Cognitive impairments in epilepsy:

Cognition is the alert quality of the mind, which is capable of thinking and reasoning. It predominantly constitutes of higher order mental functions; such as attention, intelligence, language, visual memory, psychomotor speed and execution. The major mediators of the neurobehavioural comorbidities of epilepsy underlie psychiatric, cognitive and social factors (Elger et al., 2004; Aldenkamp et al., 2005 & Berg et al., 2008). The neuropsychological impairment is an important comorbidity of chronic epilepsy (Elger et al., 2004). Its potential mediators include epilepsy syndromes, brain development and brain ageing, underlying brain disorders, and core epilepsy characteristics (eg, early age of seizure onset, longer epilepsy duration, epileptiform discharges, and seizure drugs) (Jack et al., 2012 & Malik et al., 2014).

Figure 2: Major mediators of the neurobehavioral comorbidities of epilepsy; adopted from Jack, 2012; The Lancet.
More than half of the epileptics had some sort of cognitive problems with abnormal behavioral manifestations (Rodin et al., 1977). Abnormal cognitive functions are commonly reported in people with chronic epilepsy. Adults who had developed epilepsy during their childhood tend to have less education, decreased rates of employment and employment at lower job levels, lower rates of marriage, poorer physical health and increased incidence of psychiatric disorders (Jalava et al., 1997 & Sillanpää et al., 1998). It is well established that epilepsy is often characterized by cognitive deficits in addition to seizures. Problems with cognition in adults can be manifested as reductions in attention, IQ, language and perceptual skills, motor speed, coordination, executive functions including problem solving, verbal and visual memory, dexterity, memory impairments, learning disabilities, attention deficits and in children cognitive problems are more diffuse, which includes learning difficulties, poor academic outcome, behaviour problems, poor social outcome (Rijckeversel, 2006). Many of the studies have confined that poorest cognition is associated with early age onset (Hermann & Seidenberg, 2007 and Malik et al., 2014). These abnormalities are related to multiple factors including seizure type, duration of epilepsy, especially in the presence of generalized tonic – clonic seizures, location of the foci, seizure frequency and type of EEG pattern and exposure to antiepileptic drugs (AEDs) (Galioto et al., 2015).

**Antiepileptic drugs and cognitive side effect:**

Antiepileptic drugs (AEDs) suppress membrane excitability, upsurge postsynaptic inhibition or alter synchronization of neural networks to decrease excessive neuronal excitability associated with development of seizure (Park & Kwon, 2008). Common side effects of decreasing neuronal excitability are slowed motor and psychomotor speed, poor attention and mild memory impairment (Meador et al., 2005). Studies in experimental animals have shown significant AED effects in the developing brain including apoptotic neurodegeneration. The public health impacts of these side-effects in human functions are language and working memory which are negatively affected (Bittigau et al., 2003 & Yang et al., 2016).
Adverse cognitive side effects have been established for many classes of Antiepileptic drugs (Vermeulen & Aldenkamp, 1995 and Aldenkamp et al., 2003). Older AEDs, especially Phenobarbital and benzodiazepines are associated with the greatest risk of cognitive side effects (Wolf et al., 1981 & Farwell et al., 1990). Several studies have indicated, Sodium Valproate has least negative impact on cognitive function (Aldenkamp et al., 1990 and Dodson & Pellock, 1993). The cognitive side effects of carbamazepine, phenytoin and sodium valproate are comparable and associated with modest psychomotor slowing accompanied by decreased attention and memory (Meador et al., 2005). Though the neuropsychological side effects generally emerge according to a dose-dependent relationship (Meador et al., 2005); however, both quality of life (Gilliam, 2002) and memory may be affected, even when serum blood concentrations are within standard therapeutic ranges. Around 11-20 % of patients with refractory epilepsy report some type of cognitive adverse
event when taking Topiramate (TPM) (Bootsma, 2004), but that percentage depends on whether subjects are healthy adults or patients with medical conditions such as epilepsy. The cognitive side effects of carbamazepine, phenytoin and sodium valproate are comparable and associated with modest psychomotor slowing accompanied by decreased attention and memory.

In comparison to those, newer AEDs as per example, Lamotrigine is also associated with little or no observable cognitive impairment (Martin et al., 1999 & Meador et al., 2005, 2001).

Levetiracetam, which has been associated with some reports of irritability and aggression, appears to have a favourable cognitive side effect profile (Rugino & Samsock, 2002). It has been associated with no significant increases in reaction time, but the size of reaction time slowing for levetiracetam was smaller than for oxcarbazepine and carbamazepine. Unlike these other two AEDs, levetiracetam was not associated with a change in any EEG or visual evoked potential parameter (Mecarelli et al., 2004).

There is little information about tiagabine and vigabatrin. Very less cognitive data exist about tiagabine, although any cognitive effects appear modest (Dodrill et al., 1998). In one small add-on study, tiagabine was associated with a decline in verbal memory as well as less energy (Fritz et al., 2005). Vigabatrin has a risk of visual field constriction, although its cognitive profile is reportedly good (Provinciali et al., 1996). Even though the greatest concern of cognitive side effects of the new generation of AEDs are taken care, but it is not completely devoid from the risk (as per only the cognitive blunting is concern). Despite of advanced medical management with modern anti-epileptic drugs, more than 30% of patients continue to have drug-resistant epilepsy with frequent seizures (Kwan et al., 2010). Thus, effective and safe therapy of epilepsy still remains a challenge. The need for new drugs with least or minimal side effect is still exists (Adhikari et al., 2015).

Medicinal Plants as antiepileptic action:

Historically, the majority of new drugs have been generated from natural products or the compounds derived from natural products. Bioactive compounds isolated from plants, fungi and bacteria have given rise to a wide range of therapeutics. Despite this success, the field of
natural product research has witnessed a significant decline in the past two decades. This is due to the emergence of target validation, combinatorial chemistry and high-throughput screening as new paradigms for drug discovery. Several other challenges have also contributed to this trend, including difficulties associated with isolating pure compounds from crude extracts, identifying their mechanism of action and synthesizing these highly complex structures. On the other hand, natural products are seen as privileged structures that have been evolutionarily selected on basis of their ability to functionally interact with biological macromolecules (Clardy & Walsh, 2004 and Koehn & Carter, 2005).

In the last 30 years there has been a decline in the output of R&D programs of pharmaceutical companies. Nevertheless it has been shown that natural products continue to play a role in the development of new drugs for the treatment of human diseases (Figure 4) (Newman & Cragg, 2012).

![Figure 4: All new approved drugs between 01/01/1981 and 31/12/2010.](image)

'N' Natural product, 'NB' Natural product Botanical, 'ND' Derived from a natural product, 'S' Totally synthetic drug, 'S*' Made by total synthesis, but the pharmacophore is/was from a natural product, 'V' Vaccine, 'NM' Natural product mimic (Newman & Cragg, 2012).

Historical evidence suggests that plants were used to treat convulsions as early as 6000 BC in India, and 3000 BC in China and Peru. Also Africa and South America have a long tradition
of using plants to treat various diseases including the treatment of epilepsy. Nowadays, people in developed countries use plants because they are viewed as natural and time-tested and are therefore considered safe by the patients using them. Before using plants care should be taken because some plants have been reported to be proconvulsant or can alter the metabolism of AEDs. In many of the developing countries the plants were used because of their easy accessibility than pharmaceutical drugs. Many different plants are being used by traditional healers for the treatment of epilepsy. Administration routes include oral, inhalation of smoke from burnt plant material, steam inhalation from the plant material, topical or other ways. Duration of the treatment was very variable among the healers (Moshi, 2005). Today proper clinical evidence for the use of plants in the treatment of epilepsy is less missing. But on the other hand, evidence from preclinical research shows positive activity from plant extracts. The Harvard Medical School program studied extracts and extract-derived compounds isolated from Chinese, Japanese and Indian therapies and two-third of these showed activity in vivo, in vitro or both (Wachtel-Galor et al, 2011). The most unfortunately these developing countries lose their intellectual property, despite of an excellent history of traditional and folk medicine.

*Marsilea quadrifolia* Linn.

In the traditional Indian medicine (Ayurveda) and folk medicine *Marsilea quadrifolia* Linn. is used for the treatment of behavioral and epileptic disorders (Satyavati & Gupta, 1987; Chauhan et al., 1988; Sivarajan and Balachandran, 1994; Vaidyaratnam et al., 1994). It is an aquatic fern used as vegetable in various part of India. Any phytochemical/s having antiepileptic and neuroprotective property with minimum or no adverse effect can be useful not only in chronic epilepsy, but also in the other psychiatric disorders. Several studies on *Marsilea quadrifolia* Linn. have revealed its sedative, anticonvulsant, antibacterial and antioxidant activities (Chatterjee et al., 1963; Chauhan et al., 1988; Ripa et al., 2009 & Reddy et al., 2012). MQ is an aquatic fern bearing four-part leaf resembling ‘4-leaf clover’. It is
distributed throughout the India and grows up in marshy places and along the banks of canals of rivers.

Figure 5: Photograph of whole plant *Marsilea quadrifolia* Linn. (Above) and leaf (bellow), adopted from Adhikari et al., Jr. of Ethnopharmacology, 2015.
It is called by different names in different parts of the country like; Cauptiya in hindi, Susni in Bengali, Citiginasoppu in Kannada, Sunisannah in Sanskrit. It is sweet, astringent, refrigerant, acrid, emollient, anodyne, hypnotic, diuretic, constipating, expectorant and aphrodisiac. In various parts of India, it is used as a vegetable. In folk medicine; the herb is used to induce sleep (Satyavati & Gupta, 1987; Vaidyaratnam, 1994 & Khare, 2004). Earlier, it was reported to have a CNS depressant and anti-convulsive effect in rat (Deshpande et al., 1980). Our earlier studies (Adhikari et al., 2011 & 2013) corroborated the findings, which established by other workers on its crude extracts (Sahu et al., 2012).

An earlier in vitro study revealed that crude methanolic extract of MQ had a good potential as an antioxidant (Zahan et al., 2011). This was supported by one of the previous studies where methanolic extract of MQ was proved to have anticonvulsive property when tested upon MES and PTZ induced epilepsy in rats (Adhikari et al., 2013). Therefore the present study was carried out to isolate the active component responsible for its antiepileptic property. Later on from our study it was evaluated that 1-Triacontanol cerotate; isolated from Marsilea quadrifolia Linn., was found to be most active component which ameliorates reactive oxidative damage in the frontal cortex and hippocampus of chronic epileptic rats (Adhikari et al., 2015) and also most effectively reduce epilepsy by developing the tolerance against PTZ kindling in rats.

**Epilepsy and brain inflammation:**

The immune system and the inflammatory reactions play an important role in tissue protection and repair from a variety of infectious or non-infectious insults. Inflammatory reactions are widely known to contribute to the pathogenesis in the brain in various CNS diseases, including autoimmune, neurodegenerative, and epileptic disorders (Vezzani et al., 2005).

Pro and anti-inflammatory cytokines and related molecules have been labelled in brain and plasma of various experimental models as well as clinical cases of epilepsy (Vezzani et al., 2005). Experimental studies in rodents demonstrated that inflammatory reactions in the brain can enhance neuronal excitability, impair cell survival, and increase the permeability of the blood-brain barrier to blood-borne molecules and cells (Jankowsky & Patterson, 2001 & Vezzani et al., 2005). Experimentally induced seizures in rodents trigger a prominent inflammatory response in brain areas recruited in the onset and propagation of epileptic activity. Recent tonic-clonic seizures in epilepsy patients induce a proinflammatory profile
of cytokines in plasma and CSF, consisting of higher IL-6 levels and lower IL-1Ra–to–IL-1α ratio (Hulkkonen et al., 2004; Vezzani et al., 2005 & Peltola et al., 1998, 2000, 2002).

Moreover, some antiinflammatory treatments reduce seizures in experimental models and, in some instances, in clinical cases of epilepsy. However, inflammatory reactions in brain also can be beneficial, depending on the tissue microenvironment, the inflammatory mediators produced in injured tissue, the functional status of the target cells, and the length of time the tissue is exposed to inflammation (Vezzani et al., 2005 & 2013).

Figure 6: Schematic representation of the cerebral vasculature and CSF circulation and their relation to inflammatory mediators and antigen transport (Adapted from Aloisi et al., 2000).
Oxidative Stress in Epilepsy:

The study of reactive oxygen species (ROS) has sparked great interest in clinical and experimental medicine in the last two decades (Cardenas-Rodriguez et al., 2013a). They are especially generated during the irradiation of ultraviolet (UV) light, X-rays, and gamma rays, products of reactions catalyzed by metals, present in air pollutants, produced by neutrophils and macrophages during inflammation, and by products of reactions catalyzed by the electron carriers in the mitochondria (Valko et al., 2006 & Cardenas-Rodriguez et al., 2013a). ROS is having dual role in biological systems i.e. beneficiary or harmful effect. The beneficial effects of ROS can be observed in their physiological role in numerous cellular responses and cell signalling systems. By contrast, at high concentrations, ROS can be important mediators of cell damage to various structures such as lipids, proteins, and nucleic acids. The beneficial effects of ROS can be either by the nonenzymatic or enzymatic antioxidant system. Despite the presence of the antioxidant defence system to combat oxidative damage caused by ROS, this damage accumulates throughout life (Valko et al., 2006 & Cardenas-Rodriguez et al., 2013a). The imbalance between the ROS and RNS levels (such as superoxide, radical O$_2^-$; hydrogen peroxide, H$_2$O$_2$; hydroxyl radical, HO•; and nitric oxide, NO) and the cellular antioxidant defense system (superoxide dismutase, SOD; catalase, CAT; glutathione peroxidase, GPx; glutathione reductase, GR; and glutathione-S-transferase, GST) is defined as “oxidative stress” (Valko et al., 2006 & Cardenas-Rodriguez et al., 2013a). Because this disequilibrium can appear at the cellular level (involving the mitochondria, cytochrome P450 system, peroxisomes, and activation of inflammatory cells (Inoue et al., 2003 & Cardenas-Rodriguez et al., 2013a)), it is involved in the development of several diseases such as cancer, atherosclerosis, and arthritis and in neurodegenerative disorders such as epilepsy (Valko et al., 2006; Waldbaum & Patel, 2010 & Cardenas-Rodriguez et al., 2013a). The participation of oxidative stress in diseases of the central nervous system (CNS) is well established (Ansari et al., 2004; Cardenas-Rodriguez et al., 2013a & Cardenas-Rodriguez et al., 2013b). The brain is highly sensitive to oxidative damage because this organ contains a large number of easily oxidized fatty acids (20: 4 and 20: 6) and a limited antioxidant system (Cardenas-Rodriguez et al., 2013b). Oxidative stress is strongly implicated during seizures induced by excitotoxicity, due to mitochondrial ROS generation. Since the beginning of the 1990s, oxidative stress has been associated with neuronal hyperexcitation caused by CNS diseases (Bondy, 1995). Dalton, in 1995, was the first to identify brain damage induced by the presence of oxidative stress in an animal experimental model (Dalton, 1995). The presence of
NO is known to be a cause of seizures (Patel et al., 1996). NO∙ is formed from high concentrations of inducible nitric oxide synthase (iNOS). The role of oxidative stress in pentylenetetrazole-induced epilepsy has been proven in rodents (Frantseva et al., 2000; Devi Uma et al., 2006 & Arma’gan et al., 2008). The increased activity of glutamatergic systems induces status epilepticus and causes an energy imbalance, increasing ROS formation (Schweizer et al., 2004). Several studies have linked seizures and cell damage to the excitotoxicity induced by pentylenetetrazole (Kov´acs et al., 2002 & Cardenas-Rodriguez et al., 2013a). Seizures are linked to the increased release of glutamate and NMDA receptor activation. In fact, during epileptic seizures induced by different models, there is an extracellular Ca$^{2+}$ concentration decrease and a cytosolic Ca$^{2+}$ concentration increase (White et al., 2007 & Cardenas-Rodriguez et al., 2013a). The effects mediated by Ca$^{2+}$ during excessive glutamate receptor activation (excitotoxicity) lead to neuronal degeneration and give rise to oxidative stress. The phospholipase A2-dependent activity of Ca$^{2+}$ mediated by glutamatergic receptors liberates arachidonic acid (AA), which generates O$_2^{-}$ through its metabolism by lipoxygenases and cyclooxygenases for eicosanoid formation (Singh et al., 2003 & Cardenas-Rodriguez et al., 2013a). The constant formation of NO∙ by the glia is neurotoxic because it increases the neuronal sensitivity to this reactive species. The neurotoxic action of NO∙ is likely caused by the formation of peroxynitrite (ONOO$^-$), which is rapidly formed by the reaction of NO∙ with O$_2^{-}$. Under conditions of energy deficit and elevated intracellular Ca$^{2+}$ concentration, xanthine oxidase generates O$_2^{-}$. In this environment, lactic acid is generated, which promotes the release of Fe$^{2+}$, the Haber-Weiss reaction, and the production of HO (Granger, 1988). Furthermore, ONOO$^-$ can react with tyrosine in proteins to form 3-nitrotyrosine (Hensley et al., 1997 & Cardenas-Rodriguez et al., 2013a).

Hippocampus:

The term ‘hippocampus’ is derived from the Greek word hippos means ‘horse’ and kampo means ‘sea monster’, named after its similarity to the sea horse. The hippocampus was also called as Ammon's horn by Danish anatomist Jacob Winsløw due to its resemblance to a ram's horn (the Egyptian god Ammon had ram's horns) (Jacob Winsløw, 1732). A decade later his fellow Parisian, the surgeon de Garengeot, used "cornu Ammonis" – horn of the historic Egyptian spirit. Hippocampus is a central module of human brain and other vertebrates. Humans and other mammals have two hippocampi, one in each side of the brain, positioned intensely in the medial temporal hemisphere. The hippocampus is one of a cluster
of assemblies within the limbic system classically called the hippocampal formation. Hippocampal formation is a highly differentiated structure consisting of histologically diverse sub-regions, including: the dentate gyrus, hippocampus, subiculum, presubiculum, and parahippocampus, and entorhinal cortex (Duvernoy et al., 1988). The dentate gyrus, hippocampus, and subiculum have a single cell layer with less-cellular or acellular layers set above and below. The other parts of the hippocampal formation have several cellular layers.

Figure 7: Diagram of rat Hippocampus, showing three-dimensional structure of the hippocampus and related structures. (Image adapted from, Amaral et al., 1989).
**Neuroanatomical organization of hippocampus:**

Individual fields of the hippocampus show differences in the structure and arrangement of nerve connections. Most of the afferent fibres reach the hippocampus through the tractus perforans. They form synapses with the dendrites of pyramidal cells. Some afferent fibers switch in the granular cell layer, whose axons are called mossy fibers, and form synapses with pyramidal cells. In the hippocampus, mossy fibers are only present in the CA3 and CA4 fields. Axons of pyramidal cells form afferent pathways. The main afferent pathways of the hippocampus lead to the corpora mamilaria to the front nuclei of the thalamus and to the hypothalamus. The main afferent pathways to hippocampus originate from the entorhinal cortex (EC), the other run from the amygdala and different parts of the neocortex. The EC has connections to other areas of the cerebral cortex. The apical dendrites of CA1 get a sparse projection. Thus, the perforant pathway establishes the EC as the main “interface” between the hippocampus and other parts of the cerebral cortex. The dentate granule cell axons (mossy fibers) pass on the information from the EC on thorny spines that exit from the proximal apical dendrite of the CA3 pyramidal cells. Then, the CA3 axons loop up into the region where the apical dendrites are located, extend all the way back into the deep layers of the EC - the Shaffer collaterals completing the reciprocal circuit. Field CA1 also sends axons back to the EC, but these are sparse than the CA3 projection. Within the hippocampus, the flow of information from the EC is largely unidirectional, with signals propagating through a series of tightly packed cell layers, first to the dentate gyrus, then to the CA3 layer, to the CA1 layer, and next out of the hippocampus (Andersen et al., 2007).

**Role of hippocampus in learning and memory:**

In a landmark paper, Scoville & Milner, 1957 reported the neuropsychological findings from a patient Henry Molaison, known by his initials, H.M., who underwent bilateral hippocampal removal for the treatment of uncontrolled refractory epilepsy. H.M., who is probably the most thoroughly studied neuropsychological subject in memory research, experienced a permanent loss of the ability to encode new information into long-term memory. The unexpected outcome of the surgery was severe anterograde and partial retrograde amnesia. He was unable to form new episodic memories after his surgery and could not remember any events that occurred just before his surgery, but he did retain memories of events that occurred many years earlier extending back into his childhood. This anterograde memory impairment has been seen in other patients with bilateral damage restricted to the hippocampus (Zola-Morgan et al., 1986). The intense interest in understanding the brain mechanisms involved in learning
and memory have helped foster research at the neuroanatomical, physiological, and behavior levels of analysis in the hippocampus.

**Hippocampus and Epilepsy:**
The hippocampus exhibits an extremely explicit role in the pathogenesis of epilepsy (Sendrowski & Sobaniec, 2013). The majority of patients with drug resistant temporal lobe epilepsy have a characteristic pathology of the brain structure – hippocampus sclerosis (HS), characterized by loss of pyramidal neurons, severe glial reaction and remodelling of neuronal networks. Studies on the pathogenesis of HS, supported by numerous literatures on the subject, but could not able to explore a clear answer to whether HS is a cause or consequence of repeated seizures. The process of epileptogenesis is usually explained in the literature by the two-hit hypothesis. The term epileptogenesis commonly refers to a period of time from the first hit, such as trauma or stroke, to the occurrence of the first epileptic seizure. It is a chronic process, in which a series of biochemical and structural changes take place in the nerve tissue. During this period, the following processes take place: a process of pathological “learning” of the neurons, pathological reorganization of neural activity, but also the reorganization of the nerve tissue microstructure (Sendrowski & Sobaniec, 2013). According to the latest research, three basic phases can be distinguished in the process of epileptogenesis: acute brain damage (initial insult), latent period with “maturation” of the epileptic focus, and actual epilepsy, where a process called secondary epileptogenesis takes place (Pitkanen & Lukasiuk, 2011). The most important etiological factors include severe craniocerebral trauma, where the risk of post-traumatic epilepsy depending on the severity of the injury ranges from 2% to as much as 25% (Leoekiewicz & Lasoń, 2007). High risk factors also include: stroke, epileptic state, recurrent and prolonged febrile convulsions, cerebral thrombosis and neuroinfections. Therefore, the neurobiological basis of epileptogenesis was analysed in experimental models of such damages (Holtkamp & Meierkord, 2007).

HS is characterized by selective loss of pyramidal neurons – especially of sectors CA1 and CA3 of the hippocampus – pathological proliferation of interneuron networks, and severe glia reaction. These changes occur in the course of long-term and complex epileptogenesis. Hence the hippocampus is an important structure in the pathophysiology of convulsions and epilepsy. Hippocampal sclerosis is considered as one of the major pathogenic factors of drug-resistant temporal lobe epilepsy (Sendrowski & Sobaniec, 2013).