A Review on Alzheimer Disease -its Treatment, Future Prospects and the Role of Juglans Regia in Alzheimer's Disease Based on Pathogenetic Research

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ABSTRACT
The diagnosis of the Alzheimer Disease if rendered accurately today and in the foreseeable future, comes with it a disease course that is inalterable and with no hope of cure. After the expansion of the molecular technology and with more than 100 years of identification of hallmark lesions of AD still there is absence of, or fundamentally erroneous understanding of AD pathogenesis. Hence there is absence of disease modifying therapy of AD. We are compelled to accept that modern science has provided a catalog of downstream changes in AD. These changes include: Amyloidosis; tau phosphorylation; oxidative stress including RNA modification and heavy metal toxicity; neurotrophic and neurogeneration failure; disordered energy metabolism; synaptic alteration; and autophagy. In this review we examine this aspect in the light of available evidences present in the literature. Furthermore pathology and pathophysiology of AD based on the recent observations is also described. A critical analysis of possible ABP transport across BBB is presented; animal models of AD are given in brief. Furthermore a new role of nano drug delivery to treat the AD is also discussed. Over 35000 plant species are currently used for medicinal purposes around the world. These plant contain more than 4000 flavanoid structures, terpenes, alkaloids, etc and provide numerous health benefits including anti-inflammatory, anti-oxidant, anti neoplastic, anti diabetic. Can we Identify the herbal extracts that affect AD pathomechanism, highlighting interaction of Aβ, mitochondrial anti-oxidant mechanism, inflammatory pathway, and cholinergic and glutaminergic function in pre synaptic and post synaptic neuron. This is a big question. Also Is Juglans regia (walnut) one such alternative?

Keywords: Alzheimer’s disease, amyloidosis, BBB, juglans regia, nano drug delivery, tau phosphorylation

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INTRODUCTION
Alzheimer disease is characterized by progressive cognitive decline [1] usually beginning with the impairment in the ability of forming memories [2,3]. These lossess are due to synaptic damage and neuronal loss in hippocampus,cerebral cortex and other brain regions [4]. Lower educational and occupational attainment,low mental ability in early life,reduced mental activity,reduced physical activity [5,6]. The pathological manifestations of Alzheimer include extracellular amyloid plaques and intracellularly neurofibrillary tangles [2,3,5] non cognitive symptoms like disturbance in diurnal activity, aggressiveness, symptoms of affective and paranoid schizophrenic disease are often associated with AD, these are not progressive [2].

Microvascular pathology of AD: [7] It includes atrophied thin musdes, glomerular loop formation, fragmentation with twisted or tortous vessels. The smooth muscle cell show degeneration, Astrocytic end foot swollen and atrophied pericytes, endothelial cell swollen with irregular shapes nuclei, basal lamina thickened and disrupted. This shows structural integrity of BBB is compromised. This will also be taken in the coming discussion. In the US approximately 5.5 million people are affected. Worldwide prevalence is as high as 25 milion. Accurate incidence rate of
Alzheimer’s disease is difficult to establish as the age at onset is not known [3]. The National Institute of Neurological and Communicative Disorders and Stroke and The Alzheimer’s Disease and Related Disorder Association (NINCDS-ADRDA) developed criteria to develop consistency in diagnosis which was recently updated and includes: neuropsychological assessment, brain imaging and neuropathological, biochemical and genetic understanding of the disease [3,8]. Risk factors of AD given in (Table 1) and lesions in (Table 2) respectively.

**Table 1: Risk factors** [3]

<table>
<thead>
<tr>
<th>Antecedent</th>
<th>Direction</th>
<th>Possible mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiovascular Disease</td>
<td>Increased</td>
<td>Parenchymal destruction</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Strategic location</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increase Aβ Deposition</td>
</tr>
<tr>
<td>Smoking</td>
<td>Increased</td>
<td>Cerebrovascular effects</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oxidative Stress</td>
</tr>
<tr>
<td>Hypertension</td>
<td>Increased and Decreased</td>
<td>Microvascular diseases</td>
</tr>
<tr>
<td>Type II Diabetes</td>
<td>Increased</td>
<td>Cerebrovascular effects</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Insulin and Aβ compete for clearance</td>
</tr>
<tr>
<td>Obesity</td>
<td>Increased</td>
<td>Increased risk of Type II Diabetes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>inflammatory</td>
</tr>
<tr>
<td>Traumatic Head Injury</td>
<td>Increased</td>
<td>Increase Aβ and APP deposition</td>
</tr>
<tr>
<td>Education</td>
<td>Decreased</td>
<td>Provides cognitive reserve</td>
</tr>
<tr>
<td>Mediterranean Diet</td>
<td>Decreased</td>
<td>Anti-oxidant, anti inflammatory</td>
</tr>
<tr>
<td>Physical activity</td>
<td>Decreased</td>
<td>Activates brain plasticity promotes brain</td>
</tr>
<tr>
<td></td>
<td></td>
<td>vascularisation</td>
</tr>
</tbody>
</table>

**Table 2: Cerebrovascular lesions in Alzheimer’s disease** [7]

<table>
<thead>
<tr>
<th>Pathology</th>
<th>Occurrence (%) about 300 cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Degeneration</td>
<td>100</td>
</tr>
<tr>
<td>Extravasation of serum protein</td>
<td>&gt;80</td>
</tr>
<tr>
<td>Cerebral amyloid angiopathy</td>
<td>&gt;90</td>
</tr>
<tr>
<td>Intracerebral hemorrhage</td>
<td>&gt;10</td>
</tr>
<tr>
<td>Cerebral and cortical infarcts</td>
<td>&gt;30</td>
</tr>
</tbody>
</table>

**PATHOPHYSIOLOGY:**

The Amyloid plaques and the neurofibrillary tangles are the pathological hallmarks of AD. Aβ accumulation is an upstream event which triggers tau pathology resulting in impaired function and cell loss [8].

1. **Amyloidosis:** It is altered Aβ metabolism, most prominent downstream change of AD [9]. Plaques are formed by the extracellular aggregation of Aβ (peptide of 39-42 amino acid) which is produced by the cleavage of an integral membrane protein Amyloid precursor protein(APP) by the action of β and γ Secretase [2,10,11]. This APP exist in 3 isoforms: APP 695 shortest isoform is expressed by neurons, APP751 and APP770 are expressed in Glial cells like astrocytes [5]. Aβ(40) and Aβ 42 form the majority of amyloid beta of human brain and plays important role in progression of AD. A number of studies suggest that small oligomers of Aβ are more toxic than Aβ fibrils [11]. Soluble Aβ oligomers cause rapid detrimental effects on excitatory synaptic functions leading to impaired learning and memory, also it inhibits hippocampal long term potentiation (LTP) and long term depression(LTD) that correlate to learning and memory [10]. In autosomal dominant AD-mutation causes Aβ accumulation In late onset sporadic AD there is high levels of cerebral Aβ likely to be caused by impaired clearance rather than overproduction [2,8]. It is likely that ABP is produced locally in the brain (neuronal theory) or can gain access into the brain from circulation.
(vascular theory) by breaching the BBB [7]. Taken together data support the concept that vascular system plays important role in regulating level of ABP in brain. When the level of ABP in brain extracellular space exceeds the transport capacity of clearance mechanism across BBB, an accumulation of ABP in brain will occur [7].

The **four important observations** that support the amyloid hypothesis are: [12]

1. After changes in the Aβ metabolism and plaque formation, the neurofibrillary tangles of tau are seen in the AD brains
2. Aβ toxicity is tau dependent—in the pathogenetic cascade of AD there is altered APP processing which occurs before the tau alteration
3. Genetic variability at human Apoe E locus involves Aβ metabolism with reduced cerebral Aβ deposition in the offspring produced by crossing of APP transgenic mice with that of Apolipoprotein E deficient mice.
4. Late onset AD is likely to be caused due to the genetic variability in Aβ catabolism and clearance

These four findings conclude that Aβ accumulation is primary and the rest include tau tangle formation [12]. The oligomer concept says that soluble low protein aggregates are related to cognitive function

Many cortical amyloid deposits (diffuse and no surrounding neuritic and glial pathology) are seen in humans with no symptoms of AD. Amyloid hypothesis shown in (Figure 1)

2. **TAU:** It has become equally important to amyloidosis because (i) It has superior relationship between protein deposition and cognition (ii) brain region affected is clear (iii) there is much greater specificity in diagnosis with cell to cell propagation of phosphorylated tau there is recent impact in favour of tau pathology and disease [9,13].

Tau is a phosphoprotein that contains 85 potential serine, threonine, and tyrosine phosphorylation sites. They found that almost 10 phosphorylation sites can be detected in the normal brain of humans and on the other hand 45 sites have been found in AD brain [14,15]. According to a study severity of cognitive impairment depends on the burden of the neocortical Neurofibrillary Tangles (NFTs) whose number correlate with the disease duration in both Typical and Atypical AD. This shows that distribution of Tau pathology shows the clinical picture of dementia [14].

It is a growing consensus that amyloid β peptide increases tau phosphorylation and also initiates multiple pathways that causes neuronal cell death [2,5] called as downstream changes. Brain imaging and cerebrospinal fluid biomarkers yield good specificity and sensitivity hence useful in diagnosis and also for the clinical research [16].

3. **Oxidative stress:**
Yet another potentially important pathogenic mechanism, suggested in studies that it is impossible to study in vivo. Adduct formation such as advanced glycation end products, advanced lipid per oxidation end products, and free carbonyls offer direct assessment of oxidative stress [17]. More recent is finding of RNA oxidation, which is deleterious, as it is single stranded, may induce sub lethal changes within the cell. Unfortunately it is present in all cell types and all tissues [9,10]. Several studies suggest Aβ induced oxidative stress is observed in neurodegenerative AD brain [2,10,18].

4. **Mitochondria and energy metabolism:**
Mitochondrial alterations like fission fusion abnormalities, defect in the electron transport chain protein and cytoskeleton abnormalities are all altered in AD [19]. Mitochondria shows calcium metabolism, intrinsic apoptosis pathway, activation of caspases and generation of free radicals(ROS) Thus represent the model for neurodegeneration [2,9]. Defected mitochondria cannot supply necessary cellular ATP at nerve terminals for normal neural communication, cause loss of synapse and synaptic function and ultimately will cause cognitive decline in AD patients [2].

5. **Autophagy** abnormalities contribute to load of oxidized macromolecules. Hence dietary antioxidants and enhancement of autophagy by calorie restriction improves learning and memory functions [20].
6. **Inflammation:**

   It is important component in the pathogenesis of AD, microglia and astrocytes are activated. In Aβ induced inflammation, microglia activates and differentiates into phagocytic CD 11b+ cells that secrete IL-1beta, TNF alpha, nitric oxide, free radicals and chemokines. All this activate complement innate pathway. In contrast microglia also differentiate into CD 11c antigen via adaptive pathway and secrete anti-inflammatory cytokines- (IL 4, IL10, TGF beta) [21]. The increasing evidence suggest that activated microglia via cytokines secretion exacerbate plaque pathology, enhance hyperphosphorylation of tau. Hence suppression of microglial activity is the possible strategy to treat AD [2,22].

**BLOOD BRAIN BARRIER IN ALZHEIMER’S DISEASE:**

According to the observations permeability of BBB in AD is **likely to be** altered [7]. Deposition of Amyloid beta peptides is around the larger arterial vessels and cerebral capillaries which is anatomical seat of BBB. Breakdown of BBB could affect cognitive, sensory, and structural disturbances in the CNS [7].

**Herbal extracts must pass to BBB to be effective in the CNS.** The BBB is made of dense layer of endothelial cells that create barrier between the blood and brain parenchyma. This layer has low density of pinocytotic vessels and contain efflux transporters like P-glycoprotein, multi-drug resistance associated protein and mono carboxylic acid transporters that controls flow of molecules from cerebro vascular circulation into the brain. The flavanoids either exhibit stimulatory (quercetin stimulate P-glycoprotein) or inhibitory (resveratrol inhibit P-glycoprotein) interactions with one or many of these transporters [2]. Anatomical characters shown in (Figure 2).

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**Figure 1: The hypothesis of Amyloid Cascade** [12]

**Figure 2: Anatomical characteristics of blood brain barrier** [7]
Blood brain barrier (BBB) versus brain blood barrier (bbb): Besides BBB the bbb should also be normal, this means that any substance from Brain like Aβ or any toxic elements are not allowed to enter the general circulation. i.e. from Brain to blood. BBB break down will cause rebound effects of toxins that are coming from the brain and then circulating to the whole CNS causing cellular reactions and tissue damage.

HUMAN BBB RECEPTORS FOR ABP (amyloid β peptide) 1-40:

a) Binding sites of sABP1-40 on HBMEC (Human Brain Microvascular Endothelial Cells): [7] The binding of sABP1-40 on HBMEC was only at the apical side and was absent from basolateral side. Also it is temperature dependent.

b) Influence of RAGE and SR on sABP1-40 transport: Receptor for advanced glycation end products (RAGE) and Scavenger receptor (SR) influences sABP 1-40 transport across BBB. As shown by the binding of sABP1-40 to the apical side of BBB was inhibited 60% by anti RAGE Antibody. SR is identified in brain microvasculature, in the study SR transfected Bowes cells displayed specific binding of sABP1-40 in a dose dependent manner.

c) Effect of LRP and RAP on clearance of ABP: There is abundant evidence showing that free ABP binds to RAGE and SR and the ABP in complex with apoE or apoJ will bind to LDL receptor related protein-I (LRP-1) or LRP-2.

d) Brain clearance of ABP in mice: Mathematical analysis show a higher clearance of ABP is via BBB transport than via interstitial fluid. CNS injection of ABP 1-40 and insulin to mice reached CNS, but the amount of radio labelled ABP 1-40 was at each point of time lower than the insulin. This reflects ABP clearance is from CSF. Clearance of ABP in mice is concentration dependent. The data strongly support the concept that when the levels of ABP in brain extracellular space exceeds transport capacity of clearance across BBB, accumulation of ABP in brain will occur. Hence Alzheimer Disease Pathogenesis in summary shown in (Figure 3).

ANIMAL MODELS:

A) Transgenic Animal models: [20,24,25]

When dementia is diagnosed patient already has histopathological changes in the brain so to understand the pathological process of the disease and develop disease modifying drugs animal models are being used [20]. An ideal animal model must mimic the human disease, in this rodents play a major role as they cannot develop plaques and tangles normally; these plaques and tangles can be induced in them [24]. The generation of transgenic mouse line was the breakthrough of modern Alzheimer's disease research [20].
1. To test the influence of mt DNA mutation: a mouse line named “MILAN” was made. It did not contain mitochondrial transcription factor A in forebrain neuron which is necessary for transcription and replication of mtDNA [20].

2. Genetic mouse model of autophagy impaired clearance cause neurodegeneration. UBB(+1) and Atg7 showed neurodegeneration [20].

3. Insulin/insulin like growth factor signalling (IL): Reduced levels of IGF1 in genetically modified mouse have disrupted long term potentiation in hippocampus and there is impairment in spatial learning and memory [20].

4. SAM Models: Senescence accelerated mouse prone 8(SAMP8) - Advantage of this model is that it investigates mechanisms in age related learning and memory deficits at both gene level and protein levels. It includes 9 SAMP sub strains which undergo accelerated aging, and includes 3 SAMR (Senescence accelerated mouse resistant) sub strains which undergo normal aging. Genes that are involved in ROS production, neuroprotection, signal transduction, immune response and protein folding are altered in SAMP8 mice (most reliable model). Other models given in (Table 4). The table shows that the most widely used models Tg2576, APP23 and APP/PSI only mimic initial phase of AD, [6] and when therapeutic strategies are tested they give false positive results. The 5xFAD model has less face validity, while 3x-tgAD has highest face and construct validity.

B) Chemically induced models: [24,26]

Manipulations of central neurotransmitter pathways by chemical means form the basis of these models. Scopolamine induced memory deficits, L-Methionine induced memory dementia, Colchicine induced dementia, Okadaic acid (OKA) induced memory deficit. Sodium Azide induced dementia, etc.

C) High Fat Diet induced dementia: [24]

Diet consisting of lard, casein, cholesterol, sodium chloride, vitamins, minerals, is feed to rats chronically for 90 days, causes atherosclerosis and impairment of learning and memory. There is cross relationship between CNS and peripheral cholesterol pool. Hence peripheral cholesterol can modulate CNS cholesterol and vice versa.

D) Miscellaneous Models: [24]

- Bio active phospholipid LPA (lysophosphatidic acid) causes neurite retraction in neuronal cells.
- PAF Receptor antagonist like BN50730 and BN 52031 produce amnesia.
- Nitric oxide inhibiters like L-NAME (NG-nitro-L arginine methyl ester) and...
omega-nitro-L arginine produce impairment in memory by generation of reactive nitrogen species.

- Clonidine (0.1-0.5mg/kg i.p.) α2 receptor agonist
- Lignocaine (10-80 mg/kg i.p.) Na+ channel blocker
- Cycloheximide (7.5-120mg/kg, s.c.)

Table 4: Validity of most widely used genetic mouse models of Alzheimer’s disease [20]

<table>
<thead>
<tr>
<th></th>
<th>Tg2576.</th>
<th>APP23</th>
<th>APP/PS1</th>
<th>3xtg AD</th>
<th>5xFAD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Face validity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Late onset</td>
<td>Yes</td>
<td>Yes</td>
<td>No late onset</td>
<td>Yes</td>
<td>No, late onset</td>
</tr>
<tr>
<td>Progressive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Learning/memory impairment</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Diurnal cycle disturbances</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Not tested</td>
<td>Not tested</td>
</tr>
<tr>
<td>Increased anxiety levels</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Constrictive validity</td>
<td>Amyloid plaques</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Tau hyperphosphorylation</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Neurofibrillary tangles</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Neuroinflammation</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Loss of non-adrenergic neurons</td>
<td>Yes</td>
<td>Yes/N 0</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Loss of cholinergic neurons</td>
<td>No</td>
<td>Yes/N 0</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Massive neuronal loss in cortex</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Predictive validity</td>
<td>Free from false negative findings</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

TREATMENT STRATEGY BASED ON Aβ BIOLOGY: [12]
The significance of Aβ peptide in disease pathology is highlighted by the fact that number of small compounds that inhibit the Aβ formation are undergoing clinical testing as potential therapy for AD [5].

- **By inhibiting the two proteases** either β or γ, from where Aβ is formed [12,27].
- **By immunization strategies**-[28] To prevent the oligomerisation of Aβ, by the use of active or passive immunization (antibodies to Aβ promote microglial clearance and decrease cerebral levels of the peptide) [5,14,29]. The passive immunotherapy showed occasional appearance of micro hemorrhage and occurrence of vasogenic edema in some individuals especially those apolipoprotein Eε4 genotype [5,27].
- **By regulating Neuroinflammation** with the use of NSAIDS (non steroidal anti inflammatory drugs) eg Aspirin, indomethacin, ibuprofen and COX2 inhibitors [6,12,27,28].

- **By Modulating cholesterol homeostasis** (as high cholesterol diet has been shown to increase Aβ pathology in animals) [12] cholesterol lowering drugs eg HMG-CoA reductase inhibitor eg statins [28].
- **By Chelation** of the metal ion like Cu2+ and Zn2+ as Aβ aggregation is dependent on these ions based on the observations
- **By preventing the synaptotoxic and neurodegenerative effects**
- **By restoring cholinergic deficits with use of enhancers-** cholinesterase inhibitor eg Tachrine, Donepezil, Rivastigmi [6,15,27].

According to data from animal studies **AN1792** was the first synthetic Aβ 1-42 peptide which entered the first Clinical Trial with QS21 as adjuvant but the trial was halted due to meningoencephalitis which was developed in 6% of individuals [30]. Still the drug was effective in clearing Aβ plaques and also improved performance of some neuropsychological tests [5].

TREATMENT STRATEGY BASED ON TAU BIOLOGY –[14]
• **Tau immunotherapeutic approach**- an emerging disease modifying strategy that target phosphorylated tau epitopes like phospho Ser 396;phospho Ser 404;phospho Thr231;phospho Ser 235;phospho Ser 422 etc. [13,14]. It can prevent or reduce tau hyperphosphorylation, prevent tau aggregation or clear tau oligomers. Hence reduce neurofibrillary pathology [14,31].

**Limitations** include: [14]
1. The immunotherapies target the phospho sites which are present in human brain so there is a big concern on the safety of vaccine targeting specific Tau species.
2. This therapeutic approach can target only specific one or two phospho sites among 45 of AD brain.
3. It is unknown whether all AD phospho epitopes co-exist on the same Tau molecule or whether distinct sub species of tau has a variety of different phospho codes.

First in man tau vaccine that entered Phase I clinical trial in June 2013 was AADvac. Vaccine reduced early and Late manifestations of tau pathology as seen in animals, also improved short term memory in Y maze. **Tau Vaccine is:** [14]

a. **Immunogenic**- has antibody response
b. **Specific** – target only one antigenic site
c. **Selective in nature**- differentiate between pathological and physiological tau
d. **Safe**- helper T cell epitopes are derived from carrier protein and immnr response is shifted to Th 2 phenotype
e. **Therapeutically effective**- reduces tau phosphorylation as well as oligomer
f. **Improves neurobehavioral parameters.**

b. **Anti-oxidant therapy** is safe using in vitro cell culture and mouse models of AD several labs are currently involved in targeting this [2,15].
c. **Memantine** non-competitively inhibits NMDA receptor, prevent glutamate excitotoxicity and also has minimal side effects [2,6,27].

**FUTURE PROSPECTS IN AD:** [7]

**Neurotrophic factors**- Future therapeutic strategy for AD is to use multimodel drug that effectively target brain insulin/IGF signaling cascade and also enhances IGF level or other neurotrophic factors, alternatively a mixture of different neurotrophic factors could be useful to attenuate pathogenesis of AD. Cerebrolysin, a mixture of several neurotrophic factors has multi model action on brain cells including neuroprotection, neurodegeneration and angiogenesis, a hope for this drug as potential future drug [15].

**Nanotechnology for treatment**-
There is advancement in nano technology for diagnostics and drug delivery purposes this is quite likely that therapeutic agents if delivered through nano technology will induce long term neuroprotection engineered nano particles having high specificity for brain capillary endothelial cell is another way hence further studies are needed.

But unfortunately most of these drugs have side effects like gastro intestinal bleeding, liver and renal toxicity and nausea. These drugs do not work with patients who carry ApoE gene [28]. In this respect natural herbal alternative with pleiotropic useful properties and with least adverse effects provide greater therapeutic benefit than single ingredient synthetic pharmaceutical drug having serious side effects [28]. The complex pathology of AD and heterogenous pharmacological effects of herbal extracts pose difficult challenges in using herbal drugs in AD [2].

**But studies suggest that both are at a new cross road** [2] The following two tables give anti-oxidant and anti Aβ related effects in AD (Table 5 & 6 respectively).

The mechanism of action of some of the identified anti-oxidant is known, but as the active ingredients in much plants extract possessing anti-oxidant properties remain to be identified. **Hence a complete picture is yet to emerge** [18].

**YES ONE SUCH ALTERNATIVE IS WALNUT (JUGLANS REGIA) that has been recognized for its medicinal value centuries ago It is expected to produce cumulative benefits and exhibit enhanced neuroprotection by virtue of being "natural statin", natural NSAIDS ", natural anti-oxidant", natural anti apoptotic agent", and"memory enhancer" [28].
Table 5: Herbs tested for anti-oxidant or anti apoptosis related effects in AD [2]

<table>
<thead>
<tr>
<th>Plant Extracts</th>
<th>Models and Oxidants</th>
<th>Effects of Plant Extracts</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curcumin&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Tg2576 mice; Aβ1-40, Aβ1-42</td>
<td>Blocked the aggregation, oligomer, and fibril formation in vivo and in vitro</td>
<td>Yang et al</td>
</tr>
<tr>
<td>EGB 761</td>
<td>Sprague-Dawley rats</td>
<td>Increased the release of α-APPS</td>
<td>Colciaghi et al</td>
</tr>
<tr>
<td>Huperzine A&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Sprague-Dawley rats; Aβ1-40</td>
<td>Reversed the Aβ induced down regulation of APP secretion and protein kinase C</td>
<td>Zhang et al</td>
</tr>
</tbody>
</table>

Table 6: Herbs tested for anti Aβ and related effects in AD [2]

<table>
<thead>
<tr>
<th>Plant extracts</th>
<th>Models and oxidants</th>
<th>Effects of plant extracts</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>A)Animal models</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EGB 761&lt;sup&gt;*&lt;/sup&gt;</td>
<td>Wistar rats deficient in vitamin E</td>
<td>Increased the proportion of small sized synapses and mitochondrial density</td>
<td>Bertoni-Freddari et al</td>
</tr>
<tr>
<td>Ginkgo bilobo</td>
<td>ApoE deficient mice</td>
<td>Increased the life span and reduced periodic acid Schiff positive inclusion bodies and apoptotic cells</td>
<td>Veurink et al</td>
</tr>
</tbody>
</table>

B)In Vitro models

| Aged garlic extract and S allylcysteine | Rats PC 12 cells,Aβ25-35 | Suppressed ROS, caspase 3, and DNA fragmentation, protected cell from apoptosis | Peng et al |
| Bacopa monniera extract | Hippocampal neurons from Sprague-Dawley rats,Aβ 25-35 | Protected cell against apoptosis | Choi et al |

It is very interesting that there are very few studies showing the in vivo effects of Walnut in relation to neuronal dysfunction or Alzheimer's pathology except for the reports showing improved behaviour in mice after Walnut treatment. Natural anti-oxidants mainly obtained from plants as phenolic compounds like flavanoids, phenolic acids and alcohols, stilbenes, tocoferols, tocotrienols; ascorbic acid and carotenoids [18,32,33]. The quest for natural anti-oxidants for dietary, cosmetic and pharmaceutical uses has become the most important industrial and scientific research challenges over the last 2 decades. Efforts of gaining extensive knowledge of anti-oxidant are increasedA8, synthetic anti-oxidants with potential toxicity has shifted to discovery and utilisation of natural(such as fruits and vegetables) source anti-oxidant. Several nuts such as walnuts and peanuts are known to have significant anti-oxidant contents. **Juglans regia.L:** The maximum number of diseases is treated by fruit kernels followed by leaves. Walnuts are included in the FAO list of priority of plants because of its nutritive value [34,35] higher intake of nut is associated with better cognitive function [36].

**Description:** The tree shown in (Figure 4). *Juglans regia* known as Akhrot in India,a native of eastern Europe to Nortg Asia,it is well known for its medicinal uses and is
grown in Himlayas in India.It is a member of juglandacea family [37,38]. The genus Juglans contains about 20 species ,all are edible nuts,among these English or Persian walnut is most widely cultivated [34]. It is a tree upto 25m in height,compound leaves with 5-9 leaflets.Fruit drupe with fleshy pericarp,bony endocarp which encloses 2 edible cotyledons-kernels [34]. It is woody,decidious and frost tender tree.The root,stem,bark,leaves,seeds,cotyledons and seed oil are useful in treating health complaints [37].

Figure 4: Tree of Juglans regia [34]
Constituents: It is a rich source of unsaturated fatty acids,Vitamin E,fiber,magnesium,potassium.on comparig with other nuts it contain mono unsaturated fatty acid (MUFA) and they are highly enriched in omega 3 poly unsaturated fatty acid(PUFA) which are essentail fatty acid for us [39]. Omega 3 fatty acids have beneficial effect on diseases like cardio disorder, cancer, diabetes [40] and neurological disease [41]. FDA claimed that diet including the walnut can reduce heart disease [36]. Heart benefits include lowering cholesterol, reducing inflammation and improving arterial function [36,42]. The five major phenolic compounds in walnut kernel are gallic and cinnamic acid,catechin,juglone and rutin.the phenolic compound in the seed coat are more as compared to walnut flesh: 12 It is rich in phenol which have beneficial effect on human health [43,44].

Economic importance: The creamish colur nuts are edible while black ones are not from which cooking oil is taken out/kernel are used in confection,cake,etc Epicarp of fruit produces dye used for colouring woollen blankets and polishing furniture, Endocarp used as fuel for domestic purpose and in kilns [34]. According to the data animals on walnut diet improved spatial learning and performance on morris water maze [43]. All parts of the plant are medicinally important [45]. The green husk contain ascorbic acid(Vitamin C). It can be extracted and used as supplement. The root and stem bark are anthelmintic, astringent, anti bacterial and detergent. The stem bark is dried and used as tooth cleaner [37]. In China Semen juglandis is used to tonify the kidney,warm lung and relax the bowel.

Bioavailability of phenolic compound in Juglans regia: [2] According to the studies isoflavones and gallic acid (present in walnut) are the most readily bio available which is an important criteria in selecting the herbal drugs of AD [2].

- Juglans regia as an anti-oxidant:
The walnut tree is cultivated commercially throughout southern Europe, northern Africa, eastern Asia and USA. English Walnut Seeds contains abundant phospholipids, proteins, tocopherols a vitamin E family compound, and unsaturated fatty acids, phenolic compounds [44,46] hence it is a rich nutrient food food high in anti-oxidant or essential fatty acid prevent neuronal dysfunction during aging [17,47]. Dietary intake of polyphenols attenuates the progression of the disease [48]. The walnut is unique among the nuts due to the presence of anti oxidants [39]. Lipid per oxidation, DNA and protein damage mainly occur by OH radical eg Fenton Reaction, it is reported in the study that the Juglns regia nut extract showed capacity of reducing DNA damage in a dose dependent manner [49,50]. In poly unsaturated lipid areas like brain and liver lipid per oxidation takes place by oxidative degradation and lipid radicals are formed, here also Juglans regia showed prevention of lipid peroxide generation [49]. Isolation of anti-oxidant compounds: Given in (Table 7) below.
Table 7: The DPPH scavenging activities of extracts and isolated phenolic compounds of *Juglans regia* [51]

<table>
<thead>
<tr>
<th>Test Materials</th>
<th>IC50 (mg dried material equivalents/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEF</td>
<td>20+-2.2</td>
</tr>
<tr>
<td>EEF</td>
<td>0.83+-0.25</td>
</tr>
<tr>
<td>BUF</td>
<td>0.88+-0.01</td>
</tr>
<tr>
<td>AF</td>
<td>1.7+-0.08</td>
</tr>
<tr>
<td><strong>PYROGALLOL (1)</strong></td>
<td>white colourless needles obtained{C6H6O3}</td>
</tr>
<tr>
<td><strong>P-Hydroxybenzoic acid (2)</strong></td>
<td>as white amorphous powder{C7H6O3}</td>
</tr>
<tr>
<td><strong>Vanillic acid (3)</strong></td>
<td>as white amorphous powder{C8H8O4}</td>
</tr>
<tr>
<td><strong>Ethyl gallate (4)</strong></td>
<td>as white needles{}</td>
</tr>
<tr>
<td><strong>Protocatechuic acid (5)</strong></td>
<td>as white amorphous powder{C7H6O4}</td>
</tr>
<tr>
<td><strong>Gallic acid (6)</strong></td>
<td>as white needles{C7H6O5}</td>
</tr>
<tr>
<td><strong>3,4,8,9,10-pentahydroxydibenzo-{b,d}pyran-6-one (7)</strong></td>
<td>pale yellow needles{C13H8O7}</td>
</tr>
</tbody>
</table>

PEF-Petroleum ether fraction; EEF-ethyl acetate fraction; BUF-n butanol fraction; AF- aqueous fraction of kernels of *J. Regia* [51].

So the order is:

*3,4,8,9,10-pentahydroxydibenzo-{b,d}pyran-6-one (7)> Gallic acid (6)>Ethyl gallate(4)> PYROGALLOL (1)> Protocatechuic acid (5)> Vanillic acid (3)*

Among the four fractions EEF and BUF showed greatest DPPH scavenging activity. Compound 1.4 and 7 are newly discovered.

The activity difference was likely due to the number of hydroxyls present in the aromatic ring suggested by the structure activity relationship evaluation of these phenolic compounds. Chemical structure is shown below in (Table 8) and (Figure 5).

A link between Alzheimer's disease and an excess presence of oxidant free radicals in the brain causing oxidative stress and related cellular damage is there which in turn causes inflammation in the brain, a consequence of amyloid deposition. Oxidative stress can be decreased by enhancing anti-oxidant enzymes through activation of cytoplasmic transcriptional factor(Nrf2)/ARE-anti oxidant response element, pathway and by dietary and endogenous anti-oxidant chemicals [21,52].

- **Juglans regia as an acetyl cholinesterase inhibitor:**

Alzheimers disease is associated with a decline in cholinergic function in the basal fore brain [21] and cortex and therefore cholinesterase enzyme family has become imperative in neuropathology of AD. Inhibition of cholinesterase, acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) which hydrolyse neurotransmitter acetylcholine and butyrylcholine respectively causes improvement in AD symptom (53,54). Because of the adverse effects of existing ChEs inhibiters, the development of nontoxic ChEs inhibiters as alternative to existing drug is of substantial interest.
Anticholinesterase activity of dichloromethane, acetone, ethyl acetate, methanol and water extract of leaf and fruit of Juglans regia was investigated by spectrophotometric method of Ellman et al against AChE and BChE at 50, 100 and 200µg/ml. The highest BChE inhibition was shown (32.8±1.36% at 200µg/ml). Also the tea infusion of walnut was found to possess 45% of AChE inhibition at 1.36g/l [53].

Table 8: Structure Elucidation for isolated compounds [51]

<table>
<thead>
<tr>
<th>Compound</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
<th>R5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-OH</td>
<td>-OH</td>
<td>-OH</td>
<td>-H</td>
<td>-H</td>
</tr>
<tr>
<td>2</td>
<td>-COOH</td>
<td>-H</td>
<td>-H</td>
<td>-OH</td>
<td>-H</td>
</tr>
<tr>
<td>3</td>
<td>-COOH</td>
<td>-H</td>
<td>-H</td>
<td>-OCH3</td>
<td>-OH</td>
</tr>
<tr>
<td>4</td>
<td>-COOCH2CH3</td>
<td>-H</td>
<td>-OH</td>
<td>-OH</td>
<td>-OH</td>
</tr>
<tr>
<td>5</td>
<td>-COOH</td>
<td>-H</td>
<td>-OH</td>
<td>-OH</td>
<td>-H</td>
</tr>
<tr>
<td>6</td>
<td>-COOH</td>
<td>-H</td>
<td>-OH</td>
<td>-OH</td>
<td>-OH</td>
</tr>
</tbody>
</table>

Figure 5: Chemical structure of compounds 1-7 [51]

CONCLUSION
The pathophysiological process of AD is reviewed; Aβ and NFTs cause extensive damage to CNS, such as cholinergic deficits, inordinate oxidative stress, and inflammatory processes. These processes of AD provide multifarious of targets for therapeutic or preventative agents. BBB transport equilibrium plays determining role for ABP homeostasis in the CNS.
drug therapy for AD should be targeted to achieve neuroprotection using combination of drugs, or novel therapeutic agents, for example cerebrolysin and nanoparticles. Also drug delivery by nano technology will achieve therapeutic success. Herbal drug are less toxic, can readily cross BBB and are bioavailable to exert synergistic effects including improved cognitive and cholinergic functions like Juglans regia(walnut). So the answer to the question asked in the beginning is Yes Juglans regia is one such alternative. Thus herbal drugs appear to be promising alternative medicine in treating AD patients. But most activities of Juglans regia have been seen observed in vitro. Hence further studies are needed on animal models to see their activity so that Juglans regia can emerge as an anti-Alzheimer agent in the near future.

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