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Review Article

Preventive Mechanism, Therapeutic Property, Pharmacokinetics and Benefitsof Asiatic Acid - A Triterpenoid Of *Centella Asiatica* In Alzheimer Disease: An In-Depth Review

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A B S T R A C T

Alzheimer's disease (AD) remains the most prevalent form of age-related dementia worldwide, and it has no cure. Memory loss, difficulty communicating, depression, agitation, mood swings, and psychosis all develop gradually in this disorder. Reduced physical activity, infection, smoking, and the prevalence of diseases like obesity and diabetes all pose a risk for the development of AD. Current synthetic medications only alleviate symptoms by targeting a single molecule, so they can't deal with the complex pathogenesis of AD. The scientific community is actively working to characterise therapeutic agents derived from plants that have shown promise in the literature in treating AD due to their perceived efficacy, safety, and availability. Traditional Chinese medicine includes the use of the plant *Centella asiatica* for its purported benefits to cognitive performance and memory. Pentacyclic triterpenes are largely responsible for its therapeutic and medicinal effects, which include accelerated enhanced memory. These pentacyclic triterpenoids are asiaticoside and madecassoside as saponins and their aglycones, asiatic acids and madecassic acids. Amongst other triterpenoid Asiatic acid has several therapeutic properties against AD like anti-cholinesterase, neuroprotective and anti-inflammatory. This review focused on the pathophysiology of AD followed by a detailed account on Asiatic acid and the research findings to date related to its mechanism of action on AD, advantage over other two terpenoid, BBB permeability, Pharmacokinetic, molecular docking and different therapeutic activities.

Keywords: Asiatic acid, dementia, BBB, Alzheimer disease, triterpenoid, neuroprotective, anti-cholinesterase

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INTRODUCTION

The leading cause of dementia worldwide is Alzheimer's disease (AD), which currently has no cure. After decades of research, estimates suggest that Alzheimer's disease affects 35 million people around the world, it is quite possible that this number projected to rise to roughly 65 million by 2030 and potentially multiplying by 2050 [(1-2)]. The etiological factors of AD include neuroinflammation, neuronal membrane damage, gene mutations, oxidative stress, the formation of toxic molecules, protein misfolding, and dysfunctional mitochondria [(3)]. Several factors, including decreased physical activity, infection, smoking, and the occurrence of diseases such as obesity and diabetes, all pose a threat to AD. Two main culprit free radical accumulation and acetylcholine (ACh) insufficiency in Alzheimer's patients' brain are thought to be a contributing reason to dementia and cognitive issues [(4-5)]. Beta-amyloid plaques, deposits of amyloid protein outside the neurons and the deposition of an aberrant tau protein known as tau tangle inside the neurons are two negative changes in the brain that may also contribute to the development of Alzheimer's disease [(6-7)].

Glutamate an excitatory neurotransmitter if produce excessively for overstimulation of N-methyl-D-aspartate (NMDA) receptors,intracellular free calcium ion concentration raises and activates catabolic enzymes, which results in an intracellular cascade of harmful events trigger AD [8]. Alzheimer disease can be identified through its histological features like neuritic plaque and neurofibrillary tangle (NFT) development in the brain [9].The most common drugs used to treat neurodegenerative illnesses today are donepezil, tacrine, eserine, rivastigmine, huperzine A, and galantamine; however, they all come with their own set of side effects. Meanwhile, herbal remedies have a long history of safe and effective use [10-11]. So public and scientific interests haveshifted to phytopharmaceuticals as an attractive option for new drug development with minimum side effects [12].*Centella asiatica* contains a high content of C30 pentacyclic triterpenoids, also known as centelloides[13].From the ancient era multiple Chinese medicine are used to treat different types of diseases. *Centella asiatica* or gotu kola is one of them which is entensively used in for its multiple therapeutic potential [14]. As per Indian Ayurveda in treating mild and chronic disease it is known as"cure-all herb" for thousands of years [15]. Its name is Asiatic penywort, a member of the family Apiaceae and the subfamily Mackinlayoideae[16]. China, Sri Lanka, India, Indonesia, and Malaysia, as well as other countries like South and Southeast Asia, Africa, are all places where it is found growing wild [17]. The seeds are pumpkin-shaped nutlets 3-5mm in length, and the little green or pinkish-white flora is borne in dense umbels [18]. *Centella asiatica* is classified as a "Medhya Rasayana" or brain tonic in Ayurveda, and it has a variety of effects on the CNS, including nerve stimulatory tonic, memory and learning rejuvenator, sedative, tranquillizer, and intellect enhancing characteristics [19]. *C. asiatica* has been shown to improve cognitive performance, preventing the formation of beta-amyloid and protecting brain tissue from damage by inhibiting acetylcholinesterase activity and decreasing phospholipase A2 (PLA2) activity [20]. *C. asiatica* exerts antianxiety and antidepression action by GABA facilitory action through stimulation of glutamic acid decarboxylase (GAD) enzyme, *C. asiatica* ingestion could boost acetylcholine levels in brains by blocking this enzyme (AChE) which actually improves cognition in AD [21]. The plant is rich in Pentacyclic triterpenes which are mainly documented to have positive effects in enhanced memory or mental clarity. The triterpenes are asiaticoside, madecassoside, asiatic acid, madecassic acid, betulinic acid, isothankunic acid and thankunic acid [22].

Amongst these triterpenes, Asiatic acid will be taken into account for this review. Asiatic acid is chemically an aglycone of ursane-type pentacyclic triterpenoids. It is mainly absorbed from the jejunum and has been shown to be beneficial in neuroprotection in Alzheimer's disease [23].Scientists are more interested in Asiatic acid because it already showed many neuroprotective effects in many researches one of them is protective effect against t-BHP-induced cellular damage and oxidative stress in HepG2 cells. This is achieved by modulating Nrf2 signaling through the activation of Akt and ERK second messengers. Furthermore, it has been observed to protect neuroblastoma B103 cells from amyloid-induced cell death by lowering intracellular free radical levels. In addition, Asiatic acid has shown to prevent spinal cord injury in rats by suppressing oxidative stress and inflammation [24].

In a study conducted by Panupong Puttarak et al it was proposed that Asiatic acid improves learning and memory in passive and active avoidance tests [25]. However, the particular mechanism of action of Asiatic acid in terms of their therapeutic potential is yet unknown [26]. In this review the advantage of asiatic acid over other terpenoids of centella, possible mechanism of action, pharmacokinetics, therapeutic properties, blood brain barrier permeability, molecular docking study are the point which will be discussed.

Pathophysiology

Alzheimer's disease can be precipitated bymultiple factors and physiological dysfunctioning commonly popular as hypothesis likeoxidative stress,astrogilal activation neuroinflammation accumulation of amyloid, Tau hypothesis, metal hypothesis, calcium hypothesis, cholinergic hypothesis.

Amyloid Hypothesis

Amyloid plaques are formed by the accumulation of Aβ fragments, which are polypeptides consisting of 15-20 amino acids. These fragments are located in the external and transmembrane regions of the APP glycoprotein. During the amyloid cascade, Aβ fragments are released outside the cell through enzymatic cleavage of APP. Three main enzymes participate in this breakdown process: α-secretase cuts the Aβ sequence, β-secretase acts on the external site of the APP protein, and γ-secretase works on the transmembrane portion of the same protein to release Aβ[27].Low-density lipoprotein receptor-related protein 1 (LRP-1) transporter is considered the main regulator of Aβ in the brain. LRP1 facilitates the clearance of Aβ from the brain and its transport to the bloodstream [28]. Soluble LRP-1 (sLRP-1) binds to Aβ in the periphery and promotes its elimination through hepatic and renal metabolism [29].The pathogenic processes in AD are primarily caused by A40-42, which is formed when β and γ-secretases cleave the N-terminal end of APP, resulting in the production of APP [30]. The main mechanism of Aβ plaque formation involves the conversion of disordered Aβ monomers into β-sheet structures, leading to the formation of neurotoxic fibrils, which ultimately develop into plaques [31]. The exact mechanism underlying the production of amyloid plaques remains unclear but is believed to depend on the concentration and stability conditions of Aβ. Aβ deposition is a common pathological factor in the initiation of AD [32]. Several proteases, such as PC7, ADAM-10, TACE, and aspartyl protease transmembrane BACE, may also function as β-secretases. The β-secretase complexes, composed of PSEN1 and PSEN2, cleave Aβ and release it into the body [30,33].

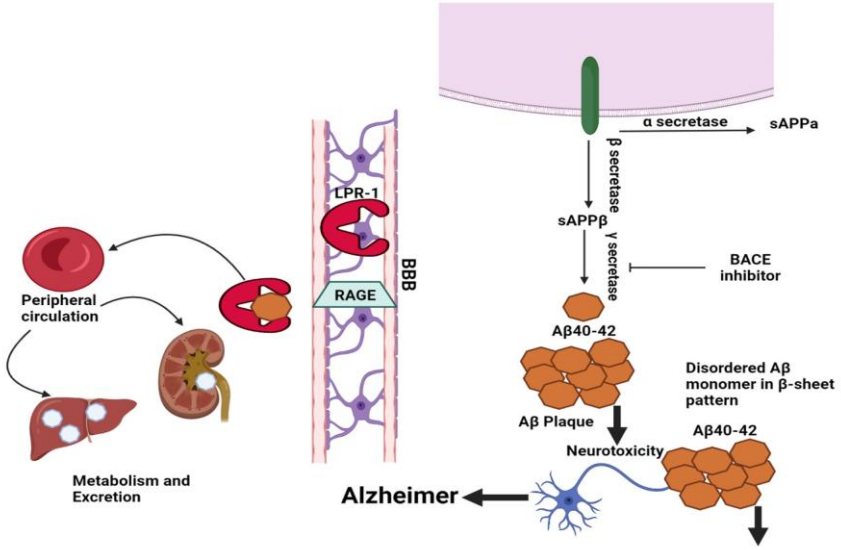

Figure: 1. Role and transportation of Amyloid β in the pathogenesis of Alzheimer Disease:Amyloid β is cleaved off from the precursor protein APP three enzyme respectively α-secretase cuts the Aβ sequence, β-secretase acts on the external site of the APP protein, and γ-secretase works on the transmembrane portion of APPand release Aβ. B and γ secretase produce Aβ40-42.Disordered manner of Aβ40-42 monomers turn into β-sheet structures to form Aβ plaque as neurotoxic element in AD. Soluble LRP-1 (sLRP-1) and RAGE are specific kind of transporter protein transports Aβ in the periphery and promotes its elimination through hepatic and renal metabolism.

Oxidative stress

Previous and current researchers have found a definitive correlation between oxidative stress and Alzheimer's disease [34]. The redox system is disrupted due to abnormalities in the levels of antioxidants and pro-oxidants, leading to biomolecular damage. Polyunsaturated fatty acids, which are present in high amounts in neuronal cells, react with reactive oxygen species (ROS) and undergo molecular apoptosis and lipid peroxidation reactions [35]. Reactive species like ROS and reactive nitrogen species (RNS) are mainly produced inside the mitochondria and release high-energy electrons that can damage every biomolecule of brain cells. Since the brain has a high demand for oxygen and comparatively low levels of antioxidants, it becomes more susceptible to oxidative stress. High oxidative stress leads to complications such as the formation of neurofibrillary tangles (NFTs), increasing the risk of AD [36].

Agarwal *et al*. have found that an excess of ROS can damage biomolecules like DNA, proteins, lipids, and fatty acids, and produce RNS and peroxynitrite (ONOO-) by regulating the expression of inducible nitric oxide synthase (iNOS) [37]. Multhaup *et al*. concluded that an increase in ROS further affects the activity of amyloid precursor protein (APP), the production of Aβ oligomers, senile plaques, hyperphosphorylated tau protein, and numerous additional neuroinflammatory pathways such as NF-κB/TNF-α, GSK-3, and WNT [38]. Elevated levels of reactive oxygen and nitrogen species (ROS, RNS, and ONOO-) highly connected to disorganised synaptic homeostasis and neurodegeneration resulted in AD [39].Tyagi *et al*. concluded that increased ROS is capable of causing lipid peroxidation anda byproduct, malondialdehyde (MDA) has been linked to neuroinflammation, neuronal apoptosis, and neurotransmitter dysfunction [40].

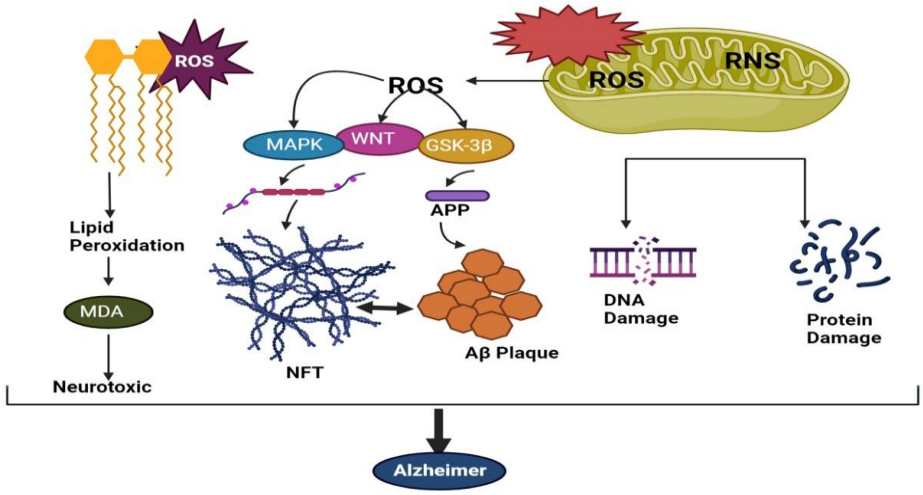

Figure: 2. Oxidative stress induced reactive species and mitochondrial dysfunction causing Alzheimer Disease.

Imbalance in antioxidant system produce ROS and (RNS) inside the mitochondria and release high-energy electrons that can damage every biomolecule of brain cells. Increase ROS induced lipid peroxidation make MDA leading to neurotoxicity.ROS further affects the activity of amyloid precursor protein (APP), the production of Aβ oligomers, hyperphosphorylated tau protein, through altered neuroinflammatory pathways such as NF-κB/TNF-α, GSK-3, and WNT.

Astroglia

Astroglia plays a crucial role in progression of Alzheimer by different inflammatory marker and certain genes. Genes, including Fermitin family member 2, Sortilin-related receptor 1, and ApoJ, expressed in glial cells helps them pathogenesis of AD [41]. Reactive astrocytes, which are found beside amyloid plaques, produce abnormal intracellular calcium signaling leading to abnormal homeostasis in brain.[42]. Activated microglia secrete different inflammatory markers, including interleukin IL-1α, tumor necrosis factor (TNFα), and complement competent 1q (C1q), which together induce the neurotoxic phenotype A1. Research concludes that A1 astrocytes in a mouse model increase the release rate of an unknown neurotoxin, causing neuronal death and decreasing neuronal survival and synapse formation[43]. The ApoE gene plays a role in the production of amyloid plaque formation, and this gene is expressed by astrocytes in the brain [44]. Expressed ApoE4 increases blood-brain barrier permeability by activating cyclophilin A-NF-kappaB metalloproteinase 9 pathways in pericytes, which, in turn, damages pericytes and the BBB. This breakdown in the BBB causes neurodegeneration and triggers AD [45]. A research study proposed that iPSC human glial cells obtained with the ApoE4 gene showed abnormal amyloid beta uptake and clearance, cholesterol deposition, and excessive acidification of endosomes, all of which damage neuronal cells [46]. Amyloid-β itself can activate the NFκB pathway in astroglia, resulting in the release of C3, which damages the dendritic structure in neurons through receptor binding and changes the phagocytosis of amyloid β in favor of AD pathogenesis [47].

Neuro inflammation

Along with Aβ and NFT, inflammation also contributes to the pathogenesis of AD. Wang *et al.* mentioned that there are notable differences between neuroinflammation and peripheral inflammation in terms of tumor, dolor, rubor and calor [48]. Inflammation is necessary for the healing process and normally goes away on its own; however, chronic inflammation results from ongoing production of cytotoxic substances and lengthy inflammation, which have adverse consequences on brain processes [49].Roughly 35% of the brain's cells are astrocytes, which are specialized glial cells [50].Under normal circumstances microglia are neuroprotective and play a key role in phagocytosis, and release neurotrophins to maintain a healthy brain environment [51].When microglia become activated, inflammatory cytokines such as interleukin-1 (IL-1), interleukin-1 (IL-1), tumor necrosis factor (TNF-α), or reactive oxygen and nitrogen species are produced and released, resulting in a pro-inflammatory response. IL-1 enhances the abnormal processing of amyloid precursor protein (APP) and generates Aβ. There is an increased concentration of IL-1 in the brain of AD patients [52].Verkhratsky *et al*. also described that in Alzheimer's disease, activated astrocytes release proinflammatory cytokines like TNF-α, IL-6, IL-1, and TGF-β, which, in turn create decrease in ATP, and an increase in intracellular Ca2+, all of which contribute to neuroinflammation and worsening of cognitive function [53]. Additionally, when microglia are activated, other signaling pathways, such as the PI3K/Akt pathway, which controls apoptosis and inflammatory responses, are also induced [54].Microglia also gets activated in response to CNS stressors, such as neuronal damage or infection, and release pro-inflammatory factors (M1 phenotype) or anti-inflammatory molecules (M2 phenotype) [55]. In the case of AD, microglia respond to PAMPs (pathogen-associated molecular patterns) or DAMPs (danger-associated molecular patterns) by adopting an M1 phenotype, which exacerbates inflammation and speeds up the course of the illness [56].Oligodendrocytes' primary role is to generate myelin sheaths around nerve fibers, which protect and insulate the axons. indeed there is selective loss of oligodendrocytes and a reduction in myelin proteins surrounding Aβ plaques. It was recently discovered by Tsai et al. that oligodendrocytes are substantially damaged in AD and make neurons prone to inflammation [57].Large amounts of mitochondrial DNA (mtDNA) are released into the cytosol from dysfunctional mitochondria that are not eliminated by mitophagy. This mtDNA, along with other ROS metabolites like ATP, fatty acids, succinate, peroxidized lipids, advanced glycation end-products, altered N-glycans, and HMGB1, are recognized as DAMPs and provoke an innate immune inflammatory response [58].

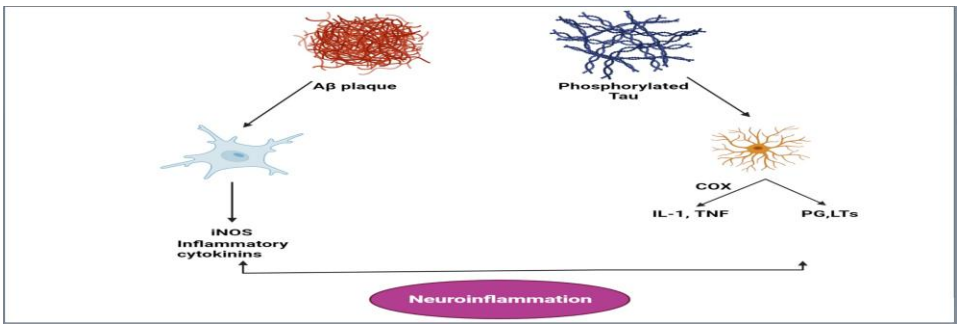

Figure: 3. The role of astroglia and neuroinflammation in Alzheimer Disease.Microglia get activated in response to CNS stressors like Aβ and phosphorylated Tau and release inflammatory cytokines such as interleukin-1 (IL-1), interleukin-1 (IL-1), tumor necrosis factor (TNF-α), or reactive oxygen and nitrogen species resulting in neuroinflammation.

Cholinergic hypothesis

The clinical symptoms of AD indicate that impaired cholinergic neurotransmission is a major pathophysiological cause of AD. Acetylcholine is a neurotransmitter associated with learning and memory function [59]. The cholinergic hypothesis is based on three pillars: one, the consumption of presynaptic cholinergic biomarkers in the cerebral cortex; two, the neurodegeneration of the NBM (nucleus basalis of Meynert), which is the main source of cholinergic innervation; and three, cholinergic antagonists causing memory disturbances while agonists are favorable medicines [60]. Lower levels of choline acetyltransferase have been found in the hippocampus and frontal cortex of AD patients [61]. Cholinesterase inhibitors and donepezil are used to restore lower acetylcholine levels and alleviate the symptoms of AD [62].

Tau Hypothesis

Tau is a protein found within the microtubule assembly. It is a major contributor to the formation of neurofibrillary tangles (NFTs), which result in neuronal structural loss and dysfunction [63-64]. Phosphorylation of the tau protein leads to its dissociation from the microtubule arrangement, creating NFTs that obstruct axonal flow within neurons [65]. These structural changes result in defects in phosphatases and protein kinase enzymes, contributing to abnormal signaling cascades [66]. Tau-focused Alzheimer's disease (AD) research is gaining popularity as it holds the potential to yield promising results in AD. Kametani et al. found that NFTs, observed in people with frontotemporal dementia and other tauopathies, but cannot be detected in healthy individuals, while Aβ can be found in healthy individuals without neurodegeneration [67]. Alasmari *et al*. identified molecules such as protein kinase A, cyclin-dependent kinase 5 (Cdk5), GSK-3, p38 MAPKs, NF-kB, and ROS as contributors to the development of hyperphosphorylated tau protein and neurofibrillary tangles [68].

Metal Hypothesis

Metal dyshomeostasis is involved in the progression and pathogenesis of neurodegenerative diseases. According to Weekly *et al*., the ionosphere and metal chelators are well-known modulators of transition metal homeostasis, and a number of these molecules are used in clinical trials. Metal-binding compounds are not the only drugs but are capable of targeting transition metal homeostasis [69]. The mammalian brain has larger concentrations of Cu, Zn, Fe, Mn, and Cr ions than other tissues, suggesting that the brain uses these metal ions more frequently in various metabolic activities [70]. In 1994, it was first suggested that when Aβ reacts with Zn2+ and Cu2+, it forms amyloids. This interaction led to the invention of the "metals hypothesis of AD" [71]. Aβ is a metalloprotein and the presence of Cu2+, Zn2+, and perhaps Fe3+ in the brain increases Aβ's toxicity by producing free radicals and H2O2. Zn2+ causes Aβ to combine and form oligomers rapidly. At pH 6.8-7.0, both Cu2+ and Fe3+ significantly tempt Aβ accumulation [72-73].Tabner *et al*. concluded that Aβ reacts with metals, creating oligomers and fibrils, but also decreases Cu2+ and Fe3+ and produces H2O2 through the sharing of electrons between these metals and oxygen atoms. This oxidation is facilitated by Aβ's methionine35 and tyrosine10, which could potentially help in the production of hazardous soluble Aβ-oligomers by causing dityrosine formation and covalent crosslinking of Aβ [74-75]. Farina *et al*. suggested that every kind of CNS cell contains iron, and due to iron's two oxidation states, it is linked to the production of reactive oxygen species (ROS), which can lead to oxidative stress and ultimately neuronal death [76].Graham et al. reported that more pro-inflammatory cytokines and free radicals are released when active microglia contain too much iron. Another fact is Copper levels gradually decline with aging and low Cu contents in the hippocampus and amygdala in AD patients's compared to controls, indicate a loss of brain mass [77]. White *et al*. found that transgenic APP mice Tg2576 and APP23 (animal models for AD) also have less copper in their brains, while APP- and APLP2-knockout mice have more Cu in their cerebral cortex [78].

Calcium Dysfunction

It is widely acknowledged that calcium is a crucial second messenger that controls the normal homeostasis of various brain cells [79]. Thus, as seen in AD, any change in the level of Ca2+ has a significant impact on the normal functioning of brain cells [80].Benarroch *et al.* reported that multiple signaling and metabolic processes, including neuronal growth, long-term potentiation, synaptic plasticity, exocytosis, energy metabolism, and enzyme activation/deactivation, have been linked to Ca2+ [81]. Schampel A, reported that voltage-gated calcium channels control the appropriate level of calcium in neurons under normal circumstances, and the ER serves as a storehouse for calcium inside the cytoplasm [82].According to Stutzmann, G.E *et al*., Ca2+ is released from the ER via RyR and IP3R receptors and the SERCA pump, which is then involved in a number of cellular processes [83]. Misquitta concluded that RYR and IP3R receptors and SERCA pump control how much Ca2+ is stored and released. Therefore, any homeostasis can be changed by things like stress, trauma, or poisonous stimuli, which leads to the progression of AD pathology and cognitive dysfunction [84].Popugaeva *et al*. reported that increased levels of Ca2+ and calcineurin trigger the activation of several enzymes, alter LTP, and impair synaptic plasticity, which impairs memory [85]. Ferreiro *et al.* reported that Aβ production is stimulated by both increasing calcium overload and APP modulation, and Aβ itself induces calcium overload through modulating IP3R [86]. Castillo *et al*. discussed that too much calcium turns on the NMDA receptor, which leads to hyperexcitation, decreases LTP, and activates pro-apoptotic proteins and inflammatory cytokines [87].

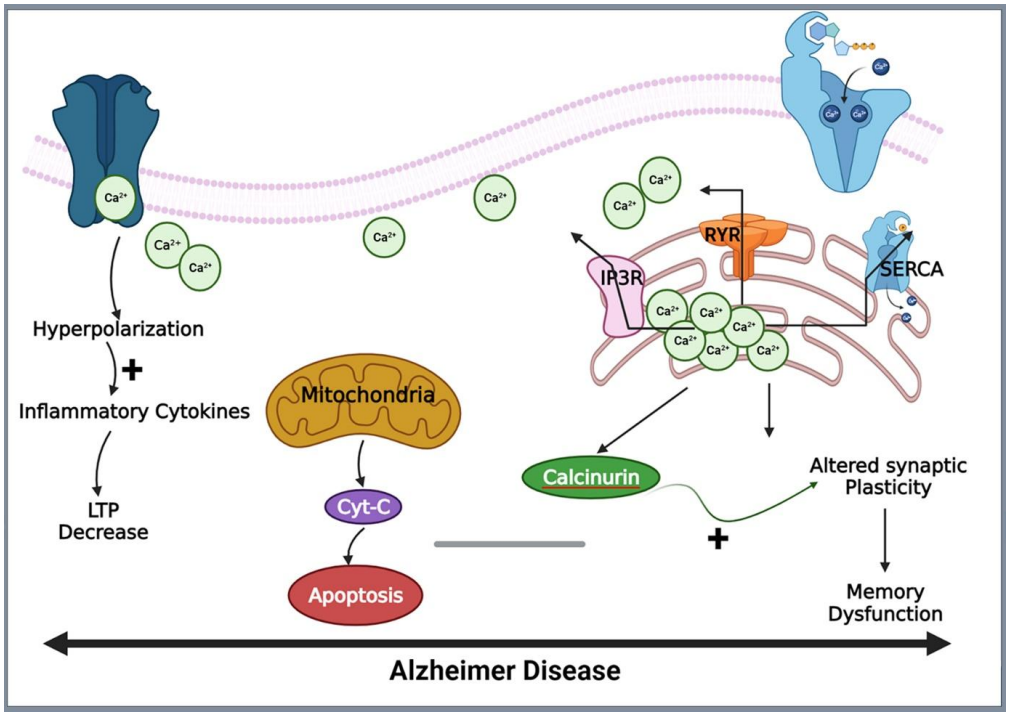

Figure: 4. Role of calcium dysfunction in the pathogenesis of Alzheimer Disease

Ca2+ is released from the ER via RyR and IP3R receptors and the SERCA pump, increased levels of Ca2+ and calcineurin trigger the activation of several enzymes, alter LTP, and impair synaptic plasticity and Aβ production through modulating IP3R. ROS mediated mitochondrial dysfunction create ER stress which also increase Ca2+.

Molecular mechanism of Asiatic acid in neuro-protection

Exact molecular mechanism of Asiatic acid is not fully understood, various studies have proposed different mechanisms of action based on the observed effects in cellular and animal models. Ji-Hyun Park et al. demonstrated thatmethamphetamine(METH)-induced cleavage of PARP was decreased through the inhibition of caspase-3 cleavage by AA[88].Mashoque Ahmad Rather *et al.* reported in their research that Asiatic acid can attenuate Al-triggered cell apoptosis by decreasing oxidative stress, mitochondrial malfunction, and modulating Aβ level, AChE level, and inflammation in an animal model of AD[89].Dhanasekaran M *et al.* proposed that the ethanolic extract of *Centella asiatica* mixture, which mainly contains asiatic acid and asiaticoside, have been shown to reduce deposition of Aβ in the hippocampus and improve mouse behavioural symptoms in an AD transgenic mouse model [90].M.H. Veerendra Kumar documented in a study that extract of *Centella asiatica* is used in the treatment of Alzheimer's to improve memory performance [91].Chien-Li Chen *et al*. concluded that *Centella asiatica* extract protects against neurotoxicity from Aβ1-40 by reducing ROS levels, which is possible through the activation of the antioxidant enzyme system [92]. CAE also has the ability to directly reduce ROS or prevent Aβ accumulation, specifically Aβ1-42 and Aβ1-40, as evidenced in transgenic mice [93].Loganathan *et al*. marked Asiatic acid as a cognition improver against quinolic acid-induced stress by reducing oxidative stress in a rat model [94].Amyloid-β is synthesized from the amyloid beta precursor protein (AβPP) by a rate-limiting enzyme, BACE1. Two other enzymes play a role in the degradation of amyloid-β. According to Patil *et al*., Asiatic acid increases IDE and NEP enzyme activity and decreases BACE1 enzyme activity to exert control over Aβ formation [95]. Dose-dependent treatment of Asiatic acid has been proven beneficial in ceramide-induced Alzheimer's with less mitochondrial damage [96].In a research conducted on a cholinergic neuroblastoma cell line, 36 derivatives of Asiatic acid showed enhancement in cognition by accelerating choline acetyltransferase activity [97].Asiatic acid has a rotatable bond and a free aliphatic side chain, making it a favorable structure. Asiatic acid binds to the hydrophobic pocket of the active site of acetylcholinesterase and offers an inhibitory role [98].In a research on the pathogenesis of Alzheimer's disease the GSK3β and PI3K/Akt signaling pathway is reported as an important component. The main culprit of AD, the Tau protein, is phosphorylated by the GSK3β enzyme, which remains in an inactivated state due to PI3K/Akt signaling. High levels of PI3K activation lead to inhibition in the storage of phosphorylated tau protein in neurons. Cheng et al. concluded that Asiatic acid increases the activation of PI3K, thus preventing Tau protein induced cell damage [99].M.N. Nasira et al. investigated that Asiatic acid competes with acetylcholine at the esteric site of AChE to inhibit this enzyme. AChE has two sites, one anionic site and one esteric site. In order to inactivate AChE, only one site of the AChE gorge must be inhibited. Asiatic acid also reveals an inhibitory effect on specific GABAB receptors, causing neuronal cells' potassium ions to exit the cell, resulting in neuronal cell hyperpolarization and a decrease in EPSP as a neuroprotective mechanism[100]. Cholinergic and GABAergic pathways are interconnected in the hippocampus and exert a combined effect on memory and cognition. Overall Asiatic acid can be a good compound for treating dementia in Alzheimer's disease [101].

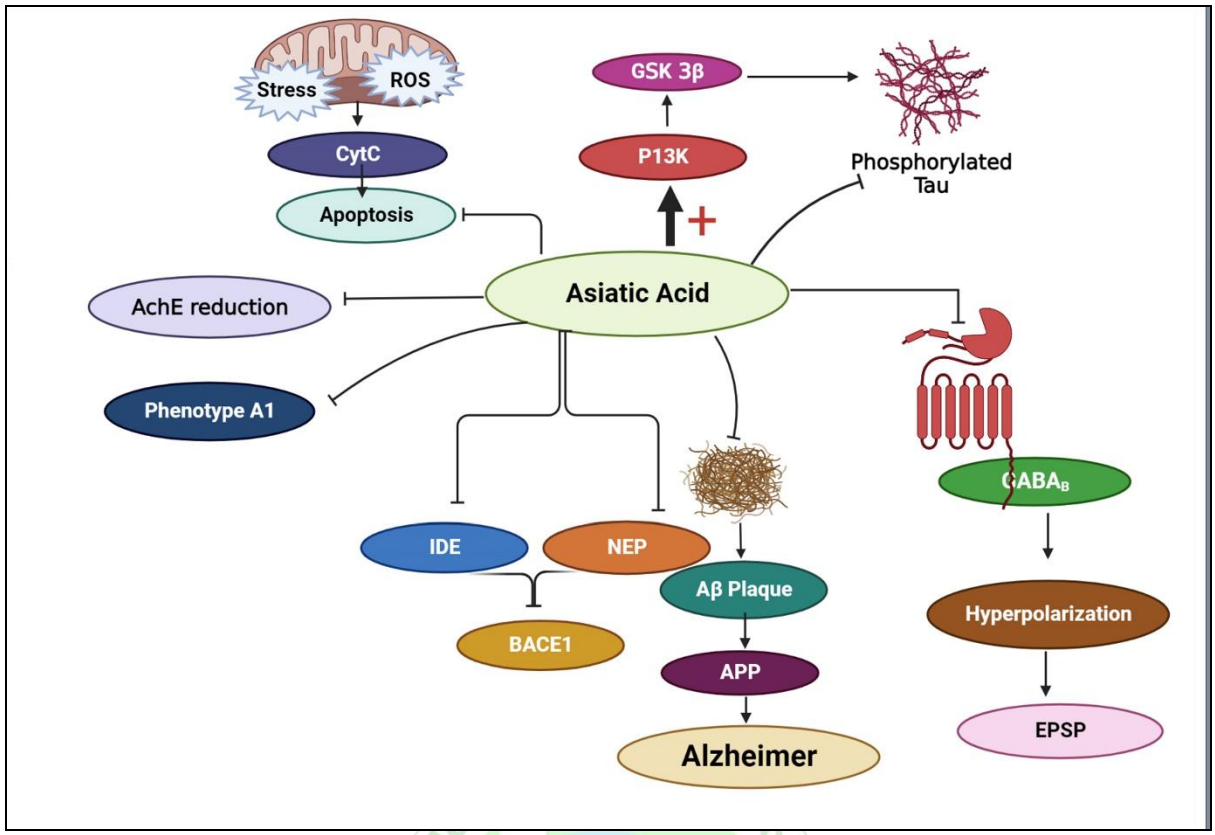

Figure: 5. Mechanism of action of Asiatic acid in treatment of Alzhiemer Disease

Asiatic acid can attenuate neuronal cell apoptosis by decreasing oxidative stress, mitochondrial malfunction, modulating Aβ level, AChE level, and inflammation andimprove behavioural symptoms in AD.Asiatic acid increases IDE and NEP enzyme activity which degrade Aβ and decreases BACE1 enzyme activity to exert control over Aβ formation.Asiatic acid increases the activation of PI3K, thus preventing Tau protein induced cell damage.Asiatic acid also reveals an inhibitory effect on specific GABAB receptors, potassium ions to exit the cell, resulting cellular hyperpolarization and decrease in EPSP, a neuroprotective mechanism. AA also reduced cytochrome c mediated cell apoptosis through mitochondria as an effect of high level of ROS produced inside it.

Antioxidant activity

Centella asiatica exerts its antioxidant potential through three main distinct events, namely inhibition of linoleic acid peroxidation, superoxide scavenging, and radical removal [102]. Methanolic leaf extractions of *Centella asiatica* serve as an enormous natural source of antioxidants [103]. Research has shown extremely satisfying results when *Centella asiatica* extracts were used in a variety of free radical reactions in in vitro experiments [104]. Asiatic acid, a pentacyclic triterpene found in *C. asiatica*, restores oxidative stress parameters to normal in MSG (Monosodium glutamate)-induced rat brains [105]. In a mouse model, the most famous pentacyclic terpenoid, Asiatic acid, derived from *Centella asiatica*, has been reported to protect mitochondria in cerebral ischemia [106]. Min-fang XU *et al*., in an in vitro research study, reported that Asiatic acid provides relief from glu-induced neurotoxicity by reducing oxidative stress [107]. Earlier studies have confirmed that Asiatic acid arrests oxidative stress by activating numerous substances such as superoxide dismutase, glutathione peroxidase, nuclear erythroyed 2-related factor, and provides a cognition-enhancing effect [108].When administered orally at a dosage of 300 mg/kg body weight for 60 days, *C. asiatica* significantly reduced regional lipid peroxidation (LPO) in the brain [109]. According to mato*et al*., aqueous extract exhibits physical stabilitydue to its antioxidant activity [110].

Anticholinesterase activity

The enzyme AChE belongs to the family of cholinesterases that are special carboxyl ester hydrolases. Following activation of postsynaptic ACh receptors, ACh is hydrolyzed by AChE to yield choline and acetate [111].

According to the cholinergic hypothesis, the hydrolysis of ACh by AChE causes a decrease in ACh level in AD brains, making AChE the target enzyme for treatment of AD[112]. *C. asiatica's* anticholinesterase action makes it a natural anti-herb. A study found that hydroalcoholic extracts of *Centella asiatica* had an effect on cholinesterase retardation after 48 hours [113]. Mashoque Ahmad Rather *et al* in a research detected that Alcl3 induced raised level of AChE enzyme in hippocampus reduced after treatment with Asiatic acid of *C. asiatica* plant [114]. According to ZettyZulikha Hafiz *et al*. raw extract of *C. asiatica* containing triterpenes like Asiatic acid was resulted in reduction of AChE in neuroblastoma cell line SH SY5Y of human after exposure for 24 hr[115]. The ethylacetate extract of date vinegar combined with 2 percent *Centella asiatica* extract inhibits the enzyme AChE and provide smooth cholinergic neurotransmission in AD [116]. An in-vitro study was carried out in which commonly used six traditional herbs were tested for anti-AChE activity.

This study revealed that at 100-150µg/mL concentration, the hydroalcoholic extract of CA has shown a 50% inhibition of AChE compared to standard physostigmine's IC50 value of 0.076±0.0042 µg/ml [117]. In an in-vitro study on six selected Malaysian plant extracts for AChE inhibitory potential, *C. asiatica* extracts from leaves and roots were tested and consequently, the leaf extract showed high AChE activity than root [118]. According to Mathew and Subramanian *et al.* in a comparative study methanolic extract of traditionally used *C. asiatica* reveals positive result in inhibition of cholinesterase amongst twenty different medicinal plants [119]. Nasir *et al* reported that Asiatic acid of *C. asiatica* exhibits anticholinergic effect at 125 ng compared to physostigmine at 1 ng and galanthamine at 10ng [120]. According to Boopathy *et al*. Asiatic acid is a potent AchE inhibitor without any toxic effect when it is studied in hippocampal cell lines [121].

Neuroprotective Effect

The neuroprotective mechanism of Asiatic acid has not been properly understood. Asiatic acid exerts neuroactive properties by reducing the levels of various chemicals such as rotenone, H_2O_2, and glutamate [122]. It has been reported that Asiatic is effective against methamphetamine neurotoxicity by inhibiting mitochondrial apoptosis and signaling pathways, such as NF-kβ/STAT3/ERK [123]. Ahmad *et al.* found that Asiatic acid offered neuroprotection in animal models by modulating factors such as AchE activity, Aβ levels, and the burden of aluminum[124]. Sun *et al.* demonstrated that Asiatic acid provided protection from C2 ceramide toxicity in cortical neurons of rats [125]. Additionally, Umka Welbat *et al.* and Xu *et al.* showed that Asiatic acid improved cognition in animal models induced by valproic acid [126].In their research, Sirichaot*et al.* reported that Asiatic enhanced learning and memory by elevating the levels of Notch1 protein, which increases cell proliferation [127]. Jiang *et al.* proposed that Asiatic acid provided neuroprotection in spinal injuries by increasing Nrf2 and its target genes while reducing reactive oxygen species [128]. It has been documented that Asiatic acid helps maintain the stability of the blood-brain barrier and promotes mitochondrial function. AA is primarily known as an inhibitor of AchE activity, excitatory postsynaptic potential (EPSP), and locomotor activity [129]. It has also been reported as an effective therapeutic regimen in certain types of dementia [130]. In a study conducted by Gadahad *et al*., it was found that administration of AA for an extended period enhanced cell proliferation in a specific region of the hippocampus known as the sub granular zone [131].According to Patil Maki, it inhibits BACE1, the rate-limiting enzyme in the amyloid β synthesis pathway, by increasing ADAM10, which is responsible for the production of non-amyloidogenic APP [132].

Pharmacokinetic Properties

It is difficult to conduct ADME studies on the therapeutically active compounds of certain botanicals yet the metabolism of Asiatic acid was first studied on a rat model in 1971 by Chassud *et al.*[133]. Based on a research Xia *et al.*mentioned about ten Asiatic acid metabolites resulting from phase I metabolism reactions, such as hydroxylation, dehyoxylation, and dehydrogenation [134-135]. Another researcher discovered asiatic acid glucuronide, which constituted about 36 % of the bile content after oral administration of a radiolabeled asiatic acid combination, using a Desagaradiochromatogram[136]. Earlier reports indicated that significant amounts of asiatic acid were found in feces after oral administration of a standardized mixture of *C. asiatica*, suggesting that intestinal esterase or gut microflora hydrolyzed the sugar moiety of the parent glycosidic compound. Furthermore, triterpenoids of ECa 233 were converted into triterpenic acids, which were excreted via faeces[137]. Lingling Gu reported that solid lipid nanoparticles (AASLN) of a salt composition of Asiatic acid had a particle size of 200-300mm. The nanoparticles were prepared using the solvent injection method, and Glycerin monostearate (GMS) and P188 were used as the solid matrix and surfactant, respectively. An increase in particle size was observed with higher amounts of solid matrix and surfactant concentration, and high lipid content cause them to unite and form a single mass [138]. According to Yong Fang Yuan *et al.*, asiatic acid is rapidly absorbed when tested orally on Sprague-Dawley rats, and faster metabolism was observed based on the short half-life of 0.348 hrs only. They also reported that Asiatic acid is transported to biological membranes through passive transport, which may affect absorption. Additionally, it was found to be non-cytotoxic up to a concentration of 2µM, as the Caco-2 cells did not die at that concentration in a research study. The rat intestinal perfusion model was used to measure the intestinal absorption of Asiatic acid [139].Ling Guo *et al.* reported that the half life of Asiatic acid decreased and clearance increased when co-administered with glycyrrhizin. They also found that glycyrrhizin accelerated the efflux of Asiatic acid by activating the P-gp transporter. Furthermore, the metabolism of Asiatic acid was reported to increase due to CYP450 enzyme induction by the same drug [140]. PhanitSongvut*et al.* documented Asiatic acid as a metabolite of madecassoside and asiaticoside, two other components of ECa 233 capsule [141]. Another study explained that asiaticoside, an unabsorbed glycoside of C. asiatica, is hydrolyzed to Asiatic acid by intestinal bacteria capable of breaking down aglycones, most likely a species of Eubacterium called Eubacterium spp. [142]. According to Rush *et al.*, human plasma concentrations of asiatic acid were found to be similar after 12 hours of treatment with equimolar doses of asiaticoside and asiatic acid given orally. Kirsten M. Wright *et al.* proposed that asiatic acids are abundantly found in human plasma even after glycoside administration alone, suggesting better absorption in humans [143].According to a study, the concentration of asiatic acid reached in the brain is sufficient to elicit neuroprotection, suggesting that asiatic acid may cross the blood-brain barrier. Chronic oral treatment of asiatic acid has been reported to increase $t_{1/2}$, Cmax, and AUC in a randomized crossover study [144]. Yongfang Yuan *et al.* reported poor Cmax (394.2 ng/ml) and a smaller $t_{1/2}$ for asiatic acid when administered via the intra-gastric and i.v. routes to Sprague-Dawley rats. The absorption was significantly higher in the jejunum compared to other segments of the intestine [145].Most research on Asiatic acid detection in plasma employs high-performance liquid chromatography with ultraviolet detection (HPLC-UV) [146]. According to a study, a selective and repeatable HPLC/ESI-MS/MS method was

developed to measure the amount of AA in rat plasma using colchicine as an internal standard. The proposed method has also been tested according to US Food and Drug Administration rules and used in a pharmacokinetic study with female albino wistar rats[147].

MolecularDocking study of Asiatic acid

Computer-aided drug design (CADD) makes use of molecular docking to investigate the binding relationship between ligands (potential inhibitors) and enzyme targets [148].

Computer-aided drug discovery techniques have recently been used in pharmaceutical research to identify potential drug candidates, their binding sites, and develop drugs that fit better in the case of different brain diseases research [149]. To do structure-based drug discovery and structural molecular biology, in silico molecular docking technology is most popular [150]. Additionally, the goal of docking ligand-protein is to forecast molecular recognition, binding patterns, and binding affinity (kcal/mol) [151].

Dang Chan Kim *et al*. studied the anti-inflammatory effect of asiatic acid through iNOS-mediated inflammatory nitric oxide generation in microglia using in silico molecular docking techniques and BV2 microglia cell-based assay system. According to this research, asiatic acid efficiently influences TLR-4 and iNOS cell signaling, leading to a significant, concentration-dependent decrease in LPS-induced NO generation. It also bound to seven different amino acid residues in the iNOS target protein, while tetrahydrobiopterin only bound to six [152].

Nor Atiqah Jusril *et al.* conducted in vitro studies which showed that asiatic acid has the highest inhibitory activity with the lowest binding energies—10.27 kcal/mol but is still less effective than eserine against AChE. The docking results obtained for asiatic acid suggest that the present hydroxyl group at the C-1, C-2, and C-3 positions theoretically improves the AChE inhibition of the 4EY7 enzyme. With regard to hydrogen bonding, asiatic acid demonstrated three potent interactions with the residues His447 (1.24), Tyr337 (3.72), and Arg296 (1.97). According to carbon-hydrogen bonding between Trp286 and Gly121, the molecules eserine and asiatic acid exhibit a similar bonding relationship [153].

The crucial residues of AChE are Tyr337 and Trp86 because they maintain the structure of the binding gorge and maintain electrostatic equilibrium [154].

Overall, the molecular interactions shown in the docking analysis suggest that asiatic acid in *Centella asiatica* may be represented as a potential inhibitor based on its very good interaction due to strong hydrogen bonds and hydrophobic interactions.

Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor is a non-NMDA type ionotropic glutamate receptor for fast transmission, but its mutant version causes Alzheimer's disease and other central nervous system illnesses [155].

Preenon Bagchi concluded that when the structure of AMPAR is reconstructed in 3D and screened against asiatic acid from *Centella asiatica* it proves to be a promising ligand for the AMPA receptor [156].

Permeability of Blood Brain Barrier and Asiatic acid

The blood-brain barrier is composed of endothelial cells that lined cerebral microvessels (BBB), together, the cells form a tight junction that completely blocks off the paracellular pathway .Therapeutics targeting the central nervous system (CNS) that are ineffective because they cannot cross the blood-brain barrier (BBB).There is a lack of information, however, on how deeply these compounds can penetrate the brain[157]. The BBB acts as a combinedphysical, enzymatic barrier to the flow of molecules between the circulatory system and the central nervous system [158]. When the BBB is broken, molecules from the blood that could be injurious to neurons can get into the brain. Alzheimer's disease and other neurodegenerative diseases may result from this [159]. Liew KF concluded that Asiatic acid had Papp value of50.94 ± 10.91 cm/s found in PBEC model to measure BBB permeation [160].

In order to increase the bioavailability, numerous innovative delivery methods have been investigated. These include solid lipid nanoparticles poly D, L-lactic-co-glycolic acid (PLGA) based NLCsand surface conjugation of albumin nanoparticles with glutathione[161]. The NMDA and AMPA receptors located in the BBB act as endogenous ligands for glutathione, it promotes asiatic acid permeability in the brain and increases the passage of asiatic acid to the brain[162]. Tripty Halder *et al.*discovered that AA loaded nanostructured lipid carriers (AAN) protect amyloid $Beta_{1-42}$ induced cognitive impairment and oxidative stress in SHSY5Y Cells [163]. According to a bio-distribution study by NisithRaval*et al* asiatic acid loaded with bovine serum albumin (BSA) nanoparticles(NPs) in conjugation with glutathione were 1.3 times more bioavailable than asiatic acid solution and ten times more bioavailable than unconjugated asiatic acid-loaded NPs. This outcomehelps in binding to the NMDA receptor at the glycine and glutamate site, which is then followed by clathrin-mediated endocytosis of the conjugated NPs-NMDA receptor complex [164].

As a result of pre-incubating procrine brain endothelial cells (PBECs) with 20 mg/mL of asiatic acid for 1 hour, Nur Aziah Hanapi found that the cells were greatly protected from oxidative damage caused by H_2O_2 and shown remarkable BBB penetration.Nur AziahHanapi*et al*. reported that Asiatic acid has higher apparent permeability values than donepezil [165].

Advantage over other terpenoids

Meeran MFN concluded that Asiatic acid has a high lipophilicity value, a low ionic strength, and a high hydrogen bond donor (HBD) and acceptor (HBA) value (log P) of 5.7, and acceptable passive permeability are all within the allowable range according to Lipinski's rule of five. In contrast to asiatic acid, the structures of other two glycosides asiaticoside and madecassoside of Centella asiatica do not follow Lipinski's rule of five [166]. Its high apparent permeability (Papp) value, andan equivalent

threshold of 90 A2 for topological polar surface area (TPSA) favours possible passive BBB permeability. However, asiaticoside and madecassoside fall beyond the acceptable physicochemical data range[167] M.N. Nasira*et al.* came to the conclusion that asiaticoside, another triterpenoid, needs to be taken in high doses over a long period of time to work as a cognition improver. In contrast, aqueous extracts of asiatic acid, which is a GABAB agonist and an AchE inhibitor, may help improve learning and memory in the short term [168].

Asiatic acid and its derivatives stop glutamate from causing excitotoxicity in neurons grown in a lab dish [169]. Notably, asiatic acid led to a higher peak plasma concentration earlier than asiaticoside did. Although asiaticoside takes longer to be absorbed because it must be hydrolyzed in vivo by intestinal enzymes, the plasma profile for asiatic acid showed a more typical "sawtooth pattern," indicating more availability [170]. Besides many advantages,oral bioavailability of AA is only 16.25% , it doesn't dissolve well in water (0.1583 mg/mL in saturated saline) and is broken down quickly by the liver ($t_{1/2}$ was 9.493 min) but AA's oral bioavailability was improved by adding hydroxypropyl—cyclodextrin and making solid lipid nanoparticles (SLN) [171]. Poovizhi T *et al.* demonstrated by both preclinical and clinical pharmacokinetic evidence, asiatic acid is expected to be distributed systemically via albumin binding and Madecassic acids can be quickly taken orally, whereas asiatic acid is absorbed immediately from jeunum. Although asiatic acid has low bioavailability, its derivatives have shown promise as a medicinal treatment for a variety of conditions [172]. Having advantages over other two terpenoid Scientists are paying more attention to asiatic acid because of its potential as a medicine. For example, it can protect rats from spinal cord injuries by reducing oxidative stress and inflammation, and in HepG2 cells, it protects against cellular damage and oxidative stress caused by t-BHP by activating Akt and ERK signals [173]. In addition to its anti-cancer properties, asiatic acid has been revealed to shield neuroblastoma B103 cells from Aβ-induced cell death by lowering concentration.Intracellular free radical caused by H_2O_2, or death of cell[174]. There was evidence that asiatic acid could boost brain BDNF levels [175].

CONCLUSION

Asiatic acid, asiaticoside, madecassoside, and caffeoylquinic acids have all been demonstrated to contribute to the herb's neurological effects to date. A great natural candidate for treating neurodegenerative diseases, depending on its advantages over other compounds lie AA is. Through its various pharmacological properties, AA could reduce Al-induced cognitive impairment, cholinergic deficits, A burden, oxidative stress, Tau pathology, inflammation, and apoptosis.The administration of A-A is efficient in improving learning and memory for acute PA and active avoidance in learning.The cognitive enhancing properties of A-A call for additional research on its chronic study and mechanism of action in larger groups of animals.. The use of AA in molecular biology has increased due to its pharmacological properties, low toxicity, and commercial availability. To establish AA (analogue and its derivatives) as an ideal therapeutic agent in a variety of diseases, more thorough studies are needed to clarify the molecular mechanisms and pharmacological effects of AA.

REFERENCES

1. Singh AK, Rai SN, Maurya A, Mishra G, Awasthi R, Shakya A. Therapeutic Potential of Phytoconstituents in Management of Alzheimer's Disease. Evidence-Based Complementary and Alternative Medicine. 2021;2021:1-19.
2. Singh AK, Mishra G, Maurya A, Awasthi R, Kumari K, Thakur A, Rai A, Rai GK, Sharma B, Kulkarni GT, Singh SK. Role of TREM2 in Alzheimer's Disease and its Consequences on β- Amyloid, Tau and Neurofibrillary Tangles. Curr Alzheimer Res. 2019;16(13):1216-1229.
3. Abbas S, Latif M, Shafie N, Ghazali M, Abidin N, Mustafa M, et al. A review of antioxidant and anti-acetylcholinesterase activities of *Centella asiatica (L.)* Urb. for the treatment of Alzheimer's disease. Food Res. 2021;5(2):1-17.
4. Awasthi M, Upadhyay AK, Singh S, Pandey VP, Dwivedi UN. Terpenoids as promising therapeutic molecules against Alzheimer's disease: amyloid beta- and acetylcholinesterase-directed pharmacokinetic and molecular docking analyses. Molecular Simulation. 2018;44(1):1-11.
5. Jusril NA, Muhamad Juhari ANN, Abu Bakar SI, Md Saad WM, Adenan MI. Combining In Silico and In Vitro Studies to Evaluate the Acetylcholinesterase Inhibitory Profile of Different Accessions and the Biomarker Triterpenes of Centella asiatica. Molecules. 2020;25(15):3353.
6. Azerad R. Chemical structures, production and enzymatic transformations of sapogenins and saponins from Centella asiatica (L.) Urban. Fitoterapia. 2016;114:168-87.
7. Phaniendra A, Jestadi DB, Periyasamy L. Free Radicals: Properties, Sources, Targets, and Their Implication in Various Diseases. Ind J Clin Biochem. 2015;30(1):11-26.
8. Cobley JN, Fiorello ML, Bailey DM. 13 reasons why the brain is susceptible to oxidative stress. Redox Biology. 2018;15:490-503.
9. Sadigh-Eteghad S, Sabermarouf B, Majdi A, Talebi M, Farhoudi M, Mahmoudi J. Amyloid-Beta: A Crucial Factor in Alzheimer's Disease. Med Princ Pract. 2015;24(1):1-10.
10. Tönnies E, Trushina E. Oxidative Stress, Synaptic Dysfunction, and Alzheimer's Disease. J Alzheimers Dis. 2017;57(4):1105-1121.
11. Xu M, Xiong Y, Liu J, Qian J, Zhu L, Gao J. Asiatic acid, a pentacyclic triterpene in Centella asiatica, attenuates glutamate-induced cognitive deficits in mice and apoptosis in SH-SY5Y cells. Acta Pharmacol Sin. 2012;33(5):578-87.
12. Mehla J, Gupta P, Pahuja M, Diwan D, Diksha D. Indian Medicinal Herbs and Formulations for Alzheimer's Disease, from Traditional Knowledge to Scientific Assessment. Brain Sciences. 2020;10(12):964.
13. Yiannopoulou KG, Papageorgiou SG. Current and future treatments for Alzheimer's disease. Ther Adv Neurol Disord. 2013;6(1):19-33.
14. Sun B, Wu L, Wu Y, Zhang C, Qin L, Hayashi M. Therapeutic Potential of Centella asiatica and Its Triterpenes: A Review. Front Pharmacol. 2020; 11.
15. Sabaragamuwa R, Perera CO, Fedrizzi B. Centella asiatica (Gotu kola) as a neuroprotectant and its potential role in healthy ageing. Trends in Food Science & Technology. 2018;79:88-97.
16. James J, Dubery I. Pentacyclic Triterpenoids from the Medicinal Herb, *Centella asiatica (L.)* Urban. Molecules. 2009;14(10):3922-41.
17. Lawal OM, Wakel F, Dekker M. Consumption of fresh Centella asiatica improves short term alertness and contentedness in healthy females. Journal of Functional Foods. 2021;77:104337.
18. Pratibha Singh; Singh, J.S.Recruitment and competitive interaction between ramets ans seedlings in a perennial medicinal herb,*Centella asiatica*,Basic and Applied Ecology.2002 3(1): 65-76
19. Ahmad Rather M, Justin Thenmozhi A, Manivasagam T, Dhivya Bharathi M, Essa MM, Guillemin GJ. Neuroprotective role of Asiatic

acid in aluminium chloride induced rat model of Alzheimer's disease. Front Biosci (Schol Ed). 2018;10(2):262-275.

20. Puttarak P, Dilokthornsakul P, Saokaew S, Dhippayom T, Kongkaew C, Sruamsiri R. Effects of Centella asiatica (L.) Urb. on cognitive function and mood related outcomes: A Systematic Review and Meta-analysis. Sci Rep. 2017;7(1)
21. Tohid H. Anti-glutamic acid decarboxylase antibody positive neurological syndromes. NSJ. 2016;21(3):215-22.
22. Orhan IE. *Centella asiatica(L.)* Urban: From Traditional Medicine to Modern Medicine with Neuroprotective Potential. Evidence-Based Complementary and Alternative Medicine. 2012;2012:1-8.
23. Li Y, Yang F, Yuan M, Jiang L, Yuan L, Zhang X, et al. Synthesis and evaluation of asiatic acid derivatives as anti-fibrotic agents: Structure/activity studies. Steroids. 2015;96:44-9.
24. Hanapi NA, Mohamad Arshad AS, Abdullah JM, Tengku Muhammad TS, Yusof SR. Blood-Brain Barrier Permeability of Asiaticoside, Madecassoside and Asiatic Acid in Porcine Brain Endothelial Cell Model. Journal of Pharmaceutical Sciences. 2021;110(2):698-706.
25. Nasir M, Habsah M, Zamzuri I, Rammes G, Hasnan J, Abdullah J. Effects of asiatic acid on passive and active avoidance task in male Spraque–Dawley rats. Journal of Ethnopharmacology. 2011;134(2):203-9.
26. Biswas D, Mandal S, Chatterjee Saha S, Tudu CK, Nandy S, Batiha GE. Ethnobotany, phytochemistry, pharmacology, and toxicity of *Centella asiatica (L.)* Urban: A comprehensive review. Phytotherapy Research. 2021;35(12):6624-54.
27. Mills J, Reiner PB. Regulation of Amyloid Precursor Protein Cleavage. Journal of Neurochemistry.1999;72(2):443-60.
28. Haque SE, Iqubal A, Iqubal MK, Fazal SA, Pottoo FH. Nutraceuticals and their Derived Nano-Formulations for the Prevention and Treatment of Alzheimer's Disease. CMP. 2021;15(1):23-50.
29. Fezoui Y, Teplow DB. Kinetic Studies of Amyloid β-Protein Fibril Assembly. Journal of Biological Chemistry. 2002;277(40):36948-54.
30. Firdaus Z, Singh TD. An Insight in Pathophysiological Mechanism of Alzheimer's Disease and its Management Using Plant Natural Products. MRMC. 2021;21(1):35-57.
31. Kumar Thakur A, Kamboj P, Goswami K, Ahuja K. Pathophysiology and management of alzheimer's disease: an overview. JAPLR. 2018;7(2).
32. Sagare A, Deane R, Bell RD, Johnson B, Hamm K, Pendu R. Clearance of amyloid-β by circulating lipoprotein receptors. Nat Med. 2007;13(9):1029-31.
33. Shibata M, Yamada S, Kumar SR, Calero M, Bading J, Frangione B. Clearance of Alzheimer's amyloid-β1-40 peptide from brain by LDL receptor–related protein-1 at the blood-brain barrier. J Clin Invest. 2000;106(12):1489-99.
34. Kim GH, Kim JE, Rhie SJ, Yoon S. The Role of Oxidative Stress in Neurodegenerative Diseases. Exp Neurobiol. 2015;24(4):325-40.
35. Liu Z, Zhang A, Sun H, Han Y, Kong L, Wang X. Two decades of new drug discovery and development for Alzheimer's disease. RSC Adv. 2017;7(10):6046-58.
36. Gella A, Durany N. Oxidative stress in Alzheimer disease. Cell AdhMigr. 2009;3(1):88-93.
37. Agrawal I, Jha S. Mitochondrial Dysfunction and Alzheimer's Disease: Role of Microglia. Front Aging Neurosci, 2020;12
38. Multhaup G, Ruppert T, Schlicksupp A, Hesse L, Beher D, Masters CL. Reactive oxygen species and Alzheimer's disease. Biochemical Pharmacology. 1997;54(5):533-9.
39. Yan MH, Wang X, Zhu X. Mitochondrial defects and oxidative stress in Alzheimer disease and Parkinson disease. Free Radical Biology and Medicine. 2013;62:90-101.
40. Tyagi E, Agrawal R, Nath C, Shukla R. Influence of LPS-induced neuroinflammation on acetylcholinesterase activity in rat brain. Journal of Neuroimmunology. 2008;205(1-2):51-6.
41. Liddelow SA, Guttenplan KA, Clarke LE, Bennett FC, Bohlen CJ, Schirmer L. Neurotoxic reactive astrocytes are induced by activated microglia. Nature. 2017;541(7638):481-7.
42. Zorec R, Parpura V, Vardjan N, Verkhratsky A. Astrocytic face of Alzheimer's disease. Behavioural Brain Research. 2017;322:250-7.
43. Hong S, Beja-Glasser VF, Nfonoyim BM, Frouin A, Li S, Ramakrishnan S. Complement and microglia mediate early synapse loss in Alzheimer mouse models. Science. 2016;352(6286):712-6.
44. Liu C, Zhao N, Fu Y, Wang N, Linares C, Tsai C, et al. ApoE4 Accelerates Early Seeding of Amyloid Pathology. Neuron. 2017;96(5):1024-1032.
45. Sweeney MD, Sagare AP, Zlokovic BV. Blood–brain barrier breakdown in Alzheimer disease and other neurodegenerative disorders. Nat Rev Neurol. 2018;14(3):133-50.
46. Prasad H, Rao R. Amyloid clearance defect in ApoE4 astrocytes is reversed by epigenetic correction of endosomal pH. Proc Natl Acad Sci USA. 2018;115(28)
47. Arranz AM, De Strooper B. The role of astroglia in Alzheimer's disease: pathophysiology and clinical implications. The Lancet Neurology. 2019;18(4):406-14.
48. Ozben T, Ozben S. Neuro-inflammation and anti-inflammatory treatment options for Alzheimer's disease. Clinical Biochemistry. 2019;72:87-9.
49. Cao J, Hou J, Ping J, Cai D. Advances in developing novel therapeutic strategies for Alzheimer's disease. Mol Neurodegeneration. 2018;13(1)
50. Thakur S, Dhapola R, Sarma P, Medhi B, Reddy DH. Neuroinflammation in Alzheimer's Disease: Current Progress in Molecular Signaling and Therapeutics. Inflammation. 2023;46(1):1-17.
51. Dhapola R, Hota SS, Sarma P, Bhattacharyya A, Medhi B, Reddy DH. Recent advances in molecular pathways and therapeutic implications targeting neuroinflammation for Alzheimer's disease. Inflammopharmacol. 2021;29(6):1669-81.
52. Sochocka M, Diniz BS, Leszek J. Inflammatory Response in the CNS: Friend or Foe?. Mol Neurobiol, 2017;54(10):8071-89.
53. Baroja-Mazo A, Martín-Sánchez F, Gomez AI, Martínez CM, Amores-Iniesta J, Compan. The NLRP3 inflammasome is released as a particulate danger signal that amplifies the inflammatory response. Nat Immunol. 2014;15(8):738-48.
54. Bartzokis G. Age-related myelin breakdown: a developmental model of cognitive decline and Alzheimer's disease. Neurobiology of Aging. 2004;25(1):5-18.
55. Onyango IG, Jauregui GV, Čarná M, Bennett JP, Stokin GB. Neuroinflammation in Alzheimer's Disease. Biomedicines. 2021;9(5):524.
56. Wang J, Song Y, Chen Z, Leng SX. Connection between Systemic Inflammation and Neuroinflammation Underlies Neuroprotective Mechanism of Several Phytochemicals in Neurodegenerative Diseases. Oxidative Medicine and Cellular Longevity. 2018;2018:1-16.
57. Siracusa R, Fusco R, Cuzzocrea S. Astrocytes: Role and Functions in Brain Pathologies. Front Pharmacol. 2019;10:1114.
58. Verkhratsky A, Olabarria M, Noristani HN, Yeh C, Rodriguez JJ. Astrocytes in Alzheimer's disease. Neurotherapeutics. 2010;7(4):399-412.
59. Greenwald BS, Davis KL. Experimental pharmacology of Alzheimer disease. Adv Neurol. 1983;38:87-102.
60. Hampel H, Mesulam M, Cuello AC, Farlow MR, Giacobini E, Grossberg GT, et al. The cholinergic system in the pathophysiology and treatment of Alzheimer's disease. Brain. 2018;141(7):1917-33.
61. Sanabria-Castro A, Alvarado-Echeverría I, Monge-Bonilla C. Molecular Pathogenesis of Alzheimer's Disease: An Update. Ann Neurosci. 2017;24(1):46-54.
62. Chase TN, Farlow MR, Clarence-Smith K. Donepezil Plus Solifenacin (CPC-201) Treatment for Alzheimer's Disease. Neurotherapeutics. 2017;14(2):405-16.

63. Braak H, Braak E. Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol. 1991;82(4):239-59.
64. Kosik KS, Joachim CL, Selkoe DJ. Microtubule-associated protein tau (tau) is a major antigenic component of paired helical filaments in Alzheimer disease. Proc Natl Acad Sci USA. 1986;83(11):4044-8.
65. Brion JP. The role of neurofibrillary tangles in Alzheimer disease. Acta Neurol Belg.1998;98(2):165-74.
66. Kametani F, Hasegawa M. Reconsideration of Amyloid Hypothesis and Tau Hypothesis in Alzheimer's Disease. Front Neurosci. 2018;12.
67. Paula VdJRd, Guimarães FM, Diniz BS, Forlenza OV. Neurobiological pathways to Alzheimer's disease: Amyloid-beta, TAU protein or both?. Dement neuropsychol. 2009;3(3):188-94.
68. Alasmari F, Alshammari MA, Alasmari AF, Alanazi WA, Alhazzani K. Neuroinflammatory Cytokines Induce Amyloid Beta Neurotoxicity through Modulating Amyloid Precursor Protein Levels/Metabolism. BioMed Research International. 2018;2018:1-8.
69. Weekley CM, He C. Developing drugs targeting transition metal homeostasis. Current Opinion in Chemical Biology. 2017;37:26-32.
70. Bush AI. The Metal Theory of Alzheimer's Disease. JAD. 2012;33(s1):S277-S281.
71. Bush A, Pettingell W, Multhaup G, d Paradis M, Vonsattel J, Gusella J. Rapid induction of Alzheimer A beta amyloid formation by zinc. Science. 1994;265(5177):1464-7.
72. Dong J, Atwood CS, Anderson VE, Siedlak SL, Smith MA, Perry G, et al. Metal Binding and Oxidation of Amyloid-β within Isolated Senile Plaque Cores: Raman Microscopic Evidence. Biochemistry. 2003;42(10):2768-73.
73. Tabner BJ, Turnbull S, El-Agnaf OM, Allsop D. Formation of hydrogen peroxide and hydroxyl radicals from A(beta) and alpha-synuclein as a possible mechanism of cell death in Alzheimer's disease and Parkinson's disease. Free RadicBiol Med. 2002;32(11):1076-83.
74. Barnham KJ, Haeffner F, Ciccotosto GD, Curtain CC, Tew D, Mavros C. Tyrosine gated electron transfer is key to the toxic mechanism of Alzheimer's disease β-amyloid. FASEB j. 2004;18(12):1427-9.
75. Gh Popescu BF, Nichol H. Mapping Brain Metals to Evaluate Therapies for Neurodegenerative Disease. CNS Neuroscience & Therapeutics. 2011;17(4):256-68.
76. Farina M, Avila DS, da Rocha JBT, Aschner M. Metals, oxidative stress and neurodegeneration: A focus on iron, manganese and mercury. Neurochemistry International. 2013;62(5):575-94.
77. Graham SF, Nasaruddin MB, Carey M, Holscher C, McGuinness B, Kehoe PG. Age-Associated Changes of Brain Copper, Iron, and Zinc in Alzheimer's Disease and Dementia with Lewy Bodies. JAD. 2014;42(4):1407-13.
78. White AR, Reyes R, Mercer JF, Camakaris J, Zheng H, Bush AI, et al. Copper levels are increased in the cerebral cortex and liver of APP and APLP2 knockout mice. Brain Research.1999;842(2):439-44.
79. Verkhratsky A. Physiology and Pathophysiology of the Calcium Store in the Endoplasmic Reticulum of Neurons. Physiological Reviews. 2005;85(1):201-79.
80. Wang Y, Shi Y, Wei H. Calcium Dysregulation in Alzheimer's Disease: A Target for New Drug Development. J Alzheimers Dis Parkinsonism. 2017;7(5)
81. Benarroch EE. Neuronal voltage-gated calcium channels: Brief overview of their function and clinical implications in neurology. Neurology. 2010;74(16):1310-5.
82. Schampel A, Kuerten S. Danger: High Voltage—The Role of Voltage-Gated Calcium Channels in Central Nervous System Pathology. Cells. 2017;6(4):43.
83. Stutzmann GE. Calcium Dysregulation, IP3 Signaling, and Alzheimer's Disease. Neuroscientist. 2005;11(2):110-5.
84. Misquitta C, Mack D, Grover A. Sarco/endoplasmic reticulum Ca2+(SERCA)-pumps: link to heart beats and calcium waves. Cell Calcium. 1999;25(4):277-90.
85. Popugaeva E, Pchitskaya E, Bezprozvanny I. Dysregulation of neuronal calcium homeostasis in Alzheimer's disease – A therapeutic opportunity?. Biochemical and Biophysical Research Communications. 2017;483(4):998-1004.
86. Ferreiro E, Oliveira CR, Pereira CM. The release of calcium from the endoplasmic reticulum induced by amyloid-beta and prion peptides activates the mitochondrial apoptotic pathway. Neurobiology of Disease. 2008;30(3):331-42.
87. Castillo C, Martinez JC, Longart M, García L, Hernández M, Carballo J, et al. Extracellular Application of CRMP2 Increases Cytoplasmic Calcium through NMDA Receptors. Neuroscience. 2018;376:204-23.
88. Park J, Seo YH, Jang J, Jeong C, Lee S, Park B. Asiatic acid attenuates methamphetamine-induced neuroinflammation and neurotoxicity through blocking of NF-kB/STAT3/ERK and mitochondria-mediated apoptosis pathway. J Neuroinflammation. 2017;14(1)
89. Ahmad Rather M, Justin-Thenmozhi A, Manivasagam T, Saravanababu C, Guillemin GJ, Essa MM. Asiatic Acid Attenuated Aluminum Chloride-Induced Tau Pathology, Oxidative Stress and Apoptosis Via AKT/GSK-3β Signaling Pathway in Wistar Rats. Neurotox Res. 2019;35(4):955-68.
90. Dhanasekaran M, Holcomb LA, Hitt AR, Tharakan B, Porter JW, Young KA, et al. Centella asiatica extract selectively decreases amyloid β levels in hippocampus of Alzheimer's disease animal model. Phytother Res. 2009;23(1):14-9.
91. Veerendra Kumar M, Gupta Y. Effect of different extracts of Centella asiatica on cognition and markers of oxidative stress in rats. Journal of Ethnopharmacology. 2002;79(2):253-60.
92. Chen C, Tsai W, Chen C, Pan T. Centella asiatica extract protects against amyloid β1–40-induced neurotoxicity in neuronal cells by activating the antioxidative defence system. Journal of Traditional and Complementary Medicine. 2016;6(4):362-9.
93. Soumyanath A, Zhong Y, Henson E, Wadsworth T, Bishop J, Gold BG. *Centella asiatica*Extract Improves Behavioral Deficits in a Mouse Model of Alzheimer's Disease: Investigation of a Possible Mechanism of Action. International Journal of Alzheimer's Disease. 2012;2012:1-9.
94. Loganathan C, Thayumanavan P. Asiatic acid prevents the quinolinic acid-induced oxidative stress and cognitive impairment. Metab Brain Dis. 2018;33(1):151-9.
95. Patil SP, Maki S, Khedkar SA, Rigby AC, Chan C. Withanolide A and Asiatic Acid Modulate Multiple Targets Associated with Amyloid-β Precursor Protein Processing and Amyloid-β Protein Clearance. J Nat Prod. 2010;73(7):1196-202.
96. Zhang X, Wu J, Dou Y, Xia B, Rong W, Rimbach. Asiatic acid protects primary neurons against C2-ceramide-induced apoptosis. European Journal of Pharmacology. 2012;679(1-3):51-9.
97. Kim SR, Koo KA, Lee MK, Park H, Jew S, Cha K, et al. Asiatic acid derivatives enhance cognitive performance partly by improving acetylcholine synthesis. Journal of Pharmacy and Pharmacology. 2010;56(10):1275-82.
98. Jusril NA, Muhamad Juhari ANN, Abu Bakar SI, Md Saad WM, Adenan MI. Combining In Silico and In Vitro Studies to Evaluate the Acetylcholinesterase Inhibitory Profile of Different Accessions and the Biomarker Triterpenes of *Centella asiatica*Molecules. 2020;25(15):3353.
99. Cheng W, Chen W, Wang P, Chu J. Asiatic acid protects differentiated PC12 cells from Aβ25–35-induced apoptosis and tau hyperphosphorylation via regulating PI3K/Akt/GSK-3β signaling. Life Sciences. 2018;208:96-101.
100. Nasir M, Abdullah J, Habsah M, Ghani R, Rammes G. Inhibitory effect of asiatic acid on acetylcholinesterase, excitatory post synapticpotential and locomotor activity. Phytomedicine. 2012;19(3-4):311-6.
101. Gregory J, Vengalasetti YV, Bredesen DE, Rao RV. Neuroprotective Herbs for the Management of Alzheimer's Disease. Biomolecules. 2021;11(4):543.

102. Zahara K. Clinical and therapeutic benefits of Centella asiatica. PAB. 2014; 3(4):152-9.

103. Abbas S, Latif M, Shafie N, Ghazali M, Abidin N, Mustafa M, et al. A review of antioxidant and anti-acetylcholinesterase activities of *Centella asiatica (L.)* Urb. for the treatment of Alzheimer's disease. Food Res. 2021; 5(2):1-17.

104. Shukla SD, Bhatnagar M, Khurana S. Critical Evaluation of Ayurvedic Plants for Stimulating Intrinsic Antioxidant Response. Front Neurosci. 2012;6

105. Xu M, Xiong Y, Liu J, Qian J, Zhu L, Gao J. Asiatic acid, a pentacyclic triterpene in Centella asiatica, attenuates glutamate-induced cognitive deficits in mice and apoptosis in SH-SY5Y cells. Acta Pharmacol Sin. 2012; 33(5):578-87.

106. Paidi RK, Sarkar S, Ambareen N, Biswas SC. Medha Plus – A novel polyherbal formulation ameliorates cognitive behaviors and disease pathology in models of Alzheimer's disease. Biomedicine & Pharmacotherapy. 2022; 151:113086.

107. Xu M, Xiong Y, Liu J, Qian J, Zhu L, Gao J. Asiatic acid, a pentacyclic triterpene in Centella asiatica, attenuates glutamate-induced cognitive deficits in mice and apoptosis in SH-SY5Y cells. Acta Pharmacol Sin. 2012; 33(5):578-87.

108. Gray NE, Zweig JA, Matthews DG, Caruso M, Quinn JF, Soumyanath A. Centella asiatica Attenuates Mitochondrial Dysfunction and Oxidative Stress in Aβ-Exposed Hippocampal Neurons. Oxidative Medicine and Cellular Longevity. 2017;2017:1-8.

109. Sabaragamuwa R, Perera CO, Fedrizzi B. *Centella asiatica* (Gotu kola) as a neuroprotectant and its potential role in healthy ageing. Trends in Food Science & Technology. 2018;79:88-97.

110. Mato L, Wattanathorn J, Muchimapura S, Tongun T, Piyawatkul N, Yimtae K. *Centella asiatica*Improves Physical Performance and Health-Related Quality of Life in Healthy Elderly Volunteer. Evidence-Based Complementary and Alternative Medicine. 2011;2011:1-7.

111. Abbas S, Latif M, Shafie N, Ghazali M, Abidin N, Mustafa M. A review of antioxidant and anti-acetylcholinesterase activities of *Centella asiatica (L.)* Urb. for the treatment of Alzheimer's disease. Food Res. 2021;5(2):1-17.

112. Orhan G, Orhan I, Sener B. Recent Developments in Natural and Synthetic Drug Research for Alzheimers Disease LDDD. 2006;3(4):268-74.

113. Mukherjee PK, Kumar V, Houghton PJ. Screening of Indian medicinal plants for acetylcholinesterase inhibitory activity. Phytother Res. 2007;21(12):1142-5.

114. Ahmad Rather M, Justin Thenmozhi A, Manivasagam T, Dhivya Bharathi M, Essa MM, Guillemin GJ. Neuroprotective role of Asiatic acid in aluminium chloride induced rat model of Alzheimer's disease. Front Biosci (Schol Ed). 2018;10(2):262-275.

115. Hafiz ZZ, Amin M'M, Johari James RM, Teh LK, Salleh MZ, Adenan MI. Inhibitory Effects of Raw-Extract *Centella asiatica* (RECA) on Acetylcholinesterase, Inflammations, and Oxidative Stress Activities via In Vitro and In Vivo. Molecules. 2020;25(4):892.

116. I Adenan M, A Jusril N, A Radzun K, Z Hafiz Z. Comparative Study on Anti-Acetylcholinesterase and Anti-Inflammatory Activities of Date and Apple Vinegars Fortified with Centella Asiatica. IJET. 2019;7(4.14):116.

117. Mukherjee PK, Kumar V, Houghton PJ. Screening of Indian medicinal plants for acetylcholinesterase inhibitory activity. Phytother Res. 2007;21(12):1142-5.

118. H. Nour, A., M. Khan, A. Z. Sulaiman, T. Batool, A. H. Nour, M. Mumtaz Khan, and F. Kormin. In Vitro Anti-Acetyl Cholinesterase and Antioxidant Activity of Selected Malaysian Plants". Asian Journal of Pharmaceutical and Clinical Research. 2014 ;7(3): 93-97,

119. Mathew M, Subramanian S. In Vitro Screening for Anti-Cholinesterase and Antioxidant Activity of Methanolic Extracts of Ayurvedic Medicinal Plants Used for Cognitive Disorders. PLoS ONE. 2014;9(1):e86804..

120. Nasir M, Abdullah J, Habsah M, Ghani R, Rammes G. Inhibitory effect of asiatic acid on acetylcholinesterase, excitatory post synapticpotential and locomotor activity. Phytomedicine. 2012;19(3-4):311-6.

121. Boopathy R, Chitra L, Srilakshmi Prabha N, Babu SA. P2-233: Effect of asiatic acid on hippocampal cell line: A novel inhibitor of acetylcholinesterase from Centella asiatica. Alzheimer's & Dementia. 2009;5(4S_Part_11)

122. Nataraj J, Manivasagam T, Justin Thenmozhi A, Essa MM. Neuroprotective effect of asiatic acid on rotenone-induced mitochondrial dysfunction and oxidative stress-mediated apoptosis in differentiated SH-SY5Y cells. Nutritional Neuroscience.2017;20(6):351-9.

123. Heikkila R. Effects of 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine on neostriatal dopamine in mice. Neuropharmacology.1984;23(6):711-3.

124. Justin Thenmozhi A. Asiatic acid nullified aluminium toxicity in in vitro model of Alzheimer rsquo s disease. Front Biosci. 2018;10(1):287-99.

125. Sun T, Liu B, Li P. Nerve Protective Effect of Asiaticoside against Ischemia-Hypoxia in Cultured Rat Cortex Neurons. Med Sci Monit. 2015;21:3036-41.

126. Umka Welbat J, Sirichoat A, Chaijaroonkhanarak W, Prachaney P, Pannangrong W, Pakdeechote P. Asiatic Acid Prevents the Deleterious Effects of Valproic Acid on Cognition and Hippocampal Cell Proliferation and Survival. Nutrients. 2016;8(5):303.

127. Nasir M, Habsah M, Zamzuri I, Rammes G, Hasnan J, Abdullah J. Effects of asiatic acid on passive and active avoidance task in male Spraque–Dawley rats. Journal of Ethnopharmacology. 2011;134(2):203-9.

128. Jiang W, Li M, He F, Bian Z, He Q, Wang X, et al. Neuroprotective effect of asiatic acid against spinal cord injury in rats. Life Sciences. 2016;157:45-51.

129. Nasir M, Abdullah J, Habsah M, Ghani R, Rammes G. Inhibitory effect of asiatic acid on acetylcholinesterase, excitatory post synapticpotential and locomotor activity. Phytomedicine. 2012;19(3-4):311-6.

130. Nasir M, Habsah M, Zamzuri I, Rammes G, Hasnan J, Abdullah J. Effects of asiatic acid on passive and active avoidance task in male Spraque–Dawley rats. Journal of Ethnopharmacology. 2011;134(2):203-9.

131. Kappettu Gadahad MR, Rao M, Rao G. Enhancement of Hippocampal CA3 Neuronal Dendritic Arborization by Centella asiatica (Linn) Fresh Leaf Extract Treatment in Adult Rats. Journal of the Chinese Medical Association. 2008;71(1):6-13.

132. Patil SP, Maki S, Khedkar SA, Rigby AC, Chan C. Withanolide A and Asiatic Acid Modulate Multiple Targets Associated with Amyloid-β Precursor Protein Processing and Amyloid-β Protein Clearance. J Nat Prod. 2010;73(7):1196-202.

133. Chassaud LF, Fry BJ, Hawkins DR, Lewis JD, Sword IP, Taylor T, Hathway DE. The metabolism of asiatic acid,-madecassic acid and asiaticoside in the rat. Arzneimittelforschung. 1971;21(9):1379-84

134. Xiao W, Jiang W, Li K, Hu Y, Li S, Zhou L, Wan R. Protective effect of asiatic acid in an experimental cerulein-induced model of acute pancreatitis in mice. Am J Transl Res. 2017;9(8):3842-3852

135. Chassaud LF, Fry BJ, Hawkins DR, Lewis JD, Sword IP, Taylor T, Hathway DE. The metabolism of asiatic acid,-madecassic acid and asiaticoside in the rat Arzneimittelforschung. 1971;21(9):1379-84.

136. Bossé JP, Papillon J, Frenette G, Dansereau J, Cadotte M, Le Lorier J. Clinical study of a new antikeloid agent. Ann Plast Surg.1979;3(1):13-21.

137. Schubert M. Solvent injection as a new approach for manufacturing lipid nanoparticles – evaluation of the method and process parameters. European Journal of Pharmaceutics and Biopharmaceutics. 2003;55(1):125-31.

138. Yuan Y, Zhang H, Sun F, Sun S, Zhu Z, Chai Y. Biopharmaceutical and pharmacokinetic characterization of asiatic acid in *Centella*

asiatica as determined by a sensitive and robust HPLC–MS method. Journal of Ethnopharmacology. 2015;163:31-8.

139. Guo L, Cui Y, Hao K. Effects of glycyrrhizin on the pharmacokinetics of asiatic acid in rats and its potential mechanism. Pharmaceutical Biology. 2018;56(1):119-23.

140. Songvut P, Chariyavilaskul P, Tantisira M, Khemawoot P. Safety and Pharmacokinetics of Standardized Extract of *Centella asiatica* (ECa 233) Capsules in Healthy Thai Volunteers: A Phase 1 Clinical Study. Planta Med, 2019;85(06):483-90.

141. KOBASHI K, AKAO T. Relation of Intestinal Bacteria to Pharmacological Effects of Glycosides. Bioscience Microflora. 1997;16(1):1-7.

142. Wright KM, Bollen M, David J, Speers AB, Brandes MS, Gray NE, Alcázar Magaña A, McClure C, Stevens JF, Maier CS, Quinn JF, Soumyanath A. Pharmacokinetics and Pharmacodynamics of Key Components of a Standardized Centella asiatica Product in Cognitively Impaired Older Adults: A Phase 1, Double-Blind, Randomized Clinical Trial. Antioxidants (Basel). 2022;11(2):215.

143. Tan SC, Bhattamisra SK, Chellappan DK, Candasamy M. Actions and Therapeutic Potential of Madecassoside and Other Major Constituents of Centella asiatica: A Review. Applied Sciences.2021;11(18):8475.

144. Yuan Y, Zhang H, Sun F, Sun S, Zhu Z, Chai Y. Biopharmaceutical and pharmacokinetic characterization of asiatic acid in Centella asiatica as determined by a sensitive and robust HPLC–MS method. Journal of Ethnopharmacology. 2015;163:31-8.

145. Zheng X, Wang S. Determination of asiatic acid in beagle dog plasma after oral administration of Centella asiatica extract by precolumn derivatization RP-HPLC. Journal of Chromatography B. 2009;877(5-6):477-81.

146. Nair SN, Menon S, Shailajan S. A liquid chromatography/electrospray ionization tandem mass spectrometric method for quantification of asiatic acid from plasma: application to pharmacokinetic study in rats. Rapid Commun Mass Spectrom. 2012;26(17):1899-908.

147. Begum S, Nizami SS, Mahmood U, Masood S, Iftikhar S, Saied S. In-vitro evaluation and in-silico studies applied on newly synthesized amide derivatives of N-phthaloylglycine as Butyrylcholinesterase (BChE) inhibitors. Computational Biology and Chemistry. 2018;74:212-7.

148. Speck-Planche A, Cordeiro MNDS. Advanced In Silico Approaches for Drug Discovery: Mining Information from Multiple Biological and Chemical Data Through mtk- QSBER and pt-QSPR Strategies, CMC. 2017;24(16)

149. Leelananda SP, Lindert S. Computational methods in drug discovery. Beilstein J Org Chem. 2016;12:2694-718.

150. R.M J, Ray A, Naik D, Sanyal D, Shah D. Review and Research Analysis of Computational Target Methods Using BioRuby and in silico Screening of Herbal Lead Compounds Against Pancreatic Cancer Using R Programming, CDM. 2014;15(5):535-43.

151. Dong -Chan KimFinding the Novel Effect of Asiatic acid as aPotential Anti -neuroinflammatory Agent Using Microglia Cell System and In Silico Molecular Docking Technology, International Journal of Engineering Research and Technology.2018;11(12):2089-2098

152. Jusril NA, Muhamad Juhari ANN, Abu Bakar SI, Md Saad WM, Adenan MI. Combining in Silico and In Vitro Studies to Evaluate the Acetylcholinesterase Inhibitory Profile of Different Accessions and the Biomarker Triterpenes of *Centella asiatica*. Molecules. 2020;25(15):3353.

153. Kua J, Zhang Y, Eslami AC, Butler JR, McCammon JA. Studying the roles of W86, E202, and Y337 in binding of acetylcholine to acetylcholinesterase using a combined molecular dynamics and multiple docking approach. Protein Sci. 2003;12(12):2675-84.

154. Shi S, Hayashi Y, Petralia RS, Zaman SH, Wenthold RJ, Svoboda K. Rapid Spine Delivery and Redistribution of AMPA Receptors After Synaptic NMDA Receptor Activation. Science. 1999;284(5421):1811-6.

155. Bagchi P, M A, Kar A. Pharmacophore Screening and Docking studies of AMPA Receptor Implicated in Alzheimer's disease with Some CNS Acting Phytocompounds from Selected Ayurvedic Medicinal Plants. Neuropsychiatry. 2018;08(03)

156. Abbott NJ, Rönnbäck L, Hansson E. Astrocyte–endothelial interactions at the blood–brain barrier. Nat Rev Neurosci. 2006;7(1):41-53.

157. Abbott NJ, Patabendige AA, Dolman DE, Yusof SR, Begley DJ. Structure and function of the blood–brain barrier. Neurobiology of Disease. 2010;37(1):13-25.

158. Sweeney MD, Sagare AP, Zlokovic BV. Blood–brain barrier breakdown in Alzheimer disease and other neurodegenerative disorders. Nat Rev Neurol.2018;14(3):133-50.

159. Li Di , Edward H. Kerns , Ian F. Bezar , Susan L. Petusky , Youping Huang Comparison of blood–brain barrier permeability assays: in situ brain perfusion, MDR1-MDCKII and PAMPA-BBBJournal of Pharmaceutical Sciences. 2009;98(6): 1980-1991

160. Chen X, Zhang Y, Zhao P, Chen Y, Zhou Y, Wang S, et al. Preparation and evaluation of PEGylated asiatic acid nanostructured lipid carriers on anti-fibrosis effects. Drug Development and Industrial Pharmacy. 2020;46(1):57-69.

161. Patel PJ, Acharya NS, Acharya SR. Development and characterization of glutathione-conjugated albumin nanoparticles for improved brain delivery of hydrophilic fluorescent marker. Drug Delivery. 2013;20(3-4):143-55.

162. Halder T, Patel B, Acharya N. Asiatic Acid Fabricated Nanoconstructs to Mitigate Amyloid Beta1-42 Induced Injury in SH-SY5Y Cells In-Vitro and Ameliorates Cognitive Impairment by Dual Cholinesterase Inhibition and Attenuation of Oxidative Stress In-Vivo. Pharm Res. 2023;40(1):197-213.

163. Raval N, Mistry T, Acharya N, Acharya S. Development of glutathione-conjugated asiatic acid-loaded bovine serum albumin nanoparticles for brain-targeted drug delivery. Journal of Pharmacy and Pharmacology. 2015;67(11):1503-11.

164. Hanapi NA, Mohamad Arshad AS, Abdullah JM, Tengku Muhammad TS, Yusof SR. Blood-Brain Barrier Permeability of Asiaticoside, Madecassoside and Asiatic Acid in Porcine Brain Endothelial Cell Model. Journal of Pharmaceutical Sciences. 2021;110(2):698-706.

165. Nagoor Meeran MF, Goyal SN, Suchal K, Sharma C, Patil CR, Ojha SK. Pharmacological Properties, Molecular Mechanisms, and Pharmaceutical Development of Asiatic Acid: A Pentacyclic Triterpenoid of Therapeutic Promise. Front Pharmacol. 2018;9

166. van de Waterbeemd H, Camenisch G, Folkers G, Chretien JR, Raevsky OA. Estimation of Blood-Brain Barrier Crossing of Drugs Using Molecular Size and Shape, and H-Bonding Descriptors. Journal of Drug Targeting. 1998;6(2):151-65.

167. Nasir M, Habsah M, Zamzuri I, Rammes G, Hasnan J, Abdullah J. Effects of asiatic acid on passive and active avoidance task in male Spraque–Dawley rats. Journal of Ethnopharmacology. 2011;134(2):203-9.

168. J. Cott, Medicinal plants and dietary supplements: sources for innovative treatment or adjuncts, Psychopharmacol Bull.1995;31(1):131–7

169. Gray NE, Alcazar Magana A, Lak P, Wright KM, Quinn J, Stevens JF, et al. Centella asiatica: phytochemistry and mechanisms of neuroprotection and cognitive enhancement. Phytochem Rev. 2018;17(1):161-94.

170. Yuan Y, Zhang H, Sun F, Sun S, Zhu Z, Chai Y. Biopharmaceutical and pharmacokinetic characterization of asiatic acid in Centella asiatica as determined by a sensitive and robust HPLC–MS method. Journal of Ethnopharmacology. 2015;163:31-8.

171. T Poovizhi , V Krishnaprabha and L Uthira Perspective study on Pharmacological and Therapeutic potential of Centella asiatica," Medicon Medical Sciences. 2022;2(5)02-11.

172. Qi Z, Ci X, Huang J, Liu Q, Yu Q, Zhou J, et al. Asiatic acid enhances Nrf2 signaling to protect HepG2 cells from oxidative damage through Akt and ERK activation. Biomedicine & Pharmacotherapy. 2017;88:252-9.

173. Mook-Jung I, Shin JE, Yun SH, Huh K, Koh JY, Park HK, Jew SS, Jung MW. Protective effects of asiaticoside derivatives against beta-amyloid neurotoxicity. J Neurosci Res. 1999;58(3):417-25.

174. Y. Boondam, P. Songvut, M. H. Tantisira, S. Tapechum, K. Tilokskulchai, and N. Pakaprot, Inverted U-shaped response of a standardized extract of Centella asiatica (ECa 233) on memory enhancement,Sci Rep.2019; 9(1):8404.