. Mice received an injection of vehicle (control group) or scopolamine (1.00 mg/kg, in SCOP and Black Maca-treated groups) 30 min before the training session. Values are presented as medians and interquartile ranges.
* $P < 0.05$ respect to control group.
† $P < 0.05$ respect to scopolamine group.
[a] $P < 0.05$ with respect to hydroalcoholic extract of Black Maca.

described when Yellow, Red and Black Maca were assessed (Gonzales et al., 2006, 2005). For instance, Black Maca presents the greatest effect on latent learning in ovariectomized mice (Rubio et al., 2006b), a model used to represent the impairment of cognitive function due to estrogen deficiency in post-menopausal women (Monteiro et al., 2005).

In this study, memory was assessed using the water Morris maze and the step-down avoidance test. The effect of Black Maca on memory impairment induced by scopolamine in male mice was performed by using aqueous and hydroalcoholic extracts. Scopolamine interferes with memory and cognitive function in humans and experimental animals by blocking muscarinic receptors (Kopelman and Corn, 1988). This experimental animal model of scopolamine-induced amnesia has been extensively used in research to screen for drugs with potential therapeutic value in dementia (Bejar et al., 1999; de Angelis and Furlan, 1995; Hiramatsu et al., 1998; Mishima et al., 2003; Rubaj et al., 2003). Here, mice treated with scopolamine showed larger swimming distance and escape latency (water Morris maze) and shorter step-down latency (step-down avoidance test) than mice from control group. These results are in accordance with those described in previous studies (Kang et al., 2003; Yamada et al., 2004).

Both aqueous and hydroalcoholic extracts of Black Maca showed inhibitory effects against scopolamine-induced memory impairment in the water Morris maze; that is, mice treated with either aqueous or hydroalcoholic extracts of Black Maca showed shorter total swimming distance and escape latency than mice treated with scopolamine. Moreover, values in Black Maca-treated mice were comparable to those of non scopolamine-treated mice. It is important to notice that the water Morris maze investigated spatial learning and memory (D'Hooge and De Deyn, 2001) and it is especially sensitive to impaired cholinergic hippocampal function (Gage and Bjorklund, 1986). The latter suggests that Black Maca improved spatial learning and memory in male mice treated with scopolamine.

In the step-down avoidance test, Black Maca extracts exhibited an increase in the step-down latency with respect to scopolamine-treated mice. Also, a reduction in the numbers of errors during the test session was observed in Black Maca-treated mice. The step-down avoidance test is used to measure the three stages of memory (learning acquisition, memory retention, and retrieval) process depending on drug-treated period (Luo et al., 2003b). In this test, administration of scopolamine 30 min before the training session is related to learning acquisition. From these outcomes, it is suggested that Black Maca extracts improve learning acquisition reaching similar values to those of control group.

As mentioned above, special attention has been given to drugs with AChE inhibitory activity. Actually, the synthetic medicines: tacrine and donepezil, and the natural product-based: rivastigmine and galantamine are commonly used to treat cognitive dysfunction and memory loss associated with AD (Oh et al., 2004). These approved drugs are limited in use due to their adverse side effects (Small et al., 1997; Melzer, 1998; Schulz, 2003). For this reason, it is necessary to search long-acting AChEI with minimal clinical side effects. Results from the present study showed that the AChE activity of control mice increased 1.50-fold by scopolamine administration. Black Maca extracts decreased AChE activity to more than 45% of scopolamine-treated mice. These results showed the relation between the inhibitory effect on AChE activity of Black Maca and learning and memory improvement in male mice. In addition, previous studies did not show any toxic effect during short- and long-term administration with Maca (Chung et al., 2005; Gonzales et al., 2006). In fact, others authors showed a nutritional effect of Maca on mice (Canales et al., 2000) and rats (Gonzales et al., 2004). Although the purpose of the present study was to evaluate the effect of Black Maca under experimental pathological condition such as memory impairment induced by scopolamine, one limitation could be the lack of a group of mice treated only with Black Maca especially for interpreting the AChE activity.

The primary role of MAO lies in the metabolism of monoamines and in the regulation of monoamine neurotransmitter levels in brain and in the systemic circulation (Holschneider et al., 1998). The results from the present study show that Black Maca extracts did not alter MAO activity at any dose tested. From this, it is suggested that the neuroprotective effect of Black Maca do not result from direct MAO inhibition. Similar effects were observed with P10358 (1-[(3-fluoro-4-pyridinyl)amino]-3-methyl-1(*H*)-indol-5-yl-methyl carbamate, a reversible AChEI) and TV3326 (*N*-propargyl-(3*R*)-aminoindan-5-yl-ethyl methyl carbamate, an aminoindan derivative of the selective irreversible MAO-B inhibitor, rasagiline) (Smith et al., 1997; Weinstock et al., 2000). Despite Black Maca did not modify directly MAO activity, an effect on monoamine neurotransmitter should not be ruled out. For instance, it was suggested that P10358 alters striatal dopamine metabolism as a direct consequence of cholinergic stimulation and does not interfere with MAO activity or possess dopamine depleting properties (Smith et al., 1997).

These findings are important since it has been suggested the possibility that (1*R*,3*S*)-1-methyltetrahydro-beta-carboline-3-carboxylic acid, a molecule which is reported to be present in Maca, may affect negatively the central nervous system (Piacente et al., 2002). Tetrahydro-β-carbolines arises from a Pietet–Splenger condensation between L-tryptophan and aldehydes and this reaction, occurring in different foods, is temperature and pH-dependent (Herraiz and Ough, 1993). The latter makes possible to suggest that the traditional preparation of Maca may be not related to the occurrence of tetrahydro-β-carbolines in the aqueous extract. Further research is needed to confirm this suggestion. Moreover, as active principle of Maca acting on learning and memory is occurring in aqueous extract in which possibility to find alkaloids is limited, then it is

suggested that aqueous extract of Black Maca is devoid of activity that could be dangerous for the brain. On the contrary, data from this study and that from previous (Rubio et al., 2006b) suggest that Black Maca may be neuroprotective.

The compounds in Black Maca related to its neuroprotective effect have not been elucidated yet. However, it was suggested that the effect of Black Maca on learning and memory may be due to the presence of polyphenolic compounds such as quercetin and anthocyanins on Black Maca hypocotyls (Rubio et al., 2006b). In fact, previous studies indicate that quercetin and anthocyanins has neuroprotective role (Singh et al., 2003; Naidu et al., 2004; Ramirez et al., 2005; Andres-Lacueva et al., 2005). In addition, it has been demonstrated that polyphenolic compounds have an inhibitory effect on AChE activity (Kim et al., 2004). Some novel compounds have been recently identified, as two new imidazole alkaloids (lepidine A and B) (Cui et al., 2003). Also, a benzylated product, named Macaridine, derivative of 1,2-dihydro-*N*-hydroxypyridine, together with the benzylated alkamides (Macamides), *N*-benzyl-5-oxo-6*E*,8*E*-octadecadienamide and *N*-benzyl-hexadecanamide, as well as the acyclic keto acid, 5-oxo-6*E*,8*E*-octadecadienoic acid have been described (Muhammad et al., 2002). However, the effect of these compounds has not been assessed for any of the biological effects described for Maca including learning and memory and AChE inhibitory activity.

5. Conclusion

In summary, Black Maca inhibits the scopolamine-induced memory impairment.

Acknowledgements

Peng Bo, Hu Qin, Xiaming Wen, Yang Keli, Jiang Zhen and Sandra Yucra are acknowledged for their help in this study. Special thanks to the joint financial support for this work from the National Nature Science Foundation of China, NSFC in 2004 (30472016/C03020701) and from Peruvian National Council of Sciences, Technology and Innovation (CONCYTEC) through the grant PROCOM 2005 and support for travel. Julio Rubio was on leave from the Universidad Peruana Cayetano Heredia, Peru (UPCH) through the Memorandum of Understanding between IMPLAD, CONCYTEC and UPCH.

References

Andres-Lacueva, C., Shukitt-Hale, B., Galli, R.L., Jauregui, O., Lamuela-Raventos, R.M., Joseph, J.A., 2005. Anthocyanins in aged blueberry-fed rats are found centrally and may enhance memory. Nutr. Neurosci. 8, 111–120.

Bartus, R.T., 2000. On neurodegenerative diseases, models, and treatment strategies: lessons learned and lessons forgotten a generation following the cholinergic hypothesis. Exp. Neurol. 163, 495–529.

Bejar, C., Wang, R., Weinstock, M., 1999. Effect of rivastigmine on scopolamine-induced memory impairment in rats. Eur. J. Pharmacol. 383, 231–240.

Canales, M., Aguilar, J., Prada, A., Marcelo, A., Huaman, C., Carbajal, L., 2000. Nutritional evaluation of *Lepidium meyenii*(Maca) in albino mice and their descendants (in Spanish). Arch. Latinoam. Nutr. 50, 126–133.

Chung, F., Rubio, J., Gonzales, C., Gasco, M., Gonzales, G.F., 2005. Dose–response effects of *Lepidium meyenii* (Maca) aqueous extract on testicular function and weight of different organs in adult rats. J. Ethnopharmacol. 98, 143–147.

Cicero, A.F.G., Bandiere, E., Arletti, R., 2001. *Lepidium meyenii* Walp. improves sexual behaviour in male rats independently from its action on spontaneous locomotor activity. J. Ethnopharmacol. 75, 225–229.

Cobo, B., 1956. In: de Mateos, Francisco (Ed.), History of the New World. Ediciones Atlas, Madrid.

Cui, B., Zheng, B.L., He, K., Zheng, Q.Y., 2003. Imidazole alkaloids from *Lepidium meyenii*. J. Nat. Prod. 66, 1101–1103.

Davies, P., Maloney, A.J.F., 1976. Selective loss of central cholinergic neurons in Alzheimer type disease. Lancet 2, 1403.

de Angelis, L., Furlan, C., 1995. The effects of ascorbic acid and oxiracetam on scopolamine-induced amnesia in a habituation test in aged mice. Neurobiol. Learn. Mem. 64, 119–124.

D'Hooge, R., De Deyn, P.P., 2001. Applications of the Morris water maze in the study of learning and memory. Brain Res. Brain Res. Rev. 36, 60–90.

Farlow, M., Gracon, S.I., Hershey, L.A., Lewis, K.W., Sadowsky, C.H., Dolan-Ureno, J., 1992. A controlled trial of tacrine in Alzheimer's disease. The Tacrine Study Group. JAMA 268, 2523–2529.

Gage, F., Bjorklund, A., 1986. Cholinergic septal grafts into the hippocampal formation improve spatial learning and memory in aged rats by an atropine-sensitive mechanism. J. Neurosci. 6, 2837–2847.

Gonzales, G.F., 2006. Biological effects of *Lepidum meyenii*, Maca, a plant from the highlands of Peru. In: Singh, V.K., Bhardwaj, R., Govil, J.N., Sharma, R.K. (Eds.), Recent Progress in Medicinal Plants, . In: Natural Products, vol. 15. Studium Press LLC, USA, pp. 217–242.

Gonzales, G., Ruiz, A., Gonzales, C., Villegas, L., Cordova, A., 2001a. Effect of *Lepidium meyenii* (Maca) roots on spermatogenesis of male rats. Asian J. Androl. 3, 231–233.

Gonzales, G.F., Cordova, A., Gonzales, C., Chung, A., Vega, K., Villena, A., 2001b. *Lepidium meyenii* (Maca) improved semen parameters in adult men. Asian J. Androl. 3, 301–303.

Gonzales, G.F., Gasco, M., Córdova, A., Chung, A., Rubio, J., Villegas, L., 2004. Effect of *Lepidium meyenii* (Maca) on spermatogenesis in male rats acutely exposed to high altitude (4340 m). J. Endocrinol. 180, 87–95.

Gonzales, G.F., Miranda, S., Nieto, J., Fernandez, G., Yucra, S., Rubio, J., Yi, P., Gasco, M., 2005. Red Maca (*Lepidium meyenii*) reduced prostate size in rats. Reprod. Biol. Endocrinol. 3, 5.

Gonzales, C., Rubio, J., Gasco, M., Nieto, J., Yucra, S., Gonzales, G.F., 2006. Effect of short-term and long-term treatments with three ecotypes of *Lepidium meyenii* (Maca) on spermatogenesis in rats. J. Ethnopharmacol. 103, 448–454.

Halbreich, U., Lumley, L.A., Palter, S., Manning, C., Gengo, F., Joe, S.H., 1995. Possible acceleration of age effects on cognition following menopause. J. Psychiatr. Res. 29, 153–163.

Heikkinen, T., Kalesnykas, G., Rissanen, A., Tapiola, T., Livonen, S., Wang, J., Chaudhuri, J., Tanila, H., Miettinen, R., Puolivali, J., 2004. Estrogen treatment improves spatial learning in APP + PS1 mice but does not affect beta amyloid accumulation and plaque formation. Exp. Neurol. 187, 105–117.

Herraiz, T., Ough, C.S., 1993. Chemical and technological factors determining tetrahydro-β-carboline-3-carboxylic acids content in fermented alcoholic beverages. J. Agric. Food Chem. 41, 959–964.

Hiramatsu, M., Murasawa, H., Nabeshima, T., Kameyama, T., 1998. Effects of U-50,488H on scopolamine-, mecamylamine-, and dizoclipine-induced learning and memory impairment in rats. J. Pharmacol. Exp. Ther. 284, 858–867.

Holschneider, D.P., Kumazawa, T., Chen, K., Shih, J.C., 1998. Tissue-specific effects of estrogen on monoamine oxidase A and B in the rat. Life Sci. 63, 155–160.

Houghton, P.J., Agbedahunsi, J.M., Adegbulugbe, A., 2004. Choline esterase inhibitory properties of alkaloids from two Nigerian Crinum species. Phytochemistry 65, 2893–2896.

Kang, S.Y., Lee, K.Y., Park, M.J., Kim, Y.C., Markelonis, G.J., Oh, T.H., Kim, Y.C., 2003. Decursin from *Angelica gigas* mitigates amnesia induced by scopolamine in mice. Neurobiol. Learn. Mem. 79, 11–18.

Kang, S.Y., Lee, K.Y., Koo, K.A., Yoon, J.S., Lim, S.W., Kim, Y.C., Sung, S.H., 2005. ESP-102, a standardized combined extract of *Angelica gigas*, *Saururus chinensis* and *Schizandra chinensis*, significantly improved scopolamine-induced memory impairment in mice. Life Sci. 76, 1691–1705.

Kim, H.K., Kim, M., Kim, S., Kim, M., Chung, J.H., 2004. Effects of green tea polyphenol on cognitive and acetylcholinesterase activities. Biosci. Biotechnol. Biochem. 68, 1977–1979.

Knapp, M.J., Knopman, D.S., Solomon, P.R., Pendlebury, W.W., Davis, C.S., Gracon, S.L., 1994. A 30-week randomized controlled trial of highdose tacrine in patients with Alzheimer's disease. JAMA 271, 985–991.

Kopelman, M.D., Corn, T.H., 1988. Cholinergic blockade as a model of cholinergic depletion. Brain 111, 1079–1110.

Lahiri, D.K., Farlow, M.R., Greig, N.H., Sambamurti, K., 2002. Current drug targets for Alzheimer's disease treatment. Drug Dev. Res. 56, 267–281.

Lee, B., Choi, Y., Kim, H., Kim, S.Y., Hahm, D.H., Lee, H.J., Shim, I., 2003. Protective effects of methanol extract of Acori graminei rhizoma and Uncariae Ramulus et Uncus on ischemia-induced neuronal death and cognitive impairments in the rat. Life Sci. 74, 435–450.

Lee, K.Y., Jeong, E.J., Lee, H.S., Kim, Y.C., 2006. Acteoside of *Callicarpa dichotoma* attenuates scopolamine-induced memory impairments. Biol. Pharm. Bull. 29, 71–74.

Liu, X.M., Wang, S., Yu, S., Wang, L., Xiao, P., Chen, S., Su, S., Xing, Z., 1994. Computer-aided automatic control system for measuring step-down performance in mice. Acta Lab. Sci. Sin. 2, 20–25.

Liu, X.M., Chen, S., Wang, S., Meng, H., Su, S., Song, J., Shang, W., Yang, J., Xiao, P., 1998. The establishment of computer-based auto-controlling and imagine-analysing processing system of circle water-maze for studying neotropic drugs from traditional Chinese medicinal herbs. Zhong Cao Yao 29, 174–177.

Lowry, O.H., Rosenbrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193, 265–275.

Luo, J., Yin, J.H., Wei, Q., 2003a. The effect of calcineurin activator, extracted from Chinese herbal medicine, on memory and immunity in mice. Pharmacol. Biochem. Behav. 75, 749–754.

Luo, J., Yin, J.H., Wu, H.Z., Wei, Q., 2003b. Extracts from Fructus cannabis activating calcineurin improved learning and memory in mice with chemical drug-induced dysmnesia. Acta Pharmacol. Sin. 24, 1137–1142.

Melzer, D., 1998. New drug treatment for Alzheimer's disease: lessons for healthcare policy. BMJ 316, 762–764.

Mishima, K., Tsukikawa, H., Miura, I., Inada, K., Abe, K., Matsumoto, Y., Egashira, N., Iwasaki, K., Fujiwara, M., 2003. Ameliorative effect of NC-1900, a new AVP(4-9) analog, through vasopressin V(1A) receptor on scopolamine-induced impairments of spatial memory in the eight-arm radial maze. Neuropharmacology 44, 541–552.

Monteiro, S.C., Matte, C., Bavaresco, C.S., Netto, C.A., Wyse, A.T.S., 2005. Vitamins E and C pretreatment prevents ovariectomy-induced memory deficits in water maze. Neurobiol. Learn. Mem. 84, 192–199.

Morris, R., 1984. Development of water-maze procedure for studying spatial learning in the rat. J. Neurosci. Methods 11, 47–60.

Muhammad, I., Zhao, J., Dunbar, D.C., Khan, I.A., 2002. Constituents of *Lepidium meyenii* "Maca". Phytochemistry 59, 105–110.

Naidu, P.S., Singh, A., Kulkarni, S.K., 2004. Reversal of reserpine-induced orofacial dyskinesia and cognitive dysfunction by quercetin. Pharmacology 70, 59–67.

National Research Council, 1996. Guide of the Care and Use of Laboratory Animals. National Academy Press, Washington, DC, p. 125.

Oh, M.H., Houghton, P.J., Whang, W.K., Cho, J.H., 2004. Screening of Korean herbal medicines used to improved cognitive function for anti-cholinesterase activity. Phytomedicine 11, 544–548.

Piacente, S., Carbone, V., Plaza, A., Zampelli, A., Pizza, C., 2002. Investigation of the tuber constituents of Maca (*Lepidium meyenii* Walp.). J. Agric. Food Chem. 50, 5621–5625.

Ramirez, M.R., Izquierdo, I., do Carmo Bassols Raseira, M., Zuanazzi, J.A., Barros, D., Henriques, A.T., 2005. Effect of lyophilised Vaccinium berries on memory, anxiety and locomotion in adult rats. Pharmacol. Res. 52, 457–462.

Rogers, S.L., Farlow, M.R., Doody, R.S., Mohs, R., Friedhoff, L.T., 1998. A 24-week, double-blind, placebo-controlled trial of donepezil in patients with Alzheimer's disease. Neurology 50, 136–145.

Rubaj, A., Zgodzinski, W., Sieklucka-Dziuba, M., 2003. The influence of adenosine A3 receptor agonist: IB-MECA, on scopolamine- and MK-801-induced memory impairment. Behav. Brain Res. 141, 11–17.

Rubio, J., Riqueros, M.I., Gasco, M., Yucra, S., Miranda, S., Gonzales, G.F., 2006a. *Lepidium meyenii* (Maca) reversed the lead acetate induced-damage on reproductive function in male rats. Food Chem. Toxicol. 44, 1114–1122.

Rubio, J., Caldas, M., Dávila, S., Gasco, M., Gonzales, G.F., 2006b. Effect of three different cultivars of *Lepidium meyenii* (Maca) on learning and depression in ovariectomized mice. BMC Complement. Altern. Med. 6, 23.

Ruiz-Luna, A.C., Salazar, S., Aspajo, N.J., Rubio, J., Gasco, M., Gonzales, G.F., 2005. *Lepidium meyenii* (Maca) increases litter size in normal adult female mice. Reprod. Biol. Endocrinol. 3, 17.

Schulz, V., 2003. Ginkgo extract or cholinesterase inhibitors in patients with dementia: what clinical trials and guidelines fail to consider. Phytomedicine 10, 74–79.

Siegel, S., Castellan, N.J., 1988. Nonparametric Statistics for the Behavioral Sciences, second ed. McGraw-Hill, New York, USA, p. 344.

Singh, A., Naidu, P.S., Kulkarni, S.K., 2003. Reversal of aging and chronic ethanol-induced cognitive dysfunction by quercetin a bioflavonoid. Free Radic. Res. 37, 1245–1252.

Small, G.W., Robins, R.V., Barry, P.P., Buckholts, N.S., Dekosky, S.T., Ferris, S.H., Finkel, S.I., Gwyther, L.P., Khachaturian, Z.S., Lebowitz, B.D., McRae, T.D., Morris, J.C., Oakley, F., Schneider, L.S., Streim, J.E., Sunderland, T., Teri, L.A., Tune, L.E., 1997. Diagnosis and treatment of Alzheimer's disease and related disorders. Consensus statement of the American Association for Geriatric Psychiatry, the Alzheimer's Association, and the American Geriatrics Society. JAMA 278, 1363–1371.

Smith, C.P., Bores, G.M., Petko, W., Li, M., Selk, D.E., Rush, D.K., Camacho, F., Winslow, J.T., Fishkin, R., Cunningham, D.M., Brooks, K.M., Roehr, J., Hartman, H.B., Davis, L., Vargas, H.M., 1997. Pharmacological activity and safety profile of P10358, a novel, orally active acetylcholinesterase inhibitor for Alzheimer's disease. J. Pharmacol. Exp. Ther. 280, 710–720.

Tello, J., Hermann, M., Calderón, A., 1992. La Maca (*Lepidium meyenii* Walp.) cultivo alimenticio potencial para las zonas altoandinas. Boletín de Lima 14, 59–66.

Wang, R., Yan, H., Tang, X.C., 2006. Progress in studies of huperzine A, a natural cholinesterase inhibitor from Chinese herbal medicine. Acta Pharmacol. Sin. 27, 1–26.

Weinstock, M., Bejar, C., Wang, R.H., Poltyrev, T., Gross, A., Finberg, J.P., Youdim, M.B., 2000. TV3326, a novel neuroprotective drug with cholinesterase and monoamine oxidase inhibitory activities for the treatment of Alzheimer's disease. J. Neural Transm. Suppl. 60, 159–169.

Yamada, N., Hattori, A., Hayashi, T., Nishikawa, T., Fukuda, H., Fujino, T., 2004. Improvement of scopolamine-induced memory impairment by *Z*-ajoene in the water maze in mice. Pharmacol. Biochem. Behav. 78, 787–791.

Yan, J.J., Kim, D.H., Moon, Y.S., Jung, J.S., Ahn, E.M., Baek, N.I., Song, D.K., 2004. Protection against beta-amyloid peptide-induced memory impairment with long-term administration of extract of *Angelica gigas* or decursinol in mice. Prog. Neuropsychopharmacol. Biol. Psychiatry 28, 25–30.

Yllescas, M., 1994. Estudio químico y Fisicoquímico de tres ecotipos de *Lepidium meyenii* procedentes de Carhuamayo. Facultad de Farmacia y Bioquímica, Universidad Nacional Mayor de San Marcos, Lima, Peru.

Yu, M.S., Leung, S.K., Lai, S.W., Che, C.M., Zee, S.Y., So, K.F., Yuen, W.H., Chang, R.C., 2005. Neuroprotective effects of anti-aging oriental medicine *Lycium barbarum* against beta-amyloid peptide neurotoxicity. Exp. Gerontol. 40, 716–727.

Zheng, B.L., He, K., Kim, C.H., Rogers, L., Shao, Y., Huang, Z.Y., Lu, Y., Yan, S.J., Qien, L.C., Zheng, Q.Y., 2000. Effect of a lipidic extract from *Lepidium meyenii* on sexual behavior in mice and rats. Urology 55, 598–602.