Introduction
INTRODUCTION

Epilepsy is a common neurological disorder characterized by epileptic seizures which are associated with complex molecular, biochemical, physiological and structural changes in the brain (Morimoto et al., 2004). The worldwide prevalence of epilepsy is 1% (10.2/1000) with an annual incidence estimated to be 50.4/100,000 (Maguire et al., 2012). In North America, the annual incidence of epilepsy is nearly 50/100,000 (Poole et al., 2000) and in India 61/100,000 with greatest rates for infants and elderly (Mani et al., 1998). Epilepsy can happen to anyone at any time of life and the potential causes of epilepsy include brain injury, stroke, brain tumor or inflammation in brain (Luhdorf et al., 1986). Recreational drugs, alcohol can also trigger seizures in some people (Brust, 2008). In addition, viral, bacterial, fungal and parasitic infections could also result in epilepsy (Singhi, 2011). Mutations in ion channel encoding genes such as KCNQ2 (potassium channel genes), SCN1A, SCN1B, SCN1A, SCN2A (voltage-gated sodium channel genes) are the main causes of idiopathic epilepsy (Steinlein, 2004). Epileptic seizures occur due to abnormal or excessive synchronous activity of neurons in the brain (Fisher et al., 2005). The mechanism of hyperexcitable state results from alteration or imbalance in the excitatory and inhibitory neurotransmission (action potential), change in voltage-gated ion channels or intra or extra-cellular calcium ion concentration in the central nervous system (CNS) (Graves, 2006). Evidence suggests that the excess accumulation of potassium ions in the extracellular space is involved in increasing neuronal excitability and epileptic discharge (Florence et al., 2009). Clinical manifestation of epileptic seizure consists of sudden and transitory abnormal phenomena which may include alteration of consciousness, motor, sensory, autonomic, or psychological disturbances (Al Sufiani and Ang, 2012).

Temporal lobe epilepsy (TLE) is one of the most common forms of chronic epilepsy where seizure originates from the temporal lobe of brain with underlying pathologies like hippocampal sclerosis, malformative lesions, ischemic lesions, old traumatic injuries, and inflammatory lesions (Eid et al., 2008). Hippocampal sclerosis (HS), is the most common pathology associated with TLE, defined as neuronal loss and gliosis in CA1 (Sommer sector) and CA4 region of the hippocampus (Wieser, 2004). It has a differential impact on verbal learning and memory in TLE (Helmstaedter and Elger, 2009). The temporal lobe is considered important for functional organization of
memory, thus any damage to this region can affect the memory function. A number of 
factors are known to be involved in cognitive impairment in epilepsy including biologic 
factor such as seizure type, seizure frequency and seizure associated lesions in the 
frontal lobe or limbic system which can result in memory, language or psychologic 
disturbances (Theodore et al., 1999).

Cognitive problems, generally decline in memory functions have been observed 
in patients suffering from TLE. Chronic epilepsy also induces processes of functional 
reorganisation and behavioural compensation (Elger et al., 2004). Lesions in entorhinal 
cortex (EC) layer III, which normally provides monosynaptic input to area CA1 of the 
hippocampus, frequently occur in TLE and may be causally related to the memory 
impairments seen in the disease (Schwarz and Witter, 2002). Learning and memory 
impairment along with depression has been observed in patients suffering from epilepsy 
(Devinsky, 2004; Helmstaedter et al., 2004). Hippocampal volume loss in TLE patients 
compared to the controls has been observed using magnetic resonance imaging 
(Seidenberg et al., 2005). In addition, neurodegeneration or loss of neurons in the 
hippocampus along with mossy fiber sprouting has been found in different models of 
TLE (Curia et al., 2008; Rao et al., 2006). In this form of epilepsy, the sprouting mossy 
fibres are generally larger in size with aberrant connection which might be responsible 
for the generation of temporal lobe seizures (Buckmaster et al., 2002).

The neuronal loss or cell death in epilepsy could be attributed to the increased 
of oxidative stress in brain following seizures. Free radicals have a role in regulation of 
biological function and pathogenesis of neurodegenerative diseases including epilepsy 
(Chang and Yu, 2010; Kong and Lin, 2010). Studies have shown that status epilepticus 
can change the redox potential and decrease adenosine triphosphate (ATP) production 
which affects overall brain energy (Wasterlain et al., 1993). Increased oxidative stress 
could result in cellular damage and cell death via oxidation of protein, lipids and nucleic 
acid. Seizure induced lipid peroxidation, protein oxidation and DNA damage has been 
observed in various experimental models of epilepsy and epileptic patients (Mohanan 
and Yamamoto, 2002). Oxidative stress up-regulates the expression of transcription 
factors such as activator protein (AP-1) and NF-κB, the activation of which further 
regulates a number of inflammatory genes (Schreck et al., 1992).
Oxidative reactions occurring in mitochondria produce oxygen free radicals cells which contributes to the pathogenesis of various neurodegenerative diseases such as Parkinson’s disease (Ferretta et al., 2014), Huntington’s disease (Chaturvedi and Beal, 2013; Quintanilla et al., 2013), Alzheimer’s disease (Blass and Gibson, 1991). Mitochondria carry out several important cellular functions such as ATP production, intracellular calcium homeostasis, neurotransmitter biosynthesis, regulating cell death and is also the major sites responsible for more than 90% of ROS generation (Rowley and Patel, 2013; Yan et al., 2013). Increased oxidative stress and mitochondrial dysfunction are the factors directly associated with pathophysiology of epilepsy (Simeone et al., 2014; Waldbaum et al., 2010). Mutations in mitochondrial DNA also contribute to pathogenesis of epilepsy (Aguiar et al., 2012; Zsurka et al., 2010; Zsurka and Kunz, 2010). However, it remains unclear whether and how mitochondrial functions are altered in epilepsy.

Activated glial cells are demonstrated to be the essential sentinels and dynamic modulator of neuronal functions and known to contribute to pathogenesis of various neurological disorders including brain injury (Belanger and Magistretti, 2009). Glial cells (mainly microglia and astrocytes) are known to be involved in maintaining the extracellular ions and neurotransmitters homeostasis, energy metabolism and provide protection from oxidative stress (Belanger and Magistretti, 2009). It has been well demonstrated that astrocyte express transporters and receptors of GABA and glutamate which potentially exert pro or anticonvulsive action by regulating extracellular glutamate and spatial potassium buffering and hence mediates seizure dynamics (Seifert and Steinhauser, 2013; Sunderam et al., 2010). Glial cells are also known to be responsible for uptake and conversion of glutamate which is released during synaptic activity via glutamate transporters present in the membrane of astrocytes. Evidence suggests that astrocytes can monitor synaptic activity and release signalling molecule that could modulate neuronal activity (Heuser et al., 2014). During last decade, the association of brain inflammation with epileptic seizure where glia cells are involved in number of inflammatory processes that contribute to or trigger epileptic seizures has been well documented (Vezzani et al., 2008a). Activated astrocytes produce a variety of inflammatory substances such as chemokines and cytokines, particularly interleukin-1β and tumor necrosis factor-alpha on association with epileptic activity (van Noort and Bsibsi, 2009). Changes in the expression of various astrocytic enzymes, such as
adenosine kinase and glutamine synthetase can increase neuronal excitability and promote epileptogenesis (Aronica et al., 2007). Direct stimulation of astrocytes leads to prolonged neuronal depolarization and epileptiform discharges (Tian et al., 2005). The exact mechanism how glia cells are involved in epilepsy and how do they modulate excitability is still not fully understood. However, few studies suggest that glia cell can increase inflammation and excitability in the brain. The disruption of glial-mediated regulation of ions, water, and neurotransmitters could also promote hyperexcitability and hypersynchrony (Devinsky et al., 2013).

Brain inflammation is considered as an intrinsic feature of hyperexcitable pathologic brain tissue which may play a role in seizure precipitation or reoccurrence and can determine seizure threshold in susceptible brain regions (Vezzani et al., 2011b; Zurolo et al., 2011). Moreover, brain inflammation could contribute to ictogenesis and the process involved in the development of epilepsy after brain lesion development (Vezzani et al., 2011a). It has been documented that an induction of array of inflammatory molecules (cytokines/chemokine) and various signaling pathways including IL-1R/TLR may promote the neuronal hyperexcitability either by inducing a) direct post-translational changes in neuronal channels/neurotransmitter receptors or b) indirectly by affecting target systems involved in neuronal network hyperexcitability which includes an inhibition of glutamate reuptake or promotion of glutamate release by astrocytes, changes in the expression of glutamate receptor subunit and by affecting BBB permeability (Friedman et al., 2009; Viviani et al., 2007).

Blood brain barrier (BBB) is a biochemical barrier formed by endothelial junctional complexes consisting of tight junctions (TJ) and adherens junctions (AJ) that regulates the entry of blood-borne molecules to brain and preserves ionic homeostasis within the brain microenvironment (Stamatovic et al., 2008). Oxidative stress and mitochondrial impairment may change the localization and structure of TJ protein occludin and can increase BBB permeability (Najjar et al., 2013). Evidence indicates that BBB is disrupted in epileptic patients and animal models of epilepsy where up regulation of efflux transporter and metabolic enzymes have been linked to seizure genesis (Li et al., 2013). Inflammatory molecules such as interleukins, TNF-α, interferon α and β, arachidonic acid and its metabolites, prostaglandins could alter BBB permeability (Oby and Janigro, 2006). Various chemical convulsants are known to disrupt BBB by several different mechanisms such as impairment of GABA
transmission, increased glutamate neurotransmission or through direct excitatory action (Ilbay et al., 2003). However, it is still controversial that increased BBB permeability is a component of etiology of epilepsy or a consequence of seizures. Seizures or BBB opening or both can alter cerebral energy metabolism which can change the levels of local functional activity in brain (Pappius et al., 1979).

Evidence is available showing therapeutic interventions such as pharmacotherapy [(anti-epileptic drugs (AED)] and TLE surgery are associated with cognitive and behavioral dysfunction in epilepsy (Meador et al., 2001; Motamedi and Meador, 2003; Tellez-Zenteno et al., 2005). These undesirable side effects of anti-epileptic treatment are of major concern as both epilepsy and AED shows adverse effects on learning and memory functions. Various anti-epileptic drugs are available in the market to treat epileptic patients, however still more than 30% patients experience seizures after AED therapy (Loscher and Schmidt, 2011). Moreover, TLE is also known to be resistant to many anti-epileptic drugs (Beleza, 2009). Therefore, there is a need to identify natural therapies to the conventional AED which could provide the favorable clinical efficacy and tolerability with no side effects.

Turmeric (Curcuma longa) is a polyphenolic compound which is member of the ginger family (Zingiberaceae). It is a yellow color agent which is daily used in the diet mainly in Asiatic countries and consists of three curcuminoids: curcumin (diferuloylmethane; the primary constituent and the one responsible for its vibrant yellow color), demethoxycurcumin, and bisdemethoxycurcumin (Anand et al., 2007). Curcumin is a highly pleiotropic molecule that has been reported to exhibit anti-inflammatory, hypoglycemic, antioxidant, anti-cancer, wound-healing, and antimicrobial and antifungal activities (Aggarwal et al., 2007). It has shown to suppress cellular transformation, proliferation, invasion, angiogenesis, and metastasis (Goel et al., 2008). The role of curcumin has been studied in various human diseases and numerous clinical trials have been completed with few in progress (Gupta et al., 2013; Ringman et al., 2005). Curcumin has been screened as potent neuroprotective agent in patients with neurological problems due to its lipophilic nature which makes it to cross blood-brain barrier effectively (Mishra and Palanivelu, 2008). Curcumin acts as a strong antioxidant by inhibiting the generation of ROS such as superoxide anions, nitrite radical generation both in vitro and in vivo due to presence of phenolic groups (Sharma, 1976). The methoxy group on the phenyl ring and the 1,3-diketone are also important
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structural features of curcumin that contribute to its antioxidant properties (Ragunathan and Panneerselvam, 2007). The mechanism by which curcumin provide neuroprotection is through direct interaction with numerous signaling molecules (Daniel et al., 2004). Curcumin has been known to mediate anti-inflammatory role through the down-regulation of transcription regulators including NF-κB, AP-1, EGR1 and STAT3 (Shishodia et al., 2007). It is well documented that curcumin inhibits pro-inflammatory gene expression (TNF-α, IL-6, IL-8, iNOS, MMP-9, COX-2) by targeting different signal pathways to combat inflammation in brain (Amor et al., 2010). In addition, curcumin has been shown to protect blood–brain barrier integrity by reducing endothelial cells damage (Jiang et al., 2007). Moreover, curcumin has been found to ameliorate cognitive deficits by reducing neuroinflammation and oxidative stress in brain (Tiwari and Chopra, 2012). It has also been shown to regulate the expression of brain-derived neurotrophic factor and other related factors involved in synaptic transmission and crucial for maintaining molecular processes underlying cognitive function (Wu et al., 2006). Recently, curcumin has shown to exhibit neuroprotection by modulating the expression of mitochondrial proteins and ameliorating the ultrastructural changes in brain mitochondria (Srivastava et al., 2014b).

Therefore, the present study was designed with an aim to examine the glial cell response, oxidative stress, mitochondrial dysfunctions and blood brain barrier permeability in PTZ induced model of chronic epilepsy. In addition, potential of curcumin in ameliorating the behavioral, biochemical and histological changes in PTZ induced chronic epilepsy was also investigated.

The study was designed with the following objectives:

1. To establish the kindling model of chronic epilepsy using pentylentetrazole (PTZ).
2. To examine the role of curcumin supplementation on PTZ-induced neuroinflammation, altered blood brain barrier permeability and neuronal damage in PTZ induced model of chronic epilepsy.
3. To evaluate the effect of curcumin supplementation on oxidative stress and mitochondrial dysfunctions in PTZ treated animals.
4. To study the effect of curcumin supplementation against PTZ induced neurobehavioral deficits.
5. To correlate the biochemical and neurobehavioral changes with histopathological changes following curcumin administration in chronic epilepsy.