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Natural extracts as possible protective agents of brain aging

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Abstract

A growing number of studies suggest that natural extracts and phytochemicals have a positive impact on brain aging. We examined the potential of the *Ginkgo biloba* extract EGb 761 and red wine-derived constituents on cell death produced by beta-amyloid (Aβ) peptides and oxidative stress, with respect to their possible deleterious role in age-related neurological disorders. We found that EGb 761, possibly through the antioxidant properties of its flavonoids, was able to protect hippocampal cells against toxic effects induced by Aβ peptides. Moreover, we showed that an exposure of rat hippocampal cells to the nitric oxide (NO) donor sodium nitroprusside (SNP) resulted in a decrease in cell survival and increase in reactive oxygen species (ROS) accumulation. However, EGb 761 and red wine-derived polyphenols protected against these events, due to their antioxidant activities, and their ability to block SNP-stimulated activity of protein kinase C (PKC). Taken together, these results support the hypothesis that dietary intake of natural substances may be beneficial in normal aging of the brain.
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Keywords: Neuroprotection; *Ginkgo biloba* extract; Red wine; Resveratrol; Quercetin, (+)-catechin; Beta-amyloid peptides; Nitric oxide; Alzheimer's disease; Brain aging; *Vitis vinifera*

1. Introduction

Extensive research provided knowledge that an excessive accumulation of beta-amyloid (Aβ) peptides is one of the leading hypotheses to explain neurodegenerative processes that occur in Alzheimer's disease (AD). To support the amyloid hypothesis, in vitro studies performed in cell culture preparations reported neurotoxic [14,28] and apoptotic [12,37] effects of Aβ-related fragments ($A\beta_{25–35}$, $A\beta_{1–40}$ and $A\beta_{1–42}$) in regions that are effected in AD, such as the hippocampus [23]. The precise mechanisms mediating the toxic properties of Aβ remain to be fully established. It has been suggested that Aβ toxicity is associated with increases in reactive oxygen species (ROS), including the peroxide H_2O_2 [5,20] and nitric oxide (NO) [33], whose overproduction may in turn initiate neurotoxic events [8]. On the other hand, oxidative stress is considered as a risk factor in the incidence and evolution of cognitive declines that occur during normal cerebral aging and dementia, and likely plays a pivotal role in various neurodegenerative processes such as ischemia and Parkinson's disease [7,24,26,54].

There is no effective cure to treat neurological disorders associated to aging. Among therapeutic interventions that are envisioned to forestall or delay the normal and pathological aging processes, nutritional interventions may be viewed as a viable approach [13,29,61].

Ginkgo biloba extract (EGb 761, Tanakan®, IPSEN Laboratories, Paris, France) is a well known plant extract obtained from green leaves of the *G. biloba* tree according to a standardized procedure [15]. The patented extract EGb 761, that was filed for all of Europe in 1990, contains the following pharmacologically active substances including 24% flavonoids that are nearly exclusively flavonol-*O*-glycosides, 6% terpenoids (known as ginkgolides A, B, C, M, J and bilobalide), 5–10% organic acids, and >0.5% proanthocyanidins defined as flavonoid-based polymers [15]. EGb 761 exhibits a broad range of biochemical and pharmacological activities such as antioxidants and free radical

Abbreviations: Aβ, beta-amyloid; AD, Alzheimer's disease; AMD, macular degeneration; Ca^{2+}, calcium; COX, cyclooxygenase; DCF, 2′,7′-dichlorofluorescein; DMEM, Dulbecco's modified Eagles medium; EGb 761, *Gingkgo biloba* extract; LOX, lipoxygenase; MTT, 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; NDGA, nordihydroguaiaretic acid; NO, nitric oxide; NR, neutral red (3-amino-7-dimethyl-amino-2-methylphenazine hydrochloride); PKC, protein kinase C; ROS, reactive oxygen species; SNP, sodium nitroprusside; U-73122, 1-[6-((17β-3-methoxyestra-1,3,5(10)-trien-17-yl)-amino)hexyl]-1H-pyrrole-2,5-dione

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scavenging [38,39] as well as nootropic and/or neurotrophic activities in the hippocampal formation [1,32]. The pleiad of effects of EGb 761 may explain, at least in part, its protective actions in animal models of hypoxia and ischemia [16,45] and in in vitro models of toxicities [44,49,58], as well as its ability to enhance cognitive behaviors in rodents [10,60]. Prospective, double-blind, placebo-controlled studies provided support for safety and significant therapeutic efficacy—including the improvement of memory, attention and social functioning of an oral administration of EGb 761 in healthy older adults [42] and AD or multi-infarct demented patients [22,34,41].

It has also been reported that diets rich in fruits, vegetables and beverages are, along with natural extracts, an important source of polyphenols. Interestingly, epidemiological studies pointed to the possible capacity of moderate consumption of red wine—an alcoholic beverage that contains a high amount of polyphenols [19,51]—and flavonoids to reduce the incidence of certain age-related neurological disorders including macular degeneration [46] and dementia [11,47]. To support this hypothesis, in vitro studies reported that red wine polyphenols such as quercetin, (+)-catechin and resveratrol displayed protective abilities in various models of toxicity [43,48,55].

Considering the purported antioxidant properties of EGb 761 and phytonutrients and their possible therapeutic efficacies [17,22,34,35,41,46,47], we aim to investigate the effects of EGb 761 and red wine-derived polyphenolic compounds known as quercetin, (+)-catechin and resveratrol on the toxicity induced by Aβ peptides ($A\beta_{25–35}$, $A\beta_{1–40}$ and $A\beta_{1–42}$) and/or by oxidative stress.

2. Materials and methods

2.1. Materials

The *G. biloba* extract EGb 761, its flavonoid fraction (CP 205) and the terpenes bilobalide (CP 160) and ginkgolide B (BN 52021) were obtained from IPSEN Laboratories (France) whereas polyphenolic constituents and sodium nitroprusside (SNP) were purchased from Sigma (St. Louis, MO, USA). The different fragments of Aβ peptides including $A\beta_{1–40}$ and $A\beta_{1–42}$ were obtained from US Peptide (Fullerton, CA). The fragment $A\beta_{25–35}$ was kindly provided by P. Gaudreau (CHUM, University of Montreal, Montreal, Canada). Materials used for cell cultures were obtained from Gibco BRL (Burlington, Ont., Canada).

2.2. Primary hippocampal cell cultures

Hippocampal cell cultures were prepared from E19 embryos obtained from Sprague–Dawley rats. Animal care was according to protocols and guidelines of the McGill University Animal Care Committee and the Canadian Council for Animal Care.

Mixed (neuronal/glial cells) hippocampal cells were plated in 96-well plates (density of about 4×10^4 viable cells per well) and were grown in DMEM high glucose medium as described previously [2–4]. Experiments were performed in 7-day-old cultures.

2.3. Experimental treatments

2.3.1. Aβ-induced toxicity

Cells were exposed to the freshly solubilized peptides $A\beta_{25–35}$ (25 μM), $A\beta_{1–40}$ (5 μM) or $A\beta_{1–42}$ (25 μM) for 24 h, in the presence or absence of different drugs, as described previously [2]. Mixed cell viability was quantified 24 h later using the MTT colorimetric assay (see below) whereas the extent of necrotic and apoptotic cells were evaluated using the fluorescence dyes propidium iodide (PI) and Hoechst 33342, respectively (see below). ROS production was estimated using the 2′,7′-dichlorofluorescein (DCF) fluorescence assay (see below).

2.3.2. SNP-induced toxicity

Cells were treated during 20 h with a HEPES-buffered MEM high glucose medium containing SNP (100 μM) and the different test drugs, as described previously [3,4]. Following this incubation period, cell viability was determined using both the MTT and the neutral red (NR) colorimetric assays, whereas ROS production was evaluated using the DCF fluorescence assay (see below).

2.4. Assessment of cell viability and cell injury

MTT (an indicator of the mitochondrial activity of living cells) and NR (a dye which is taken up by lysosomes of living cells) are used as indexes of cell survival [18,40]. MTT reduction and NR uptake into living cells were quantified at 570 and 540 nm, respectively, using a micro-plate reader (Bio-Tek Instruments® Inc., Ville St. Laurent, Que., Canada).

Necrotic cell death was evaluated by assessing the extent of cell uptake of the PI dye, as described previously using hippocampal cell cultures [6]. PI uptake into dead cells of each well was automatically quantified (excitation = 485 nm; emission wavelength at 640 nm) using a fluorescence plate reader (Bio-Tek Instruments® Inc., Ville St. Laurent, Que., Canada).

2.5. Measurement of ROS

The accumulation of intracellular ROS was determined by measuring DCF fluorescence [40]. Briefly, 25 μM 2,7-dichlorofluorescein diacetate (DCFH-DA; Molecular Probes Inc., Eugene, OR) was applied to the culture medium at the onset of either Aβ or SNP exposure, as described previously [2–4]. DCF fluorescence was quantified (excitation = 485 nm, emission = 530 nm) using a

fluorescence multiwell plate reader (Bio-Tek Instruments® Inc., Ville St. Laurent, Que., Canada).

2.6. Assessment of apoptotic cell death

Nuclear staining was performed using the fluorescent nuclear dye Hoechst 33342, as described previously using hippocampal neurons [28]. Briefly, cells were incubated after a 24 h exposure to $A\beta_{25–35}$ in phenol red-free Hank's for 15 min containing the Hoechst 33342 dye (1 μg/ml, 15 min). The number of apoptotic neurons was then quantified using a micro-plate fluorescence reader (UV excitation and emission = 360 and 450 nm, respectively).

2.7. Nitrite assay

Accumulation of nitrite (NO_2^-), the end-product of NO production that is used as an indicator of NO synthase (NOS) activity, was measured in the culture medium by the Griess reaction using the NO colorimetric assay kit (Calbiochem, San Diego, CA), as described previously [4]. Briefly, hippocampal cells were exposed to SNP with or without polyphenols. Concentrations of NO_2^- will be quantified 24 h later at 540 nm using a micro-plate reader (Bio-Tek Instruments® Inc., Ville St. Laurent, Que., Canada).

2.8. Measurement of protein kinase C activity

The activity of protein kinase C (PKC)—an enzyme that has been shown to block the toxic effects of NO—was assessed on 7-day-old mixed hippocampal cells using a PKC assay kit (SignaTECT™ PKC Assay System, Promega, Madison, WI, USA) according to the protocol described previously [3,4]. Briefly, cells were exposed for 5 min to either vehicle or SNP (100 μM) in the presence or absence of either EGb 761 (100 μg/ml) or polyphenols (10 μM).

2.9. Statistical analyses

Survival of vehicle-treated control groups not exposed to either Aβ-derived peptides, SNP, or different drugs was defined as 100% and the number of surviving, dead and apoptotic cells in the treated groups was expressed as percent of control groups. One-way ANOVA followed by a Newman Keuls' multiple comparison test was used to compare control and treated groups with P values <0.05 being considered statistically significant. An unpaired t-test was used to compare vehicle- and drugs-treated control groups with P values <0.05 being considered statistically significant.

3. Results

3.1. EGb 761 protects hippocampal cells against Aβ peptide-induced toxicity

The MTT assay revealed that a co-treatment with EGb 761 (100 μg/ml) protected hippocampal cells against toxicity induced by the Aβ fragments $A\beta_{25–35}$ (25 μM), $A\beta_{1–40}$ (5 μM) and $A\beta_{1–42}$ (25 μM) (Table 1). The protective effects of EGb 761 were confirmed by the finding that the number of necrotic (as evaluated by the PI assay) and apoptotic (as evaluated by the Hoechst 33342 assay) cells treated with $A\beta_{25–35}$ diminished in the presence of EGb 761 (100 μg/ml) (Table 1). The MTT assay revealed that EGb 761 (100 μg/ml) was even able to protect hippocampal cells

Table 1
Comparative effects of EGb 761, its flavonoid and terpenoid constituents, and various drugs against toxicity and ROS production induced by Aβ peptides in rat hippocampal cell cultures

Treatment	Cell survival (MTT assay)	Cell injury (PI assay)	Apoptosis (Hoechst assay)	ROS production (DCF assay)
$A\beta_{1–40}$	61 ± 2	nd	nd	123 ± 14
$A\beta_{1–40}$ + EGb 761	101 ± 3*	nd	nd	62 ± 6*
$A\beta_{1–42}$	61 ± 3	nd	nd	nd
$A\beta_{1–42}$ + EGb 761	103 ± 4*	nd	nd	nd
$A\beta_{25–35}$	58 ± 2	118 ± 2	116 ± 3	151 ± 11
$A\beta_{25–35}$ + EGb 761	88 ± 2*	100 ± 5*	101 ± 3*	63 ± 8*
$A\beta_{25–35}$	58 ± 2	nd	nd	165 ± 6
$A\beta_{25–35}$ + CP 205	74 ± 3*	nd	nd	97 ± 6*
$A\beta_{25–35}$	58 ± 2	nd	nd	167 ± 7
$A\beta_{25–35}$ + bilobalide	52 ± 2	nd	nd	165 ± 11
$A\beta_{25–35}$ + ginkgolide B	50 ± 1	nd	nd	140 ± 11
$A\beta_{25–35}$	67 ± 2	nd	nd	nd
$A\beta_{25–35}$ + nitrendipine	64 ± 2	nd	nd	nd
$A\beta_{25–35}$ + chelerytrine	66 ± 2	nd	nd	nd
$A\beta_{25–35}$ + U-73122	74 ± 4	nd	nd	nd
$A\beta_{25–35}$ + NDGA	65 ± 4	nd	nd	nd

Modified with permission from [2]; nd: not determined.
* $P < 0.01$ compared to groups treated with Aβ alone.

Table 2
Comparative effects of the EGb 761, its flavonoid and terpenoids constituents, as well as various drugs against toxicity and ROS production induced by SNP in rat hippocampal cell cultures

Treatment	Cell survival (MTT assay)	Cell survival (NR assay)	ROS production (DCF assay)
SNP	42 ± 3	40 ± 3	532 ± 73
SNP + EGb 761	99 ± 5*	98 ± 3*	222 ± 22
SNP	46 ± 2	50 ± 5	583 ± 85
SNP + CP 205	73 ± 10*	76 ± 6*	321 ± 38
SNP	63 ± 3	58 ± 3	340 ± 54
SNP + bilobalide	57 ± 2	73 ± 8	356 ± 57
SNP + ginkgolide B	62 ± 2	63 ± 6	344 ± 62
SNP	23 ± 4	29 ± 4	nd
SNP + ebselen	87 ± 6*	87 ± 3*	nd
SNP + SOD	81 ± 8*	96 ± 6*	nd
SNP	37 ± 4	28 ± 3	nd
SNP + chelerythrine	76 ± 5*	77 ± 4*	nd
SNP	43 ± 6	37 ± 6	nd
SNP + U-73122	52 ± 6	36 ± 3	nd
SNP + OBAA	42 ± 10	33 ± 6	nd

Modified with permission from [3]; nd: not determined.
* $P < 0.01$ compared to groups treated with SNP alone.

that were pre-exposed 8 h before to $A\beta_{25–35}$ and $A\beta_{1–40}$ (69 ± 1 ($A\beta_{25–35}$) versus 92 ± 2 ($A\beta_{25–35}$ + EGb 761), $P < 0.05$; 61 ± 3 ($A\beta_{1–40}$) versus 97 ± 6 ($A\beta_{1–40}$ + EGb 761), $P < 0.05$). Similar, but albeit less potent, protective effects were obtained with the flavonoid fraction of the total extract (i.e. CP 205), with a maximal effect at 25 μg/ml (Table 1). In contrast, neither the terpenoid constituents of EGb 761 (i.e. bilobalide; ginkgolide B, 10 μg/ml), nor a blocker of L-type calcium channel (nitrendipine, 50 μM), inhibitors of PKC (chelerythrine chloride, 1 μM), phospholipase C (U-73122, 5 μM) and lipoxygenase (NDGA, 10 μM), offered protection (Table 1). EGb 761 (100 μg/ml) was also able to protect hippocampal cells from toxicity induced by H_2O_2, the major peroxide that possibly mediates Aβ toxicity (data not shown, see [2]), and shared with CP 205 (25 μg/ml) the ability to reduce $A\beta_{25–35}$-induced intracellular ROS accumulation (Table 1). However, the terpenes bilobalide and ginkgolide B (10 μg/ml) failed to reverse the increase in DCF fluorescence stimulated by $A\beta_{25–35}$ (Table 1).

3.2. EGb 761 and red wine-derived polyphenols protect hippocampal cells against SNP-induced toxicity

A co-treatment with EGb 761 (100 μg/ml), its flavonoid fraction CP 205 (25 μg/ml) (Table 2), quercetin (10 μM), (+)-catechin (10 μM) and resveratrol (10 μM) (Table 3) were capable of attenuating hippocampal cell death (as estimated by the MTT and NR assays) and intracellular ROS accumulation (as estimated by the DCF assay) generated by SNP (100 μM). Similar protective effects were obtained with

Table 4
Effects of EGb 761, quercetin, (+)-catechin and resveratrol on PKC activity and nitrite accumulation induced by SNP in rat hippocampal cells

Treatment	PKC activity	Nitrite accumulation
SNP	127 ± 7	nd
SNP + EGb 761	105 ± 6**	nd
SNP	130 ± 4	154 ± 12
SNP + quercetin	104 ± 9*	178 ± 17
SNP	126 ± 4	154 ± 12
SNP + (+)-catechin	132 ± 6	160 ± 9
SNP	126 ± 4	154 ± 12
SNP + resveratrol	123 ± 6	180 ± 19

Modified with permissions from [3,4]; nd: not determined.
* $P < 0.05$ compared to groups treated with SNP alone.
** $P < 0.01$ compared to groups treated with SNP alone.

Table 3
Summary of the effects of red wine polyphenols and inhibitors of cyclooxygenase and lipoxygenases against toxicity and ROS production induced by SNP in rat hippocampal cell cultures

Treatment	Cell survival (MTT assay)	Cell survival (NR assay)	ROS production (DCF assay)
SNP	50 ± 6	57 ± 5	226 ± 15
SNP + quercetin	76 ± 5*	77 ± 4*	162 ± 8*
SNP	41 ± 5	44 ± 6	288 ± 23
SNP + (+)-catechin	94 ± 5*	89 ± 3*	154 ± 14*
SNP	44 ± 4	39 ± 5	265 ± 23
SNP + resveratrol	94 ± 4*	84 ± 3*	172 ± 18*
SNP	42 ± 9	38 ± 2	239 ± 26
SNP + trolox	96 ± 3*	96 ± 2*	112 ± 9*
SNP	34 ± 4	42 ± 6	177 ± 12
SNP + indomethacin	28 ± 4	37 ± 5	153 ± 8
SNP	43 ± 3	39 ± 5	228 ± 17
SNP + MK-886	33 ± 4	27 ± 4	216 ± 18
SNP	55 ± 8	56 ± 6	255 ± 15
SNP + 2-(1-thienyl)ethyl 3,4-dihydroxybenzylidenecyanoacetate	53 ± 7	46 ± 4	225 ± 10

Modified with permission from [4].
* $P < 0.01$ compared to groups treated with SNP alone.

various antioxidant drugs (i.e. ebselen (1 μM); superoxide dismutase (500 UI/ml); trolox (100 μM)) and chelerythrine (1 μM) (Table 2). In contrast, the terpenoids bilobalide (1 μg/ml) and ginkgolide B (1 μg/ml), as well as selective inhibitors of phospholipases (i.e. U-73122 (5 μM), OBAA (5 μM)), cyclooxygenases (i.e. indomethacin (5 μM)) and lipoxygenases (i.e. MK-886 (5 μM); 2-(1-thienyl)ethyl 3,4-dihydroxybenzylidenecyanoacetate (10 μM)) were not effective (Tables 2 and 3). In addition, among the drugs tested here, only EGb 761 (100 μg/ml) and quercetin (10 μM) were able to block the stimulatory effect of SNP (100 μM) on the activity of PKC (Table 4), an enzyme that is involved in a number of cellular responses including free radical production. Finally, none of red wine polyphenols (10 μM) were able to attenuate nitrite accumulation caused by SNP (100 μM) (Table 4).

4. Discussion

The present study has shown that EGb 761 and red wine-derived polyphenols completely protected hippocampal cells against Aβ peptides- and/or oxidative stress-induced toxicities. These data may be of particular interest given the deleterious role of the accumulation of Aβ peptides and ROS in hippocampal dysfunction/neurodegeneration that possibly occurs during normal brain aging, as well as neurodegenerative diseases [23,53].

The mechanisms of action underlying the protective effects of EGb 761 were examined. We observed that treatments with EGb 761 and its flavonoid fraction were able to strongly inhibit $A\beta_{25–35}$- and NO-induced ROS accumulation and that the total extract protected cells against the harmful effects of H_2O_2, a purported mediator of Aβ toxicity [5]. These data altogether suggest that the hydroxyl radicals scavenging properties of EGb 761 are likely to be involved in its neuroprotective actions against the toxic effects of Aβ [49]. Moreover, and in accordance with previous data [44,58], EGb 761 inhibited the number of $A\beta_{25–35}$-induced apoptotic events, a process that may be relevant to neurodegeneration occurring in AD [27]. We hypothesized that the anti-apoptotic effect of EGb 761 is related to its inhibitory effect on H_2O_2-induced toxicity since this natural extract has been reported to prevent hydroxyl radicals-induced apoptosis in cultured neurons [44,58]. Finally, we showed that the inhibition of intracellular effectors are not directly involved in the neuroprotective actions of EGb 761 since none of tested inhibitors of intracellular effectors were able to protect against $A\beta_{25–35}$-induced toxicity. Nevertheless, we cannot exclude other properties of EGb 761 since its flavonoid fraction only partially protected hippocampal cells. Interestingly, preliminary data obtained in rodents showed that the *G. biloba* extract was able to inhibit $A\beta_{1–40}$ fibril formation and to disrupt Aβ fibrinilolysis [9,57], while a recent animal study revealed that EGb 761 increased gene expression for transthyretin, a protein that may play a neuroprotective role by Aβ sequestration [59].

EGb 761 shares with its flavonoid fraction and inhibitor of PKC and L-type calcium channels, the capacity of blocking SNP-induced toxicity, while its terpenoid constituents and inhibitors of phospholipases failed to display any effects. These data suggest that the protective effects of EGb 761 against NO-induced toxicity are not only attributable to the antioxidant properties of its flavonoids but also via its ability to modulate other mechanisms associated to NO toxicity such as PKC and Ca^{2+} channels. To support this hypothesis, we showed that EGb 761 inhibited the stimulatory effect of SNP on PKC activity [3].

In addition to EGb 761 protective effects, our data demonstrated that red wine-derived constituents such as the flavonoids quercetin and (+)-catechin, and the stilbene resveratrol were capable of protecting hippocampal cells against SNP-induced toxicity. Interestingly, the concentrations of polyphenols used in the present study are similar to those present in red wine, depending upon the vintage and type of grapes [19,51]. These effects appear to be mainly attributable to antioxidant properties and do not seem to involve intracellular enzymes such as cyclooxygenase, lipoxygenase, NO synthase, and, with the exception of quercetin, PKC. These findings are in agreement with previous studies showing that these polyphenols displayed, mainly through their antioxidant properties, neuroprotective activities in cell cultures and animal models of ischemia [25,36,43,48,52,56].

These data considered altogether support the growing idea that polyphenol intake that are present in high amounts in natural extracts, fruits, vegetables and beverages may have beneficial effects against disorders that are associated with aging. An animal study performed by Joseph's group showed that, among natural extracts tested in aged rats, supplementation of extracts of blueberry, a fruit that contains high amounts of polyphenols, was found to be the most effective in delaying and even reversing age-related cognitive behavioral impairments [30,31]. Moreover, the properties—in particular their free radical scavenging/antioxidant—of the flavonoid fraction of EGb 761 could account for much of the pharmacological effects of the total extract [38,39] including its in vitro [2,3] and in vivo [16,45] protective effects. Recent epidemiological studies performed in relatively large cohorts of elderly subjects suggested that a few glasses (i.e. 250–500 ml) of red wine (which contains a high amount of polyphenols) appear to be associated with diminished risk of macular degeneration, AD, cognitive deficits and cerebral infarction [35,46,47,50]. Moreover, adult drinkers aged 55–88 years who consume moderately (two to eight drinks per day) alcoholic beverages (in particular wine) performed better in various cognitive tests compared to abstainers [17]. Moreover, a recent 5-year follow-up epidemiological study performed in a large cohort of 1367 subjects aged 65 and older showed a significant inverse association between flavonoids (mean consumption of 14.4 mg per day

with fruits as the most important source) and the risk of dementia [11]. Since the inverse correlation between wine consumption and dementia was not significant after adjustment for flavonoid intake, the authors suggested that the association between red wine consumption and AD may be related to their polyphenolic constituents. In addition to the possible direct effect of phytochemicals in the brain function, it is possible that their beneficial effects may be attributable, at least in part, to their purported cardioprotective effects [21].

In conclusion, the data reported here support the hypothesis that dietary intake or supplementation of polyphenols that are widely present in food and in various natural extracts may prevent or delay the incidence of age-related neurological disorders. Further epidemiological studies are necessary to confirm this hypothesis and to recommend polyphenols as a prophylactic means to forestall and/or to delay the incidence of neurological dysfunctions that are associated with pathological brain aging.

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