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REVIEW

A review of antioxidant and anti-acetylcholinesterase activities of *Centella asiatica* (L.) Urb. for the treatment of Alzheimer's disease

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Abstract

Of many neurodegenerative diseases, Alzheimer's disease (AD) is the most common cause of dementia globally, which is still incurable even after decades of extensive research to find a definite and permanent cure. Accumulation of free radicals and acetylcholine (ACh) deficiency in the brains of Alzheimer's patients are considered as the factors leading to dementia and cognitive problems. This is by mechanisms involving disturbance in the balance between the amount of free radicals and the antioxidant defense system along with cholinergic deficit leading to impaired cholinergic neurotransmission. Currently available anti-AD drug therapy carries only the partial benefit of slowing down the progression of disease besides having various side effects and, hence, there is a developing interest to search for new drugs. Plants have always been of special interest in the field of new drug discovery research by virtue of their enormous biological diversity and tremendous potential of bioactive molecules. Many herbs are known to ameliorate the symptoms related to memory and cognitive disorders. *Centella asiatica* (L.) Urb. is one of the widely used plants claimed by the traditional system of medicine to have its positive effects on memory and brain functioning. The objective of this review is to present a comprehensive account on *C. asiatica* by summarizing the research until date related to its medicinal potential with a special focus on antioxidant and anti-acetylcholinesterase (anti-AChE) properties reflecting their potential therapeutic application for the treatment of AD.

1. Introduction

Alzheimer's disease (AD) is the most common worldwide dementia disorder of old age that is still incurable even after more than a hundred years of its discovery. Due to its worldwide prevalence and progressive nature, AD has high social and economic impacts on society (Franceschi *et al.*, 2018). Referring to 50 million dementia patients worldwide in 2018, it is expected that the number of dementia patients will rise to 82 million by 2030, and 152 million by 2050. In 2018, US$ 1 trillion were spent worldwide on the treatment of dementia and it is expected that this number would double by 2030. There were 9.9 million newly reported dementia cases around the globe, that is, a new dementia case every 3 seconds (Patterson, 2018). The adverse changes in the brain for the development of AD include extraneuronal deposition of protein amyloidβ (known as beta-amyloid plaques) along with the deposition of an abnormal tau protein inside the neurons (known as tau tangles). These pathological brain changes lead to neurodegeneration (Chen and Mobley, 2019) and have been linked to a variety of etiological factors like neuroinflammation, neuronal membranes damage, gene mutations (below 5%), oxidative stress, formation of toxic molecules, protein misfolding, and dysfunctional mitochondria (Wider and Wszolek, 2008).

The oxidative stress and cholinergic hypotheses for the onset and development of AD are widely accepted for their relevance to anti-AD drug discovery based on plant-derived natural products (Habtemariam, 2019). As per oxidative stress hypothesis, the accumulation of excessively high levels of free radicals or reactive oxygen species (ROS) in the brain leads to a state of oxidative stress that may have deleterious effects culminating in the neurodegeneration. This is a characteristic feature of Alzheimer's brains. The literature has strong evidence to show the relationship between oxidative attack on the brain and the etiopathology of Alzheimer-related dementia (Huang *et al.*, 2016). As speculated by many research findings, the

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pathological accumulation of free radicals in the brain overburdens the antioxidant defense system of body thereby disturbing the balance between the amount of ROS and available antioxidant reserves which, in turn, is profoundly detrimental to the brain. It may result in the formation of senile plaques in the brains of Alzheimer patients leading to dementia (Abdel Moneim, 2015; Wojsiat *et al.*, 2018). On the other hand, cholinergic hypothesis deals with the disturbance in neurochemical homeostasis resulting in a persistent decline in cholinergic neurotransmission. The impairment of cognitive functions in AD has been shown to occur as a result of cholinergic deficit and subsequently decreasing levels of a neurotransmitter, the acetylcholine (ACh), in the brain due to its enzymatic hydrolysis by cholinesterases which is linked to amyloid plaques (Hampel *et al.*, 2018; Sultzer, 2018). These findings suggest a putative role of oxidative stress and cholinergic deficit in the development, progression and chronicity of AD and their importance in defining anti-AD therapeutic goals.

The scientific community is actively engaged in the characterization of plant-derived therapeutic agents for the treatment of AD for which there are promising results in the literature (Sarris *et al*., 2011; Kumar and Khanum, 2012; Naaz *et al*., 2013; Yusoff *et al*., 2014; Zainol Abidin *et al*., 2020). *Centella asiatica* (L.) Urb (*C. asiatica*) is one of the important medicinal plants used by traditional system of medicine for the treatment of a diverse range of health problems including cognitive disorders (Cheng and Koo, 2000; Hamid *et al*., 2002; Subathra *et al*., 2005; Jamil *et al*., 2007; Pittella *et al*., 2009; Saha *et al*., 2013). In addition, *C. asiatica* has been shown to have antioxidant and anti-acetylcholinesterase (anti-AChE) properties (Jamil *et al*., 2007). This review attempts to summarize the research findings up to date related to the therapeutic potential of *C. asiatica* for the treatment of AD specifically for its antioxidant and anti-AChE activities. In this review, we have discussed the etiopathology of AD followed by a detailed account on *C. asiatica* describing the research findings to date related to its medicinal properties, particularly the antioxidant and anti-AChE activities. The potential therapeutic application of *C. asiatica* for the treatment of AD including the future prospects has also been discussed.

2. Alzheimer's brain: the neurochemical aspects

The brain is made of billions of nerve cells, the neurons, which act as structural and functional units of the nervous system. All the activities of the nervous system are conferred to the health and integrity of these fundamental brain units. Alzheimer's brains present a complex state which may be a combination of neurodegeneration, cholinergic deficit, characteristic neuronal Aβ plaques, and neurofibrillary tangles of tau protein (Sadigh-Eteghad *et al.*, 2015). A review has indicated that vascular abnormalities may lead to the neurodegeneration in AD (Zlokovic, 2011). Irrespective of the causative factors, cholinergic deficit and the oxidative stress appear to be the hallmarks of Alzheimer's brains (Francis *et al*., 1999; Zhao and Zhao, 2013). A decline in cholinergic neurotransmission has been proposed due to the degenerative cholinergic neurons in basal forebrain resulting in the cognitive decline in AD patients (Mann, 1996). Cholinergic neurotransmission involves acetylcholine, a neurotransmitter. In this context, the research evidence suggests that cholinergic deficit is related to either the reduced activity of enzymes involved in the synthesis of ACh or the hyperactivity of AChE (Sims *et al*., 1983; DeKosky *et al*., 1992). AChE is not only involved in the excessive breakdown of ACh, but it also contributes to the formation of Alzheimer's fibrils by increasing the process of assembly of amyloid peptides (Inestrosa *et al*., 1996) besides increasing their neurotoxicity by making stable complexes with Aβ (Alvarez *et al*., 1998).

In addition to the cholinergic impairment, oxidative stress is also another important factor for the onset and progression of AD (Padurariu *et al*., 2013; Zhao and Zhao, 2013). Oxidative stress arises when there is an insufficiency of physiological antioxidant defense system to combat with the accumulated free radicals or reactive oxygen species (ROS) in the body. Although ROS may originate from exogenous factors like tobacco smoking, radiations, etc., most of the ROS are intracellular products of normal physiological working of the body (Phaniendra *et al.*, 2015). Free radicals have the ability to interact with almost all types of biological molecules and may disrupt their normal functions with adverse effects on cellular machinery (Nimse and Pal, 2015). Susceptibility of different body tissues to oxidative damage is variable but brain tissue is considered as more vulnerable to oxidative stress on account of being rich in lipid content and high rate of oxidative metabolism (Cobley *et al.*, 2018). Elevated levels of ROS in AD are attributed to defects in the mitochondrial electron transport chain and extracellular Aβ deposition which leads to localized inflammation and microglial activation both acting as potential sources of ROS (Guo *et al.*, 2013; Tönnies and Trushina, 2017). Linked to AD, model mice experiments have shown high oxidative stress and increased deposition of Aβ in response to a defective antioxidant defense system (Tönnies and Trushina, 2017; Martins *et al.*, 2018). The fibrillar pathogenesis, one of the hallmarks of AD, is also linked with a relatively high rate of fatty acids oxidation

which acts as a facilitator for the hyperphosphorylation and polymerization of tau protein (Butterfield and Boyd-Kimball, 2018). Also, the accumulation of a complex of peroxidized lipid and protein, the lipofuscin, has been found in neurons of Alzheimer's brains (Perry *et al*., 2002).

3. *Centella asiatica*

3.1 Geographical and botanical description

Centella asiatica (L.) Urban (syn. *Hydrocotyle asiatica* L.) belongs to the family Apiaceae (Umbelliferae) (Table 1), known by many names such as "gotu kola, Indian pennywort, Asiatic pennywort, tiger herb, mandukaparni, brahmi", is a tropical, herbaceous plant belonging to the family Apiaceae and native to low, wet swampy areas of Southeast Asia including Malaysia, Indonesia, Sri Lanka, China, India and also found in Africa, Australia and Madagascar. In view of its great medicinal significance, it is specially cultivated in some areas of the world like India, Africa, and Turkey (James and Dubery, 2009; Shinomol and Ravikumar, 2010; Orhan, 2012).

Table 1. Taxonomy of *C. asiatica*

Kingdom	Plantae
Division	Tracheophyta
Subdivision	Spermatophyta
Class	Magnoliopsida
Order	Apiales
Family	Apiaceae (Umbelliferea)
Genus	Centella
Species	*Centella asiatica*

The plant is greenish in color with a greyish tinge, having a characteristic smell and sweet but slightly bitter in taste. The plant body is herbaceous, slender, creeping, nodular rooting with 1.3-6.3 cm in diameter leaves having 2-5 cm long leaf stalks without pedicel and contains an umbel type inflorescence having 1-5 flowers which are reddish and sessile. The plant contains 0.8 cm long, small, but compressed fruit with rectangular mericarps, thick pericarp and laterally compressed seeds (Vohra *et al.*, 2011; Chandrika and Kumara, 2015).

3.2 Phytochemical constituents

C. asiatica is known to contain a wide variety of phytochemicals, the secondary metabolites, which signifies the tremendous medicinal importance of this ancient herb. It has been reported to contain the phytochemicals including triterpenoids or saponins, volatile and fatty acids, glycosides, flavonoids, alkaloids, and certain vitamins and free amino acids (Jamil *et al.*, 2007; Latif *et al*., 2019; Orhan, 2012). A concise summary of the important phytochemical constituents of *C. asiatica* has been presented in Table 2 along with representative examples of each main group of *C. asiatica* phytochemicals.

3.3 Medicinal importance of Centella asiatica – an overview

C. asiatica is well supported for its medicinal importance in a range of health problems due to its diverse biological actions and is claimed to be successfully practiced in Ayurvedic and Traditional Chinese Systems of Medicine for centuries for a number of medical problems including the cognitive disorders (Gohil *et al*., 2010; Orhan, 2012). Many research reports have indicated that the alkaloids and flavonoids present in different extracts of *C. asiatica* possess antimicrobial activity against a range of pathogenic microbes (Brinkhaus *et al.*, 2000; Zaidan *et al.*, 2005; Ullah *et al.*, 2009; Yasurin *et al.*, 2016; Jayaprakash and Nagarajan, 2016; Aftab *et al.*, 2017; Idris and Nadzir, 2017; Viera *et al.*, 2017; Prakash *et al.*, 2017; Mahalik *et al.*, 2019; Selvam *et al.*, 2019). Different *C. asiatica* extracts have been found medicinally effective as gastroprotective against cold-induced, stress-induced, ethanol-induced and acetic acid-induced gastric ulcers in rats (Chatterjee *et al.*, 1992; Cheng and Koo, 2000; Sairam *et al.*, 2001; Cheng *et al.*, 2004; Jamil *et al.*, 2007). Many studies have suggested the hepatoprotective role of *C. asiatica in vitro* as well as *in vivo* (Lin *et al.*, 2002; Antony *et al.*, 2006; Pingale, 2008; Hussin *et al.*, 2014). There are multiple research reports claiming the efficacy of *C. asiatica* as an anti-inflammatory agent by virtue of its diverse range of bioactive compounds (George and Joseph, 2009; Nhiem *et al.*, 2011; Saha *et al.*, 2013; Wan *et al.*, 2013). Anti-tumor and cytotoxic activities have also been reported for *C. asiatica* (Babu *et al.*, 1995; Bunpo *et al.*, 2004; Babykutty *et al.*, 2009; Pittella *et al.*, 2009; Ullah *et al.*, 2009).

The topical application of *C. asiatica* has been found efficacious for the treatment of skin ageing in addition to its beneficial role in wound healing (Shetty *et al.*, 2006; Haftek *et al.*, 2008; Kim *et al.*, 2011; Kwon *et al.*, 2012; Ruszymah *et al.*, 2012; Bylka *et al.*, 2014). The methanolic fraction of *C. asiatica* has proved its efficacy for wound healing in vitro as well as in vivo (Azis *et al.*, 2017). Triterpenoid saponins of *C. asiatica* showed immunomodulatory function while aqueous, methanolic, ethanolic and dichloromethane extracts of *C. asiatica* exhibited enhancement of immune function as depicted by various immunity assessment parameters (Jayathirtha and Mishra, 2004; Punturee *et al.*, 2005; Wang *et al.*, 2005; Pan *et al.*, 2010). These studies indicate the therapeutic potential of various extracts of *C. asiatica* for the management and treatment of different ailments.

Table 2. Phytochemical constituents of *C. asiatica*

Phytochemical Group	Representative Compounds	Reference
Alkaloid	Hydrocotylin	(Jamil *et al.,* 2007)
Carotenoids	β-carotene Neoxanthei Violaxanthin Leutein	(Chandrika and Kumara, 2015)
Fatty Acid Glycerides	Palmitic acid Stearic acid Lignoceric acid Oleic acid Linoleic acid Linolenic acid	(Jamil *et al*., 2007)
Flavonoids	3-glucosylquercetin 3-glucosylkaempferol 7-glucosylkaempferol Catechin Epicatechin Castilliferol Castillicetin Apigenin Quercetin Rutin Luteolin Naringin	(Miean and Mohamed, 2001; Vohra *et al*., 2011; Roy *et al*., 2013; Gray *et al*., 2018)
Minerals	Calcium Phosphrus Iron Magnesium Manganese Potassium Copper Zinc Sodium	(Chong *et al*., 2011; Chandrika and Kumara, 2015)
Phenylpropanoids	Rosemarinic acid Chlorogenic acid Isochlorogenic acid Caffeoyl quinic acids	(Chong *et al*., 2011)
Saponin Glycosides	Asiaticosides Brahminoside Brahmosides Centellosides Centellin Cenetillicin Madecassosides	(Jamil *et al*., 2007; Siddiqui *et al*., 2007)
Tannins	Tannin Phlobatannin	(Chong *et al*., 2011)

Table 2 (Cont.). Phytochemical constituents of *C. asiatica*

Phytochemical Group	Representative Compounds	Reference
Terpenoids	Asiatic acid Asiaticin Brahmic acid Brahminoside Madecassic acid Thankuniside Sesquiterpenes: α-Humulene, β-caryophyllene, bicyclygermacrene, germacrene B and germacrene D Monoterpenes: myrcene, γ-terpinene and α-pinene	(Singh and Rastogi, 1969; Aziz *et al.,* 2007; Siddiqui *et al*., 2007; Akula and Odhav, 2008; Hashim *et al*., 2011)
Vitamins	Ascorbic acid Nicotinic acid β-carotene Vitamin A Thiamine Riboflavin Niacin	(Chong *et al.,* 2011, Chandrika and Kumara, 2015)
Miscellaneous	Phytosterols Resin Vallerine Limonene Menthone Bornyl acetate α-elemene β-elemene γ-curcumene Spauthulenol Caryophyllene oxide Mintsulfide	(Akula and Odhav, 2008; Chong *et al*., 2011; Roy *et al.,* 2013; Chandrika and Kumara, 2015)

The animal studies on rats and rabbits have investigated the cardioprotective effect of *C. asiatica* for the treatment of experimentally induced cardiac pathological conditions (Pragada *et al.*, 2004; Li *et al.*, 2007; Bian *et al.*, 2008). A number of research findings have signified the medicinal importance of *C. asiatica* in the treatment of diabetes and related diabetic complications (Rao and Mastan, 2007; Ramachandran and Saravanan, 2013; Alqahtani *et al.*, 2013; Kabir *et al.*, 2014; Supkamonseni *et al.*, 2014). The radioprotective function of *C. asiatica* against γ- and UVB-irradiation of experimental animals has also been investigated and proved to be efficacious in radiation-induced damage (Shobi and Goel, 2001; Sharma and Sharma, 2002; Sharma and Sharma, 2005; An *et al.*, 2012; Joy and Nair, 2009). A study investigated the anabolic potential of *C. asiatica* and found significant effects as indicated by an overall increase in hemoglobin, blood protein nitrogen and an associated decrease in the mean value of blood urea levels (Jamil *et al.*, 2007). Antiprotozoal, antifilarial, antiviral, antiallergic, antipruritic, antifertility, antitubercular and antispasmodic effects of *C. asiatica* have also been reported in the literature (Zaidan *et al.*, 2005; Ullah *et al.*, 2009).

A diverse range of various therapeutic effects on nervous system and related ailments has been reported in literature for *C. asiatica* as anticonvulsant, antidepressant, antiepileptic, anxiolytic, tranquilizing, sedative and neuroprotective (Jamil *et al.*, 2007; Kumar *et al.*, 2009; Gohil *et al.*, 2010; Visweswari *et al.*, 2010; Orhan, 2012; Mathew and Subramanian, 2014). In terms

of the neuroprotective role of *C. asiatica*, its functional properties related to AO and anti-AChE activities have been a promising area of research specially related to their role in ameliorating the symptoms related to neurodegenerative disorders like AD. The following part of the review will exclusively discuss the therapeutic potential of *C. asiatica* in terms of its antioxidant and anti-AChE activities as has been reported in the literature.

3.4 Potential anti-AD therapeutic activity

There are numerous research reports in the scientific literature that show the neuroprotective effects of *C. asiatica* and emphasize its immense potential as a candidate for anti-AD therapeutic agent (Brinkhaus *et al.*, 2000; Gohil *et al.*, 2010; Vohra *et al.*, 2011). As mentioned earlier, oxidative stress and cholinergic deficit are major factors related to AD pathology so *C. asiatica* will be reviewed in connection to the antioxidant and anti-AChE activities reported in the literature.

4. Antioxidant activity of *C. asiatica* extracts

C. asiatica has tremendous potential of being a natural source of antioxidant as its antioxidant activity has been found comparable to that of Rosemary and sage (Jamil *et al.*, 2007). Protective role of *C. asiatica* extract against γ-radiation induced DNA damage in vitro has been revealed by plasmid relaxation assay and the radioprotective function of *C. asiatica* has been suggested to its antioxidant property (Joy and Nair, 2009). In a comparative study involving 43 edible plants of Thailand, *C. asiatica* showed a comparatively high level of natural antioxidant compounds including vitamin C, vitamin E, carotenes, tannins and total phenolics (Chanwitheesuk *et al.*, 2005).

The leaves of *C. asiatica* have been tested in vitro for superoxide free radical activity, inhibition of linoleic acid peroxidation, and 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging activity and appeared to possess high antioxidant potential (Shukla *et al.*, 2012) which correlate with the findings that *C. asiatica* leaves possess a higher level of natural antioxidant (Odhav *et al.*, 2007; Akula and Odhav, 2008). Zainol *et al.* (2003) performed a comparative study to investigate the antioxidant activity of extracts from different parts of *C. asiatica* and the highest antioxidant activity was found in *C. asiatica* leaf extract. Their finding positively correlated with the phenolic contents of *C. asiatica* leaves which were found to have the highest phenolic content as compared to the other parts of *C. asiatica*. The results of this study suggested that phenolics might be the most potent bioactive compounds in terms of *C. asiatica* antioxidant activity. Contrary to this study, root extract showed the maximum antioxidant activity irrespective of the type of solvent used in a study that determined the antioxidant potential of *C. asiatica* in extracts from different plant parts including root, petiole and leaf. In particular, the ethanolic extracts of root and leaf showed antioxidant activity fairly comparable to α-tocopherol (Hamid *et al.*, 2002).

The statistically significant values of free radical scavenging activity in ethanolic extracts of *C. asiatica* leaves were found attributable to polyphenols and flavonoids as the plant secondary metabolites are likely to be more concentrated in this fraction. Also, the methanolic extract of *C. asiatica* leaves was found to have DNA damage protective activity (Anand *et al.*, 2010) and the finding is interesting in view of the concept that the accumulation of free radicals may lead to DNA damage and ageing, the risk factors for the onset and development of Alzheimer's disease.

The free radical scavenging activity of the aqueous extract of *C. asiatica* with an IC_{50} value of 31.25 μg/mL was found statistically significant against the IC_{50} values of 2.50 μg/mL and 7.58 μg/mL generated by ascorbic acid and butylated hydroxytoluene (BHT), respectively. The significantly high antioxidant activity of *C. asiatica* aqueous extract was hypothesized to the flavonoids (Pittella *et al.*, 2009) on account of having numerous hydroxyl groups, the hydrogen-donors, best suited to possess powerful free radical scavenging activity (Cao *et al.*, 1997; Mensor *et al.*, 2001). Ferric thiocyanate (FTC) test and thiobarbituric acid (TBA) test were used to find the antioxidant activity in aqueous extracts of six Malaysian herbs including *C. asiatica* and the highest antioxidant activity was shown by *C. asiatica* extract in TBA test (Huda-Faujan *et al.*, 2007).

4.1. Centella asiatica antioxidant activity: animal studies

As insufficiency of body's AO defence mechanism is considered as a hallmark of AD, an experimental mice model study elucidated the association of sporadic AD in humans with intracerebroventricular (ICV) streptozotocin (STZ) in rats and the production of free radicals with the cognitive impairment in this model. The cognitive dysfunction and oxidative stress were induced in male Wistar rats after bilaterally injecting ICV STZ (3 mg/kg) on days one and three followed by evaluation of therapeutic effects of *C. asiatica* aqueous extract for twenty-one days. After that, the behavioural task was completed and rats were killed to assess the level of oxidative stress in the whole brain. Interestingly, there was a dose-dependent improvement in cognition level as well as an increase in free radical scavengers in *C. asiatica* treated rats (Veerendra Kumar and Gupta, 2003).

The oral administration of 50 mg/kg/day crude methanolic extract of *C. asiatica* to a lymphoma-bearing mouse resulted in a significant increase in antioxidant enzymatic activity notably of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSHPx) (Jayashree *et al.*, 2003). Therapeutic effect of *C. asiatica* as antioxidant was studied on administering the *C. asiatica* extract (300 mg/kg body weight/day) for a period of 60 days by monitoring lipid peroxidation (LPO) and protein carbonyl (PCO) contents in selected five anatomical parts of aged rat brains viz. cortex, hypothalamus, striatum, cerebellum and hippocampus having low antioxidant status and high LPO and PCO contents in all five regions of brain compared to the control rats. The treatment with *C. asiatica* extract was found to be efficacious in decreasing LPO and PCO contents in regional brain areas with a relative increase in antioxidant level (Subathra *et al.*, 2005).

The alleviation of oxidative stress in Sprague-Dawley female rats pre-treated with monosodium glutamate was observed on administration of *C. asiatica* extract (100 and 200 mg/kg body weight) by monitoring SOD and CAT levels which showed a post-treatment increase while glutathione levels were unaffected (Hussin *et al.*, 2007). CV colchicine (15 µg/5 µL) was injected to male Wistar rats that produced a profound impairment to their memory and antioxidant level. Starting four days before colchicine treatment, administration of *C. asiatica* extract (150 and 300 mg/kg) spanning over twenty-five days resulted in a significant decrease in memory impairment and oxidative damage induced by colchicine (Kumar *et al.*, 2009). The same researchers investigated the role of *C. asiatica* as potent antioxidant in mice model with memory impairment and oxidative damage induced by a chronic administration of D-galactose (100 mg/kg) to mice over six weeks followed by *C. asiatica* treatment (150 and 300 mg/kg) for six weeks that had a significantly protective effect as shown by cognitive improvement and reversal of oxidative damage compared to control (Kumar *et al.*, 2011).

Male Wistar rats were kept on aluminium (100 mg/kg body weight) and *C. asiatica* (150 and 300 mg/kg body weight). *Centella asiatica* proved to have a neuroprotective function as reflected by a significant improvement in mitochondrial dysfunction in the experimental animal as compared to control (Prakash and Kumar, 2013). The aqueous extract of *C. asiatica* in doses of 200 and 300 mg/kg was tested for oxidative stress that significantly decreased the malondialdehyde (MDA) levels in the brain with a parallel increase in glutathione levels. At 300 mg/kg, *C. asiatica* extract was effective in increasing the CAT levels but SOD levels were not significantly increased (Kumar and Gupta, 2002).

The parameters of oxidative stress including LPO, ROS production, hydroperoxide and PCO synthesis were shown to reduce in four selected brain areas (cortex, cerebellum, hippocampus and striatum) of prepubertal mice on administering the *C. asiatica* leaf powder in contrast to control animals. Also, the mitochondrial and cytosolic parts of various brain regions exhibited an increased antioxidant level in experimental animals on diet supplemented with *C. asiatica* leaf powder. The potent action of *C. asiatica* administration was evident by up-regulation of glutathione reduction, total thiols, non-protein thiols and antioxidant enzymatic activities in the mentioned brain areas (Shinomol, 2008b; Shinomol and Ravikumar, 2010).

The aqueous extract of *C. asiatica* was found to have considerable prophylactic neuroprotective action in animal models kept on *C. asiatica* prophylaxis (5 mg/kg body weight) for 10 days coupled with the injection of 3-nitropropionic acid (3-NPA) only for last 2 days (i. p., 75 mg/kg body weight/day). A complete attenuation of oxidative stress in brain areas including striatum induced by 3-NPA was observed due to *C. asiatica* prophylaxis in experimental models as compared to the control animals (Shinomol, 2008a).

The effect of *C. asiatica* extract on AD pathology related to Aβ plaque formation in a double transgenic mouse carrying presenilin 1/amyloid precursor protein (PSAPP) mutations were studied and positive therapeutic effects of *C. asiatica* extract were confirmed by observing a decline in Aβ and fibrillar amyloid plaques on treatment with *C. asiatica* extract at 2.5 mg/kg body weight and 5 mg/kg body weight, respectively. Additionally, the antioxidant function was also confirmed in vitro (Dhanasekaran *et al.*, 2009).

A pentacyclic triterpene of *C. asiatica*, the asiatic acid (AA), has been found to restore the parameters related to oxidative stress to the normal values in hippocampus and cortical regions of rat brains with monosodium glutamate (MSG) induced oxidative stress as revealed by quantifying the amounts of lipid peroxidation, superoxide dismutase and glutathione (Xu *et al.*, 2012).

The hamster model with hyperlipidemia induced by high-fat diet (HFD) was used in an investigation to explore the antioxidant potential of *C. asiatica* ethanolic extract in vivo. The antioxidant activities of SOD and GSHPx were found to increase while that of MDA was significantly decreased in hyperlipidemic golden hamsters after 35 days' treatment with 95% ethanolic

extract of *C. asiatica* (Zhao *et al.*, 2014).

An investigation has shown the radioprotective property of *C. asiatica* extract in case of whole-body exposure to γ-radiations as assessed by the experiment on mouse bone marrow cells using an alkaline comet assay (Joy and Nair, 2009). The oxidative stress was induced in experimental rats by administering 20 ppm arsenic (III) for 4 weeks in drinking water coupled with *C. asiatica* supplementation and there was a significant increase in the level of brain thiobarbituric acid reactive substance (TBARS) (Gupta and Flora, 2006) supported by another study on the effect of *C. asiatica* extract on arsenic-induced oxidative stress in experimental rats (Flora and Gupta, 2007).

5. Cognitive effects of *Centella asiatica* extract: studies on mouse models

Multiple studies on rodents have evaluated the cognitive effects of *C. asiatica* water extracts and shown their efficacy on improvement in cognition level of experimental animal models as assessed by standard behavioral tests. Mouse models with MSG induced dementia were produced to investigate the role of antioxidant activity by giving subcutaneous injection of MSG in the neck (2.5 mg/g body weight) to neonatal mice (the experimental group) from 7^{th} to 13^{th} postnatal days while the control group was administered with only 0.9% sodium chloride (NaCl). On 28^{th} postnatal day, the animals of the same gender receiving the same treatment were grouped together. From 14^{th} to 30^{th} postnatal days, oral treatment with antioxidant activity (50 mg/kg and 100 mg/kg body weight) was given to the drug group followed by the assessment of cognition level through standard behavioral test in which the animals receiving high dose of antioxidant activity showed significantly better performance compared to the control (Xu *et al.*, 2012). Neonatal mice showed efficient learning and improvement in dimensional memory when treated with *C. asiatica* water extract from 15^{th} to 30^{th} postpartum days (Rao *et al*., 2005).

The cognitive effects of *C. asiatica* water extract were studied in the most popular transgenic animal model for AD, Tg2576 mouse, in which age and space-dependent AD pathology were produced by inserting a mutant human APP gene into its genome. Following *C. asiatica* extract treatment, the experimental animal model showed improvements in AD-associated behavioral deficits (Soumyanath *et al.*, 2012). In continuation to it, researchers investigated for the bioactive compound of *C. asiatica* extract and confirmed several mono- and dicaffeoylquinic acids (CQAs) in *C. asiatica* extract with neuroprotective properties as assessed and evaluated in MC65 and SH-SY5Y, the neuroblastoma cell lines (Gray *et al.*, 2014) supported by a previous study that showed the neuroprotective effect of dicaffeoylquinic acids against Aβ-induced neurotoxicity in SH-SY5Y (Deng *et al.*, 2013).

6. Cell culture assays: the molecular insight of *Centella asiatica* medicinal potential

The neuroprotective role of *C. asiatica* was investigated on human neuroblastoma cells SH-SY5Y pretreated with antioxidant activity (0.1-100 nmol/L) that resulted in attenuation of glutamate-induced (10 nmol/L) toxicity with a promoting effect on upregulation of peroxisome proliferator-activated receptor-gamma co-activator-1α (PGC-1α) and silent information regulator 2 family of protein 1 (Sirt1) (Xu *et al*., 2012). In order to elucidate the underlying molecular signaling pathway involved in neuroprotective effect of *C. asiatica*, a standardized *C. asiatica* extract (Eca 233) (Wanasuntronwong *et al.*, 2012) was used at the dose of 1-100μg/ml in a study on IMR-32 neuroblastoma cells which caused the hyperphosphorylation of ERK and Akt proteins reflecting their activation under Eca 233. In addition, the role of ERK/Akt signaling pathway was later confirmed by using specific inhibitors of MEK or P13K that abrogated the nuritogenic effect of Eca 233 suggesting its nuritogenic property based on MEK/ERK and P13K/Akt signaling pathways in human neuroblastoma IMR-32 cells (Wanakhachornkrai *et al.*, 2013).

Another study on Aβ expressing neuroblastoma cells linked the memory-enhancing property of *C. asiatica* extract with the involvement of ERK/RSK signaling pathway as a plausible molecular array (Xu *et al.*, 2008). In a recent study based on cell culture assays, the researchers found that aqueous extract of *C. asiatica* profoundly decreased the level of ROS in cells having Aβ-induced oxidative stress. They also reported *C. asiatica* aqueous extract-induced expression of antioxidant response gene NEF2L2 in MC65 and SH-SY5Y neuroblastoma cells. There was a significant increase in mitochondrial electron transport chain genes expression in primary neurons of rat hippocampus with a parallel induction in antioxidant response gene expression in primary neurons as well (Gray *et al.*, 2015).

7. Antiacetylcholinesterase activity of *Centella asiatica*

The ACh has a neuromodulatory effect in the brain and acts as a neurotransmitter for cholinergic synaptic transmission. The enzyme AChE belongs to the family

of cholinesterases that are special carboxyl ester hydrolases. Activation of postsynaptic ACh receptors is followed by the AChE catalyzed hydrolytic cleavage of the ester bond in ACh into choline and acetate, the event which eventually terminates the cholinergic nerve transmission governed by ACh (Barbosa Filho *et al.*, 2006; Lionetto *et al.*, 2013).

As proposed by the cholinergic hypothesis, the abnormally high activity of AChE results in a decline in ACh concentration in the brain leading to a state of cholinergic deficit that contributes to the development and progression of AD. In view of its pathological role, AChE has become a promising target in drug discovery research to find its natural inhibitors. In the following account, an attempt has been made to give a comprehensive view of the importance of *C. asiatica* as a promising candidate to investigate for the novel, natural anti-AChE based on the research findings in the literature.

The cell culture assay has shown an up-regulation of AChE expression in cultured embryonal carcinoma P19 cells in response to Aβ peptide accumulation (Sberna *et al.*, 1997). AChE is considered as the target enzyme for the treatment of AD by virtue of its hydrolytic action on ACh leading to a decline in ACh level in AD brains (Orhan *et al.*, 2006). The ethanolic extract of *C. asiatica* was tested for anti-AChE activity in-vitro and the results confirmed that it possessed a significant level of anti-AChE activity (Rahman *et al*., 2012). An in-vitro study was carried out in which six traditional herbs commonly practiced in Indian system of medicine 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 IC_{50} value of 0.076±0.0042 µg/mL (Mukherjee *et al.*, 2007).

In a comparative study on ethanolic extracts of aerial parts of *C. asiatica* of Turkey and India together with a standard *C. asiatica* extract of Chinese origin, only the standard *C. asiatica* extract at a concentration of 200 µg/mL showed 48.28±1.64% inhibition of AChE (Orhan *et al*., 2013). In another comparative study involving twenty different medicinal plants, traditionally used in Ayurvedic system of medicine for memory problems, were tested for their AChE inhibitory activity. The study resulted in the IC_{50} value of 890±67.4 in AChE inhibition assay for the methanolic extract of *C. asiatica* (Mathew and Subramanian, 2014).

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 inhibitory activity compared to the extract of the roots (Nour *et al.*, 2014). An in-vitro study has investigated the AChE inhibitory activity of asiatic acid which is a triterpenoid isolated from *C. asiatica* in context to its role in learning and memory enhancement. TLC bioautographic AChE inhibitory assay was performed to test the AChE inhibitory potential of asiatic acid in which a two-fold serial dilution of asiatic acid showed an anti-AChE property at the concentration of 125 ng compared with the AChE inhibition by physostigmine and galanthamine at the concentration of 1 ng and 10 ng, respectively (Nasir *et al.*, 2012).

The anticonvulsant property of different *C. asiatica* extracts relative to cholinergic property was studied in rats with pentylenetetrazole (PTZ)-induced seizures. The rats were kept on oral treatment with different aqueous extracts of CA at a dose of 200 mg/kg body weight for one week followed by PTZ treatment to induce seizures. Different brain regions of seized rats showed increased ACh level and decreased AChE activity during the period of PTZ-induced seizures as compared to the control group (Visweswari *et al.*, 2010).

An experiment revealed that ICV administration of colchicine at a dose of 15 µg/5 µL injection volume resulted in significantly high level of AChE activity in the brain of male Wistar rats. However, chronic oral treatment of ICV-colchicine treated rats with aqueous *C. asiatica* extract (150 mg/kg and 300 mg/kg, P.O.) administered as 0.5 mL/kg body weight resulted in a significant attenuation of increased AChE activity as compared to the colchicine-treated animals (Kumar *et al.*, 2009).

The whole-brain anti-AChE activity of lyophilized leaf-water extract of *C. asiatica* was investigated in male albino Wistar rats in which stress was induced by containing the animals in plastic tubes of size 20 cm x 7 cm for two hours a day over a period of total twenty-one days. On day 21, the animals were killed by cervical displacement and whole-brain anti-AChE activity was determined by Ellman's method. The results of this experiment revealed that AChE activity was significantly higher in stress condition as compared to the control group. On co-administering the stressed animals with lyophilized leaf-extract of CA (200 mg/kg bodyweight for 21 days), the AChE activity was observed to be nearly normal. In addition, the stress-free control group did not show any significant change in AChE level on the administration of lyophilized leaf-extract of *C. asiatica* (200 mg/kg bodyweight for 21 days) (Sarumathi and Saravanan, 2013). Monoterpenes identified in 0.1% essential oil extracted from *C. asiatica* (Brinkhaus *et al.*, 2000) were reported as AChE inhibitors (Miyazawa and

Yamafuji, 2005).

The role of asiatic acid as anti-AChE inhibitor was investigated in hippocampal cell lines in which the asiatic acid was applied to the cell lines followed by the analyses of AChE activity and probable toxicity of asiatic acid. This study reported asiatic acid as an effective AChE inhibitor without any toxic side effects on hippocampal cell lines (Boopathy *et al.*, 2009). It is, however, worth mentioning that literature does cite a reference showing a significant increase in the level of AChE in Swiss albino mice during postnatal period on *C. asiatica* treatment besides showing a positive nootropic effect of *C. asiatica* as indicated by enhancement in learning and memory functions of the experimental animals (Rao *et al.*, 2005).

8. Future prospects and conclusion

The antioxidant therapy has been linked to the suppression of AD-related symptoms by retarding the oxidative damage as shown in mouse models on AD. In view of it, the use of antioxidant as a therapeutically active agent might prove beneficial in devising effective treatment strategies for AD. Anti-AChEs have been shown to be the multifunctional molecules that not only have their direct role in AChE inhibition but also work as free radical detoxifiers and anti-inflammatory agents. These diverse therapeutically important biological functions of anti-AChEs make them a good candidate as part of the AD treatment regimen. Also, a huge body of research data suggests the promising nature of drug discovery related to AD therapy in context to new antioxidants and anti-AChE from natural sources including plants. To date, the majority of the antioxidants and therapeutically active biomolecules against a number of diseases including cognitive disorders have been identified, isolated and purified from plant sources that exhibit the therapeutic potential of the plant world. In view of its tremendous medicinal importance for a large number of medical problems including nervous system ailments and claimed to be successfully practiced in different traditional systems of medicine across the world since centuries, *C. asiatica* appears to be a good candidate for new drug discovery research in order to search for novel antioxidants and AChE inhibitors. Despite the enormous therapeutic potential of alkaloids for the treatment of AD, it may be concluded that investigative research particularly related to *C. asiatica* alkaloids appears to be the most neglected area of drug discovery.

Alzheimer's disease is a type of neurodegenerative disorders affecting behavioural, social and economic aspects of not only the patients but also the society at large. AD is considered as a multifactorial problem involving the accumulation of high level of free radicals or ROS as well as the cholinergic deficit in AD brains that contribute to its development and progression. A number of research studies emphasize the impact of oxidative stress as one of the key factors in the development and progression of various neurodegenerative disorders including AD. Increasing research evidence indicates that development of AD is strongly correlated to the exposure of Alzheimer's brains to oxidative stress which represents a disturbance in the delicate balance between the production of ROS and oxidant detoxification mechanism leading to a dysfunctional biological system. In addition, the role of AChE in cholinergic neurotransmission has been intensively studied in the past several decades highlighting the therapeutically important status of anti-AChE in the treatment of AD. Low levels of ACh in Alzheimer's brains implicate the need to suppress the activity of AChE in order to restore the cholinergic neurotransmission and hence the importance of anti-AChE becomes evident. Multiple studies have shown the therapeutic potential of anti-AChE for the reduction of AD-related symptoms. Since decades, plants have been the focus of active research as sources of natural antioxidants and other therapeutic molecules and *C. asiatica* is one of the widely used traditional plants with proven efficacy in cognitive disorders as claimed by folk medicine. This review has summarized the research related to the same and concludes that there are many missing links in *C. asiatica* research aiming at anti-AD drug discovery that still needs to be established to elucidate the comprehensive biochemical nature of its therapeutically active biomolecules so as to reach a definite cure for AD.

Conflict of interest

The authors declare no conflict of interest.

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