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

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ABSTRACT

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 (9). 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 have shifted 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 extensively 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, tranquilizer, 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 antidepressant action by GABA facilitatory 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, isothnunkic acid and thakninc 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 by multiple factors and physiological dysfunctioning commonly popular as hypothesis like oxidative stress, astroglial 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: ß-secretase cuts the Aβ sequence, ß-secretase acts on the external site of the APP protein, and ß-secretase works on the transmembrane portion of the same protein to release Aβ. 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 ß 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).
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-kB/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 and a byproduct, malondialdehyde (MDA) has been linked to neuroinflammation, neuronal apoptosis, and neurotransmitter dysfunction (40).

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 APP and 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.

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 help them pathogenesis of AD. Reactive astrocytes, which are found beside amyloid plaques, produce abnormal intracellular calcium signaling leading to abnormal homeostasis in brain. 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. The ApoE gene plays a role in the production of amyloid plaque formation, and this gene is expressed by astrocytes in the brain. 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. 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. 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.

Neuroinflammation

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. 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. Roughly 35% of the brain’s cells are astrocytes, which are specialized glial cells. Under normal circumstances microglia are neuroprotective and play a key role in phagocytosis, and release neurotrophins to maintain a healthy brain environment. 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. 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. Additionally, when microglia are activated, other signaling pathways, such as the PI3K/Akt pathway, which controls apoptosis and inflammatory responses, are also induced. 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). 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 speed up the course of the illness. 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. 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.

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-κB, 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 moderators 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).
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 that methamphetamine (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 Aβ 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 quinolinic 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).
Asiatic acid can attenuate neuronal cell apoptosis by decreasing oxidative stress, mitochondrial malfunction, modulating Aβ level, AChE level, and inflammation and improve 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 matoet al., aqueous extract exhibits physical stability due 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 phystostigmine’s IC50 value of 0.076±0.0042 µg/ml [137]. 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 phystostigmine 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, H2O2, and glutamate [122]. It has been reported that Asiatic is effective against methamphetamine-neurotoxicity by inhibiting mitochondrial apoptosis and signaling pathways, such as NF-kB/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, Sirichao 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, dehydroxylation, 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 Desagaru-chromatogram [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 (ASLNa) of a salt composition of Asiatic acid had a particle size of 200-300nm. The nanoparticles were prepared using the solvent injection method, and Glycer 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]. PhanitSongvuet 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 t1/2, Cmax, and AUC in a randomized crossover study [144]. Yongfang Yuan et al. reported poor Cmax (394.2 ng/ml) and a smaller t1/2 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\(^{147}\).

### Molecular Docking 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 tetrahydrobipterin 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 combined physical, 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 of 50.94 ± 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-lactico-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 Nisith Ravalet 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 proronic 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\(_2\)O\(_2\) and shown remarkable BBB penetration. Nur Aziah Hanapiet al. reported that Asiatic acid has higher apparent permeability values than donepezil \(^{(165)}\).

### Advantage over other terpenoids

Meeran MF 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, and an 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. Nasirae 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%\(^{(171)}\), 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)\(^{(172)}\). 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 jejunum. Although asiatic acid has low bioavailability, its derivatives have shown promise as a medicinal treatment for a variety of conditions\(^{(173)}\). 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 excitotoxicity in neurons grown in a lab dish\(^{(174)}\). 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\(_2\)O\(_2\), or death of cell\(^{(175)}\). There was evidence that asiatic acid could boost brain BDNF levels\(^{(176)}\).

**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 Alzheimer's disease. 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.

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**SYNOPSIS**

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 Alzheimer's disease. 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.


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