INTRODUCTION

Alzheimer’s disease (AD) is an irreversible, progressive brain disorder that occurs gradually and results in memory loss, unusual behaviour, personality changes and a decline in thinking abilities and is the most common cause of dementia in old people. (Standart et al, 2002). The course of this disease varies from person to person, as does the rate of decline. The prevalence of this disease doubles every 5 years beyond the age 65 on average. AD patients live for 8 to 10 years after they are diagnosed (Wurtman, 1985). Clinically, it is characterized by loss of memory, inability to learn new things, loss of language function, a deranged perception of space, inability to do calculations, indifference, depression, delusions, and other manifestations.

The brain, in AD, shows a loss of cholinergic neurons in the basal forebrain, decreased acetylcholine (Ach) levels, and a decrease in the acetylcholine synthesizing enzyme choline acetyltransferase (CHAT) in the cerebral cortex. Ach plays a crucial role in information processing and memory. Although other neurotransmitter systems (noradrenalin, serotonin, Dopamine) are also deficient in AD, the cognitive impairment correlates best with the loss of cholinergic input.

In Alzheimer’s disease, progressive neurodegeneration occurs in multiple areas of the brain, including relatively selective involvement of the nuclei basalis, hippocampus, amygdala, entorhinal cortex, and eventually the high-order association cortex of the temporal, frontal, and parietal regions. The neuronal damage and the attending loss of synaptic density disable several neural systems essential to learning and retrieval of memories.

Experimental Alzheimer rat models can be prepared by different methods. Some of these include- stereotaxical infusion of different chemicals like colchicine and
streptozocin (in lateral ventricle and hippocampus), ibotenic acid (in nucleus basalis magnocellularis) (Bhattachaya et al. 1995).

Studies on animal models of AD have contributed greatly in understanding the etiology and etiopathogenesis of the disease. There are many advantages of using animal model in research work on AD as the etiology, pathogenesis of the disease and its complication can be clearly understood. It also helps in the development and evaluation of newer agents for the treatment of AD. But till date there is no such model yet discovered which mimics all the pathophysiological symptoms of AD. Of these models colchicine is a very common, well standardized and effective model of AD.

Colchicine is a microtubule disrupting agent. It is a potent inhibitor of microtubule aggregation and produces marked destruction of hippocampal granule cells, mossy fibres and septohippocampal pathways. It blocks axoplasmic transport and also induce neurofibrillary degeneration by binding to tubuline, the principal structural protein of microtubule (Veerendra kumar and Gupta, 2002) which is associated with loss of cholinergic neurons and decrease in acetylcholinetransferase, thereby resulting in impairment of learning and memory.

Thus intracerebroventricular colchicine model is relevant to AD as both are characterized by degeneration of cognitive functions, microtubule destruction and decrease in cholineacetyltransferase activity.

Treatment of Alzheimer's is a complex and multifactor process, involving both pharmaceutical and non-pharmaceutical approaches.

Several pharmacologic strategies have been used in attempts to treat the cognitive deficits in Alzheimer's disease including (a) Cholinesterase inhibitors, (b) Nootropic
agents, (c) Improved blood flow / psycho-stimulation, (d) Nerve growth factors, (e) Anti-inflammatory drugs, (f) Antioxidants, (g) Inhibitors of amyloid formation, (h) Inhibitors of tau protein deposition etc. and some non pharmacological treatments includes (a) Reality orientation, (b) Reminiscence therapy, (c) Behavioural modification, (d) Occupational activities and many others.

The reduction in barometric pressure and the consequent fall in the partial pressure of oxygen (PO2) at higher altitudes lead to hypobaric hypoxia (HBH), a unique extreme environmental condition, faced by humans at times. When HBH is mild to moderate, several physiological adaptations in the respiratory, vascular, hematological and metabolic functions ensure adequate oxygen availability in the brain. However, if the hypoxia is severe or sustained, a drop in the oxygen saturation in brain is inevitable leading to neuropsychological dysfunction. Alterations in mood, psychomotor performance, perceptive processes and cognitive functions including learning and memory have been reported to be associated with HBH (Roach and Hackett, 2001; Rodway et al., 2003; Shukitt-Hale et al., 1991).

The effects of HBH on cognitive impairments in humans are well documented at different altitudes and durations (Bartholomew et al., 1999; Bolmont et al., 2000; Li et al., 2000; Shukitt-Hale et al., 1990, 1991). The hypoxia exposure in these studies ranges from a few hours to almost a month.

It has been demonstrated that hypobaric hypoxia at the altitude of around 5400 meters could distort normal sleep mechanism (Bhatia et al., 1969). Rats exposed to hypoxia undergoes obstructive sleep apnea (OSA). OSA is a condition characterized by repeated upper airway obstruction during sleep. OSA causes neurocognitive and behavioral deficits including excessive daytime sleepiness, impaired short-term
memory, and problems with language comprehension and expression, all of which are compatible with global executive dysfunction (Gozal et al., 2001).

Some market available drugs are used to protect against such neurocognitive deficiencies in hypobaric hypoxia. Some of these include Flunarizine (Silverstein et al., 1986), Oxotrimorine and N-Acetyl cystein (Jayalakshmi et al., 2005).

There exist at least three different subtypes of nicotinic receptors in the human frontal cortex. The nicotinic receptors can be divided into three types, termed super-high, high, and low affinity. Brains of patients with Alzheimer's disease demonstrate decrements in the high-affinity nicotinic sites. The nicotinic and muscarinic systems appear to jointly modulate performance in learning and memory. Presynaptic nicotinic receptors mediate a positive feedback mechanism that modulates cholinergic activity. Trials with nootropics that presumptively alleviate the symptoms of mental aging and cerebral insufficiency exemplify non specific empirical treatments. Acetylcholinesterase inhibitors (tacrine) and Ach receptor agonists, including nicotine, have been used to treat AD. The marginal success of this approach suggests that, in addition to Ach deficiency, there are other profound alterations that contribute to the cognitive dysfunction. The use of acetylcholine releasers is based on their enhancement of stimulus-induced acetylcholine delivery into the synapse. Such an action would improve the signal-to-noise ratio during neuronal transmission without the toxicity associated with cholinesterase inhibitors or the distorted temporal pattern of neurotransmission observed with cholinergic agonists.

The development and implementation of safe and effective pharmacologic treatments have not yet fully utilized the above basic scientific findings.

The approaches of slow progression in Alzheimer's disease is now on the rise and it generally offer palliative treatment to augment the functioning of deficient
neurotransmitter systems in Alzheimer's disease. However, the suggestion that restoring either cholinergic transmission or some other property of cholinesterase inhibitors may slow the course of the illness needs to be pursued. Advances in the understanding of the biology of Alzheimer's disease permit the development of strategies that interfere with the underlying pathophysiology of the illness. The rationale and description of potential neuroprotective strategies will have to be studied.

Some of these include antioxidant therapy, antiamyloid drugs, chelating agents, immunological approaches etc.

Although there have been many recent advances in the understanding of the pathological process of Alzheimer's disease and hypoxia induced memory loss, there still remain limited therapeutic approaches some yet even to be tested in humans. The use of herbal therapies is widely in use through out the World now a days. Although from ancient times different indigenous plants are used for therapeutic management, yet little information is available regarding their mode of action. So this aspect has evoked a pleothora of investigations. India has a rich source of medicinal plants and a great history of using those plants in treatment of wide variety of diseases from time immemorial. So exploration of one of such plant of Indian origin is an emerging area of research which can provide some protection in Alzheimer's disease.

*Moringa oleifera* (MO), commonly called 'sajna', a multipurpose tree found almost all over the Asian and African countries and are consumed as food by the people. This plant has several medicinal properties. In rural areas of India, the leaf of MO is
chewed as it is believed to act as a brain booster. But there is no scientific evidence for this.

This plant has several medicinal properties including intermittent fever, in paralytic affections, palsy, chronic rheumatism (Chatterjee et al., 1992), carminative stomach ache, abortifacient, cardiac (Bhattacharya et al., 1969) and circulatory toxicity. The leaf of this plant has anti-inflammatory and hypotensive effect. In rural areas of India, the leaves of MO are chewed as it is believed to act as a brain booster. But there is no scientific evidence for this.

It was reported that MO leaf can provide protection against oxidative stress generated in AD by providing necessary antioxidants. (Ganguly et al., 2005) and can provide protection in hypobaric hypoxia by alteration of the brain monoamines which are associated with memory loss. (Ganguly and Guha, 2006)

MO leaves are a storehouse of all essential amino acids, vitamin A, B, C, E etc, choline, minerals like phosphorous, magnesium, iron, selenium etc which are believed to improve memory functions by different mechanisms to overcome memory deficit. (Parle and Dhingra, 2003), (Cantuti-castelvetri et al., 2000), (Martin et al., 2000). (Martinez et al., 2000), (Buchman et al., 2001).

So, the present study has been undertaken to show whether this plant extract can modulate the neuronal functions or possess any Neuroprotective effect to combat the neuronal loss in different experimental models of Alzheimer’s disease as well as the deficits of memory caused by chronic hypobaric hypoxia in rats.

Purpose of the Present Investigation

Since time immemorial man has been using herbs or plant products as ayurvedic medicine for the treatment of various diseases. The rural people with traditional folk
belief use MO to boost memory. However very scanty scientific study has been done to show the nootropic activity of *Moringa oleifera*. This plant in our country is easily available and cheap. Their wide uses in tribal practice make this to be potential medicinal plants to improve the mental health. Based on this information, the present research was designed to undertake detailed scientific investigation of MO as a neuroprotective agent in AD. To fulfil the purpose the work has been designed and divided into two portions. The first portion contains two chapters in which a well known standard model of Alzheimer’s disease has been chosen and the neuroprotective role of MO has been shown by different biochemical and histological parameters. In the second portion, consisting of three chapters, the memory loss during chronic hypobaric hypoxia has been identified and the role of MO to combat the memory loss on exposure to hypobaric hypoxia has been shown. The chapters designed are as follows:

**Chapter I**- In this chapter, the effective dose of MO for memory improvement has been evaluated by behavioural training in rats. In our study, radial arm maze task has been used to determine the effective dose of MO.

**Chapter II**- Oxidative stress is one of the well known causes of AD and MO, rich in antioxidants should be able to scavenge the free radicals generated in AD condition. So, our aim is to show the antioxidative property of MO in experimental model Alzheimer’s disease.

**Chapter III**- The third objective is to show the neuroprotective property of MO in colchicine infused experimental Alzheimer rats. Colchicine causes selective degeneration of cholinergic neurons together which may be evident from cholinergic enzymes activity like cholineacetyltransferase and acetylcholinesterase as well as from histomorphological studies together with accumulation of amyloid precursor
protein (APP) and neurofibrillary tangles (NFT) and whether following treatment with MO, the degeneration of neurons was significantly less together with the accumulation and size of APP and NFTs.

Chapter IV- Monoaminergic systems are also involved in modulatory functions of memory processing in brain and following colchicine infusion these neurons are also damaged together with cholinergic neurons, so in this chapter the areas of brain suffering from monoaminergic deficits were studied along with the EEG wave pattern in surface cortex and protection by MO in monoaminergic system was studied.

Chapter V- Chronic hypobaric hypoxia is also a well-known cause of dementia and hence leads to loss of cholinergic neurons, which may be evident from the cholinergic enzyme activity. The neuronal changes can well be observed in hippocampal areas that form the memory circuitry. Along with the cellular loss, accumulation of amyloid precursor protein as plaque and formation of neurofibrillary tangles due to high altitude hypoxia may develop in rats that may be important markers of Alzheimer's disease and whether MO can provide protection by its antioxidative and neuroprotective property was observed in this study.

Chapter VI- Additionally, the loss of monoaminergic system in hypobaric hypoxia was observed along with EEG wave pattern and protective role MO in memory impairment by high altitude hypoxic exposure was noticed by alteration of different brain monoamines like norepinephrine, dopamine and serotonin associated with loss of memory.