CHAPTER-1

INTRODUCTION
Introduction

The nervous system comprises the brain, the spinal cord, and a vast array of neurons that control major body functions. Every organ can be adversely affected by toxic substances, but the nervous system is particularly vulnerable. The toxic damage to the brain or spinal cord is usually permanent as unlike other cells of the body, neurons normally cannot regenerate once lost. Even minor alterations in the structure or function of the nervous system may have profound consequences for neurological, behavioral and related body functions. Toxicants-induced alterations in nervous system can be reflected in changes in neurobehavioral output. This fact suggests that nervous system should be among the first to be thoroughly assessed in cases of exposure to known or potentially hazardous agent.

Neurotoxicity has been defined as any adverse effect on the structure and function of the central and peripheral nervous system produced by a biological, chemical or physical agent (Tilson, 1990). The neurotoxic effect can be identified and characterized by doing various studies involving biochemical, anatomical, physiological, neurobehavioral etc. The onset of neurotoxicity can vary from immediate to delayed effects following exposure to a toxic substance, and whose duration may be transient or persistent. The brain is particularly vulnerable to oxidative stress. Oxidative damage plays an important role in the pathogenesis of various neurological disorders (Quereshi et al., 2004) and neurobehavioral impairments (Sharma et al., 2009). Oxidative stress is also believed to be the central element in the regulation of the apoptotic pathways triggered by environmental stressors (cytotoxic agents, pollutants or toxicants) (Franco et al., 2009; Takaheshi et al., 2004). Neurotoxicology is an important discipline of neurosciences and significant research is being carried out. The thrust of neurotoxicology is to understand and delineate the basic mechanisms of neurotoxicity caused by various chemicals and other neurotoxic agents using modern biological tools. Toxic damage of the nervous system in human beings may occur following abuse of huge variety of substances and
other certain other types of chemicals encountered in the environment (Botha and Penrith, 2008).

Many plants are used in traditional medicine from time immemorial and they are commonly assumed to be safe. This safety is based on their long usage in the treatment of diseases according to the traditional knowledge accumulated over years. However, recent scientific research has shown that many plants used as food or in traditional medicine are potentially toxic, mutagenic and carcinogenic (Popat, 2001). Both medicinal and poisonous plants possess secondary metabolites such as cardiac glycosides, alkaloids, flavonoids, tannins, proteins, amino acids etc (Poppenga, 2010) and exhibit beneficial or adverse effects based on it (Adediwura et al., 2012). A number of studies have been reported on the neurotoxic effects of herbal medicines (Jaouad et al., 2004; Taziebou et al., 2008).

In the traditional Ayurveda and Siddha system of medicine, *Coscinium fenestratum* has been mainly used for treating diabetes mellitus (Neelesh et al., 2010). The decoction of the stem is useful in snake-bites and the decoction of stem bark is used to treat intermittent fevers (Selvam, 2010). It is suggested to have thomogenic, anti-inflammatory, antiseptic, tonic effects and is used against ophthalmopathy and inflammations (Chitra et al., 2011). The alcoholic extract of *C. fenestratum* stem, a medicinal plant reputed for health promoting and various therapeutics effects in the Indochina region induced neurotoxicity in cerebral cortex, straitum and hippocampus and also increased stereotype behaviours in rats (Jintanaporn, 2006). *Asimina triloba* (pawpaw), a temperate plant contains a high concentration of annonacin, which is toxic to cortical neurons (Potts et al., 2012). There is an annonacin-containing commercial supplement made from twig extracts of pawpaw and is marketed as a safe complement to cancer therapy (Coothankandaswary et al., 2010). The fruit of *A. triloba* is used as a laxative and leaves are diuretic (Foster and Duka, 1990). Neurotoxicity of the raw tuber of a Chinese medicinal plant, *Alocasia macrorrhiza* have also been reported. The tuber is known to contain a neurotoxin sapotoxin (Chan et al., 1995). *A. macrorrhiza* has been used as folk medicine in different district of Bangladesh. The leaves are used to prevent iron deficiency, to enhance eye sight and as a good source of protein. It is used as functional food which is specially included in diet as a
preventive measure for jaundice or constipation (Rahamatullah et al., 2010). *Conium maculatum* is native to Europe and naturalized in Colombia. It has been found to contain alkaloids. The initial clinical signs of *C. maculatum* poisoning in domestic animals and human include muscle weakness, tremors, incoordination and mydriasis, followed by depression, coma and death from respiratory failure (Frank and Reed, 1987). *C. maculatum* has been traditionally used in the treatment of spasmodic disorders, and to relieve nervous excitation, pain in stomach, nervousness and alertness (Madaan and Kumar, 2012). *Ipomea carnea* subspecies fistulosa, a subspecies present in Colombia, affects the central nervous system of cattle, sheep, and goats in Brazil. The toxic compound of this plant was found to be the indolizidine alkaloid swainsonine (Armien et al., 2007). The milky juice of *I. carnea* is used for the treatment of Leucoderma. It is used to decrease the teratogenic effect resulting from cyclophosphamida (Auudy et al., 2003). *Ricinus communis* is a potent phytomedicine for diabetes (Shokeen et al., 2008). The oil of *R. communis* seed possess significant antiulcer properties at a dose of 500 mg/kg and 100 mg/kg (Rachhadiya et al., 2011). Ricin is the toxic lectin isolated from this plant and have been suggested to mimic the syndrome of human motor neuron disease and affects motor neurons and sensory neurons (Rosa et al., 1995). *Artemisia absinthium* contains a monotropene ketone called thujone in its essential oil. If threshold concentrations are exceeded, this compound exhibit neurotoxic properties leading to dose-dependent tonic-clonic seizures in animals (Lachenmeier, 2010). This plant has been used as traditional herbal medicine in China, Europe and Pakistan for the treatment of gastric pain, cardiac stimulation, to improve memory for the restoration of declining mental function (Bora and Sharma, 2010). *Myristica fragrans* (nutmeg) is widely used as flavoring agents, and are used in higher doses for their aphrodisiac and psychoactive properties in male rats (Tajuddin et al., 2005). Medicinally, it is known for its stimulative and carminative properties (Madsen and Bertelsen, 1996; Lagouri and Boskou, 1995). Nutmeg oil has been shown to have anticonvulsant at lower doses. It was found to be effective in preventing seizure spread in a set of established animal models (Wahab et al., 2009). The active ingredients in nutmeg is called Myristicine and is a naturally occurring insecticide and acaride with possible neurotoxic effects on dopaminergic neurons and a monoamine oxide (Truitt et al., 1963; Lee et al., 2005).
The Indian subcontinent is well known for its diversity of medicinal plants and its health care traditions (Wu, 1988). The indigenous people are well acquainted with the properties and uses of plants of their surroundings and they depend on the forest resources for various purposes like for wood, timber, non-timber forest products, medicines, food etc (Pushpangadan, 1995). Manipur, one of the easternmost border states (22,327 km² geographical area) of India is a part of Indo-Myanmar Hot spots of bio-diversity (Myers et al., 2000) and possess rich floral diversity. It has a vast forest cover and its biodiversity results in a wide range of traditional knowledge including the use of ethnomedicinal plants. It is important to be aware of plants used for medicinal purposes that also have tendencies to cause poisoning in man and animals. The fact that the plants identified as toxic are being used in folk medicine for the treatment of diseases could be due to the therapeutic efficacy of such plants at a low dose (Botha and Penrith, 2008). Based on the above facts regarding damage to the nervous system by some medicinal and useful plants, there is an increasing need to study the neurotoxicity of these plants. They may be used as medicine or consumed for other purposes. Keeping in view the above findings, the purpose of the present study is to provide an understanding of the neurotoxicological effects induced by some selected useful plants from Manipur, India. The study also includes if there is amelioration of neurotoxicity by N-methyl-D-aspartate (NMDA) receptor antagonist, MK-801 (Dizocilpine).

The N-methyl-D-aspartate (NMDA) receptor is one of the widely distributed glutamate receptors in the brain (Skerry and Genever, 2001). They belong to a family of glutamate receptors that contribute to signal transmission in the central nervous system (Traynelis et al., 2010). NMDA receptor are ligand-gated ion channels critical for fast signaling in the CNS. NMDA receptors are tetrameric assemblies composed of two glycine-binding NR1 subunits combined with two glutamate-binding NR2 subunits (Dingledine et al., 1999; Erreger et al., 2004). In various instances, antagonists of NMDA have been demonstrated to have neuroprotection, anticonvulsant and muscle relaxant action (Johnson and Snell, 1985; Martin et al., 1988; Martin et al., 1990). NMDA receptors are also the major mediators of excitotoxicity. Although physiological activation of this receptor is necessary for cell survival, overactivation
is a signal for cell death. The excitotoxic cell death involves necrosis and apoptosis (Choi, 1992). MK-801 [(+)-5-methyl-10, 11-dihydro-5H-dibenzo(a,d)cyclohepten-5,10-imine-maleate] or dizocilpine is an antagonist of N-methyl-D-aspartate receptor which exerts its action by binding non-competitively to associated ion channels (Hargreaves and Cain, 1995). It could significantly inhibit the apoptosis of neurons in damaged brain areas (Han et al., 2009). Dizocilpine have been evaluated for use in treatment of diseases associated with excitotoxicity (Ayala and Tapia, 2005). It has been reported to reduce calcium accumulation in cells and histologic damage following focal cerebral ischemia (Greenberg et al., 1990).

In this study, we have taken two regions of the mice brain viz. cerebral cortex and midbrain. The cerebral cortex is responsible for performing various important functions which includes general movements, perception, attention and behavioral reactions. Many areas of the cerebral cortex are concerned with processing of the sensory informations and motor functions. Midbrain is a portion of the central nervous system associated with vision, alertness and auditory. There are various neurons present in the midbrain which are responsible for forming connections between components of the motor systems.