ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Neuro-therapeutic Benefits of *Centella asiatica* on Some Neurodegenerative Diseases: A Review.

Samaila M Chiroma[1, 2], Mohamad Aris Mohd Moklas[1]*, Che Norma M[1], Mohamad Taufik H[1], Zulkhairi Amon[3], Saravanan Jagadeesan[1], and Bulama Ibrahim[1, 2].

[1]Department of Human Anatomy, Faculty of Medicine and Health Sciences Universiti Putra Malaysia, Serdang Selangor, Malaysia.
[2]Department of Human Anatomy, College of Medical Sciences University of Maiduguri, Borno state, Nigeria.
[3]Faculty of Health Sciences UiTM Campus Puncak Alam, Puncak Alam Selangor, Malaysia.

ABSTRACT

Medicinal herbalism has become popular because of its documented efficacy, synergistic interactions against complex conditions and lesser side effects. *Centella asiatica* (CA) is a traditional Ayurveda medicine widely used in India, China, and Malaysia and across countries in south-eastern Asia for treating a range of diseases. The whole plant or its parts (leaf or roots) are used for medicinal purpose and its chemical constituents have been proven scientifically to have neuro-protective, wound healing, anti-inflammatory and antioxidants activities. In this review we have retrieved and evaluated available literatures on neuro-therapeutic properties of CA on neurotoxic and transgenic models of Alzheimer's diseases (AD), Parkinson's disease (PD) and Huntington's disease (HD). The extract was found to have neuroprotective ability through modifying a key signaling pathways and events concerned with neuro-degeneration. Further research is required to discover more bioactive compounds of CA and their exact mode of action.

Keywords: *Centella asiatica*, Neurodegenerative disease, Neuro-Therapeutic, Ayurveda medicine.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Centella asiatica (CA), also locally known as pegaga in Malaysia is a plant with a wide range of usage in Ayurvedic medicine and traditional medicine of countries in South-eastern Asia including Malaysia [1]. The herb is in the apiaceae family and being used as a source of food, medicine and beverages globally. Formulations from the plant is used as a remedy for gastrointestinal tract disorders, skin diseases and for revitalizing brain cells[2]. It has been estimated that CA is among the most soughed plants in the International market of medicinal plants trades and because of its high demand this species have been depleted, therefore the International Union for Conservation of Nature and Natural Resources (IUCN) has listed it among the endangered plants species.

All over the world there are numerous preparations of CA in different pharmaceutical forms being recommended for several neurological indications. Taking this fact into consideration, the plant has significantly drawn the attention of several scientific groups to focus on its neuro-protective properties in order to confirm its traditional usage scientifically. For this reason online literature search has been conducted using different electronic data bases to get recent information on CA. This review aims to cover neuro-therapeutic benefits of CA on some selected neurodegenerative diseases which will be described first followed by its neuro-regenerative properties.

Morphology and Characteristics

Centella asiatica, up till now it is considered as a wild plant because it has not undergone domestication to crop cultivation. In their work on morphology of pegagan leaves in North Sumatera Noverita *et al*[3] listed the following macroscopic characteristic of CA; it is an annual spreading herb and not trunked. The leaves are singly arranged in a rosette root, ranging from two to ten in number, kidney shaped, the base rounded, the edge serrated, with diameter of 1-9 cm, green in colour, and sometimes slightly hairy (figure 1). The stem is also green in colour and 10-40 cm in length. The flowers are fascicled umbel with each umbel comprising of white to purple colour flowers; the fruits are oblong and globularly shaped and approximately 2 inches long; the seeds have pendulous embryo with a particular odour and a bittersweet taste[2,3]. CA grows well in marshy and damp places covering the surface like a carpet and it flourishes very well in sandy loam soil which is very fertile[4]. Geographically, this plant is indigenous to warmer regions of the globe, so it is native to India, Sri Lanka, South-East Asia, China, Mexico, USA, South Africa, Columbia, Eastern South America, Venezuela and Madagascar[1,2,5].

A **B**

Figure1: *Centella asiatica* (a) plant body (b) leaf

Chemical Constituents

Reports have shown that CA contains a huge number of compounds belonging to different chemical classes. The major bioactive chemical compounds in the plant are the triterpenes, volatile oils, alkaloids,

glycosides and flavonoids. The plant is also affluent in other constituents like mesoinositol, sitosterol, oligosaccharide centellose, carotenoids and vitamins [1,5]. Triterpenes saponisides have been found to be the most abundant and the major triterpenes components include: madecassic acid (6-hydroxy-asiatic acid), asiatic acid, madecassoside, asiaticoside, madasiatic acid, thankunic acid, betulinic acid and isothankunic acid [6,7].

Essential oils purified from plants are being used in large scale by food and cosmetic industries due to the sweet fragrance and aroma [8]. One of the chemical constituent of CA is the essential oil as reported by different authors. Ali analyzed some selected Malaysian traditional plants in Peninsular Malaysia, he identified 23 oil compounds in CA with α-humulene, γ-murolene , β-cubebene, β-caryophyllene as the major constituents [8]. Another author in Sabah Malaysia reported 20 oil compounds from CA[9]. In another study conducted by Oyedeji in South Africa, he reported the following; α-humulene, β- caryophyllene, bicyclogermacrene, germacrene B and myrcene as the major constituents of *centella aciatica*[10]. Wongfhun in Thailand characterized the flavours in CA juice and reported humulene, β-caryophyllene, a-copaene, β-farnesene, β-elemene and alloaromadendrene as the major essential oil constituents [11].

CA have a quite number of bioactive compounds which are responsible for its wide range of therapeutic activity even though a significant variation exits in its active compound among samples grown in different localities [12]. Govarthanan compared the active compounds between wild propagated and in vitro grown CA and found a sharp decrease in the total phenolic compounds, flavonoids and ascorbic acids in the in vitro propagated plant compared to the wild type[12]. Sekar et al [13] reported the yield of different phytochemical compounds after using different extraction solvents. In a studies conducted by Krishnaiah[14] on six Malaysian medicinal plants he reported terpenoids, flavonoids and tannins as the major phytochemical constituents of the Malaysian species of CA.

Effects of CA on neurodegenerative diseases

Among many uses of CA, literatures have shown that many cultures and traditions uses CA (part or whole) as a remedy in brain related disorders: in Malaysia for mental fatigue[15]; Nepal for poor memory [5]; Java and Malay Peninsula as brain tonic; Thailand as brain tonic [16], and Bangladesh for mental illness [17]. In addition CA have been used to take care of neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD) and mild cognitive impairments (MCI) [18]. Neurodegenerative disease is a general terminology given to a variety of conditions which principally affects the neurons in the individual brain and the spinal cord. Neurons are the smallest structural units' which serves as construction blocks of the nervous system. Whenever neurons are damaged they don't have the capacity to regenerate, which is why neurodegenerative diseases are not curable and incapacitating conditions that leads to progressive disintegration and / or loss of nerve cells. Commonly known examples of neurodegenerative diseases includes: Alzheimer's, Parkinson's, Prion and Huntington's diseases (HD), others are spinocerebellar ataxia (SCA) and Spinal muscular atrophy (SMA).Discussed below are some neurodegenerative diseases:

Alzheimer's disease

Alzheimer's disease (AD) is the most common progressive, dementing neurodegenerative disease associated with old age, which affects numerous people every year, and these figures are expected to increase as the populace becomes older[19]. The neuropathological hallmark of AD includes the presence of senile plaque formed by the deposit of β-amyloid protein and the neurofibrillary tangles formed by hyper phosphorylated tau protein, both in the hippocampus and the cerebral cortical regions of the brain. Alzheimer's patients suffer from impaired cholinergic functions, induction of amyloid cascade, oxidative stress, steroid hormones deficiencies and the presence of glutamate mediated exicitotoxicity [20]. Any substance that can reduce the β-amyloid burden and stops the hyper phosphorylation of tau (micro tubular protein) would be a good candidate in the battle of Alzheimer's scourge. The neuroprotection aspect of CA majorly involves reducing oxidative stress, prevention of beta amyloid plaque formation in AD, dopamine neurotoxicity in PD and enzyme inhibition [7]. Soumyanath et al. [21] investigated the possible mechanism of action of aqueous extract of CA in Tg2576 mouse, an AD mice model with high β-amyloid burden. Oral administration of water extract of CA showed a great improvement in behavioural abnormalities associated with β-amyloid burdened mice.In vitro, water extract of CA protected MC65 human neuroblastoma cells and SH-SY5Y cells from toxicity induced by endogenously generated and endogenously added β-amyloid respectively. The extract also prevented the formation of intracellular β-amyloid aggregation in MC65 cells. Nevertheless, the extract did not

protect the cells from oxidative damage, glutamate toxicity or show anticholinesterase activity which are the current mechanisms for AD therapies. Phenolic compounds are the major chemical constituent of the extract, but it does not contain asiatic acid a known CA neuroprotectant triterpene. Therefore water extract of CA contain a novel active compounds that have a unique therapeutic potential for AD treatment. Other studies have shown that water extract of CA can protect against Aβ toxicity in vitro [22], increases mitochondrial respiration as well as induces the expression of mitochondrial and anti-oxidant genes[23], improve cognitive performance in healthy aged mice [24] and improve dendritic aboritezation and synaptic differentiation [25].Review conducted by Alfarra and Omar [26] has shown that asiaticoside is one of the major the derivatives of triterpenes that is responsible for therapeutic activities of CA. Improved cognitive behavior was observed in male Sprague-Dawley rats after acute administration of asiatic acid [27].

Amjad and Umesalma [28] explored the possible neuroprotective efficacy of CA on a chronic aluminium chloride ($Alcl_3$) exposed rats. The exposure leads to oxidative stress, cognitive impairments, biochemical and histo-morphological changes in rat brain similar to neurodegeneration in AD and PD. Significant improvements in memory performance, decrease in acetylcholineterase (AChE) activity, attenuation of histo-morphological changes and oxidative defense was observed after CA administration. The active constituents of CA that are believed to have neuro-protective potentials in modern medicine include; brahmaside, madecassic acid, asiatic acid, as well as flavonoids madesiatic acid and madecassoside[29].

Parkinson's disease

Parkinson's disease (PD) is an age dependent neurodegenerative disorder associated with degeneration of dopaminergic neurons in the substantia nigra that is presented by movement disorders such as gait disturbance, resting tremor, bradykinesia, rigidity and postural instability[1,30].The specific means of neurodegeneration PD is still mysterious but mitochondrial dysfunction, oxidative stress and generation of reactive oxygen species (Ros)are thought to be part of it[31]. Neuroprotective effect of aqueous extract of CA was evaluated in 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) challenged aged Sprague Dawley rat. After 21 days of administration, homogenate of corpus striatum and hippocampus were examined for oxidative biomarkers levels. In all the parameters evaluated CA extract reversed the neurotoxic effect of MPTP to near normal condition. The study revealed that CA aqueous extract has a protective effect on the brain in neurodegenerative disorders like Parkinson's disease. This neuroprotective effect of CA extract could be due to its richness in polyphenols and triterpenes which are useful xenobiotics detoxifying agents as it could enhance brain antioxidants, decrease lipid perioxidation and significantly protect the brain from MPTP neuro-toxicity [31]. In a similar studies, the neuroprotective effects of asiaticoside, a triterpenoid soponin isolated from CA was investigated in rat model of PD induced by MPTP. The data revealed treatment with asiaticoside protected dopaminergic neurons by opposing MPTP induced neurotoxicity and improved locomotor dysfunction. Asiaticoside markedly weakened the MPTP induced decrease in dopamine (DA) level, increased the level of glutathione (GSH), reduced the levels of malondialdehyde (MDA) as well as increased Bcl2/Bax ratio. These studies have shown that asiaticoside is effective in reversing MPTP induced PD through its neuroprotective effects which includes; maintenance of metabolic balance of DA, antioxidant activity and increasing the Bcl2/Bax ratio [32].

The effect of acetone extract of CA on Drosophila model of PD (expressing human α-synuclein in neurons) was investigated by administering the extract as a dietary supplement, the flies fed on the extract for 24 days. After the treatment, the activity patterns and climbing abilities of the transgenic Drosophila flies were evaluated. While after sacrifice the effect of the extract was further evaluated on glutathione content, protein carbonyl content, lipid peroxidation, and glutathione-S-transferase activity in the brains of the flies. A remarkable delay was observed in the loss of climbing ability and the activity pattern of PD model flies treated with the CA supplemented food compared to the untreated PD model flies, consequently a reduced oxidative stress was observed in the brains of the treated PD flies in comparison to the untreated PD flies. These results indicated that the acetone leaf extract of CA is effective in reversing PD symptoms in the Drosophila model of PD [33].

Jansen et al. [34]compared five Ayurvedic herbs (Zandopa, centella asiatica ,Withaniasomnifera, Bacopa monnieri and Sidacordifolia)to see their effect on climbing ability in Drosophila melanogaster PD model based on loss of function of phosphatase and tensin-induced putative kinase 1 (PINK1). A surprising result was seen

in CA treated group; because a remarkable reduction in climbing ability in WT flies was observed. The negative effect of CA on the climbing ability of WT and no positive effect on PINK1 flies could be due to its toxicity on Drosophila or the dosage chosen in the experiment was inappropriate [34]. This study showed a small draw back on neurotherapeutic effect of CA, but Jansen et al. recommended more studies on this great Ayurvedic herb. Researchers from Indonesia [35] reported a positive outcome after evaluating the effects of standardized methanol extract of CA on rotenone induced PD zebrafish model. The data revealed an increased in motility as well as increase in the level of Brain-derived Neurotrophic Factor (BDNF). Many studies have used rotenone to induce PD in different animal models that mimic the human symptoms[36]. Rotenone induces neurotoxity by inhibiting complex 1 of mitochondrial electron transport chain. The rotenone model is characterized by decreased motility, decreased level of BDNF, decreased DA and increased apoptosis which are the hallmark of PD. Data from these study suggested methanol extract of CA exerts neuno-therapeutic effect by increasing neurotrophic factor BDNF and conserving dopamine levels.

Huntington's disease

Huntington's disease (HD) is a progressive, deadly neurodegenerative, hereditary disorder that causes a continuous breakdown of nerve cells in the brain. For now HD has no known treatment, it is characterized by deterioration of a person's physical and psychological abilities during the peak years of their working carrier[37]. Exposure to 3-Nitropropionicacid (3-NPA) in experimental rats causes discriminatory degeneration of neurons in striatum , induces mitochondrial toxicity, enhances MDA levels in the brain and encourages ROS generation similar to Huntington's disease[38]. George et al. [39]conducted in vitro investigation to evaluate the effectiveness of aqueous extract of CA in 3-NPA-induced oxidative stress in brain mitochondria. The 3-NPA exposed mitochondria showed a momentous concentration reliant increase in levels of hydro-peroxide, MDA and ROS indicating induction of oxidative stress. CA treatment reversed the concentration of ROS and hydroperoxide in mitochondrial fraction to normalcy, indicating it has the capacity to modulate neuronal dysfunction at sub cellular level. The extract also provided a defense to mitochondria from different parts of brain in vitro. The study concluded by suggesting that CA modulates neuroprotection by its antioxidative properties. In a similar work prophylactic effect of CA on 3-NPA induced HD like mice model was investigated. The neurotoxin induced an oxidative stress in the mice that were not treated as evidenced by significant increase in the levels of hydroperoxides, ROS and MDA in the cytosol and mitochondria of the striatum. On the other hand the CA prophylaxis totally controlled the 3-NPA-induced oxidative stress. 3-NPA also elicited marked protein oxidation and oxidative stress in mitochondria/cytosol of other brain regions also, but were completely attenuated by CA prophylaxis. The striatum and the other brain regions of the 3-NPA administered mice showed a major depletion in antioxidant enzymic defenses which were also protected completely by CA prophylaxis. They concluded the study by suggesting that; the prophylactic efficacy of CA could be related to its capability to increase the levels of thiols and GSH and to alter the antioxidant machinery in the brain regions of juvenile mice[40].

Enhancement and regeneration of nervous tissues

From traditional medicine to Ayurvedic medicine and coming through to the orthodox medicine, literatures have revealed the memory and cognitive enhancing ability of CA. To prove this point, scientist have worked tirelessly by investigating the potency of CA on the regeneration of both central and peripheral nervous tissues. The neurites elongation ability of asiatic acid a fraction of CA was revealed by Soumyanath et al. [41]who demonstrated it through in vitro experimental model of brain cells. They also noticed that the elongating capacity could be stimulated by other chemical constituents found in the plant that have synergistic end product with asiatic acid. Rao et al.[42]investigated the role of fresh leaf extract of CA on dendritic morphology of amygdaloid in 7 days old rat pups. After 6 weeks of administration at different doses (2ml/kg, 4ml/kg and 6 ml/kg body weight), the animals were sacrificed, brain removed and amygdaloid neurons were processed by silver staining. When the obtained data was compared with age-marched control rats, a noteworthy increase was observed in the length of dendrites and the branching points of dendrites in the amygdaloid neurons of the treated rats. The dendritic aboritezation observed was dose dependent and also period reliant because only the neurons of rats administered with 4ml/kg and 6 ml/kg body weight of CA and for a longer duration of 4 weeks - 6 weeks showed a marked increase in length. In a similar research, the efficacy of standardized extract of CA (ECa233) was evaluated in IMR-32 human neuroblastoma cells by Wanakhachornkrai et al.[43]. A neutrite outgrowth promoting activity was demonstrated by the extract in human IMR-32 neuroblastoma cell line which is mediated by ERK1/2 and Akt signaling pathways. The extract

was further evaluated on viability the human neuroblastoma IMR-32 cells and it has no negative effect, this finding is in agreement with [46] who reported a dose of up to 10g/kg of ECa233 caused no any lethality and significant adverse effect on experimental animals[44].

CONCLUSION

As can be observed, the present review demonstrated the therapeutic benefits of CA in both in vivo and in vitro studies of some major neurodegenerative diseases. This is being achieved through its neuroprotective properties which includes anti-oxidative, anti-inflammatory, conservation of neurotransmitters as well as neuroregenerative properties. Other essential properties of CA includes attenuation of cognitive impairments and nerve cell proliferation. The major chemical constituents of CA with the therapeutic benefits are triterpenes saponis and asicositides, even though the crude extract have a good memory enhancing effect suggesting a synergistic effect by the various constituents. Other therapeutic properties of CA include anticancer, anti-fungal, anti-bacterial, antidepressant, wound healing to mention just but few. As an endangered species mass cultivation of CA plants in farms must be encouraged, mass propagation through tissue culture would be helpful while callus and suspension culture techniques can be used to produce the secondary metabolites [29]. Additional studies is required on this wonder plant in order to harness its potentials.

REFERENCES

[1] Lokanathan Y, Omar N, Ahmad Puz NN, Saim A, Hj Idrus R. Recent updates in neuroprotective and neuroregenerative potential of C*entella asiatica*, Malaysian J Med Sci, 2016;23:4–14.

[2] Zahara K. Clinical and therapeutic benefits of *Centella asiatica,* Pure Appl, Biol, 2014;3:152–9.

[3] Vinolina NS, Siregar LAM, Justin A. Morphology of leaves and content of secondary metabolites asiaticoside in some accession of pegagan (*Centella asiatica* l . Urban) in North Sumatera, Proceedings of the annual international conference, Syiah Kuala University-life sciences and engineering chapter, 2012;2:22–4.

[4] Singh S, Gautam A, Sharma A, Batra A. *Centella asiatica* L.: A plant with immense medicinal but thretened,International journal of pharmaceutical sciences review and research,2010;4:9–17.

[5] Shakir Jamil S, Nizami Q, Salam M. *Centella asiatica* (Linn.) urban óa review. Indian J Nat Prod Resour, 2007;6:158–70.

[6] Pan J, Kai G, Yuan C, Zhou B, Jin R, Yuan Y., Separation and Determination of Madecassic Acid in Extracts of *Centella asiatica* Using High Performance Liquid Chromatography with β-Cyclodextrin as Mobile Phase Additive, Chinese J Chromatogr 2007;25:316–8.

[7] Orhan IE.,*Centella asiatica* (L.) Urban: From traditional medicine to modern medicine with neuroprotective potential, Evidence-Based Complement Altern Med, 2012;2012.

[8] Mohd SMA, Analysis of phenolics and other phytochemicals in selected Malaysian traditional vegetables and their activities in vitro,PhD diss., University of Glasgow, 2008.

[9] Lee TK, Vairappan CS. Antioxidant, Antibacterial and Cytotoxic Activities of Essential Oils and Ethanol Extracts of Selected South East Asian Herbs, J Med Plants Res 2011;5:5284–90.

[10] Oyedeji OA, Afolayan AJ. Chemical composition and antibacterial activity of the essential oil of *Centella asiatica* growing in South Africa, Pharm Biol, 2005;43:249–52.

[11] Wongfhun P, Gordon MH, Apichartsrangkoon A. Flavour characterisation of fresh and processed pennywort (C*entella asiatica* L.) juices, Food Chem 2010;119:69–74.

[12] Govarthanan M, Rajinikanth R, Kamala-Kannan S, Selvankumar T, A comparative study on bioactive constituents between wild and in vitro propagated C*entella asiatica*. J Genet Eng Biotechnol 2015;13:25–9.

[13] Arumugam T, Ayyanar M, Koil Pillai YJ, Sekar T, Phytochemical screening and antibacterial activity of leaf and callus extracts of C*entella asiatica*. Bangladesh J Pharmacol, 2011;6:55–60.

[14] Krishnaiah D, Devi T, Bono A, Sarbatly R. Studies on phytochemical constituents of six Malaysian medicinal plants, J Med Plants Res, 2009;3:67–72.

[15] Hamid AA, Shah ZM, Muse R, Mohamed S, Characterisation of antioxidative activities of various extracts of C*entella asiatica* (L) Urban, Food Chem 2002;77:465–9.

[16] Punturee K, Wild CP, Vinitketkumneun U, Thai medicinal plants modulate nitric oxide and tumor

necrosis factor in J774.2 mouse macrophages, J Ethnopharmacol 2004;95:183–9.
[17] Ahmad F, Khan MA, Ahmad M, Zafar M, Nazir A, Marwat SK. Taxonomic studies of grasses and their indigenous uses in the salt range area of Pakistan, African J Biotechnol, 2009;8:231–49.
[18] Tiwari S, Singh S, Patwardhan K, Gehlot S, Gambhir IS, Effect of *Centella asiatica* on mild cognitive impairment (MCI) and other common age-related clinical problems, Dig J Nanomater Biostructures, 2008;3:215–220.
[19] Lin X, Huang R, Zhang S, Wei L, Zhuo L, Wu X, et al., Beneficial effects of asiaticoside on cognitive deficits in senescence-accelerated mice, Fitoterapia 2013;87:69–77.
[20] Shah, R. S.; Lee, H.; Xiongwei, Z.; Perry, G.; Smith, M.A.; Castellani RJ, Current approaches in the treatment of Alzheimer's disease, Biomed Pharmacother 2008;62:199–207.
[21] Soumyanath a, Zhong YP, Henson E, Wadsworth T, Bishop J, Gold BG, et al.,C*entella asiatica* Extract Improves Behavioral Deficits in a Mouse Model of Alzheimer's Disease: Investigation of a Possible Mechanism of Action, Int J Alzheimers Dis 2012;2012
[22] Gray NE, Morré J, Kelley J, Maier CS, Stevens JF, Quinn JF, et al. Caffeoylquinic acids in CA protect against amyloid-β toxicity. J Alzheimer's Dis, 2014;40:359–73.
[23] Gray NE, Sampath H, Zweig JA, Quinn JF, Soumyanath A. *Centella asiatica* Attenuates Amyloid-beta-Induced Oxidative Stress and Mitochondrial Dysfunction, J Alzheimer's Dis 2015;45:933–46.
[24] Gray NE, Harris CJ, Quinn JF, Soumyanath A,CA modulates antioxidant and mitochondrial pathways and improves cognitive function in mice, J Ethnopharmacol, 2016;180:78–86.
[25] Gray NE, Zweig JA, Murchison C, Caruso M, Matthews DG, Kawamoto C, et al,*Centella asiatica* attenuates Aβ-induced neurodegenerative spine loss and dendritic simplification, Neurosci Lett, 2017;646:24–9.
[26] Alfarra HY, Omar MN,*Centella asiatica*: from folk remedy to the medicinal biotechnology – a state revision, Int J Biosci, 2013;3:49–67.
[27] Nasir MN, Habsah M, Zamzuri I, Rammes G, Hasnan J, Abdullah J. Effects of asiatic acid on passive and active avoidance task in male Spraque-Dawley rats, J Ethnopharmacol, 2011;134:203–9.
[28] Syed USA, Protective Effect of *Centella asiatica* against Aluminium-Induced Neurotoxicity in Cerebral Cortex, Striatum, Hypothalamus and Hippocampus of Rat Brain- Histopathological, and Biochemical Approach, J Mol Biomark Diagn, 2015.
[29] Prakash V, Jaiswal N, Srivastava M, A review on medicinal properties of *Centella asiatica*, Asian J pharm Clin Res, 2017:10;69-74.
[30] Olanow CW, Tatton WG. Etiology and pathogenesis of Parkinson's disease, Annu Rev Neurosci, 1999;22:123–44.
[31] Haleagrahara N, Ponnusamy K. Neuroprotective effect of CA extract (CAE) on experimentally induced parkinsonism in aged Sprague-Dawley rats, J Toxicol Sci, 2010;35:41–7.
[32] Xu CL, Wang QZ, Sun LM, Li XM, Deng JM, Li LF, et al, Asiaticoside: Attenuation of neurotoxicity induced by MPTP in a rat model of Parkinsonism via maintaining redox balance and up-regulating the ratio of Bcl-2/Bax, Pharmacol Biochem Behav, 2012;100:413–8.
[33] Siddique YH, Naz F, Jyoti S, Fatima A, Khanam S, Rahul, et al, Effect of *Centella asiatica* leaf extract on the dietary supplementation in transgenic drosophila model of parkinson's disease, Parkinsons Dis 2014;2014.
[34] Jansen RLM, Brogan B, Whitworth AJ, Okello EJ, Effects of five ayurvedic herbs on locomotor behaviour in a Drosophila melanogaster Parkinson's disease model, Phyther Res, 2014;28:1789–95.
[35] Khotimah H, Sumitro SB, Ali M, Widodo MA, Standardized *Centella asiatica* Increased Brain- Derived Neurotrophic Factor and Decreased Apoptosis of Dopaminergic Neuron in Rotenone- Induced Zebrafish, GSTF J Psychol 2015;2:8–15.
[36] Sonia Angeline M, Chaterjee P, Anand K, Ambasta RK, Kumar P, Rotenone-induced parkinsonism elicits behavioral impairments and differential expression of parkin, heat shock proteins and caspases in the rat, Neuroscience, 2012;220:291–301.
[37] Ross CA, Tabrizi SJ, Huntington's disease: From molecular pathogenesis to clinical treatment, Lancet Neurol, 2011;10:83–98.
[38] Beal MF, Brouillet E, Jenkins BG, Ferrante RJ, Kowall NW, Miller JM, et al, Neurochemical and histologic characterization of striatal excitotoxic lesions produced by the mitochondrial toxin 3-nitropropionic acid, J Neurosci 1993;13:4181–92.
[39] Shinomol GK, Muralidhara, Effect of *Centella asiatica* leaf powder on oxidative markers in brain regions of prepubertal mice in vivo and its in vitro efficacy to ameliorate 3-NPA-induced oxidative stress in mitochondria, Phytomedicine 2008;15:971–84.

[40] Shinomol GK, Ravikumar H, Muralidhara, Prophylaxis with *Centella asiatica* confers protection to prepubertal mice against 3-nitropropionic-acid-induced oxidative stress in brain, Phyther Res 2010;24:885–92.

[41] Soumyanath A, Zhong Y-P, Yu X, Bourdette D, Koop DR, Gold SA, et al,*Centella asiatica* accelerates nerve regeneration upon oral administration and contains multiple active fractions increasing neurite elongation in-vitro, J Pharm Pharmacol, 2005;57:1221–9.

[42] Mohandas RKG, Muddanna RS, Gurumadhva RS, Enhancement of amygdaloid neuronal dendritic arborization by fresh leaf juice of *Centella asiatica* (Linn) during growth spurt period in rats, Evidence-Based Complement Altern Med, 2009;6:203–10.

[43] Wanakhachornkrai O, Pongrakhananon V, Chunhacha P, Wanasuntronwong A, Vattanajun A, Tantisira B, et al, Neuritogenic effect of standardized extract of *Centella asiatica* ECa233 on human neuroblastoma cells, BMC Complement Altern Med 2013;13.

[44] Chivapat S, Chavalittumrong P, Tantisira MH, Acute and sub-chronic toxicity studies of a standardized extract of *Centella asiatica* ECa 233, Thai J Pharm Sci, 2011;35:55–64.