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REVIEW ARTICLE

Can Nutraceuticals Prevent Alzheimer's Disease? Potential Therapeutic Role of a Formulation Containing Shilajit and Complex B Vitamins

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Alzheimer's disease (AD) is a brain disorder displaying a prevalence and impact in constant expansion. This expansive and epidemic behavior is concerning medical and public opinion while focusing efforts on its prevention and treatment. One important strategy to prevent this brain impairment is based on dietary changes and nutritional supplements, functional foods and nutraceuticals. In this review we discuss the potential contributions of shilajit and complex B vitamins to AD prevention. We analyze the status of biological studies and present data of a clinical trial developed in patients with mild AD. Studies suggest that shilajit and its active principle fulvic acid, as well as a formula of shilajit with B complex vitamins, emerge as novel nutraceutical with potential uses against this brain disorder. © 2012 IMSS. Published by Elsevier Inc.

Key Words: Alzheimer's disease, Tau protein, Nutraceuticals, Functional foods, Shilajit, Fulvic acid.

Introduction

Alzheimer's disease (AD) is a brain disorder affecting the elderly with an increasing incidence due to the high rate of increase in the aging population, especially in developing countries. The economic impact of this disease is expanding due to indirect and direct costs derived from AD (1). However, the lack of effective treatments against this devastating disease has led pharmaceutical companies to increase their budgets in research, development and innovation in order to achieve plausible solutions. Nevertheless, based on recent clinical trials, many molecules chosen as potential targets for AD treatment have yielded negative results (2,3). This is a consequence of the fact that most strategies have been driven to the wrong targets (4,5). However, current efforts in increasing our knowledge on AD pathogenesis as the basis to define therapeutic strategies is stimulating clinical research in this field, albeit timidly and with few available results, to the search of news targets as potential drugs for treatment of AD (6). On the other hand, the use of natural products such as nutraceutical compounds has a promising future (7). In this article we review some of the characteristics of known nutraceutical compounds based on natural products and their potential use against AD, with emphasis on preclinical investigations and a clinical trial using a formulation containing shilajit and vitamin B complex.

Summarized State of the Art in AD

AD is the leading cause of dementia worldwide and the fourth cause of death in developed countries after cancer, cardiovascular diseases and stroke. Although senile plaques with the amyloid β (Aβ) and neurofibrillary tangles (NFT) are found in the extra- and intracellular domains in brains of AD patients, this disorder is referred to as a tauopathy, i.e., a disease arising from a tau protein dysfunction (8). Tau protein belongs to the family of

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microtubule-associated proteins (MAPs) and is involved in the assembly and stabilization of microtubules as well in the interaction with other cytoskeletal filaments (8,9). As a tauopathy, a cumulative set of reports suggests that hyperphosphorylation of tau protein is one of the key processes in the chain of events that leads to neuronal degeneration and cellular death in the pathway to AD (10,11). Although hyperphosphorylated tau does aggregate into homomultimeric structures (PHF and straight filaments), there are also very high levels of soluble oligomeric phosphorylated tau in AD brain. There is evidence that suggest that soluble hyperphosphorylated tau is the main contributor to the disordered cytoskeletal architecture associated with neurofibrillary tangles. Hyperphosphorylated tau self-aggregates but also associates with other MAPs to inhibit their ability to stabilize microtubules. Further, mouse model studies support the notion that oligomeric hyperphosphorylated tau is the signal that triggers neuronal degeneration.

According to this, new noninvasive diagnosis techniques are being developed (12–14). Moreover, several new molecules of a natural origin (15) have been assayed as potential therapeutic avenues. This approach defines an interesting path in the search of natural compounds as part of dietary supplements, functional foods or nutraceuticals that may have preventive and/or therapeutic actions against AD.

Emergence of Nutraceuticals for Human Health

The links between diet and human disease are gaining increasing interest among investigators. The term nutraceutical is defined as any food (fruits, vegetables, nuts, tea and botanical products, among others) or part of a food such as a dietary supplement that produces a medical or health benefit including the prevention and treatment of a disease (16,17). Generally, nutraceutical products are administered in dosages that exceed those that could be obtained from normal foods, considering the absence of very low toxicity to the organism. On the other hand, functional foods are now being examined for their physiological benefits and for their ability to reduce risk factors in chronic diseases (18). Even though functional food is not to be accepted as a definition, this concept was coined in Japan in the 1980s approving certain foods with documented health benefits to improve the health of the population (19). The principal difference between nutraceuticals and functional foods is the customary presentation of the product (nutraceuticals such as pills, capsules, beverages, whereas functional foods are those such as whole vegetable, fruit or natural product). Despite the fact that the concept has no legal definition (20), marketing of nutraceuticals as nutritional supplements is booming. According to specialized studies, the global market for nutraceuticals generated an estimated $49 billion in 2011 and should reach $67 billion in 2016, with an annual growth rate of 6.4% (21). This derives from the high consumer demand for natural products. Furthermore, natural products have generated a growing interest because they generally have the advantage of not being associated with side effects or adverse reactions, even at high doses.

Potential Actions of Nutraceuticals Against AD

Dietary supplements are highly recommended and suggested as a strategy against several diseases, together with other nutritional programs (7,22). In this context, epidemiological evidence linking diet, modifiable environmental factors, and the risks of AD is rapidly increasing (23). A non-balanced diet appears to impact on the risk of AD. There is a belief that the customary diet in Eastern countries has an important contribution to the health of that population as well as for resisting and preventing age-related diseases (24). In the following text we will review a formulation of the phytocomplex called shilajit and complex B vitamins, which appears as a potential dart against AD and other neurodegenerative disorders, in which a scientific basis as pre-clinical investigation and clinical trials exist.

Vitamins of the B Complex

Certain B vitamins (folic acid, B6 and B12) have a critical importance in human health because they are involved in homocysteine metabolism as cofactors or substrates for enzymes in this metabolic pathway (25). Homocysteine is a neurotoxic molecule (5) and its high levels in plasma are related to neurodegenerative disorders in the elderly (26). High concentration of this molecule in plasma and tissues is largely determined by the body's status of these vitamins (27), regarding it as a sensitive marker of folate and vitamin B12 deficiency (28). Several studies indicate that supplementation of vitamins such as folic acid and vitamin B12 significantly reduces homocysteine levels in patients (27,29), thus decreasing the possibility of acquiring sporadic forms of AD (7).

Shilajit and Fulvic Acid

Shilajit, also known in northern India as salajit, shilajatu, mimie or mummuyo, is a blackish brown substance either in powder form or an exudate found in rocks in the Himalaya mountains between India and Nepal, but also found in Russia, Tibet, Afghanistan. Shilajit, named for the Andean shilajit, has also been found in northern Chile (30). Shilajit has been known for centuries in ayurvedic medicine as a rejuvenating and anti-aging compound (31). Considering its unique composition as a phytocomplex, researchers have hypothesized that shilajit is produced by the decomposition of many plant species (32). Molecularly, shilajit

consists mainly of humic substances, which are the result of degradation of plants by several microorganisms, especially fungi (33). This leads to the generation of a phytocomplex rich in humic substances, fulvic acid (FA), and selenium, among others. Humic substances are operationally divided into humin, humic acid and FA according to its solubility in water at different pH values. Humin are not soluble in water under any pH condition. Humic acid is soluble in water under alkaline conditions and has a molecular weight of 5−10 kDa (30). FA is soluble under all conditions of pH and has a molecular weight ~800 Da (15). It is well absorbed from the intestinal tract and eliminated from the body within a few hours (34). Today, the medicinal properties are being investigated with positive results, attributing to FA many of the medicinal properties of shilajit (5,15).

Our laboratory has developed an in-depth study on the effects of shilajit and a proposed formulation containing shilajit (200 mg/capsule) and vitamins B6 (14.5 mg), B9 (200 μg) and B12 (3 μg) on human health. Our findings indicate the safety of this formulation in cell murine lines at increasing concentrations, showing also that shilajit is nontoxic in neuronal cell cultures (data not shown). Moreover, morphometric studies were carried out that show that the formulation promotes neuritogenesis in hippocampal cells in primary cultures (30). These results indicate that shilajit and this formulation consisting of shilajit plus B complex vitamins can increase the outgrowth of neurites and other cell prolongations in hippocampal cells in culture. Additionally, increasing concentrations of FA evidences neuritogenesis as revealed by immunofluorescence microscopy in N2a cell line cultures. These findings suggest that FA contained in shilajit may have neurotrophic effects in cells in culture. This effect seems to be intensified in the presence of other known neuroprotective compounds as B complex vitamins. To ascertain whether synergic effects between B complex vitamins and FA exist, studies demonstrated that the formulation has more potent effects than shilajit alone (Carrasco et al., unpublished results). Other studies performed with FA *in vitro* show that this compound not only exerts an action such as an anti-oxidant but also binds specifically to tau and prevents pathological self-association (15). Our laboratory analyzed the anti-aggregation effects of FA of 4RMBD tau protein fragment in presence of heparin (a known tau protein aggregation inducer) *in vitro*. Current evidence indicates that tau aggregation into paired helical filaments (PHF) and in NFT causes cell death in AD and other tauopathies. On the other hand, interaction between humic substances and proteins is largely described in detail in studies made in the soil science field (35−37). According to Tomaszewski et al., this interaction is made possible by the presence of very negative charges in humic substances that interact with positive charges of proteins (38), which are widely present in tau protein structure (39). Another evidence of the interaction between FA and tau protein is given in cell culture experiments in N2a line performed in our laboratory that show that FA inhibits the formation of trimers and tetramers in nonproliferative conditions, increasing the availability of monomers of tau protein (Figure 1). Together this body of evidence indicates that FA, a humic substance present in the phytocomplex shilajit, could disassemble pathological filaments or oligomers of tau protein into monomers, leaving this monomer available to form new neuritic prolongations in cultured cells (see scheme in Figure 2).

Other preclinical studies indicate potential uses of shilajit in other pathologies. Various properties have been ascribed including (a) antiulcerogenic properties (40); (b) antioxidant properties (41,42); (c) activator of complement in immune system (43); (d) antidiabetic properties (31); (e) anxiolytic (44); (f) immunomodulator (31); (g) anti-inflammatory (45); (h) analgesic (46); antifungal properties (47); (i) ability to interact positively with other drugs (48) and (j) protective properties in high altitudes (49).

Clinical Studies with the Shilajit-based Formulation in AD

In a clinical study the nutraceutical formulation composed of Andean shilajit and complex B vitamins was administered to a group of healthy volunteer subjects as a memory enhancer. Informed consent was obtained from all subjects. The reported effects of this treatment were mainly related to improvements in memory and as an energy enhancer (Figure 3).

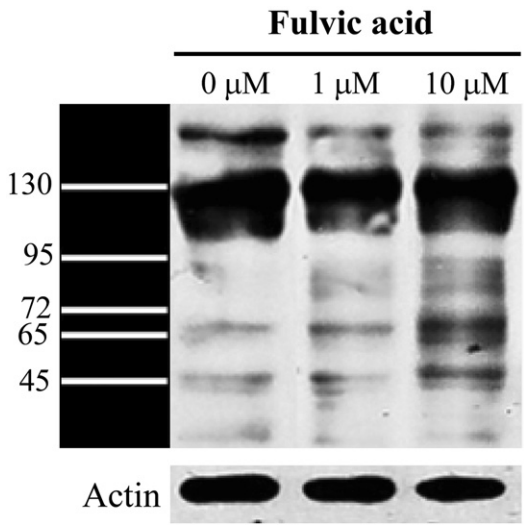

Figure 1. Fulvic acid (FA) promotes the disaggregation of tau protein in cells cultured in nonproliferative conditions. Immunoblot assayed using tau5 antibody in different protein extracts of N2a cell culture exposed to increasing FA concentrations. Increase in the availability of monomeric forms of tau (45−65 kDa) and the reduction of high molecular weight variants (>130 kDa) are noteworthy.

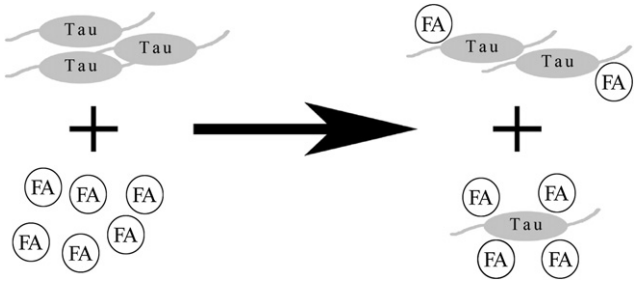

Figure 2. Schematic representation of the effects of FA in tau aggregates. Considering that tau protein is positively charged and that FA is strongly negatively charged, FA interacts with tau protein aggregates, disassembling these structures. FA and humic substances exhibit the capacity to encapsulating positively charged proteins (38).

Table 1. Characteristics of AD subjects treated either with an Andean shilajit-based nutraceutical or with placebo

	Number	%
Age		
Mean	76.38	
Standard deviation	5.68	
Gender		
Female (*n*)	10	63
Male (*n*)	6	38
Group		
Formulation of Andean shilajit plus vitamins B6, B9 and B12 (*n*)	9	56
Placebo (*n*)	7	44

AD, Alzheimer's disease.

We carried out a placebo-controlled pilot study evaluating the cognitive effect of the Andean shilajit-based formulation in a group of 16 patients with probable Alzheimer's disease dementia. Patients were recruited from the Cognitive Neurology & Dementia Unit (Unidad de Neurología Cognitiva y Demencias-UNCD) of the Neurology Service, Hospital del Salvador in Santiago, Chile. Diagnosis was performed according to NINCDS/ADRDA criteria (50). A set of tests were performed for all subjects at week 0, week 12, and at the end week 24 of the study. The tests included mental examination performed with the Chilean's version of the Mini-Mental test of Folstein (MMSE) (51), Boston naming test (60 figures), cognitive portion of the Alzheimer's Disease Assessment Scale (ADAS-Cog) (52), Trail-making Test A (TMT-A), and Neuropsychological Inventory (NPI-12) (53). Staging of dementia severity was established with the Global Deterioration Scale (GDS) (54). Characteristics of the study participants are shown in Table 1. Statistical analysis in the clinical study was performed with repeated measures analysis of variance (ANOVA) using SPSS statistical package v.17.0 for windows.

Data showed that there was a clear tendency toward a less cognitive deterioration of patients as measured by GDS in comparison with the cognitive deterioration increase in the placebo group (Figure 4A). Moreover, a significant decrease in NP-12 scores was observed in AD patients as compared with placebo administered to controls, indicating that the formulation results in fewer neuropsychological symptoms with less distress for the caregiver (Figures 4B and 4C). More interesting, data on the biochemical tau platelet biomarkers for diagnosis of AD indicated that treatment with the formulation resulted in an effective control of the increase in the HMW/LMW ratio of tau species related to AD. Studies suggest that the nutraceutical formulation controls the formation of the oligomeric tau marker in the blood of AD patients (Table 2). Considering the small population included in the study, we did not find significant differences between groups for the studies carried out with the other neuropsychological tests.

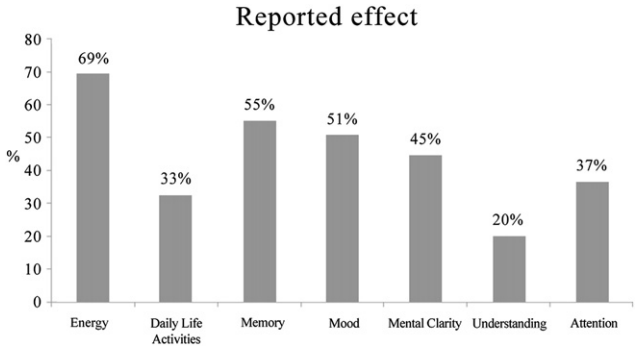

Figure 3. Reported effect of the formulation based in Andean shilajit plus complex B vitamins in 50 cognitively healthy subjects (ages 60.88 ± 16.29, 39.5% males) based on a structured survey and written informed consent. Two capsules of 300 mg each (200 mg shilajit, plus vitamins) of the formulation were administered to subjects for 6 months. The most common action reported is improvement in energy followed by a better memory performance.

Discussion

There are divided opinions about the uses of nutraceuticals against certain diseases. The first issue is the nonexisting legal framework to regulate their trade because there is concept confusion. Legal bodies in most countries classify nutraceuticals as dietary supplements; therefore, regulation is not as stringent as for drugs (16). Otherwise, three categories of claims on labels of these kinds of products exist in most countries: (i) nutrient content claims describing the percentage of a nutrient in a product relative to the daily value, (ii) structure/function claims expressing the effect of a dietary supplement on structure or function of the body and (iii) health claims describing the relationship between a food or bioactive ingredient and reduction in disease risk (16). Usually, health claims attributable to most biologically active ingredients when ingested in the form of

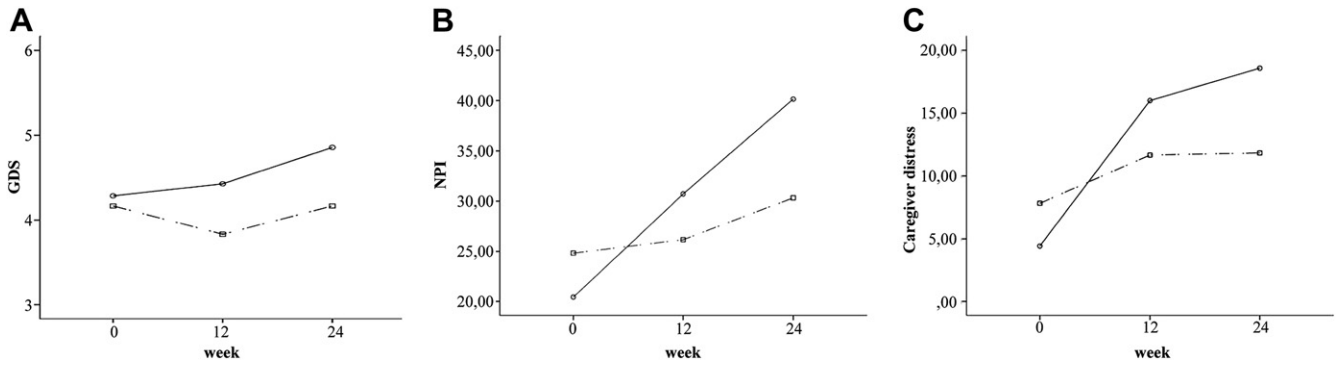

Figure 4. Differences between AD subjects treated with an Andean shilajit-based formulation or placebo. (A) Global Deterioration Scale (GDS) graphic shows less deterioration in the group treated with the formulation, whereas there are less neuropsychological symptoms (NPI) (B) and caregiver distress (C) in the treatment group as compared with placebo group. Dashed line and squares: Andean shilajit-based treatment group; continuous line and circles: placebo group.

isolated compounds as nutraceuticals have often little or doubtful scientific foundation (55). Therefore, limited data available are mainly from *in vitro* assays and preclinical trials, which declare its results inconclusive (56).

In this review we have shown some effects at *in vitro* and *in vivo* levels from a nutraceutical formulation or dietary supplement. Our research in the field of nutraceuticals has led to an increase of scientific knowledge about pharmacological and medical benefits of such formulations, which together with toxicity studies and clinical trials may lead to compounds that are safe to human health and work efficiently in the control of cognitive disorders such as AD.

In conclusion, nutraceutical compounds are becoming a new hope for AD therapeutics. Because new drugs for AD have no proven efficiency in AD treatment, physicians, patients and families are currently gradually turning towards food supplements and natural compounds that offer hope of preventing and eventually impeding cognitive decline. Some of those compounds have been known and used for many years with the expectation of obtaining enhanced energy and a better cognitive performance and to ultimately mitigate the effects of aging. Nevertheless, well-designed and methodologically correct studies are scarce. Fortunately, several serious investigations are on course to demonstrate the possible roles and mechanisms of action of nutraceuticals in the aging population.

Table 2. Changes in platelet tau biomarker upon treatment of AD patients with the nutraceutic formulation

	Clinical trial groups		
	AD patients[a]		Control group[b]
	+ formulation	+ placebo	Normal
Ratio HMW/LMW[c]	Mean ± SD	Mean ± SD	Mean ± SD
Time zero	2.967 ± 0.368	2.879 ± 0.567	1.076 ± 0.421
Time 24 weeks	2.320 ± 0.541	3.369 ± 0.697	1.107 ± 0.239

AD, Alzheimer's disease; HMW, high molecular weight; LMW, low molecular weight.
n for group formulation: 9/*n* placebo group placebo: 7/*n* normal group.
[a]Patients with mild/moderate AD incorporated in the study and with approved incorporation criteria.
[b]Healthy controls of same age to whom samples were withdrawn for platelet tau studies.
[c]Ratio HMW/LMW between oligomerized (HMW tau) and normal tau (LMW tau). An increase in this marker correlates with cognitive impairment (12,13).

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