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Mangifera indica L. extract (Vimang) improves the aversive memory in spinocerebellar ataxia type 2 transgenic mice

[*El extracto de* Mangifera indica *L. (Vimang) mejora la memoria aversiva en ratones transgénicos portadores de ataxia espinocerebelosa tipo 2*]

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Abstract

Context: The spinocerebellar ataxia type 2 (SCA-2) is a progressive neurodegenerative disorder without specific therapy identified, and it is related to the loss of function in the cerebellum, mitochondrial dysfunction, oxidative stress and neurotoxic processes. Scientific evidence indicates that *Mangifera indica* L. aqueous extract (MiE) and its major constituent (mangiferin) display antioxidant, anti-inflammatory and neuroprotective actions.

Aims: To investigate the MiE and mangiferin effects on behavioral outcomes of neurological function in SCA-2 transgenic mice.

Methods: The SCA-2 transgenic mice were daily and orally administered during 12 months with MiE (10, 50, and 100 mg/kg), mangiferin (10 mg/kg) or vehicle. It was evaluated locomotion (open-field), aversive memory (inhibitory avoidance) and declarative memory (object recognition). To explore possible cellular mechanisms underlying the in vivo effects was also evaluated their effects on nerve grow factor (NGF) and tumor necrosis factor-α (TNF-α) levels in the human glioblastoma cell line U138-MG supernatant.

Results: MiE administration did not affect the object recognition memory, but mangiferin did. The natural extract improved selectively the aversive memory in SCA-2 mice, indicating that MiE can affect behavioral parameters regarding fear-related memory. MiE also induced a significant increase in supernatant levels of NGF and TNF-α in vitro in human U138-MG glioblastoma cells.

Conclusions: The results suggest that MiE enhances the aversive memory through a mechanism that might involve an increase in neurotrophin and cytokine levels. These findings constitute the basis for the use of the natural extract in the prevention/treatment of memory deficits in SCA-2.

Keywords: *Mangifera indica;* memory; nerve growth factor; spinocerebellar ataxia type 2; tumor necrosis factor; Vimang.

Resumen

Contexto: La ataxia espinocerebelosa tipo 2 (SCA-2) es una enfermedad neurodegenerativa progresiva, sin una terapia específica. Se asocia con pérdida de la función del cerebelo, disfunción mitocondrial, estrés oxidativo y neurotoxicidad. Evidencias científicas indican que el extracto acuoso de *Mangifera indica* L. (MiE) y su componente mayoritario (mangiferina) poseen propiedades antioxidantes, anti-inflamatorias y neuroprotectoras.

Objetivos: Investigar los efectos de MiE y mangiferina sobre parámetros conductuales de la función neurológica en ratones transgénicos portadores de la SCA-2.

Métodos: Los animales se trataron diariamente y por vía oral, durante 12 meses con MiE (10, 50, y 100 mg/kg), mangiferina (10 mg/kg) o vehículo. Se evaluaron la locomoción, la memoria aversiva y la memoria declarativa. También se evaluaron sus efectos sobre los niveles del factor de crecimiento neuronal (NGF) y el factor de necrosis tumoral- α (TNF- α) en un cultivo de células neuronales.

Resultados: El extracto no afectó la memoria de reconocimiento de objetos, pero la mangiferina sí la modificó. También mejoró selectivamente la memoria aversiva de los ratones SCA-2, indicando que puede afectar la memoria asociada al temor en esta patología. Además, el MiE indujo significativamente los niveles de NGF y TNF- α in vitro, en los sobrenadantes del glioblastoma humano U138-MG.

Conclusiones: Los resultados sugieren que el MiE mejora la memoria aversiva por medio de mecanismos que pueden involucrar un incremento en los niveles de neurotrofinas y citocinas y constituyen las bases para el uso del extracto natural en la prevención/tratamiento del déficit de memoria en la SCA-2.

Palabras Clave: Ataxia espinocerebelosa tipo 2; factor de crecimiento neuronal; factor de necrosis tumoral; *Mangifera indica;* memoria; Vimang.

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INTRODUCTION

The spinocerebellar ataxia type 2 (SCA-2) is a progressive and rather incapacitating disorder, with no treatment available. The principal clinical manifestations of SCA-2 are cerebellar gait ataxia, dysmetria, adiadochokinesis and dysarthria, as well as saccadic and slow ocular movements, hypotonia and abnormal tendon reflexes (Orozco et al., 1990). SCA-2 is caused by the expansion of a CAG repeated in the coding region of the ataxin-2 gene to more than 31 repetitions. It is relatively rare worldwide, but common in the eastern part of Cuba, with a prevalence of 50 patients per 100,000 (Pulst, 2003). The Spinocerebellar ataxias are related to the loss of function in the cerebellum, mitochondrial dysfunction, and neurotoxicity processes. Oxidative stress is a key factor in the pathogenesis of inherited ataxias, but the mechanisms involved vary between different conditions. So, there is a rationale for testing antioxidants in ataxias attributable to DNA repair defects, autosomal-dominant spinocerebellar ataxias and ataxias with primary mitochondrial dysfunction (Pandolfo, 2008).

Polyphenols represent a group of secondary metabolites that widely occur in plants and have been studied for their strong antioxidant capacities and other properties, by which cell functions are regulated (Han et al., 2007). The composition of *M. indica* includes a mixture of polyphenols, terpenoids, steroids, fatty acids and microelements. A detailed phytochemical investigation of mango stem bark extract has led to the isolation of seven phenolic constituents: gallic acid, 3,4-dihydroxy benzoic acid, gallic acid methyl ester, gallic acid propyl ester, mangiferin, (+)-catechin, (-)-epicatechin, benzoic acid and benzoic acid propyl ester; mangiferin was found to be the predominant component (Núñez-Sellés et al., 2002).

The polyphenol mangiferin, a xanthone glucoside, has been reported to have multiple biological effects, as antioxidant and immunomodulatory (Leiro et al., 2003), anti-allergic (Rivera et al., 2006), anti-inflammatory and antinociceptive (Garrido et al., 2004a). Mangiferin enhances recognition memory in rats through a mechanism that might involve cell proliferation and an increase in supernatant levels of the neurotrophin nerve growth factor (NGF) and the cytokine tumor necrosis factor (TNF-α) in vitro in human U138-MG glioblastoma cells (Pardo-Andreu et al., 2010). Mangiferin also produced an impairment of fear memory consolidation in rats (Andreu et al., 2011).

Based on phytochemistry composition and on the ethnopharmacological knowledge, a standardized aqueous extract from the bark of selected species of *Mangifera indica* L. (MiE) has been developed in Cuba as a food supplement, under the brand name of Vimang® (Guevara-García et al., 2004). This extract is proposed as a nutritional supplement (antioxidant) and an anti-inflammatory, analgesic and immunomodulatory treatment to prevent disease progress or to increase the patient's quality of life in gastric and dermatological disorders, AIDS, cancer and asthma (Núñez-Sellés et al., 2002). MiE has shown potent in vitro and in vivo antioxidant activities, in a range of experimental models apparently due to phenolic components (Pardo-Andreu et al., 2008). In addition, it produces analgesia and anti-inflammatory effects in rodent models (Garrido et al., 2004a), modulates macrophage function (Leiro et al., 2004) and mouse humoral immune responses (Garcia et al., 2003), and protects against septic shock in a rodent model (Garrido et al., 2004b). Cellular mechanisms mediating the actions of Vimang® might include inhibitions of T cell proliferation, tumor necrosis factor (TNF-α)-induced activation of nuclear factor kB (NF-κB), interleukin-1beta (IL-1β), nitric oxide synthase (iNOS), and cyclooxygenase-2 (COX-2) expressions (Núñez-Sellés et al., 2007). Furthermore, it was found that MiE prevents glutamate-induced excytotoxicity in primary cultured neurons of the rat cerebral cortex (Lemus-Molina et al., 2009).

The Center for Genetic Engineering and Biotechnology, Havana, Cuba has generated a unique SCA-2 transgenic animal mouse, microinjecting a human purified SCA-2 transgenic fragment (Aguiar et al., 2006). Given the several biological actions of MiE and mangiferin, especially those related with their antioxidant and neuroprotective potentials, we investigated their effects

on behavioral outcomes of neurological function in SCA-2 transgenic mice.

MATERIALS AND METHODS

Plant material and preparation

Mangifera indica L. stem bark was collected from a cultivated field located in the region of Pinar del Rio, Cuba. Voucher specimens of the plant (Code 41722) were deposited at the Herbarium of the Academy of Sciences, guarded by the Institute of Ecology and Systematics from the Ministry of Science, Technology and Environment, Havana, Cuba and authenticated by MSc Ramona Prieto, curator, and MSc Isora Baró, Director of the Herbarium.

Stem bark extracts of *Mangifera indica* L. were prepared by decoction in water, concentrated by evaporation and spray dried to obtain a fine brown powder, coded as 112, that melted at 210–215 ºC with decomposition and contained the active ingredient used in Vimang® pharmaceutical formulations. Planar, liquid and gas chromatographic methods, mass spectrometry and UV/VIS spectrophotometry, showed them to contain polyphenols as major (45%) fraction (Núñez-Sellés et al., 2002). Mangiferin (2-β-d-glucopyranosyl-1,3,6,7- tetrahydroxy-9H-xanthen-9-one), supplied by the Centre of Pharmaceutical Chemistry (Cuba), had been purified from MiE by extraction with methanol; HPLC showed it to be 95% pure.

Drugs and pharmacological procedures

Experimental procedures were carried out in accordance with European regulations on animal protection (Directive 86/609), the Declaration of Helsinki, and/or the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the US National Institute of Health (NIH Publication № 85-23, revised 1996).

All experimental protocols were approved by the Institutional Animal Care and Ethical Committee from the Centro de Estudio para las Investigaciones y Evaluaciones Biológicas, Instituto de Farmacia y Alimentos, Universidad de La Habana, Cuba.

Adult female mice F066 transgenic (with SCA-2) and the founder animals B6D2F1 (wild-type control) (Aguiar et al., 2006) were obtained from the Center for Genetic Engineering and Biotechnology (Havana, Cuba). After division into seven groups of at least five animals, the mice were treated daily, during 12 months, with oral administration of water (untreated control wild-type or untreated SCA-2 transgenic groups); as well as 10, 50, 100 mg/kg of MiE and 10 mg/kg of mangiferin in the SCA-2 transgenic animals, and the higher dose of MiE (100 mg/kg) in the founder animals (control wild-type) in order to perform the memory tests. MiE and mangiferin solutions for the animal's treatment were daily prepared in distilled water. The animals were kept on a 12 h light/dark cycle with food (Standard diet for rodents, CENPALAB) and water available *ad libitum*, housed in a controlled environment at 20 ± 2°C. The transgenic SCA-2 male mice also were used (data not shown). Given the probable greater severity of disease in males, some animals died, reducing the sample size, not allowing consistent data. All procedures were conducted from 10 a.m. to 5 p.m. and were approved by the institutional animal care committee. The same animals were used in all tests, at one week interval.

Experimental protocol of behavioral tests

Open-field behavior

Open-field exploration was carried out as previously described (Maurmann et al., 2011). The open-field was performed in a 60 × 45 × 50 cm arena (Ugo Basile, Italy). The floor of the arena was divided into 12 equal squares by black lines.

The mice were put in the arena, placed on its left rear quadrant and left to explore it freely for 5 min. Latency to start locomotion, crossings of the black lines, rearings performed and the number of fecal pellets were counted. The number of crossings and rearings were used respectively as measures of locomotor activity and exploratory behavior, whereas the latency to start locomotion and the number of fecal pellets were used as a measure of anxiety (Maurmann et al., 2011). Twenty-four hours later, the animals were left to explore

the apparatus again for another 5 min, and the same measures were recorded to evaluate habituation to the open-field.

Novel object recognition

The novel object recognition task was performed as previously described (Pardo-Andreu et al., 2010; Maurmann et al., 2011). Object recognition training and test trials took place in the same arena used for the open-field.

Training was conducted by placing individual mice for 5 min into the arena, in which two identical objects (objects A1 and A2) were positioned in two adjacent corners, 10 cm from the walls. In a long-term memory retention test given 24 h after training, the same mice explored the field for 5 min in the presence of a familiar object A1 and a novel object B. Between trials the objects were washed with 70% ethanol solution. All objects presented similar textures and sizes, but distinctive colors and shapes. Exploration was defined as sniffing or touching an object with the nose and/or forepaws. The exploratory preference or the object recognition index was defined as the percentage of the total exploration time that the animal spent investigating object A2 (in the training trial) or the novel object.

Inhibitory avoidance

Inhibitory avoidance in rodents is a widely used animal model of fear-related learning and memory. The step-down inhibitory avoidance apparatus and procedures were described in previous studies (Maurmann et al., 2011; Andreu et al., 2011). The inhibitory avoidance test apparatus was a box of 30 x 21 x 18.5 cm (Insight®, Riberão Preto, SP, Brazil) whose floor consisted of parallel stainless steel bars (1 mm diameter) spaced 1 cm apart with a 2 cm high platform placed on the right side.

In the training trial, animals were placed on the platform, and their latency to step-down on the grid with all 4 paws was recorded; immediately after stepping down on the grid, animals were given a 0.3 mA/3 s foot shock. In the retention test session carried out 24 h after training (long-term memory retention), no foot shocks were given when stepping-down on the grid, and ceiling of 180 s was imposed in the test latency.

Cell culture

The human glioblastoma cell line U138-MG was used in this study to explore possible cellular mechanisms underlying the effects of MiE in the brain. Cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, penicillin (100 U/mL), and streptomycin (100 µg/mL) (Farias et al., 2008; Pardo-Andreu et al., 2010).

Measurement of nerve growth factor levels in cell culture supernatants

After 72 h of incubation, cell supernatants were collected for determination of nerve growth factor (NGF) levels using sandwich enzyme-linked immunosorbent assay (ELISA) commercial kits according to the manufacturer's instructions (Chemicon, USA), as previously described (Farias et al., 2009; Pardo-Andreu et al., 2010). Briefly, micro-titer plates (96-well flat-bottom) were coated for 24 h with the samples diluted 1:2 in sample diluents and standard curve ranged from 7.8 to 500.0 pg of NGF. Then, plates were washed four times with sample diluents. Monoclonal anti-NGF rabbit antibody diluted 1:1000 in sample diluent was incubated for 3 h at room temperature. After washing, a second incubation with anti-rabbit antibody peroxidase conjugated diluted 1:1000 for 1 h at room temperature was carried out. After addition of streptavidin-enzyme, substrate and stop solution the amount of NGF was determined for absorbance in 450 nm. The standard curve demonstrates a direct relationship between optical density (OD) and NGF concentration.

Measurement of tumor necrosis factor-α concentration in cell culture supernatants

The amount of TNF-α in the culture supernatants after 72 h of incubation were determined using Ready-To-Go Cytokine Elisa kit (eBioscience, catalog number 88-7346, San Diego, CA, USA) according to the manufacturer protocol. Recombinant human cytokine was

employed for generating a standard curve, which demonstrated a direct relationship between the log OD and the log of TNF-α concentration (Pardo-Andreu et al., 2010).

Statistical analysis

Quantitative data were expressed as mean ± SEM. Comparisons among groups were performed using Kruskal–Wallis analysis of variance followed by Mann–Whitney U (non-parametric data) post-hoc tests when necessary. Comparisons among behavioral trials within the same group (comparisons between open-field behavior session and habituation session in the open-field test, comparisons between training and test sessions in the novel object recognition and the inhibitory avoidance) were made by Wilcoxon test. The rest of the data were analyzed with unpaired Student t tests. *P* values of less than 0.05 were considered statistically significant. Statistical analysis was performed using the statistical software package SPSS (9.0, IBM, USA).

RESULTS

Open-field behavior and open-field habituation

In the open-field behavior, there were not significant differences among groups in the latency to start locomotion (P=0.12) (Fig. 1A). MiE and mangiferin treatments did not affect the number of crossings (Fig. 1B), but showed a statistical trend (P=0.09). They also did not influence the number of rearing (P=0.40) and fecal pellets (P=0.15).

There was a significant decrease between latencies among the open-field session and the habituation session in the SCA-2 treated with MiE-100 and mangiferin, indicating habituation memory (P<0.05, Wilcoxon test) (Fig. 1A). There were not significant differences among groups in the number of crossings, although a statistical trend (P=0.06) (Fig. 1B), indicating a trend in alterations in locomotion. There were not signifycant number of fecal pellets (P=0.08) or number of rearings (P=0.50), indicating a trend in reduction of exploratory behavior between groups in the habituation session, compared with the SCA-2 untreated animals.

Novel object recognition

Results for novel object recognition memory are shown in Fig. 2. There were no significant differences among groups in the exploratory preference between objects in the training trial (P=0.89). In addition, there was no significant difference among groups in long-term memory retention test, carried out 24 h after training (P=0.14). In this no aversive memory, there were significant training-test differences only in the mangiferin group (P=0.046). The results indicate that oral administration of *Mangifera indica* extract did not affect, whereas mangiferin affects the novel object recognition memory in SCA-2 mice.

Inhibitory avoidance

Results for inhibitory avoidance are shown in Fig. 3. There were significant statistical differences among treatments in the training (P=0.004) and in the test (P=0.0001) by Kruskal–Wallis analysis. The MiE treated animals in all doses (10, 50 and 100 mg/kg) showed learning in the aversive memory (inhibitory avoidance task) – an effect that was not observed in mangiferin treatment. Curiously, the ability of the control wild-type mice to remember the aversive stimulus they received during training (see the statistical differences between training and test tasks in healthy control in Fig. 3) is not observed in the transgenic mice that probably have lost their capacity to learn or build up memory from an aversive stimulus. The MiE treatment restored this ability. The facilitator effect of the extract is not related to non-mnemonic influences of MiE by itself on locomotor performance, since the open-field results showed no difference between training and test crossings (Fig. 1B).

Effects of MiE on U138-MG glioblastoma cell proliferation, nerve growth factor and tumor necrosis factor α levels

In order to explore possible cellular mechanisms mediating the beneficial effects of MiE on fear

memory was performed in vitro experiments using cultured U138-MG human glioblastoma cells as an experimental model. It was found that the incubation with MiE induced a significant increase in the concentration of NGF (at 5 and 50 µg/mL) and TNF-α (at 50 µg/mL) in culture supernatant (Fig. 4AB).

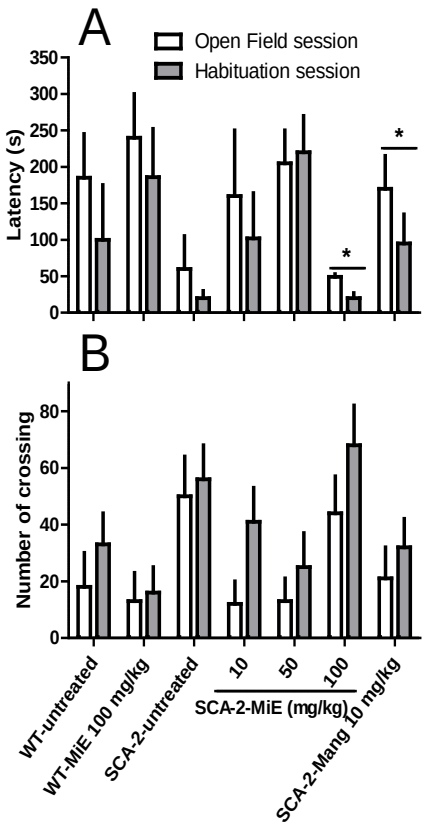

Figure 1. Open-field behavior and habituation in healthy wild-type mice (WT) or spinocerebellar ataxia type 2 (SCA-2, transgenic) orally treated with the aqueous extract from the bark of *Mangifera indica* L. (MiE, 10, 50 or 100 mg/kg) or mangiferin (Mang, 10 mg/kg) for 12 months. Animals were left to freely explore the arena for 5 min a day, during two days. Bars are mean ± SEM of latency to start locomotion (**A**) and number of crossings (**B**) during training (open-field, light columns) or test (habituation, gray columns). In (**A**), **P*<0.05 indicates a significant difference between open-field and habituation sessions.

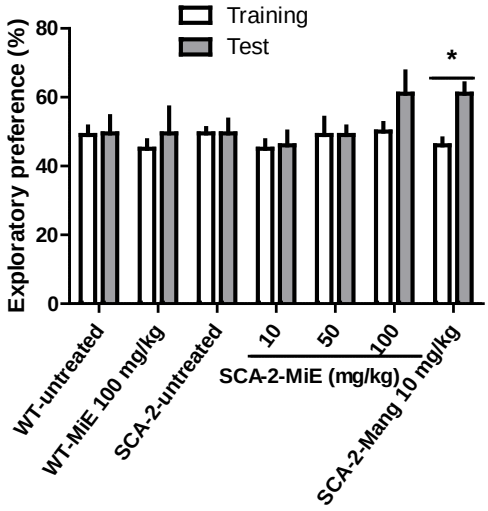

Figure 2. Novel object recognition memory in healthy wild-type mice (WT) or spinocerebellar ataxia type 2 (SCA-2, transgenic) orally treated with the aqueous extract from the bark of *Mangifera indica* L. (MiE, 10, 50 or 100 mg/kg) or mangiferin (Mang, 10 mg/kg) for 12 months. Data are mean ± SEM. Exploratory preferences was the % of the time exploring one object during training (light columns) or the novel object during test (gray columns). Memory retention was tested 24 h after training. **P*<0.05 indicates a significant difference between training and test.

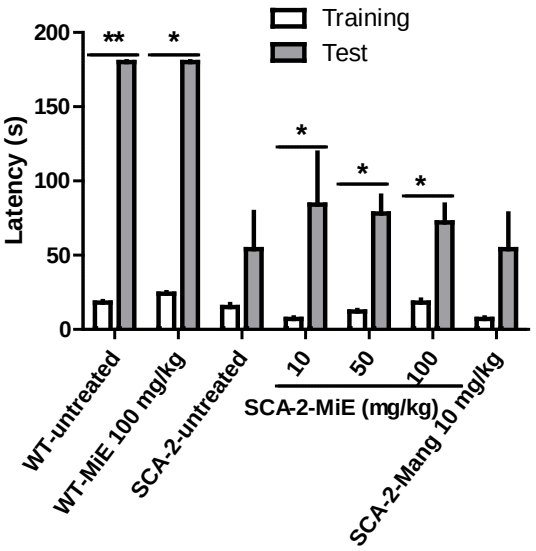

Figure 3. Fear-related memory assessed in an inhibitory avoidance task in healthy wild-type mice (WT) or spinocerebellar ataxia type 2 (SCA-2, transgenic) orally treated with the aqueous extract from the bark of *Mangifera indica* L. (MiE, 10, 50 or 100 mg/kg) or mangiferin (Mang, 10 mg/kg) for 12 months. Memory retention was tested 24 h after training. Bars are mean ± SEM. Latencies to step-down (s) of training (light columns) or test (gray columns). **P*<0.05 and ***P*<0.01 indicate a significant difference between training and test.

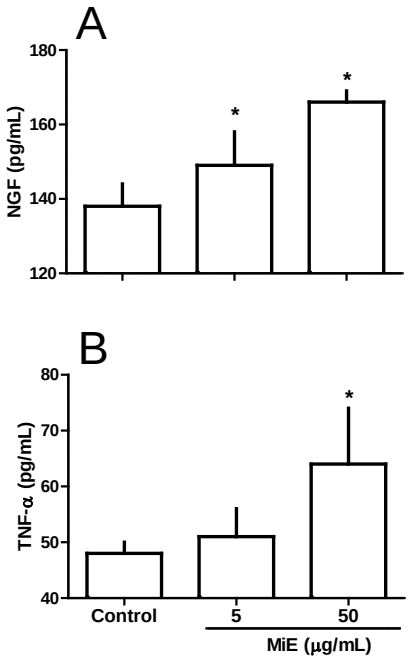

Figure 4. Effect of the aqueous extract from the bark of *Mangifera indica* L. (MiE) on nerve growth factor (NGF) (**A**) and tumor necrosis factor α (TNF-α) (**B**) levels in U138-MG culture supernatants. After 72 h of incubation with MiE (5 and 50 µg/mL) or DMSO 1/1000 dilution (control) cell supernatants were collected for NGF and TNF-α determinations using commercial kits according to the manufacturer's instructions (see Materials and Method section). Data are expressed as mean ± SEM of at least three independent experiments. **P*<0.05 vs. control with DMSO.

DISCUSSION

Mangiferin is the main compound in the *Mangifera indica* extract (approximately 7 g/100 g dry weight of MiE) (Núñez-Sellés et al., 2002). In this work, the only group that showed learning in the novel object recognition memory was SCA-2 female mice treated with mangiferin (the MiE did not influence the object recognition memory). Mangiferin improved object recognition memory in healthy rats through a mechanism that might involve an increase in neurotrophin and cytokine levels (Pardo-Andreu et al., 2010). This xanthone did not affect the inhibitory avoidance memory in SCA-2. These results are consistent with recent experiments in which mangiferin impaired the fear memory in rats (Andreu et al., 2011). Although mangiferin is the main polyphenolic compound in MiE, it presents a profile of biological activity distinct from MiE, probably due to the numerous molecules with biological activities present in the whole extract. Along with the xanthone mangiferin, MiE contains other polyphenols, including phenolic acids (gallic, 3,4-dihydroxybenzoic, and benzoic acids), phenolic esters (methyl gallate, propyl gallate, propyl benzoate) and flavan-3-ols (catechin/epicatechin). The effect of the substances mentioned above could be ascribed to catechin and epicatechin, which are the second and third principal components of MiE (Núñez-Sellés et al., 2002). In this regard, it has been shown that a catechin-enriched tea extract reversed scopolamine-induced retention deficits in both step-

through passive avoidance and spontaneous alteration behavior tasks (Kim et al., 2004). Nevertheless, the additive or synergistic effects of the complex mixtures of phytochemicals, instead of a single component, are probably responsible for the learning observed in the inhibitory test elicited by MiE treatment, something that mangiferin alone did not produce.

Together, the data suggest that acute oral administration of MiE (a mixture of active molecules) improve emotionally-motivated memory without affecting other aspects of behavior, namely the non-associative memory, locomotion and exploratory behavior. In fact, clinical interventions with the extract in AIDS patients or elderly persons did not provide visible evidences of neurological deterioration, even at 2-6 months of daily treatment (Núñez-Sellés et al., 2007). Since memory deficits have been reported for SCA (Bürk et al., 2003), aging and Alzheimer disease persons (Buckner et al., 2004), the present findings, viewed together with the evidenced lack of toxicity and clinical safety of MiE (Núñez-Sellés et al., 2007), constitute a rationale in the use of the natural extract for the prevention/treatment of memory deficits in SCA-2 and probably in other neurodegenerative diseases, including aging.

There is a well documented relationship between brain levels of NGF and cognitive ability in different animal models, including aversive memory tasks (Fiore et al., 2002; Barichello et al., 2013). Here it is showed for the first time that MiE increases the amount of NGF secreted by human glioma cells, an action that might be involved in the observed enhancing effect of the extract on aversive memory.

Neurotrophic factors guarantee the survival, maintenance and regeneration of specific neuronal populations in the adult brain. In this regard, they have used to treat a variety of chronic and acute disorders of the CNS. The clinical use of neurotrophins, although encouraging, has been limited for their failure in crossing the blood-brain barrier. Therefore, the identification of potential drugs like MiE that can mimic or even increase the levels of endogenous neurotrophic factors could have significant potential for treating CNS disorders.

Although we do not know the mechanism by which MiE incubation increased the U138-MG supernatant concentration of TNF-α, the increased levels of this cytokine might explain the higher levels of NGF in U138-MG glioblastoma cells supernatant after incubation with MiE. In this regard, it was shown that TNF-α stimulates the synthesis and secretion of biologically active NGF in the quiescent mouse and human fibroblasts and in human glioblastoma cells, as a result of the increased transcription or/and stability of the NGF mRNA (Hattori et al., 1993). Moreover, Beattie et al. (2002) demonstrated that glial-derived TNF markedly influenced synaptic efficacy by upregulating surface expression of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic (AMPA) receptors, an effect mediated through TNF-R1 and phosphatidylinositol 3 (PI3) kinase-dependent processes (Stellwagen et al., 2005).

Such findings support a neuromodulatory role for TNF within the brain and suggest a possible role for TNF in synaptic plasticity and possibly learning and memory.

Further investigations are required to clarify the mechanisms underlying the improving effect of MiE on aversive memory and the effect of mangiferin in the recognition memory.

Biological evidence indicates that synaptic plasticity and memory consolidation depend on early molecular mechanisms, including the activation of calcium-calmodulin-dependent protein kinase II (CaMKII), the phospholipase C (PLC)/protein kinase C (PKC), the cyclic adenosine monophosphate (cAMP)/protein kinase A (PKA), the mitogen-activated protein kinase (MAPK)/ extracellular signal-regulated protein kinase (ERK), and the phosphatidylinositol 3-kinase (PI3K) signaling pathways (Roesler and McGaugh, 2010). It is possible that mangiferin and other biologically active compounds of MiE influence these molecular mechanisms involved in synaptic plasticity.

CONCLUSIONS

Systemic and chronic administration of Mie enhances the aversive memory in SCA-2 transgenic mice, without affecting declarative memory. The memory improving mechanism might

involve an increase in neurotrophin and cytokine levels that may prevent the neural damage in the SCA-2 brains. Although further experiments are necessary to clarify the effect of MiE, these findings constitute the basis for the use of the natural extract in the prevention/treatment of memory deficits in SCA-2.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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