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ANTI EPILEPTIC BIOACTIVE FACTORS IN A MEDICINAL PLANT *CENTELLA ASIATICA*

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Abstract: This study mainly aimed at to investigate the antiepileptic property of a medicinal plant *Centella asiatica* against the Pentylenetetrazole (PTZ) induced epilepsy. Epilepsy is a convulsive episode and is the most frequent neurodegenerative disorder affecting about 50 million people world-wide. The epileptogenesis is a dynamic process with a tendency to develop hyperexcitability in one or other regions of the central nervous system. Epilepsy is not normally life-threatening, although physical injury can occur as a result of seizures. In rare cases, epilepsy can cause sudden, unexplained death. The primary treatment for epilepsy is the use of antiseizure medicines called anticonvulsant or antiepileptic drugs (AED) to bring seizures under control. Though considerable work has emerged during past several years from other countries, much is awaited from developing countries which are endowed with rich heritage of flora and fauna. It is in this context, efforts have been made in the present study to the neuropharmacological action of *Centella asiatica* with particular reference to anticonvulsant and neuroprotective activity. *Centella asiatica* is referred to as one of the great multipurpose miracle herbs of oriental medicine. It is considered as one of the most powerful rejuvenating herbs in Indian Ayurvedic medicine. The active constituents of Centella include triterpenoid glycosides i.e. asiatic acid, asiaticoside, madecassic acid, madecassoside, oxyasiaticoside, and centelloside. Saponin glycosides i.e. brahmiside, brahminoside. Flavonol glycosides i.e. quercetin-3-glycoside and Kampferol-3-glycoside and elements Calcium, Magnesium and Sodium.
Key words: *Centella asiatica*, Epilepsy, Pentylenetetrazole, Triterpenoids.

I. INTRODUCTION

The primary treatment for epilepsy is the use of antiseizure medicines called anticonvulsant or antiepileptic drugs (AED) to bring seizures under control. AEDs can either reduce the occurrence of seizures or prevent them from occurring, but they do not cure epilepsy. The selection of an appropriate AED is based on diagnosis of the epileptic syndrome of the patient. AEDs have an initial starting doses and subsequent titration is based on response to medication and side-effect profile. Volprate and Clonazepam may be active agents which are effective against Myoclonic, Akinetic and Atonic seizures in young children (McNamara, 1995). Approximately 50% of all women with epilepsy have increased seizure frequency during pregnancy. Infant mortality is higher for epileptic mothers. Children of epileptic mothers who received antiseizure medication during the early months of pregnancy have an increased incidence of a variety of birth defects (McNamara, 1995). AEDs like carbamazepine and phenytoin induce the malformation in offspring's of epileptic mothers (Lindhout *et al*., 1984; Jones *et al*., 1989).

In patients who do not respond to medication, epilepsy surgery is a potential mode of treatment that can offer up to a 70% to 90% chance of seizure freedom in some patients. Other novel modes of therapy include the vagal nerve stimulator (VNS), which is usually reserved for those patients with intractable epilepsy and are not surgical candidates (Dileep, 2003). Neurocognitive side effects include dizziness, drowsiness, unsteadiness, blurred vision, ataxia, tremor, nystagmus, impaired memory and fatigue (Dileep, 2003). Though various AEDs are available clinically management of epilepsy is a very complex task due to co-existing neuropsychiatric complications (Krishnamoorthy, 2001).

The success of treatment depends on many factors which include

- The type of epilepsy
- How accurate the diagnosis is
- Whether the right type of treatment is being taken and if it is being taken correctly
- Whether the person has any other associated disability.

The initial evaluation in patients who present with spells or seizures is to determine whether these episodes are epileptic in nature. Approximately 30-50% of epileptic cases has a known cause and is termed as acquired epilepsy (Sun *et al*., 2001). It can be induced by repeated application of short electrical stimuli (kindling) to amygdale or hippocampus. Some synaptic changes in kindling rats include enhancement of 1) Voltage sensitive calcium conductance 2) Glutamate release in CA3 region of hippocampus 3) GABA release in CA1 region of hippocampus and 4) Sensitivity of NMDA receptors and glutamate metabo receptors (Akiyama *et al*., 1992). Once epileptic seizures are diagnosed the next step is to determine the epileptic syndrome and then the seizure type. This is helpful for choosing medications as well as for evaluating a patient for surgical treatment. The epileptic syndrome is determined based on the history, physical examination, EEG findings, and neuroimaging studies (Nair, 2003). The primary treatment for epilepsy is the use of antiseizure medicines called anticonvulsant or antiepileptic drugs (AED) to bring seizures under control.

Plant Description

Centella asiatica is referred to as one of the great multipurpose miracle herbs of oriental medicine. It is considered as one of the most powerful rejuvenating herbs in Indian Ayurvedic medicine, where it is called **"Brahmi"** meaning **"Greatest of the great"**. It has been used in ayurvedic preparation either in the fresh or in the extract form (Rao *et al.*, 2005).

Leaf of *Centella asiatica*

REGULATION OF EPILEPSY

Glutamate is required for normal brain function, the presence of excessive amounts of glutamate can lead to excitotoxic cell death (Lipton and Rosenberg, 1994). Excitotoxicity is mediated by various types of glutamate receptors (Glu-R) particularly N-methyl-D-aspartate (NMDA), AMPA or kainic acid in different neurodegenerative disorders. Activated NMDA receptor channels allow an influx of Ca^{2+} which in excess can activate a variety of potentially destructive processes (Standaert and Young, 1995). By Blockade of voltage dependent Ca^{2+} channels and release of extracellular Mg^{2+} channels, can regulate the activity of NMDA Channels and thereby release of transmitters. Prevention of repetitive firing of a neuron is possible by blocking the voltage-activated Na^{+} channels in epileptics (McNamara, 1995). Through the inhibition of inhibitory neurotransmitter (GABA), the resting membrane potential can be regulated and thus can reduce the probability of glutamate excitation (McNamara, 1995).

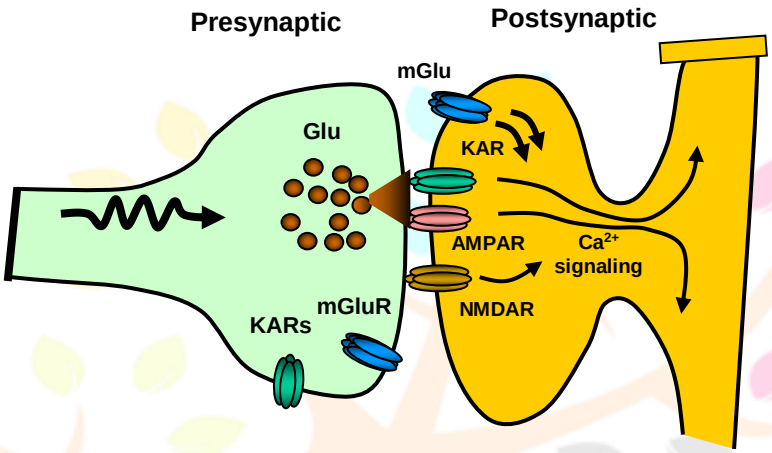


Figure showing the regulation of epilepsy during synaptic transmission.

DIAGNOSTIS OF EPILEPSY

Electroencephalogram (EEG)

An EEG, electroencephalogram, is a test that can help to diagnose epilepsy. During an EEG, the electrical signals of the brain are recorded. This electrical activity is detected by electrodes or sensors placed on the patient's scalp. The electrical signals produced by the brain neurons are picked up by the electrodes and transmitted to a polygraph, where they produce separate graphs on moving paper using an ink writing pen or on a computer screen.

Hormone Test

An epilepsy blood test measures the amount of the hormone prolactin in the blood. It helps to determine whether a seizure was caused by epilepsy or another disorder. The test must be done within 10 to 20 minutes after a seizure, measures levels of the hormone prolactin in the blood. Prolactin is produced by the pituitary gland, but an area of the brain called the hypothalamus controls its release. Epileptic seizures are thought to affect the hypothalamus and may alter the release of prolactin, causing levels of the hormone to rise.

PENTYLENETETRAZOLE

Pentylenetetrazole (PTZ), a tetrazol derivative, has been shown to induce convulsions presumably by impairing GABA-mediated inhibition at the GABA receptor (Olsen, 1981). It acts as a central nervous system and respiratory stimulant. It is considered a non-competitive GABA antagonist. PTZ can induce seizures at high doses and might have other serious side effects. It has been used experimentally to study seizure phenomenon and to identify pharmaceuticals that may control seizure susceptibility. It is generally believed that PTZ exerts its effects by binding to the picrotoxin-binding site of the post-synaptic $GABA_A$ receptor (Macdonald and Barker, 1977). Overall effect of PTZ should be an increase in glutamate-GABA ratio, which may contribute to the triggering of convulsions (Lacoste *et al.*, 1988).

Herbal Treatment (Antiepileptic Therapy)

The herbs and their extracts showed effect on epileptic models directly or indirectly. The decoction of *Mimosa pudica* leaves protected mice against PTZ and strychnine-induced seizures (Bum *et al*., 2001)**.** Methanol extract of *Viscum capense* also protected the mice against PTZ-and bicuculline-induced tonic seizures (Amabeoku, 1998). Ethanolic extract of *Maprounea africana* and *Trema guinunsis* delayed the onset of clonic convulsions induced by PTZ in mice (N'gouemo, 1994). Methanolic extract of *Vitex negundo* Linn. also showed significant protection against strychnine and leptazole induced convulsions. The fractions of *Albizzia lebbeck, Hibiscus rosa sinesis* and *Butea monosperma* protected the animals from maximum electroshock, electrical kindling and PTZ-induced convulsions (Kasture *et al.*, 2000).

II. MATERIALS AND METHODS

Procurement and Maintenance of Experimental Animals

Male adult Wistar rats weighing 150±25 grams were used as the experimental animals in the present investigation. The rats were purchased from the Indian Institute of Science (IISc), Bangalore, maintained in the animal house of the department in polypropylene cages under laboratory conditions of 28 ± 2^0C temperature with photoperiod of 12 hours light and 12 hours dark and 75% relative humidity. The rats were fed with standard pellet diet (Hindustan Lever Ltd., Mumbai) and water *ad libitum*. The rats were maintained according to the ethical guidelines for animal protection and welfare bearing the CPCSEA 438/01/a/cpcsea/dt:17.07.2006 in its resolution No:09/(i)/a/ CPCSCA/ IAEC/ SVU/ WR/KSP/Dt. 04.03.2006.

Collection of the plant material

Centella asiatica (CA) plant was collected from Tirumala hills and indentified by a botanist, Department of Botany, S.V.University, Tirupati. A voucher specimen was deposited in the herbarium of the Department of Botany, S.V.University, Tirupati (Voucher no. 1688). The leaves were separated from the plant, dried in shade, powdered and powder was used for the extraction of anticonvulsant principle/s using different solvents.

Preparation of Plant Extracts

The active principles of the leaves of plant were extracted into different solvents, Methanol, Water, n-Hexane, Chloroform, Ethyl acetate and n-Butanol, since these solvents were predominantly used by several investigators for extracting anticonvulsant principle(s) from various plants (Sowmyalakshmi *et al*., 2005; Vattanajun *et al.*, 2005). Powdered plant material was soaked in methanol for 2 days at room temperature and the solvent was filtered. This was repeated 3-4 times until the extract gave no coloration. The extract was distilled and concentrated under reduced pressure in the Buchi rotovapour R-114 yielding a gum-like residue, which was then suspended in water and extracted with various organic solvents of increasing polarity (starting with the lipophilic solvent n-Hexane, ending with the more hydrophilic n-Butanol). The solvent from each extract was distilled and concentrated under reduced pressure in the Buchi rotavapour. Finally the extracts were freeze dried and were used for further studies.

Induction of Epilepsy

Convulsions were induced by an intraperitoneal (i.p.) injection of Pentylenetetrazole (60mg/Kg body weight) in saline (Ray and Poddar, 1985; Gupta *et al.*, 1999; Santos *et al.*, 2002; Rizwan *et al*., 2003).

Administration of Test substance

Each fraction of CA extract (200mg/Kg body weight) was dissolved in saline and given to the animals for one week prior to the injection of PTZ (Saxena and Flora, 2006). A gavage tube was used to deliver the substance by the oral route, which is the clinically expected route of administration of CA (Vattanajun *et al.*, 2005). The volume of administration was kept at 1ml to the animal. Diazepam, an anticonvulsant drug, was dissolved in normal saline and given intraperitoneally (2mg/Kg bw i.p.) for one week to the experimental animals (Reference control).

Procurement of Chemicals

All chemicals used in the present study were Analar grade (AR) and obtained from the following scientific companies: Sigma (St. Louis, MO, USA), Fisher (Pittsburg, PA, USA), Merck (Mumbai, India), Ranbaxy (New Delhi, India), Qualigens (Mumbai, India). In the present investigation Barnstead Thermoline

water purification plant for nanopure water, Kubota KR centrifuge and Hitachi U-2000 Spectrophotometer and other standard equipments were used for biochemical/physiological analyses.

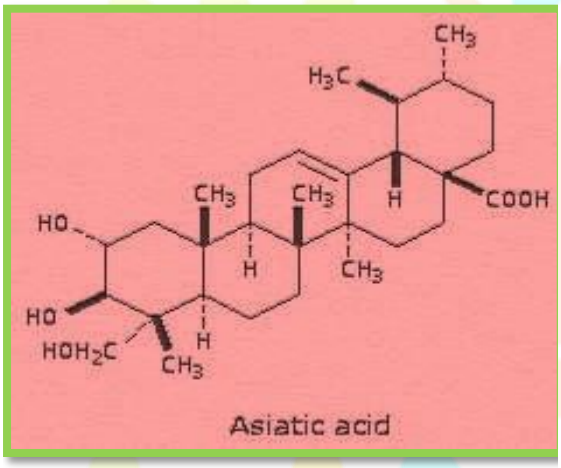
Statistical treatment of data

All assays were carried out with six separate replicates from each group. The mean, standard error (SE) and Analysis of Variance (ANOVA) were done using SPSS statistical software for different parameters. Difference between control and experimental assays was considered as significant at $P<0.05$.

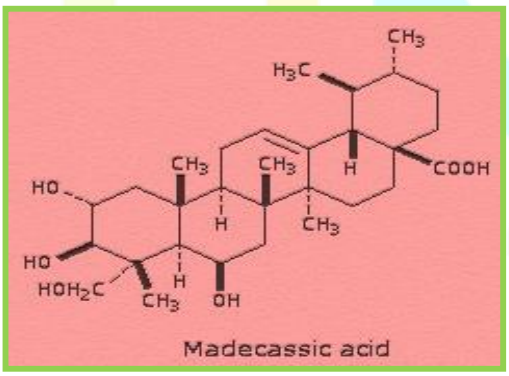
III. RESULTS AND DISCUSSION

Chemical Constituents

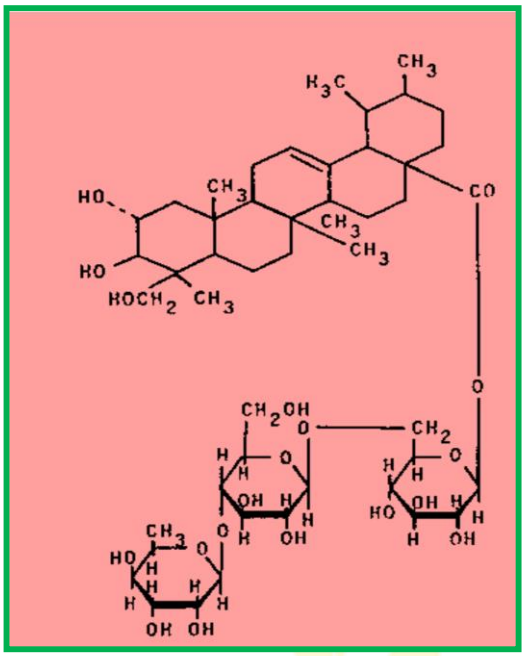
The active constituents of *Centella* include triterpenoid glycosides (asiatic acid, asiaticoside, madecassic acid, madecassoside, oxyasiaticoside, and centelloside) (Inamdar *et al.*, 1996; Maquart *et al.*, 1999); saponin glycosides (1.4-3.4%) (brahmiside, brahminoside); flavonol glycosides (quercetin-3-glycoside and Kampferol-3-glycoside); flavonoids viz., naringin, quercitin, rutin, cathecin, kampeferol and apigenin; phytosterols such as ß-sitosterol, stigmasterol and camposterol and a volatile oil consisting of vallerin, camphor, cineole and terpene acetate that comprises 35% of the total oil content (Gotu kola, *centella asiatica*, the Goddes of the Supreme Wisdom). Gotukola also contains naturally occurring vitamins A, B, C, G, K, tannins (24.5%); essential oils (0.8-1%); monoterpenes (I-pinene, ß-pinene, myrcene, γ-terpineol, borneol); sesquiterpenes (I-copanene, ß-elemene, ß-caryophyllene, trans-ß-farnesene, germacrene, bicyloelemene); several aminoacids (lysine, alanine, phenylalanine, serine, aspartic acid, glutamic acid); fatty acids (palmitic, oleic and linoleic acids); resin (8.9%); an alkaloid named hydrocotyline and elements Calcium, Magnesium and Sodium.



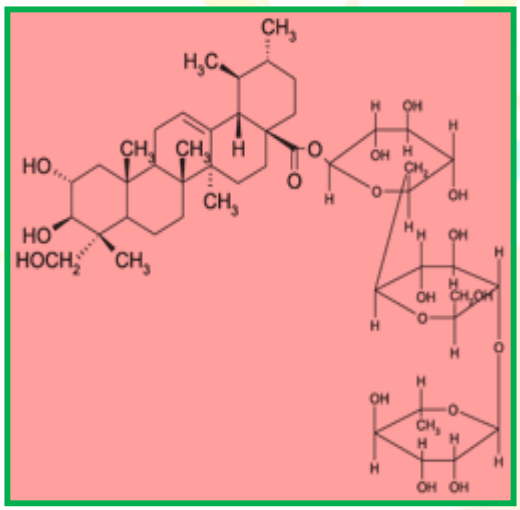
Asiatic acid
Molecular Formula : $C_{30}H_{48}O_5$
Molecular Weight : 488.70



Madacassic acid
Molecular Formula : $C_{30}H_{48}O_6$
Molecular Weight : 504.70



Asiaticoside
Molecular Formula: $C_{48}H_{78}O_{19}$
Molecular Weight: 943.12



Madecassoside
Molecular Formula: C48H78O20
Molecular Weight: 959.12

Pharmacological Properties of *Centella asiatica*

Centella asiatica appears to be non-toxic but mild allergic causing contact dermatitis in sensitive individuals (WHO, 1999). *Centella* showed sedative effect; increased mean RBC count, haemoglobin concentration, blood sugar, serum cholesterol, total serum proteins in adults (Ramaswamy *et al.*, 2000). It also showed reduction in lipid peroxidation, spontaneous motor activity, potentiation in diazepam withdrawal-induced hyperactivity, hypothermia and phenobarbitone sleeping time. Sunilkumar *et al*. (1998) reported increased vascularization of connective tissue, increased collagen biosynthesis due to aqueous extract of *Centella asiatica*. Oral treatment of methanol extract of *Centella asiatica* increased the anti-oxidant enzymes like superoxide dismutase, catalase and decreased glutathione and ascorbic acid (Jayashree *et al.*, 2003). Shukla *et al.* (1999) also reported the same increased antioxidants namely SOD, catalase, glutathione peroxidase, vitamin E and ascorbic acid in newly formed tissues. Studies on immunomodulatory activity of *Centella asiatica* revealed that the significant increase in phagocytic index, total WBC count (Jayathirtha and Mishra, 2004). It also showed effect against herpes simplex viruses (Yoosook *et al.*, 2000), *Mycobacterium leprae* and *Mycobacterium tuberculosis* (Medda *et al.*, 1995).

Extracts of *Centella asiatica* have also successfully treated in surgical wounds, skin grafts, gangrene, and traumatic injuries (Kartnig, 1988); chronic skin lesions and leprosy wounds (Lawrence, 1967*). Centella asiatica* showed wound healing activity (Suguna *et al.*, 1996; Shukla *et al.*,1999; Coldren *et al.*, 2003); antitumor activity (Babu *et al.*, 1995); anti-anxiety activity (Bradwejn *et al.*, 2000); anti-hepatoma activity (Lin *et al.*, 2002); cognition-enhancement in rats (Veerendra kumar and Gupta, 2002; Gupta and Veerendra Kumar, 2003). *Centella asiatica* was effective in improving microcirculation in venous hypertension and diabetic microangiopathy (Incandela *et al*. 2001). It was also used in the treatment of tuberculosis, syphilis, amoebic dysentery and common cold, also known as anti-aging plant (Mehmood and Mohammed Ahmad,

1998). Somchit *et al*. (2004) reported antinociceptive and anti-inflammatory effects of *Centella asiatica*. It also showed protection against radiation induced damage in liver (Sharma and Sharma, 2005), lead poisoning in CNS (Saxena and Flora, 2006), age related oxidative damage (Subathra *et al.*, 2005) and colon tumorigenesis (Bunpo *et al.*, 2004). It was also used in the treatment of anaemia, blood disorders, bronchitis, urinary disorders and splenomegali (Duke, 2001). It is also an active constituent in ayurvedic formulations like Mentat, Memorin, Mentalin, Mental Alertness, Abana (Heart care), Geriforte (Gericare), Anxocare etc.

The ethanolic extract of *Centella asiatica* significantly increased the wound breaking strength in animals (Shetty *et al.,* 2006). Triterpenes such as asiatic acid, madecassic acid and asiaticoside extracted from *Centella asiatica* stimulate extracellular matrix accumulation in the way of wound healing (Maquart *et al.,* 1999). Aqueous extract of *Centella asiatica* showed faster gel formation on open wounds when compared to ointment and cream formulation (Sunil Kumar *et al.,* 1998). Pre-co-treatment with aqueous extract of *Centella asiatica* effectively counteracted the cardiotoxicity caused due to the disorganization of mitochondrial structure and systolic failure (Gnanapragasam *et al.,* 2007). Asiaticoside, an active compound isolated from CA induces human collagen I synthesis via the activation of the TbetaRI kinase – independent smad pathway (Lee *et al.,* 2006). The water extract of CA and asiaticoside reduce the gastric ulcers in rats (Cheng *et al.,* 2004).

Total triterpenic fraction of *Centella asiatica* showed a combined improvement of the microcirculation and capillary permeability in patients with venous hypertension (Belcaro *et al.,* 1990). The histological findings of *Centella* reduce acute radiation in Sprague-Dawley rats by their anti-inflammatory activity (Chen *et al.,* 1999). *Centella* has been used as a brain tonic and sedative in the clinics of India (Handa *et al.,* 1998). In India and Africa, some preparations of the *Centella asiatica* have been employed in the treatment of leprosy (CSIR 1948-1985; Watt and Breyer, 1962).

Centella showed decrement in seizure score, improvement in learning deficits induced by PTZ and increased latencies in passive avoidance behaviour (Gupta and Veerendrakumar, 2003). It showed protection against electroshock induced convulsions, Pentylenetetrazole and strychnine induced chemoconvulsions (Ganachari *et al.,* 2004). It showed significant increment in biogenic amines (Chen *et al.,* 2005). It was also beneficial in improving memory, treating mental fatigue and anxiety (Hamid *et al.,* 2002). It also showed antidepressant activity in rats (Chen *et al.,* 2005). Lee *et al*. (2000) reported the protective effect of *Centella* against glutamate induced excitotoxicity in cultured cortical neurons due to the cellular oxidative defense mechanism. Dendritic growth in the hippocampal CA3 neurons and increased dendritic aborization during administration of *Centella* fresh leaf extracts may be implicated to the improved learning and memory (Mohandas Rao *et al.,* 2005). Accelerating capacity of *Centella* against axonal regeneration and recovery of damaged nerve cells also examined by Soumyanath *et al*. (2005). It was useful in increasing the learning capacity, memory and promoting brain growth (Anbuganapathi, 1995). It also showed a reduction in lipid peroxidation, spontaneous motor activity, potentiation in diazepam withdrawal-induced hyperactivity, hypothermia and potentiation of pentobarbitone sleeping time (Ganachari *et al.,* 2004). *Centella* showed a significant reduction in frequency of seizures of generalized tonic-clonic seizures, partial seizures, psychogenic attacks and alcoholic excess (Moharana and Moharana, 1994). Methanol and Ethylacetate extracts of *Centella* and the pure asiaticoside imparted the anxiolytic activity (Wijeweera *et al.,* 2006). Treatment with aqueous extract of *Centella* during postnatal period enhances learning and memory influencing the neuronal morphology (Rao *et al.,* 2005). It was also reported that gotu kola might cause drowsiness and gastrointestinal upset and nausea (Jonathan Klemens, 2006).

Summary

The present findings suggest significant neurochemical alterations during PTZ-induced epilepsy which might be implicated to generation of free radicals through altered antioxidant defense mechanisms. The augmented free radical attack is quite evident from the enhanced lipid peroxidation which may damage the cellular membrane architecture and alter the associated enzymes, receptors and physiological factors ultimately leading to altered metabolic functions and tissue dysfunction. The magnitude of membrane damage and the impairment in the functioning of antioxidant defense system might be considered to play significant role in the seizure susceptibility. Different extracts of *Centella asiatica* were found to modulate the activities of different neurochemical abnormalities that have occurred during epilepsy, suggesting that the CA extracts or the bioactive compounds present in the extracts may be beneficial in the antiepileptic treatment. Hence, the information gained from the present study can be used for proposing a better pharmacological utensil to treat the epilepsy and related disorders. The present study also helps in the discovery of new neuroprotective components from the medicinal plants.

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