Review Article

Recent Updates in Neuroprotective and Neuroregenerative Potential of *Centella asiatica*

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Abstract

***Centella asiatica*, locally well known in Malaysia as pegaga, is a traditional herb that has been used widely in Ayurvedic medicine, traditional Chinese medicine, and in the traditional medicine of other Southeast Asian countries including Malaysia. Although consumption of the plant is indicated for various illnesses, its potential neuroprotective properties have been well studied and documented. In addition to past studies, recent studies also discovered and/or reconfirmed that *C. asiatica* acts as an antioxidant, reducing the effect of oxidative *stress in vitro and in vivo*. At the in vitro level, *C. asiatica* promotes dendrite arborisation and elongation, and also protects the neurons from apoptosis. In vivo studies have shown that the whole extract and also individual compounds of *C. asiatica* have a protective effect against various neurological diseases. Most of the in vivo studies on neuroprotective effects have focused on Alzheimer's disease, Parkinson's disease, learning and memory enhancement, neurotoxicity and other mental illnesses such as depression and anxiety, and epilepsy. Recent studies have embarked on finding the molecular mechanism of neuroprotection by *C. asiatica* extract. However, the capability of *C. asiatica* in enhancing neuroregeneration has not been studied much and is limited to the regeneration of crushed sciatic nerves and protection from neuronal injury in hypoxia conditions. More studies are still needed to identify the compounds and the mechanism of action of *C. asiatica* that are particularly involved in neuroprotection and neuroregeneration. Furthermore, the extraction method, biochemical profile and dosage information of the *C. asiatica* extract need to be standardised to enhance the economic value of this traditional herb and to accelerate the entry of *C. asiatica* extracts into modern medicine.**

Keywords: *antioxidant, neuroprotective, neurological disease, neuronal injury, pegaga*

Introduction

The nervous system, consisting of the brain, spinal cord, and peripheral nerves, is made of complex and specialised structures which are vulnerable to various diseases and injury that reduce sensorimotor and cognitive functions, and may also be the cause of life-threatening problems in acute cases. Unfortunately, spontaneous regeneration and healing processes occur very minimally in damaged tissues due to their high complexity. Our team of researchers at the Tissue Engineering Centre, Universiti Kebangsaan Malaysia has been conducting research on the regeneration of various tissues, and studies on nerve regeneration for development of cell and tissue therapies have been going on for almost eight years (1,2). We have identified various cell sources, ranging from stem cells to adult nerve cells, and developed various scaffolds, ranging from biological tissue to synthetic hollow tubes, to enhance in vitro and in vivo nerve regeneration (3–6). Realising the historical nature of medicinal herbs, we were attracted to scrutinise the pharmacological effects of herb extracts in synergy with the provided cells and scaffolds to further enhance nerve regeneration, and also to improve the utilisation of tissue-engineered nerve grafts and cell therapy in clinical applications for nerve degeneration and injury.

It has been reported that there are 250 000 plant species on the earth, and approximately 5

000 species have specific therapeutic value (7). Generally, *C. asiatica* is a traditional medicinal herb that belongs to the Apiaceae family and is known as pegaga in Malaysia. It has also been identified by different names such as Indian pennywort in English, gotu kola in Sri Lanka, brahmi in Hindi and mandukaparni in the Ayurvedic medicine of India, buak bok in Thailand, kaki kuda in Indonesia and yuhong-yuhong in the Philippines (8,9). This review focuses on recent discoveries, preferably within the past five years, on the properties of *C. asiatica* (L.) in protection against neural-related diseases and regeneration of injured nerves, from in vitro studies to in vivo and clinical studies.

Geographically, this particular herb is native to India, China, Sri Lanka, Madagascar, Indonesia and Malaysia, and grows well in swampy and damp areas of fields (10). Pertaining to its medical benefits, it has been found broadly across its boundaries in Turkey, South America, and the West Indies (11,12). Over thousands of years, it had been used by people all around the world as a remedy for many types of disease. It had been used widely in Ayurvedic medicine to rejuvenate the body, improve the intellect and combat cognitive disorders (13). It is known as a brain tonic and has also been applied in traditional Chinese medicine. In addition, it has also been incorporated as a drug in other medical practices such as the German Homeopathic Pharmacopoeia (GHP) and the European Pharmacopoeia (14).

C. asiatica is getting more popular in the modern world due to its versatility and efficacy that have been proven by many scientific studies. *C. asiatica* is well known as a wound-healing agent, due to its ability to heal small wounds, scratches, burns, and skin irritation (15,16). As well as skin re-epithelisation, Ruszymah and her colleagues (17) remarked on the capacity of this herb to treat cornea epithelial wounds. The few innovations using *C. asiatica* extracts in healthcare applications by Ruszymah and her colleagues won awards from the International Exposition of Research and Inventions of Institutions of Higher Learning (PECIPTA) as early as 2007. As technology develops, the undiscovered potential of this herb is further scrutinised and it has been documented to have anti-inflammatory, antimicrobial and antifungal, antidepressant, antioxidant and anticancer effects (18–21). *C. asiatica* was also found to have an antiproliferative effect against human respiratory epithelial cells in vitro and it also reduced sperm count, motility, and viability in male rats, besides having other antifertility effects (12,22). Even though many preclinical investigations have been performed, further detailed clinical studies are highly needed to scientifically evaluate pharmacological values and standardise the biochemical profile of the extract before use in various therapeutic applications. Since the toxicity effects of *C. asiatica* vary according to its geographical distribution, standardising its bioactive components during extract preparation would be appear to be momentous. This is because such fluctuations can lead to various complications which would consequently limit its therapeutic value.

Chemical Composition and Preparations of *C. asiatica*

Brinkhaus et al. (10) adequately summed up the chemical composition of *C. asiatica*, discovered as early as 1956 (23). About 0.1% is essential oils, while 1–8% is saponin-containing triterpene acids and their sugar esters. Further studies on chemical constituents were done on hydroponically grown *C. asiatica* by Othman (24). The author studied the nutritional content, the total polyphenol content between air-dried samples and fresh samples, salicylic acid content and flavonoid content, and found that the nutritional content is about the same throughout the plant from the leaf to the root; however, the leaf contains higher P, Fe, Na, Mg and ascorbic acid, while the roots contain higher Ca and K. The polyphenol and salicylic acid content was higher in fresh samples compared to air-dried samples. Analysis of samples with HPLC showed that the major flavonoid compounds were catechin, rutin, quercetin, and naringin.

Next, Oyedeji and Afolayan (25) investigated the content of *C. asiatica* essential oils from plants grown in South Africa, and found that the major essential oils are a-humulene (21.06%), b-caryophyllene (19.08%), bicyclogermacrene (11.22%), germacrene B (6.29%) and myrcene (6.55%). Siddiqui et al. (26) isolated and characterised the structure of three novel compounds in *C. asiatica*: centellin, asiaticin and centellicin with the structure of 6-acetoxy-trideca-1,7-dien-4-yn-3-ol, p-benzoyloxy methyl-butyl benzoate, and 1-(20,30-dihydroxypropyl)-2-en-3-methyl-6-hydroxy-9-yn-undecanoate, respectively. Zainol et al. (27) profiled two accessions of *C. asiatica* grown in Johor Bharu and found that it showed different phytochemical content in different parts, with leaves containing the most phytochemicals. Zhang et al. (28) evaluated content of the herb from different

locations in China and developed a chemical fingerprinting method for quality evaluation. Chong and Aziz (29) reviewed the chemical components of *C. asiatica* from 1949 to 2011. They found that the chemical components were diverse and that triterpenes were the major constituents. Hashim et al. (30) specifically studied the triterpene constituents and their biological activities in ethanolic-aqueous extract from *C. asiatica* grown in a forest reserve in Malaysia. They found that there were significant amounts of madecassoside and asiaticoside, but low amounts of asiatic and madecassic acid. Finally and most recently, Devkota et al. (31) studied the content of essential oils from *C. asiatica* grown in different habitats in Nepal. They found that the essential oils varied from the ones found in South Africa. The habitat also had an influence, with *C. asiatica* grown in shady grasslands containing 39 oils, open grassland 34, and open agricultural lands 36.

Traditional and modern preparations vary between countries. In Malaysia, herbal remedies such as *C. asiatica* are prepared traditionally by traditional healers and tailored to individual needs. Most are in the form of juice and oil, and modern preparations are in tablet form (32). In India, *C. asiatica* is used as a paste applied to the skin, cold poultice used for rheumatism, leaf juice rubbed on the forehead for headache, or ointment or powder mixed with bath water (33). The leaves are also cooked and eaten, while in Malaysia it is eaten raw as a salad (34).

Enhancement and Regeneration of Nerve Cells

As synthetic drugs can lead to lung and kidney toxicity, many clinicians and scientists have searched vigorously for other alternatives to treat their patients. Herbal plants which are naturally rich in therapeutic value, more eco-friendly and have lesser side effects have been subsequently studied for utilisation in medical applications. This has brought *C. asiatica*, which is known to have memory and cognitive enhancement, into scientific investigations for nerve regeneration and neurological functions before therapeutic use.

Research on the neuroregenerative capacity of *C. asiatica* on the central nervous system has been widely conducted, focusing on brain cells. Soumyanath et al. (35) revealed the ability of asiatic acid (AA) to promote the elongation of neurites using an in vitro experimental model. They also perceived that this action could be co-stimulated with the other active compounds found in the herb that have synergistic effects with AA. In parallel with this, Rao et al. (36) reported that fresh *C. asiatica* leaf extract significantly increases the dendritic arborisation of hippocampal CA3 neurons in vivo. In view of *C. asiatica* as a nerve tonic (37), it is believed to have therapeutic effects on the peripheral nervous system too. However, further investigations are needed to bring it into evidence, as the literature on such nervous system benefits is far too limited to date.

Since variation of extract constituents can lead to various complications, scientists from Thailand have recently established a standardised extract of *C. asiatica* known as ECa 233 (38). The extract was clearly demonstrated to have stimulatory effects on the elongation of neuroblastoma cell neurites at a maximum dose of 100 μg/mL (39). In spite of dendritic arborisation, this particular herb also exhibits neuroprotective properties. Zhang and his colleagues (40) have recently disclosed that AA ameliorates the action of C2-ceramide in inducing neuronal cell death in a concentration-dependent manner.

Though the underlying mechanisms whereby the herb exerts its effects are poorly understood, the aforementioned properties synchronise with the mastery of this herb to phosphorylate several signalling pathways to mediate its functions. Indeed, the mechanisms involved in neurite development are quite complex, but the MEK/ERK and PI3/Akt signalling pathways have gained significant attention (41). A previous report elucidated that AA in *C. asiatica* elicits its neuroregenerative effect via the MAP kinase pathway (35). In agreement with this, Wanakhachornkrai et al. (39) also revealed that ECa 233 is MEK/ERK- and PI3K/AKT-dependent in promoting the elongation of neurites.

Besides that, Omar et al. (42) recently disclosed the regulation of the caspase-9 pathway by *C. asiatica* in modulating neuron cell survival against apoptosis. In this study, l-buthionine-(S,R)-sulfoximine (BSO)-induced human neuron cell death was treated with ethanolic extract of *C. asiatica* in the range of 5–500 μg/mL. They found that, at low concentrations, this extract is able to protect neuron cells against oxidative stress. This may point to the capacity of this herb in inhibiting the caspase-9 pathway by hindering the production of proapoptotic proteins, promoting antiapoptotic proteins and imitating the latter (43,44). Though this mechanism is apparently focused on neuroprotective effects against apoptosis, such a mechanism could be a subset in the regeneration and enhancement of nerve

cell injuries. All the aforementioned mechanisms overlap each other in maintaining nerve cell growth.

Effects of *C. asiatica* on Systemic Diseases

Among the many uses of *C. asiatica* in traditional medicine is as a brain tonic to increase memory performance (45,46). Also, *C. asiatica* has been used to treat neurodegenerative diseases (47) such as Alzheimer's disease, characterised by a decline in cognitive functions, and in Parkinson's disease, characterised by loss of locomotor control due to a decrease of neurotransmitters in the brain. The common denominator in these two diseases is the purported mechanism underlying their pathologies, which is oxidative stress. In this review, the effect of *C. asiatica* on ameliorating oxidative *stress in vitro and in vivo* will be described first, followed by the effect of *C. asiatica* on Alzheimer's disease and Parkinson's disease. Other mental illnesses of interest are depression and anxiety, and epilepsy.

Oxidative stress occurs when more reactive oxygen species (ROS) than antioxidants are present in the body. ROS protect the body from foreign insults such as from xenobiotics which enter the body. However, when in excess or when the antioxidant level in the body is reduced, ROS cause damage to native nucleic acids, proteins and lipids and have been implicated in neurodegeneration, cancer and ageing (48).

The effect of *C. asiatica* in improving mental performance has been attributed to its property as an antioxidant and as a promoter of antioxidant production. This has been validated through many ROS scavenging assays (radical scavenging assays) in vitro and in vivo. Anand et al. (49) reported that, with the 1, 1 diphenyl–2–picrylhydrazyl (DPPH) free radical scavenging activity assay, the methanol extract of *C. asiatica* has the highest free radical scavenging activity followed by the hexane extract. This can be attributed to the higher levels of polyphenols and flavonoids present in this fraction, which impart the antioxidant function. Anand et al. also reported on the reducing capability of *C. asiatica* extract to inactivate hydroxyls, the most reactive ROS. Again, methanol extract showed the highest reducing power.

Three assays were conducted by Chippada and Vangalapati (50) on the methanol extract of *C. asiatica*: DPPH, reducing power and nitric oxide (NO) radical scavenging assays. They found that methanolic extract of *C. asiatica* has concentration-dependent DPPH scavenging, reducing power and NO radical scavenging activity up to 2000 µg/mL. The ethanolic extract of *C. asiatica* was also confirmed, using the DPPH and superoxide radical scavenging activity assays, to have significant antioxidant activity (51). Ariffin et al. (52) tested *C. asiatica* prepared as herbal teas with the DPPH and ferric-reducing antioxidant potential (FRAP) assays. They found that *C. asiatica* herbal teas possess significantly high antioxidant properties, with non-fermented teas having a higher antioxidant activity than fermented ones.

A study on reproductivity in male rats by Sainath et al. (53) found antioxidant activity of *C. asiatica* against lead-induced oxidative stress. The rats were given access to water containing lead ad libitum and a group was also given *C. asiatica* extract. After 70 days, the rats were sacrificed and the liver, brain, kidney, testis, epididymis, prostate gland, vas deferens and seminal vesicles were examined. They found that, in the group given the extract, there was a decrease in lipid peroxidation and an elevation of antioxidant enzyme activity. The extract showed antioxidant activity in all three assays. On comparison of the methanol extract with ascorbic acid, Singh et al. (54) found the extract to have concentration-dependent antioxidant activity. All of these studies clearly indicated the antioxidant potential of *C. asiatica* extract, specifically of the methanolic extract.

There are two hypotheses for the development of Alzheimer's disease: the cholinergic hypothesis and the amyloid cascade hypothesis (52). In the first, it is hypothesised that cognitive decline in Alzheimer's patients is due to degeneration of cholinergic neurons or cholinergic transmission. Therefore, an increase in the availability of acetylcholine is advantageous and is the reason for current medical treatment with acetylcholinesterase inhibitors. The other hypothesis is that neuronal damage is due to deposition of amyloid plaques formed from beta-amyloids (Aβ). These plaques are thought to be formed due to oxidative insults which lead to inflammation, lipid peroxidation and plaque formation and deposition in the brain.

To investigate this theory, Ramesh et al. (55) studied the effect of *C. asiatica* aqueous extract on beta-amyloid fraction 42 (β42) in vitro. They found that the extract did not inhibit Aβ aggregation and did not disintegrate already formed fibrils. They therefore proposed that the beneficial effect of *C. asiatica* seen in Alzheimer's patients could be due to antioxidative or anti-inflammatory properties of *C. asiatica*, or due

to an enhanced secretase pathway, an enzyme pathway that degrades the formed plaques. Defillipo et al. (56) found that water extract of *C. asiatica* inhibited phospholipase A2 (PLA2) enzymes, particularly cPLA2 and sPLA2, which are the key players in Aβ-induced neurotoxicity. Further studies on the water extract done by Gray et al. (57,58) found caffeoylquinic acids as the active substances preventing Aβ-induced cell death, having antioxidative properties and enhancing mitochondrial biogenesis. Thus, *C. asiatica* was suggested as a prospective remedy for Aβ-induced neuroinflammation and oxidative stress. Then, Rahman et al. (51) proved that *C. asiatica* has antioxidant properties by further testing the ethanolic extract on the activity of acetylcholinesterase (AchE) and butyrylcholinesterase (BchE). They found that the extract inhibited these two enzymes and could therefore increase availability of acetylcholine for cholinergic transmission. Focusing at the molecular level, *C. asiatica* was found to increase phosphorylation of cAMP response element-binding protein (CREB) in neuroblastoma cells expressing Aβ, possibly mediated by the ERK/RSK signalling pathway (59).

Soumyanath et al. (35) experimented on the Tg2576 transgenic mouse model of Alzheimer's disease. This model contains Aβ plaques, astrocytic and microglial activation and dystrophic changes. On behavioural analysis with the Morris water maze test, the mice showed impaired spatial memory. On treatment with aqueous extract of *C. asiatica*, there was an improved learning curve comparable to wild-type mice. On dissection, however, there was no significant difference of Aβ content. In this preliminary study, they found that the aqueous extract of *C. asiatica* does not show inhibition of AchE and BchE, offers no protection against hydrogen peroxide oxidative stress and has no effect on glutamate neurotoxicity. Aqueous extract of *C. asiatica* is known to be void of AA, its main antioxidant. This could be the cause of the absence of antioxidative functions. However, since the aqueous extract does show behavioural improvements comparable to wild-type mice, the authors proposed the ability of the aqueous extract to affect the pathology of Alzheimer's disease through a different mechanism or pathway, and also the presence of novel compounds yet undetermined in the extract.

Xu et al. (60) tested AA in vitro on glutamate-induced neurotoxicity in SH-SY5Y cells and in vivo on a monosodium glutamate (MSG)-induced dementia animal model. Oxidative stress can cause apoptosis of cells and compromise mitochondrial function. AA reduced the incidence of apoptosis by 5% compared to untreated SH-SY5Y cells. AA also prevented the decline in mitochondrial membrane potential (MMP) which is a measure of mitochondrial function. In gene expression analysis, AA treatment causes upregulation of Sirt1 and PGC-1 genes responsible for mitochondrial biogenesis and functions, and hence positively affects their survival and longevity.

Parkinson's disease, on the other hand, is a neurodegenerative disease associated with the loss of dopaminergic neurons in the basal ganglia. The exact mechanism of neurodegeneration is currently unknown, but oxidative stress is thought to be one of the causes. Mitochondrial dysfunction and ROS generation is also thought to be involved. Haleagrahara and Ponnusamy (61) evaluated the effect of aqueous extract of *C. asiatica* on 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced Parkinsonism in aged Sprague-Dawley rats. MPTP binds in mitochondrial complex 1 and causes dopamine depletion in the striatum. The rats received the extract orally for 21 days, and were then sacrificed and analysed for lipid hydroperoxides, protein carbonyl content, xanthine oxidase, superoxide dismutase, glutathione peroxidase, catalase and total antioxidants. In all the parameters, *C. asiatica* when given together with the MPTP-induced oxidative stress, reversed the neurotoxic effect of MPTP towards values in the normal condition. Focusing more on the bioactive constituent of *C. asiatica*, Xu et al. (62) evaluated the effect of asiaticoside on an MPTP-induced rat model of Parkinson's disease. They performed two behavioural tests: open-field testing and ladder walking. Then, they sacrificed the rats to measure dopamine (DA) and its metabolite malonyldialdehyde (MDA), reduced glutathione (GSH) and gene expression. They found that treatment with asiaticoside for 7 and 14 days resulted in attenuation of the effects of MPTP on the behavioural tests, reduced MDA which is a marker of oxidative stress, and modulated the reduction of DA with increased concentration of GSH. Xu et al. (63) repeated the same protocol with madecassoside and found similar results. In addition, they evaluated expression of BDNF (brain-derived neurotrophic factor), which is usually reduced in Parkinson's disease. Indeed, madecassoside upregulates BDNF expression.

The role of *C. asiatica* has not been widely researched for its application in mental stress or illness. However, it has been used in Ayurvedic

medicine as a rasayana, a health tonic. In this regard, *C. asiatica* is hypothesised to act as an adaptogen to the daily stresses impacting the mind (64). Segen's Medical Dictionary defines adaptogens as a family of natural substances that compensate for fluctuations in homeostasis. Jana et al. (64) studied 33 individuals from the age of 18 to 60 with diagnosis of generalised anxiety disorder (GAD); they were withheld from antidepressant medication and given *C. asiatica* in 500 mg capsules for 60 days. Scales of stress, anxiety, depression, adjustment and attention were evaluated. All the scale scores improved after 60 days, with no side effects of vertigo, nausea and dizziness as experienced with the common medications. Kalshetty et al. (65) evaluated the antidepressant effect of *C. asiatica* extract standardised to asiaticoside (INDCA) on an animal model of chronic behavioural depression. An olfactory bulbectomy was done on the rats to induce the chronic depression. Treatment with INDCA showed dose-dependent reversal of the body weight, body temperature and heart beat reduction seen in behavioural depression, with normal activity in open-field and elevated plus maze tests. It was proposed that INDCA exerts an antidepressant effect in these rats.

The effect of ethanolic extract of *C. asiatica* on an acute depression rat model (forced swim test model) was investigated by Selvi et al. (21). They found that *C. asiatica* has a similar effect to the commercial antidepressants imipramine and diazepam, but at a lower level. They caused reduced immobility time and increased exploratory behaviour. Evaluating parameters of behaviour, Wanasuntronwong et al. (38) tested standardised *C. asiatica* extract, ECa 233, on chronically immobilised mice, a model of anxiety. They tested the mice in elevated plus maze, light-dark box and open-field tests and measured their body weight and serum corticosterone. Diazepam, a benzodiazepine used to treat anxiety, acts by binding to the GABAA receptor and inhibits its activity while downregulating corticotrophin releasing hormone (CRH), leading to a reduced corticosterone level in the blood. Based on the behavioural test, ECa 233 showed an anxiolytic effect, and blood analysis showed a reduced corticosterone level. Therefore, there is a possibility that the anxiolytic effect of ECa 233 works by the same mechanism as diazepam. Saravanan and Sarumati (66) further investigated the effect of aqueous extract of *C. asiatica* on immobilisation-induced stress in rats. They measured erythrocyte and leucocyte counts and AchE activity. These parameters were reduced on stress, but with co-administration of *C. asiatica*, the values reverted back to normal, suggesting its anti-stress property.

As for epilepsy, Visweswari et al. (67) evaluated the effect of pre-treatment for one week with n-hexane, chloroform, ethylacetate, n-butanol and water extracts of *C. asiatica* on pentylenetetrazol (PTZ)-induced seizures. During the seizures, there is a high content of acetylcholine and reduced AchE. However, in the rats pre-treated with n-hexane, ethyl acetate and n-butanol extract of *C. asiatica*, there were reduced Ach and increased AchE levels. This is considered as anti-seizure activity. In another paper, Visweswari et al. (68) again evaluated different extractions of *C. asiatica* on Na+/K+, Mg2+ and Ca2+ ATPases in rat brains during PTZ-induced epilepsy, because these enzymes play a crucial role in maintaining membrane potential to prevent neuron hyperexcitability and are inhibited during seizures. Pre-treatment with *C. asiatica* was found to increase ATPase activity levels throughout the brain.

In Vivo Studies of *C. asiatica* on Nerve or Brain Tissue Regeneration

In a study by Soumyanath et al. (35), ethanolic extract of *C. asiatica* was supplemented in the drinking water of Sprague-Dawley rats at a concentration of 2 mg/mL. The study demonstrated that *C. asiatica* promotes more rapid functional recovery (toe-spread and walking onset) and increased axonal regeneration (larger axon diameter and greater number of myelinated axons) in rats with sciatic bilateral nerve crush. Studies on the effect of *C. asiatica* as crude extract or its components on periphery nerve repair and regeneration are very limited as no in vivo studies on periphery nerve regeneration besides this study have been conducted to date.

A study on amygdaloid neurons and hippocampal CA3 neurons of rat pups (7 days old) fed with 2, 4 and 6 mL/kg body weight of fresh of *C. asiatica* leaf juice for 2, 4 and 6 weeks showed a significant increase in dendritic length and dendritic branching points. The effects were dose- and duration-dependent as only the neurons of rats treated with 4 and 6 mL *C. asiatica*/kg body weight/day for longer periods of time (4 and 6 weeks) showed significant increases (36,69). Enhancement of neuronal dendritic arborisation by fresh *C. asiatica* leaf juice supplementation in neonatal rats has been found to correlate with improved learning and memory (70).

Effects of *C. asiatica* on Neurotoxicity and Brain Injury

A study in rats with middle cerebral artery occlusion (MCAO) showed that *C. asiatica* improves neurobehavioral activity and reduces tissue death due to lack of oxygen. The rats were orally fed with *C. asiatica* extract (100, 200 and 300 mg/kg body weight) for 21 days. Subsequently, the rats underwent MCAO for 2 h followed by 22 h reperfusion. Neurobehavioral analysis by flexion test, spontaneous motor activity evaluation, grip strength and muscular coordination showed that the rats administrated with *C. asiatica* (200 and 300 mg/kg body weight) were significantly less affected by MCAO compared to the control group. The infarction volume upon MCAO was also shown to be significantly decreased in both the 200 and 300 mg *C. asiatica*/kg body weight groups. Furthermore, rats in these groups also had significant protection from neuronal injury, evidenced by more intact hippocampal and cortical neurons (71).

Learning and Memory Enhancement by *C. asiatica*

Memory enhancement effects of *C. asiatica* have been documented in Ayurvedic medicine since ancient times (72). Few randomised, placebo-controlled and/or blinded clinical studies have proven the effect of *C. asiatica* in improving cognitive function and enhancing memory (73–76). The studies found that *C. asiatica* intake for a period ranging from 2 to 6 months reduced age-related decline in cognitive function in healthy middle-aged adults and the elderly with mild cognitive impairment (MCI). Furthermore, other MCI-related conditions such as hypertension, insomnia, loss of appetite and constipation were also found to be improved following *C. asiatica* intake (76). Recent animal studies have also confirmed that *C. asiatica* supplementation improves memory performance in rats with memory dysfunction due to oxidative stress (77,78). However, a study by Jared (79) showed that *C. asiatica* uptake by rats only enhanced retention of memory but did not improve the learning process.

Recent preclinical studies have started to explore the compounds of *C. asiatica* that are responsible for cognitive and memory enhancement function. AA, a pentacyclic triterpene in *C. asiatica*, was found to significantly reduce glutamate-induced cognitive deficits. The administration of oral AA (100 mg/kg) was found to restore levels of lipid peroxidation and GSH, and the activity of superoxide dismutase in the hippocampus and cortex to control levels (55). It was also found that AA protects against glutamate-induced dementia and enhanced recovery from learning and memory deficit due to glutamate uptake in a mouse model (48). Another study by Nasir et al. (80) also found that supplementation of AA in normal rats significantly improved memory and learning in a dose-dependent manner.

Future Direction

As can be observed from the studies mentioned above, *C. asiatica* does have proven neuroprotective and neuroregenerative properties in animal models and also in humans. However, more studies are still needed to identify the compounds of *C. asiatica* that are particularly involved in neuroprotective and neuroregenerative properties and also the mechanism of action of these compounds. Besides that, the extraction method, biochemical profile, dosage information and influence of geographical and other factors in determining the biochemical profile of the plant need to be ascertained and standardised by more scientific studies. Furthermore, the effects of the *C. asiatica* extract on in vitro mesenchymal stem cell differentiation into neural lineage cells and on the in vivo model of nerve injury still remain to be explored. Thus, further studies on those areas of *C. asiatica* need to be undertaken to greatly enhance the economic value of this traditional herb and to accelerate the usage of this herb in clinical applications.

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Conflicts of Interest

The authors declare they have no conflict of interest.

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Authors' Contributions

Conception and design: YL, RHI
Analysis and interpretation of the data, collection and assembly of data: YL, NO, NNAP, AS
Drafting of the article: YL, NO, NNAP, AS, RHI
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