GENERAL DISCUSSION

The present study reported that *Moringa oleifera* (MO) increased the performance in radial maze (RAM) task dose dependently. The improved performance in RAM is due to neuroprotective action of MO on cholinergic neurons as well as on the monoaminergic system including norepinephrine, dopamine and serotonin. Impairment of RAM task in colchicine group can be related with the destruction of cholinergic neurons in hippocampal region in medial septum and ventral limb of diagonal band (VLDB) and dentate gyrus (*Dwaine and Thomas, 1990*). Other than Cholinergic neurons brain monoamines that are involved in modulatory functions in memory processing are also damaged on ICV infusion of colchicine. These include glutamatic acid, dopamine, serotonin, norepinephrine and GABA. Loss of neurons, both cholinergic and other monoaminergic systems lead to loss of memory (*Myhrer, 2003*).

Numerous epidemiological and experimental studies provide many risk factors of this disease, of which oxidative stress is the key one (*Singh and Pathak, 1990*). Induction of Alzheimer disease can occur when the generation of free radicals is augmented, scavenging of free radicals or repair of oxidative modified macromolecule decreases, or both (*Siobhan et al., 2005*).

Treatment with MO leaves increased SOD and Catalase activity and decreased lipidperoxidation. Since it is a rich source of antioxidants, specially β carotene, vitamin B, C and E it may be capable of scavenging peroxyl and superoxyl radicals (*Tagami et al., 1998*) (non enzymatic defences). Besides this, the major bioactive compound of phenolics found in MO leaves is of group such as quercetin and kaempferol. MO leaves may help to scavenge free radicals either by non-enzymatic defenses like vitamins or by bioactive compounds like flavonoids or both.
The neurons involved in memory processing lie in hippocampus which extend from dentate gyrus region and project to CA3 region which through Schaffer collaterals and project to the CA1 area. The colchicine infusion in lateral ventricle diffuses into the septal region and thus destroy the cholinergic pathways which is called septo hippocampal cholinergic pathways (SHC) (Dwaine and Thomas, 1990). The cholinergic loss in these regions is evident from the loss of activity of cholineacetyltransferase (ChAT), the enzyme involved in formation of acetylcholine. The AchE activity was also significantly lowered in the hippocampal region which may be due to non availability of Ach from the cholinergic neurons which are damaged after colchicine infusion. (rate limiting step). Apart from cholinergic loss in SHC pathways, the cholinergic neurons are also damaged in the cerebral cortex as is evident from the loss of activity of cholineacetyltransferase (ChAT) in cortical areas. Treatment with MO for 14 days revealed less destruction of cholinergic neurons in both SHC and in cerebral cortex regions. This is apparent from the increased activity of ChAT and AchE enzyme in hippocampus as well as cerebral cortex. This was also proved by histomorphological studies where the cells were more in numbers in dentate gyrus of hippocampus compared to colchicine infused group.

Colchicine destroys the neurons by disrupting the axoplasmic transport by binding and depolymerizing microtubules. APP protein, which is normally involved in normal functioning of neurons, is generally accumulated in perikaya and in the proximal neurons after colchicine administration. This aberrant accumulation of APP in the neurons of CNS may be caused by disturbances of fast axonal flow (Shigematsu and Mc Geer, 1992).

In Alzheimer brain, APP is concentrated in degenerating neurites and neurophil threads as amyloid plaques. Overproduction or accumulation of APP may play a role
in formation of amyloid plaque (Aβ) and intracellular fibrillary tangles or 
neurofibrillary tangle of paired helical filaments (PHF) which is one of the 
characteristic cellular and molecular changes in AD. The accumulation of PHF in the 
neuron might be due to impairment of axoplasmic flow (Shigematsu and Mc Geer, 

The present study also reported that MO leaf extract exerts differential effects on 
monoamine levels in different regions of central nervous system after chronic 
administration. Treatment with MO for 14 days, (250mg/kg b.w) helped to improve 
memory with a significant increase in NE level in CC, HC and CN. Previous work 
from this laboratory has reported MO root exerts inhibitory action on CNS by 
alteration of 5-HT, DA and NE (Ray et al., 2003). Norepinephrine is mostly 
concentrated in hypothalamus, in certain zone of limbic system and dentate gyrus of 
hippocampus (Ganong, 1991). In higher cortical structures such as the hippocampus, 
norepinephrine, via beta-adrenergic receptor (AR) activation, has been shown to 
reinforce the cognitive processes of attention and memory (Hermann and Lanctot, 
1997). Noradrenergic deficits is linked to depression, dementia, diminished alertness 
and concentration (Eagle et al., 1999). DA-mediated neurotransmission has also been 
related to response selection and habit learning in rats. Thus, DA can be involved in 
plural process supporting learning and memory (Myhrer, 2003). In our study, ICV 
infusion of Colchicine decreased DA level in CC, HC and CN significantly. Studies 
reveal that decreased DA level in the caudate nucleus is associated with AD 
(Daberkow et al., 2005; Joyce et al., 1998) where as excess of DA can lead to 
psychosis, elation and confusion (Cummings and Coffee, 1994). Recent studies have 
reported that reduced dopamine turnover in cortex and medial striatum leads to 
impairments in acquisition process (Joyce et al., 1998). MO treatment for 14 days,
increased DA level in CN and HC whereas no such effect was observed in CC. This region specific effect may be due to differential effect of this drug in various brain regions.

Serotonergic projections are very complex, highly branched system, which embrace wide areas of the cortex and limbic system. Most of the brain 5-HT is localized in the thalamus, hypothalamus, midbrain and raphe nuclei of the lower brain stem (Page and Carlson, 1970). There has been growing evidence that the 5-HT system is important in the regulation of memory and thus might be associated with AD while research results on this issue have been inconsistent (Ha et al., 2005). In our results, colchicine infusion in lateral ventricle produced a significant decrease in 5-HT level in CC and HC but increased in CN. MO treatment increased 5-HT level in CC only. Our work is supportive from EEG studies in which alpha, beta and gamma wave frequency was markedly suppressed in colchicine infused Alzheimer model in rats and spike wave pattern was prominently increased. Beta waves reflect desynchronized active brain tissue. It is usually seen on both sides in symmetrical distribution and is most evident frontally. It may be absent or reduced in areas of cortical damage. In Alzheimer model rats, the coordinated function of the brain is lost and also the thinking abilities as evident from our EEG results where beta wave frequency was markedly lowered. Treatment with MO showed an increased in beta wave frequency with a few alpha waves implicating that it has got an effective role to improve coordinated and integrated function of the brain.

Cognitive functions are sensitive to changes in oxygen availability. Lieberman evaluated the behavioural effects of hypoxia as a function of time of exposure and altitude achieved with various standardized tests of cognitive performance and that adverse changes in cognitive performance increased with higher altitude and longer
durations (Liebermann et al., 1999). Central mechanism responsible for the effect of hypoxia on cognitive performance is not known. It is likely that the direct effects of mild transient hypoxia on the brain causes variations in the level of specific neurotransmitters because the experimental studies done on rats showed a decline in synthesis of monoamines under simulated hypoxia (Freeman and Gibson, 1988). ACh is the primary neurotransmitter involved in the regulation of learning and memory processes (Freeman and Gibson, 1988). The rates of synthesis of other neurotransmitters (e.g., dopamine, serotonin and the amino acids) are also sensitive to hypoxia but perhaps lesser than the rate of ACh synthesis (Freeman et al., 1986).

In the present study, the impairment of radial arm maze task is related with a clear decrease in ChAT together with the presence of extracellular neuritic plaques (NP) and intracellular neurofibrillary tangles (NFT) activity followed by chronic intermittent hypobaric hypoxia which are major markers for AD (Liu et al., 2004). NFTs are composed of hyperphosphorylated tau protein in paired helical filaments (PHF) whereas plaques are composed of fibrillar amyloid beta (Masters et al., 1985). Although the precise mechanisms of pathogenesis of AD remain undefined, it is well established that the 42 amino acid form of the β amyloid peptide (Aβ42) plays a central role in mediating neurotoxicity and the formation of senile plaques. Elevated level of Aβ42, forms, can be directly cytotoxic to neuronal cells; and has been implicated for neuronal loss at the early stages of AD. MO is a very rich source of ascorbic acid (AA) which is a potent memory enhancer. 220mg of AA is present in 100 gm dried MO leaf powder. It is one of the richest source of AA. Although AA cannot penetrate the blood brain barrier, its oxidized form, dehydroascorbic acid readily enters the brain by means of facilitative transport (GLUT 1) present on endothelial cells at the blood-brain barrier is responsible for
transport of both glucose and dehydroascorbic acid into the brain (Parle and Dhingra, 2003).

Vitamin E, another antioxidant enzyme present in MO may also help to fight against dementia. It limits free-radical formation, oxidative stress, and lipid peroxidation (Halliwell and Gutteridge, 1992; Yoshida et al., 1985) and promotes survival of neurons exposed to aβ. Vitamin E prevents the oxidative damage induced by β-amyloid in cell culture and delays memory deficits in animal models. It was seen in his study that experiments performed in both cell culture and in animals indicated vitamin E and other antioxidants can prevent free radical-mediated cell death and diminish cognitive deterioration.

Exposure to hypobaric hypoxia produces behavioural and electroencephalographic changes as well as differential changes in brain monoamines in different regions of brain in rat. In our study, NE level decreased in CC, CB and in MB where as in CN, NE level increased. Treatment with MO, for 14 days, increased the NE level in CB and MB but has no effect in CC. In CN, MO decreased the NE level significantly. Our work corroborates the work of Ohkuwa who reported that epinephrine, norepinephrine, and dopamine levels decrease in the frontal lobe, cerebellum and striatum following hypoxic stress (Ohkuwa et al., 2003). In higher cortical structures such as the hippocampus, norepinephrine, via beta-adrenergic receptor (AR) activation, has been shown to reinforce the cognitive processes of attention and memory. Hypoxic stress decreased the DA level in CC and CN whereas increased the DA level in CB and MB. MO treatment led to increase in DA level in CN whereas no change was observed in CC. In CB and MB, DA level decreased markedly. Thus effect of MO is region specific. Exposure to hypoxia, led to decrease in 5-HT level in CC where these neuronal projections exist whereas in CB, CN and MB, 5-HT level
was markedly increased. Studies reveal that hypoxia alters the concentration of 5-HT in an age specific manner. Serotonergic activity has been linked to emotional aspect of behaviour. It has been shown that high anxiety in rats has been related to decreased levels of serotonin. Treatment with MO, increased the 5-HT level in CC significantly and decreased the 5-HT level in CB and MB. No change was observed in CN. Monoamines play an important role in behavioural depression, sleep and other brain activities. Our results further confirmed that MO seems to combat monoamine depletion by increasing norepinephrine in CC and MB, DA level in CN and 5-HT level in CC. while simultaneously decreasing the dopamine level of midbrain region from where the RAS arise. One may tentatively conclude that one of the effects of hypoxia in adult rats is a lesion producing long-term behavioural disorders, which are partly ascribed to dopaminergic and, possibly noradrenergic dysfunction.

Our result is supportive from our EEG studies in which low voltage fast waves or β waves were observed in control rats predominantly. Beta waves reflect desynchronized active brain tissue. But in hypoxic condition, high voltage spike discharges along with high voltage fast waves are observed whereas number of β waves prominently decreased.

Following hypoxic stress the ability to make decision and judgment diminishes along with memory loss thus leading to less β wave frequency. Chronic administration with MO showed a marked increase in β waves with a few α waves but high voltage fast waves and spike wave discharges were prominently reduced. So, our EEG results corroborate with our biochemical and behavioural parameters which suggest that monoamines like norepinephrine, dopamine and serotonin are involved in hypoxia induced memory loss. Our EEG results also corroborate the work of Speiser who reported that there is a central catecholaminergic dysfunction and behavioural
disorders following hypoxia in adult rats (Speiser et al., 1990). It is difficult to assign behavioural effects by alteration in single neurotransmitter due to the complex nature of the brain neuronal circuitry, interaction of neurotransmitters and different effects and the different effects that these transmitters have at different synapses. But MO leaf extract helps to combat the high altitude induced hypoxic memory disorder by alteration of neurotransmitters. The mechanism is a big mystery, which will be solved with further research. Thus it can be suggested that MO helps to improve memory loss by changing its electrical activity and altering monoamine level in discrete brain regions.
Moringa oleifera

Inhibits by its anti-inflammatory property

Destabilization of ER calcium homeostasis

Increased Ca\textsuperscript{2+}

Increase Calpain

p35

cdk5

TAU Hyperphosphorylation

NFT formation

Cholinergic neuronal Dysfunction

Altered APP processing in hippocampus and neocortex

Increased Aβ production and decreased Aβ clearance

APP accumulation

Loss of neurites

Necrosis/apoptosis

Altered synaptic plasticity

Dementia

Protect against Alzheimer's disease