Introduction
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Epilepsy is a chronic disorder characterized by periodic recurrent seizures, which varies from a brief lapse of attention or muscle jerks to severe and prolonged convulsions. A seizure results from sudden excessive electrical discharge of cortical neurons and is characterised by changes in electrical activity. A convulsion implies violent involuntary contractions of the voluntary muscles (Wells, et al., 2006).

The estimated proportion of the general population with epilepsy according to the World Health Organization (WHO) in the year 2002 was about 4 to 10 per 1,000 people. The recent WHO estimate shows that there are about 50 million people suffering from epilepsy across the world, among which 85% of the patients belong to developing countries. An estimated 2.4 million new cases occur each year globally (Satishchandra, et al., 2001). The reports of meta-analysis studies revealed that the overall prevalence of epilepsy among Indians as 5.59 per 1000 and both males and females are equally prone to epilepsy (Sridharan and Murthy, 1999; Radhakrishnan, et al., 2000; Sureka and Sureka, 2007; Goel, et al., 2009; Das, et al., 2006a).

Control of seizures is the major therapeutic objective in the management of epilepsy. The most common treatment modalities include both conventional and newer antiepileptic drugs (AED), ketogenic diet, vagus nerve stimulation and surgery (Devivio, 1983). The rationale of selection of AED is usually based on two presumptions; one directly related to the anticonvulsant activity of the drug, while the other considers the side effect profile of the administered AED.

The goal of AED therapy is to achieve freedom from seizures without side effects. Even with the advent of new generation AEDs, it is known that significant number of epileptic patients still struggle with adverse effects (Brodie and Dichter, 1996;
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Mattson, et al., 1996). Phenytoin, a hydantoin anticonvulsant is a most common and
effective AED prescribed for a prolonged period to achieve seizure control in all types
of generalized as well as partial seizures and status epilepticus (Leppik, 2005;
McNamara, 2006; Wilder, 1995; Walker, 2005).

The AEDs account for about 4.5-11.5% cases of aplastic (Shigeru, et al., 1985;
Robins, 1962), megaloblastic (Carl and Smith, 1992), haemolytic anaemia
(Blackburn, et al., 1998) and thrombocytopenia (Holtzer and Reisner-Keller, 1997).
Phenytoin causes haematological toxicities such as thrombocytopenia (Holtzer and
(Salzman and Smith, 1998), neutropenia, agranulocytosis (Sharafuddin, et al.,1991;
Matsuzaki, et al., 1990; Ito, et al., 2009), pancytopenia and rarely, hemolytic anemia,
aplastic anemia (Shigeru, et al., 1985) and pure red cell aplasia (Blackburn, et al.,
1998). The arene oxide metabolites of phenytoin covalently bind to macromolecules,
induce stem cell death leading to aplastic anemia followed by toxicity to lymphocytes
(Dwivedi, et al., 2004; Gerson, et al., 1983) and depress the cellular as well as
humoral immunity (Sorrell and Forbes, 1975). Phenytoin was reported to decrease
serum reduced glutathione (GSH) concentration and increase lipid peroxidation in
human beings (Liu, et al., 1997). Phenytoin was also found to reduce the total
antioxidant capacity in epileptic patients (Mahle and Dasgupta, 1997). Long term
treatment with phenytoin reduced the activities of endogenous antioxidant enzymes
like superoxide dismutase (SOD), glutathione reductase, glutathione peroxidase (GPx)
and non enzymatic antioxidants like Vitamin C, Vitamin E and concurrently increased
thiobarbituric acid reactive substances (TBARS) (Sobaniec, et al., 2007). Thus,
haematotoxicity induced by phenytoin was believed to have an etiological background
of oxidative stress.

Brain is especially vulnerable to free radical damage owing to its rich polyunsaturated fatty acid (PUFA) and iron content, high oxygen consumption and scanty availability of antioxidant enzymes when compared to other tissues (Skaper, et al., 1999). The high level of iron is essential for brain development but the iron ions form reactive oxygen species (ROS), which rearranges the double bonds of PUFA and generates a number of degradation products like lipid alkoxy, peroxy radicals and lipid hydroperoxides (Gutteridge and Halliwell, 1990). ROS increases the permeability of the blood brain barrier (Gilman, et al., 1993) and influences gene expression resulting in apoptosis and neuronal death (Gilgun-Sherki, et al., 2002). High oxidative metabolism especially in catecholamine rich areas such as basal ganglia makes neurons vulnerable to membrane lipid peroxidation (Ohkawa, et al., 1979).

Phenytoin is enzymatically bioactivated to reactive intermediates that generate ROS, which in turn damages essential macromolecules including DNA (Winn, et al., 2003) and induces lipid peroxidation. This adversely affects the membrane signal transduction systems relevant to cognition and produces neurodegenerative disorders like Alzheimer’s disease (AD), Parkinsonism, Epilepsy, Stroke, Cerebral ischemia, Multiple Sclerosis, Huntington’s Chorea, Tardive dyskinesia and Amyotrophic Lateral Sclerosis (Keller, et al., 1997; Bondy, 1995).
Phenytoin was also reported to cause liver damage. It was observed that phenytoin induced hepatotoxicity (Walia, et al., 2004) caused fatal outcome in 10-38% of cases (Dreifuss and Langer, 1987). Phenytoin induced hepatotoxicity was confirmed by elevated levels of biomarkers such as serum bilirubin, serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP) and Gamma glutamyl transferase (GGT) in epileptic patients under phenytoin therapy (Aldenhovel, 1988; Kazamatsuri, 1970; Smythe and Umstead, 1989). Clinical abnormalities related to liver appeared within 6 weeks of post phenytoin therapy in majority of patients (Harden, 2000). The most common presenting symptoms observed were fever, rashes, lymphadenopathy, anorexia, myalgia, arthralgia, jaundice, hepato-splenomegaly and hemorrhagic complications (Smythe and Umstead, 1989; Mullick and Ishak, 1980). The drug as well was found to precipitate morphological and pathological abnormalities including primary hepatocellular degeneration and necrosis (Harden, 2000). Ninety five percent of phenytoin is biotransformed by the liver and less than five percent is eliminated unchanged in the urine (Bajpai, et al., 1996). During biotransformation, phenytoin generates reactive metabolites which are implicated in the etiology of hepatotoxicity (Park, et al., 2005).

Oxidative stress is involved in the pathogenesis of many types of liver injuries including drug induced hepatotoxicity (Nitti, et al., 2008). Arene oxides are generated during metabolism of AEDs both in humans and in rodents (Shear and Spielberg, 1988; Roy and Snodgrass, 1988; George and Farrell, 1994; Madden, et al., 1996; Kalapos, 2002; Zaccara, et al., 2007). Arene oxides are non radical oxidants that oxidise thiol groups of enzymes, membranes, macromolecules and other vital constituents of the cell (Jones, 2008) and induce oxidative stress as a consequence of
the disruption of thiol redox circuits. GSH, an endogenous antioxidant offers cellular protection against oxidative stress by regulating the turnover of thiols. Phenytoin was observed to deplete the GSH levels resulting in reduction of cellular defence against oxidative stress induced by its metabolites. Defective detoxification of arene oxides by the epoxide hydrolase results in hepatotoxicity (Bavdekar, et al., 2004; Kass, 2006). Phenytoin metabolites in vitro were reported to induce intense oxidative stress on the rat hepatic mitochondria and cause mitochondrial dysfunction (Santos, et al., 2008b). Thus, phenytoin induced hepatotoxicity is believed to be mediated via oxidative stress.

Phenytoin is known to induce hyperglycemia. The mechanism of phenytoin induced hyperglycemia is considered primarily due to its inhibitory effect on insulin release. Administration of phenytoin (100 mg, p.o., thrice a day) in nondiabetic patients developed hyperglycemia (Rubeaan and Ryan, 1991) and insulin resistance. Phenytoin induced free radical generation and oxidative stress was believed to be the basic reason for the development of insulin resistance. It was also observed that long term phenytoin therapy had a contributory role in the development of chronic pancreatitis (Pezzilli, et al., 1992). Oxygen radicals mediate an important role in the initiation of acute pancreatitis (Schoenberg, et al., 1995). Thus, phenytoin via oxidative stress induced insulin resistance and pancreatic dysfunction, which in turn resulted in hyperglycemia.

Long term phenytoin therapy was observed to be associated with an increase in serum triglyceride (TG) and total cholesterol (TC) levels (Luoma, et al., 1979). Hypercholesterolemia and hypertriglyceridemia accelerate the development of atherosclerosis and progression of atherosclerotic lesions (McKenney, 2001).
Phenytoin also increased the low density lipoprotein cholesterol (LDL-C) and decreased the high density lipoprotein cholesterol (HDL-C). In addition, it has been demonstrated that increased intracellular generation of ROS plays an important role in atherosclerosis (Chisolm and Steinberg, 2000). Phenytoin induced hyperlipidemia substantially increases the risk of cardiovascular and cerebrovascular diseases.

Antioxidants offer protection against drug induced oxidative stress and toxicity without causing side effects on their own. In the present investigation, antioxidants such as Vitamin C, Vitamin E, Alpha Lipoic Acid and N Acetyl Cysteine were selected and their protective effect against phenytoin induced toxicity was investigated. The rationale behind selection of the above antioxidants was their diverse characteristics, easy availability and economic viability. Vitamin C is a water soluble antioxidant, Vitamin E is lipid soluble, Alpha Lipoic Acid is a universal antioxidant i.e both water and lipid soluble, in addition, it has antioxidant regenerating potential and N Acetyl Cysteine is a GSH precursor. Further, the study was designed to evaluate the comparative effectiveness of antioxidants on various adverse effects of phenytoin.