



UNDERSTANDING PHYTOTHERAPY OF ALZHEIMER'S DISEASE: LAST DECADE AND COMING FUTURE

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ABSTRACT

Years of research have not been able to tame the relentless progression of Alzheimer's disease (AD). With some of the most promising forerunner therapeutic molecules failing in late phase clinical trials recently and the existing modern therapies yielding only modest, short term benefits at the cost of different side effects, the focus has more strongly shifted on the plant derived molecules which have been claimed to exert significant anti dementic effects over the ages and as a matter of fact have come up with impressive findings in rigorously conducted experimental laboratory studies and randomised clinical trials. The review summarizes the core salient findings in this domain in the past ten years.

INTRODUCTION

Hundred years after Alzheimer's disease (AD) was officially documented by Alois Alzheimer in 1907, it still remains a challenge to biomedical researchers. Drugs which retard the underlying progression of the disease are yet to see the light of the day. One of the reasons is the cross talk of various molecular determinants which ultimately lead to the development of the disease are not fully defined even after decades of research and millions of dollars which have been pumped into this project.

With clinical treatment of this ravaging degenerative disease eluding researchers since its discovery, there has been a steady growing interest in reports of herbal remedies demonstrating potential pharmacological effects in various aspects of pathophysiology of AD and many of them showing multipronged mechanisms of action in combating dementia. However, relatively less has been done to actively extend the preliminary findings further to know their true potential. Since herbals are finding popular use in therapeutic armamentarium, a risk benefit assessment based on systematic reviews of

randomized clinical trials is the need of hour. Encouraging emergent facts about some phytoconstituents which have been put through the rigours of well planned advanced laboratory studies and randomized double blind controlled trials calls for full fledged scientific attention.

The review discusses putative plant derivatives in the context of the underlying pathomechanics of AD and sheds light on the plethora of naturally occurring substances which could be explored scientifically for an answer to AD.

Potential natural products

Acetylcholinesterase inhibiting agents

Anti acetylcholinesterase agents have been the mainstay of Alzheimer's therapeutics so far which exert their action by sustaining the levels of acetylcholine (ACh) in central synapses, particularly in hippocampus and neocortex. This approach has been moderately effective in mild to moderate AD. There are phytoconstituents in abundance which have anticholinesterase activity

and their efficacy vis-a-vis the standard marketed therapies stand to be evaluated clinically.

Bacopa monnieri which richly grows in India along the Gangetic plains contains bacosides and has tested anticholinesterase and antioxidant effects in vitro (Limpeanchob et al. 2008) and in rodents (Das et al. 2002) which are hypothesized to be the potential mechanisms among others of its utility in AD. Its beneficial effects in patients of AD have been demonstrated in a study of six months on AD patients which yielded data of markedly improved scores of orientation, attention, language and overall score on the standardized and computerized Mini Mental State Examination Scale (MMSE). Women participants of the drawn sample population presented better improvement than their men counterparts (Goswami et al. 2011). Emergent data from another study on healthy elderly volunteers, mostly above 55 years of age, is in the herb's favour pointing at improved memory retention and acquisition which concurs with similar findings and its traditional use (Morgan and Stevens 2010). Essential oils and individual monoterpenoids obtained from *Salvia lavandulaefolia* (Spanish sage) have shown inhibitory activity on the enzyme acetylcholinesterase in-vitro and in-vivo which is relevant to the treatment of AD. Other activities relevant in this context are its antioxidant, anti-inflammatory effects. Several other phytochemicals from different herbs e.g. *Acorus calamus*, *Epimedium coreanum* (Oh et al. 2004), peels of *Citrus medica* (Conforti et al. 2007), *Salvia lerrifolia* (Loizzo et al. 2010), *Phagnalon saxatile* (Conforti et al. 2010a) which possess significant anticholinesterase activity which warrant a detailed follow up with clinical trials.

Antioxidants

Neural oxidative insult is an invariable finding in patients of AD, and is observed within every class of biomolecule, ranging from nucleic acids, proteins, lipids to carbohydrates. Oxidative injury may develop secondary to excessive oxidative stress resulting from β -amyloid induced free radicals, mitochondrial abnormalities, inadequate energy supply, inflammation or altered antioxidant protective mechanisms. Consonant with this line of thought is the prevalence of free radical

oxidative stress, particularly of neural lipids, proteins and DNA, extensively in brain regions with a heavy A β burden. Treatment with antioxidants is a promising approach for decelerating disease progression by decreasing phospholipid peroxidation, protein and DNA oxidation (Markesberry, 1999).

Citrus medica peel extract is reported to contain limonene and γ -terpinene. The extract showed significant antioxidant activity verified by different assays (DPPH test, β -carotene bleaching test and bovine brain peroxidation assay). Oxidative damage, caused by the action of free radicals, may initiate and promote the progression of AD which can be attenuated by these constituents (Conforti, 2010b).

The dietary omega-3 fatty acid docosahexaenoic acid (DHA) is another promising member in this category with proven beneficial effects in mitigating oxidative damage and synaptic cognitive deficits in transgenic rodent models of AD (Cole et al. 2005).

Ginkgo biloba leaf extract has shown strong beneficial effects in treating neurodegenerative diseases like AD, geriatric complaints like vertigo and psychiatric disorders like schizophrenia (Ramassamy et al. 2007). This multitude of activities of Ginkgo leaf extract may work through various mechanisms. The proposed mechanisms of the extract containing EGb 761 are its antioxidant effect, inhibition of beta amyloid peptide (A β) aggregation to reduce Alzheimer's progression (DeFeudis and Drieu, 2000; Smith and Luo, 2004).

The basis of therapeutic action of the Ginkgo leaf extract on chronic pathological progressions like neurodegenerative diseases has more to do with its antioxidant properties. There are two proposed mechanisms of action. The extract can scavenge directly scavenges reactive oxygen species (ROS) such as hydroxyl radicals (OH \cdot), peroxy radical (ROO \cdot), superoxide anion radical (O $_2^{\cdot-}$), nitric oxide radical (NO \cdot), hydrogen peroxide (H $_2$ O $_2$), and ferryl ion species (Mahady, 2002; DeFeudis, 2003). It can indirectly also enhance activities of antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase, catalase, and/or heme-oxygenase-1, thereby indirectly contributing as an antioxidant (DeFeudis et al. 2003).

Alternatively, the Ginkgo leaf extract inhibits ROS accumulation induced by A β (particularly flavonol quercetin) and also reduces neuronal apoptosis, wherein apoptosis is considered to be one of the main causes for neurodegenerative diseases (Bastianetto et al. 2000) and thus relieves AD. Bilobalide prevents DNA fragmentation due to hydroxyl radical β -amyloid and hydrogen peroxide (Ahlemeyer and Kriegelstein 2003). Combinations of antioxidants might be of even greater potential benefit for AD, especially if the agents acted by different mechanisms or their activities complemented each other (e.g. vitamins E, C and ubiquinone). However, we are waiting for clinical data for unambiguous interpretation (Grundman and Delaney 2002).

Lesser known *Ligusticum wallichii* extract containing tetramethylpyrazine has proved that it can remarkably enhance the learning and memory abilities in D-galactose and sodium nitrite induced AD mice models as well as aluminium chloride induced AD mice, presumably by raising the activity of superoxide dismutase (SOD), reducing the activity of acetylcholinesterase (AChE) and MDA and decreasing expressions of A β and NF- κ B in the brain (Lin et al. 2008).

Antiexcitotoxic agent

Recent evidence supports that disturbance in systems with the excitatory amino acid L-glutamate (L-Glu) may have an involvement in the pathogenesis of AD. Almost all neurons in the CNS carry the N-methyl-D-aspartate (NMDA) subtype of ionotropic L-glutamate receptors, which can mediate post-synaptic calcium influx. Of late, these receptors have come in focus and Group I GluRs (Glutamate receptors) control the levels of second messengers, inositol 1,4,5-triphosphate (IP₃), calcium ions and cAMP. They promote the release of arachidonic acid via intracellular Ca²⁺ mobilization from intracellular stores which in turn facilitates the release of glutamate and could trigger the formation of neurofibrillary tangles, in AD. GluRs regulate neuronal health, possibly through a series of downstream protein kinase and cysteine protease signalling cascade that affect apoptosis mediated by mitochondria. They may also have a role in glutamate induced neuronal death by facilitating calcium mobilization (Tsai et al. 2005).

Phytoestrogens like genistein, genistin, daidzein, daidzin, formononetin, and equol, when tested in-vitro for their neuroprotective efficacy against the toxic insult of glutamate, registered a modest but significant reduction in glutamate induced excitotoxicity (Zao et al. 2002). Eugenol too prevents calcium influx and attenuates excitotoxicity and could be useful in this connection (Irie and Yoshifumi 2006).

Ginsenoside from *Panax ginseng* reportedly has a neuroprotective effect against glutamate-induced neurotoxicity by virtue of its anti-oxidation and anti-apoptotic mechanisms. Also, the inhibitory effect of ginsenoside against the formation of A β 1–40 in vitro (Li Na et al. 2007) and results of salutary enhancement of cognitive performance in clinical trials (Soon Tae Lee et al. 2008) present it to be a potential treatment strategy for AD.

Lycium barbarum, commonly known as Wolfberry, is a common ingredient in oriental cuisines and is rich in β -carotene and zeaxanthin. A recent trend has caught up of using dried Wolfberry as a food supplement in the west following reports of its anti aging potential. It has been demonstrated that a fraction of polysaccharide from Wolfberry provides impressive neuroprotection against β -amyloid peptide induced cytotoxicity in primary cultures of rat cortical neurons. Further investigations into factors causing neurotoxicity elicited by glutamate in primary cultured neurons confirmed its protective effects were comparable to memantine, a non-competitive NMDA receptor antagonist. In addition to glutamate, the phytoconstituent attenuated N-methyl-D-aspartate (NMDA)-induced neuronal damage. All these underscore its neuroprotective effects (Shan Ho et al. 2009). There is another facet to its action in terms of in vitro attenuation of homocysteine induced neuronal apoptosis, phosphorylation of tau-1 and cleavage of tau, all crucial factors in the pathogenesis of AD. It could thus serve as a disease modifying agent (Shan Ho et al. 2010). Other natural remedies coming to the fore, for instance, *Gossypium herbaceum* extract which afford beneficial inhibition of pro-apoptotic protein expression, increased calcium pump, calbindin D28k expression suggesting

maintenance of calcium homeostasis thereby preventing excitotoxic cell death due to calcium (Chao et al. 2009)

Anti amyloidogenesis agents

The amyloid cascade hypothesis of AD proposes contra normal processing of amyloid precursor protein (APP) into synaptotoxins of 4kDa amyloid β peptides, the deposition of which is the pathological hallmark of AD. Therefore, either retarding formation of $A\beta$ or preventing destabilization of pre formed $A\beta$ fibrils are both prospective therapeutic goals against AD. In this regard, *Paeonia suffruticosa* which has been used in humans in Chinese traditional medicine for years with virtually no adverse effects was found to inhibit $A\beta$ fibril formation as well as dismantle pre-formed $A\beta$ fibrils. Furthermore, the experiments with Tg 2576 mice showed that 1,2,3,4,6-Penta-O-galloyl- β -D-glucopyranose (PGG), a high molecular weight tannin type polyphenol isolated from the plant improved memory deficits in the animals by inhibiting $A\beta$ oligomerization and hence toxicity (Fujiwara et al. 2009a). Almost similar findings of elimination of senile plaques in a concentration dependent manner exist for *Uncaria rhynchophylla* (Fujiwara et al. 2009b). Therefore, they could prove to be newer therapeutic or preventive agents in AD or mild cognitive impairment.

Another study on Amazonian nootropic herb *Ptychopetalum olacoides* extract presents interesting results against $A\beta$ peptide induced neurotoxicity, i.e. $A\beta$ deposition and astrogliosis in mice but awaits a more elaborate and long term investigation (Figueiro 2010).

$A\beta$ is a major component of senile plaques in the brains of AD patients. Ginkgo leaf extract is known to inhibit the formation of $A\beta$ from β -amyloid precursor protein (APP), a crucial process in the pathogenesis of AD (Yao et al. 2004a). Formation of amyloid precursor protein has been indirectly linked to elevated cholesterol levels (Puglielli et al. 2003). It has been suggested that the inhibition of $A\beta$ is through the Ginkgo leaf extract's ability to compete with free cholesterol for interaction with $A\beta$ and thereby decrease their aggregation (Yao et al. 2004b).

Resveratrol (trans-3,4',5-trihydroxystilbene), a naturally occurring polyphenol mainly found in

grapes and red wine, markedly lowers the levels of secreted and intracellular amyloid- β ($A\beta$) peptides produced from different cell lines. It promotes proteasome dependent intracellular degradation of $A\beta$ and is a potential candidate molecule against AD (Marambaud et al. 2005).

Centella asiatica extract too have reported selective decrease of β amyloid in rodent hippocampus as well as significant in vitro free radical scavenging, lipid peroxidation and DNA damage preventing properties (Dhanasekaran et al. 2008).

Anti neuroinflammatory agents

The amyloid hypothesis states that formation of amyloid peptides ($A\beta$) by neurons is the prime trigger of the pathogenesis of AD. Evidence supporting the amyloid hypothesis has been recently reviewed (Walsh and Selkoe, 2004). However, there is still mist over how $A\beta$ causes cell damage. Several mechanisms have been proposed of which one view states that $A\beta$ protofibrils activate microglia, inciting an inflammatory response and release of neurotoxins or neurotoxic cytokines (Tan et al. 1999). It has been speculated that this inflammatory response associated with the presence of neuritic plaques is secondary to $A\beta$ accumulation and could be involved in neuronal damage and with the progression of the disease. Activated microglia and reactive astrocytes surrounding extracellular deposits of amyloid β -protein initiate an inflammatory response characterised by a local cytokine-mediated acute phase response, activation of the complement cascade and subsequent further cell damage (McGeer et al., 1993). The main inflammatory players in AD are microglia, astrocytes, inflammatory cytokines, chemokines.

Curcumin from *Curcuma longa*, apart from exerting its anti AD effects through many different mechanisms has a very important and potent anti-inflammatory effect. Curcumin inhibits $A\beta$ -induced expression of Egr-1 protein and Egr-1 DNA-binding activity in THP-1 monocytic cells. By inhibition of Egr-1 DNA-binding activity by curcumin, it attenuates inflammation. The chemotaxis of monocytes, which can occur in response to chemokines from activated microglia

and astrocytes in the brain, can be decreased by curcumin.

It also inhibits cyclooxygenase (COX-2), phospholipases and enzymes involved in metabolizing the membrane phospholipids into prostaglandins. The reduction of the release of ROS by stimulated neutrophils, inhibition of AP-1 and NF-Kappa B inhibit the activation of the pro-inflammatory cytokines TNF (tumor necrosis factor)-alpha and IL (interleukin)-1 β . In summary, curcumin decreases the main chemical for inflammation and the transcription of inflammatory cytokines. The exposure to curcumin also impairs the production of pro-inflammatory cytokines (IL-1, IL-6 and TNF- α). These studies indicate a potent inhibition of pro-inflammatory cytokine production by curcumin which is of import in AD (Mishra and Palanivelu 2008).

Mitochondrial protectants

The connection between mitochondrial abnormalities and disease has long been known. Presently, a large number of studies implicate reduced rate of neuronal metabolism in AD. (Blass et al. 2000). The most consistent defect in mitochondrial electron transport enzymes in AD is deficiency in cytochrome- c oxidase. Preliminary studies demonstrate somal accumulation of cytochrome-c oxidase protein. It is suspected that enhanced degradation of mitochondria occurs in AD, leaving behind lysosomal debris containing non functioning mitochondrial proteins. Initial finding of diminished numbers of microtubules in AD, with nitration of microtubules and alterations in tau, are consonant with altered mitochondrial trafficking and enhanced degradation in AD. Mitochondria supposedly play a vital role in apoptosis. There is clinching evidence that mitochondria contain a molecular switch for the initiation of apoptosis in the form of a nonspecific mitochondrial inner membrane channel, mitochondrial permeability transition pore. Opening of the pore causes nullification of the electrochemical gradient and activation of apoptosis inducing factor caspases, leading to apoptosis. Opening of the pore in turn, can be affected by excessive calcium uptake, reactive oxygen species or a decline in energy production. Thus, a marked reduction in mitochondrial energy

production and a chronic rise in oxidative stress could trigger mitochondrial permeability transition pore and initiate apoptosis, potentially contributing to neurodegeneration in AD (Castellani et al. 2002).

Perilla frutescens seed oil (PFSO), a rich source of unsaturated fatty acids, especially of omega-3 linolenic acid which is commonly used as herbal food supplement for vascular health has come up with encouraging results in guinea pigs. It was found to decrease reactive oxygen species, stabilize mitochondrial membrane potential on dissociated neuronal cells (Eckert et al. 2010).

A neuroprotective, particularly for the early stages of disease is lipoic acid (LA) which may fulfil the therapeutic need. A naturally occurring cofactor for the mitochondrial enzymes pyruvate dehydrogenase and α -ketoglutarate dehydrogenase, LA has been shown to have a variety of properties apart from mitochondrial protection which can interfere with the pathogenesis or progression of AD (Mackzurek et al. 2008).

It has also been suggested that ginkgo leaf extract increases expression of mitochondrial enzymes like NADH dehydrogenases, which can influence ROS generation in the mitochondria. This is a protection against uncoupling of oxidative phosphorylation, thereby increasing ATP levels regulating energy metabolism (Tendi et al. 2002). Compared to other antioxidants, the Ginkgo leaf extract (EGb 761) is known to be regulatory and controlling neurochemicals or neuroendocrine indicators according to the circumstances (Smith and Luo 2003). The main constituents implicated in all these actions are the flavonoids (quercetin and kaempferol) and the terpenoids (ginkgolides and bilobalide) (Bastianetto et al. 2000), where each active principle contributes its antioxidant property differently. The bilobalide increases the activities of the antioxidant enzymes (SOD and catalase) and improves cell viability (DeFeudis 2003).

CONCLUSION

In ethno pharmacology, numerous plant sources have been tapped to treat cognitive disorders, including neurodegenerative diseases and it has provided putative leads for AD. The use of herbal

medicines in AD is dictated by cultural traditions and social practices prevailing in a particular region. Traditional cognitive or memory enhancing plants have not been scrupulously investigated in the context of current experimental models of AD. With major strides in the knowhow of the neurobiology of AD, and limited efficacy of the current therapies, it is time we re-examined

potential naturopathic compounds. Integrative medicine if allowed by the law of the land, with an aim of cross fertilization of seminal ideas and knowledge from traditional and modern biomedical research could open newer vistas for development of novel therapeutics of this disease which has a relentless course.

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