Drugs affecting renin angiotensin system (RAS), the angiotensin receptor (ATR) blockers and angiotensin converting enzyme (ACE) inhibitors, have clinically been widely used as antihypertensive agents. Beside peripheral RAS which control blood pressure, a separate RAS exist in central nervous system (Wright et al., 1997) where it regulates numerous physiological and behavioral processes including cognitive function (Gard, 2002). In the recent years, it has been found that drugs affecting RAS i.e. ACE inhibitors and ATR blockers showed memory improvement in animals and human being (Raghavendra et al., 1998; Fogari et al., 2004; Jenkins and Chai, 2007). Further, it has been found that angiotensin II (Ang II) is associated with disturbed cholinergic function (Barnes et al., 1989) and oxidative stress (Griendling et al., 1994), the important factors implicated in pathophysiology of dementia (Pratico et al., 2002).

Though, previous studies reported beneficial effects of ACE inhibition and ATR blockers in dementia using several models but its involvement in colchicine and AF64A induced impairment in memory function has not been investigated. Therefore, the present study explored role of central ACE and ATR by utilizing intracerebral (IC) colchicine and AF64A induced model of memory impairment in mice. The IC colchicine and AF64A induced dementia models were developed in mice and validated by using clinically used acetylcholinesterase inhibitor, donepezil. The RAS affecting drugs ACE inhibitor and ATR blockers were used as experimental pharmacological tools to explore involvement of RAS components in memory impairment. The effects of ACE inhibitor, perindopril, and selective blockers of AT1 receptor, candesartan was studied on memory function and associated factors like oxidative stress and cholinergic function in mice brain. These RAS affecting drugs were reported to cross blood brain barrier (Tota et al., 2009).
6.1. Studies with colchicine induced memory impairment in mice

In the present study, we investigated role of central RAS in memory function and its relation with changes in biochemical markers of cholinergic function and oxidative stress in IC colchicine induced model of memory deficit in mice. The present study showed that ACE and AT1 receptor have crucial role in preventing memory deficit induced by intracerebral (IC) colchicine, since treatment with perindopril, an ACE inhibitor and candesartan, an AT1 receptor blocker prevented memory impairment, oxidative stress and cholinergic dysfunction in mice.

Colchicine is a cytotoxic agent which binds irreversibly to microtubules and causes their depolymerization, thereby inhibiting their assembly. Microtubules are vital components of the neuronal cytoskeleton and play a crucial role in cell growth and differentiation, axonal and dendritic transport. It has been reported that central administration of colchicine induce memory impairment in rodents by causing cholinergic neurodegeneration and oxidative stress (Fogari et al., 2004). In the present study IC administration of colchicine at a dose of 3 \( \mu \)g/mice induced spatial memory impairment as indicated by no significant reduction in escape latency time in Morris water maze test. However, lower dose of colchicine failed to induce memory deficit. Therefore, further studies were carried out by using colchicine at 3 \( \mu \)g/mice dose. This finding is in agreement with previous studies reporting impairment in memory following colchicine administration (Emerich DF et al., 1991). Further, the colchicine induced memory impairment model was validated by clinically used antidementic-anticholinesterase drug donepezil. Preventive treatment with donepezil for 14 days ameliorated colchicine induced memory impairment in mice. Kumar et al.,
(2007) also reported that chronic administration of acetylcholinesterase (AChE) inhibitor rivastigmine prevented colchicine induced dementia in rats. To study the involvement of central RAS in colchicine induced memory impairment, an ACE inhibitor perindopril and AT1 receptor blocker candesartan were used as an experimental pharmacological tool. Perindopril and candesartan were administered chronically for 14 days in colchicine injected mice and memory function was tested by Morris water maze. Perindopril and candesartan ameliorated colchicine induced dementia in mice implicating role of central ACE and AT1 receptor in memory function. Further, per se treatment of perindopril improved memory function as shown by significantly reduced retention latencies as compared to control animals. There was no significant effect on locomotor activity excluding the possibility that alteration in locomotor activity may have contributed to the observed behavioral effects. The cognitive effects of ACE inhibitors and AT1 receptor blockers have previously been investigated in various models of memory deficit (Kerr DS et al., 2005, Tota et al., 2009, 2010; Raghavendra V et al., 2001) however, to the best of our knowledge; this is the first study reporting antidementic effect of ACE inhibitor and AT1 receptor blocker in colchicine induced model of dementia. Clinical studies have shown an elevated ACE activity in various brain regions of Alzheimer's disease (AD) patients (Braszko JJ et al., 2003; Fogari R et al., 2004). Further, it has been reported that memory impairment induced by streptozotocin (STZ) or amyloid beta was associated with increased ACE activity in brain (Tota et al., 2010; Wang J et al., 2007). In the present study, we also got similar findings as colchicine significantly increased ACE activity in mice brain without affecting serum ACE activity indicating involvement of central ACE in memory deficit. Administration of perindopril decreased ACE
activity in serum and brain. Further, perindopril *per se* treatment significantly decreased ACE activity in brain. The exact mechanism of ACE activation is not known but it may be due to elevated oxidative stress by colchicine. Recently, it has been reported that STZ induced oxidative stress leads to upregulation of central ACE activity and mRNA expression in rat brain regions (Plaschke and Hoyer, 1993). Further, Usui et al., (1999), showed an elevated serum ACE activity in rats treated with nitric oxide synthase inhibitor N(omega)-nitro-L-arginine methyl ester and this effect was prevented by an antioxidant drug, N-acetylcysteine. These observations suggest that oxidative stress plays an important role in the regulation of serum and brain ACE activity.

In agreement with previous reports, we found elevated nitrosative (increased nitrite level) and oxidative stress (decreased GSH and increased MDA) in colchicine treated mice brain. This enhancement in oxidative stress markers may be due to increased formation of Ang II, due to increased ACE activity, which can stimulate NADPH oxidase that plays a pivotal role in the development of oxidative stress (Griendling et al., 1994). NADPH oxidase uses NADH and/or NADPH as substrates for the production of superoxide anion (Griendling et al., 1994, 1997) and gets activated by binding of Ang II to AT1 receptors (Griendling et al., 1994).

ACE inhibition by perindopril and blockade of AT1 receptor by candesartan showed anti oxidative action as evidenced by reduced MDA and elevated GSH levels. ACE inhibition, by decreasing Ang II, may limits the stimulation of NADPH oxidase, thereby preventing the increased oxidative stress associated with activation of the RAS. Whereas, antioxidative action of AT1 receptor blockers may be due to blockade of Ang II binding to AT1 receptors which activates NADPH oxidase (Griendling et al., 1994, 1997).
Besides it, chemical structure also seems to contribute in antioxidative action of AT1 receptor blockers. Miyata et al., (2002) demonstrated that AT1 receptor antagonist, olmesartan, scavenges different types of free radicals particularly hydroxyl radicals. All AT1 receptor antagonists share a common chemical core and the ability to quench hydroxyl free radicals is a common feature of other AT1 receptor antagonists that contain the core chemical structure, 5-(40-methylbiphenyl-2-yl)-1H-tetrazol (Miyata T et al., 2002). Pretreatment with perindopril and candesartan decreased nitrite level also.

Central cholinergic system plays an important role in memory formation and retrieval. The neurotransmitter acetylcholine is degraded by the enzyme AChE. Therefore, the use of AChE inhibitors is the most effective pharmacological approach for the symptomatic treatment of AD (Saxena et al. 2008). Colchicine has been reported to cause destruction of hippocampal granule cells and septohippocampal pathways with a reduction in AChE activity (Veerendra KMH et al., 2002). In the present study also, cholinergic system got affected in colchicine induced memory deficit as there was a significant reduction in AChE activity in mice brain. Perindopril and candesartan devoid of any inherent anti AChE activity indeed they normalized the decreased AChE activity in IC colchicine treated mice. However, perindopril and candesartan per se had no significant effect on AChE activity in brain. Better performance of perindopril and candesartan treated mice in Morris water maze test can be attributed to improved cholinergic system due to decreased activity of AChE resulting in increased acetylcholine. This observation is supported by the studies which have demonstrated that Ang II inhibits in vitro release of acetylcholine from the entorhinal cortex associated with cognitive performance and that this effect is reversed by Ang II receptor antagonists (Barnes et al, 1989).
In conclusion, the present study showed that treatment with perindopril and candesartan ameliorated colchicine induced memory impairment in mice implicating role of central RAS in memory function. Further, the beneficial effects of ACE inhibition and AT1 receptor blockade may be attributed to reduced oxidative stress and cholinergic dysfunction in brain. This study corroborated number of clinical findings that inhibition of central ACE and AT1 receptor blockade could be neuroprotective. Since hypertensive patients may suffer from cognitive decline also (Starr JM et al., 2005; Fogari R et al., 2004), AT1 receptor antagonism and ACE inhibition could be better choice in such patients.

6.2. Studies with Ethylcholine mustard aziridinium ion (AF64A) induced memory impairment in mice

The central cholinergic system plays an important role in the regulation of cognitive function (Christensen et al., 1992; Giacobini, 2004). Patients with Alzheimer’s disease (AD) have marked degeneration of cholinergic neurons (Power et al., 2003; Sivaprakasam, 2006). At present, the most effective therapeutic strategy against AD is to increase endogenous acetylcholine level through AChE inhibitors (Kakinuma et al., 2010; Ago et al., 2011). Therefore, central cholinergic hypofunction based experimental models are very commonly employed to study memory disorders.

Ethylcholine mustard aziridinium ion (AF64A) has been used as a specific cholinergic neurotoxin, because intracerebroventricular (ICV) administration of AF64A to rats and mice selectively destroys the central cholinergic system and reduces the number of presynaptic cholinergic markers, such as high affinity choline uptake, choline acetyltransferase activity, acetylcholine (ACh) release
and ACh level (Hortnagl et al., 1993). Therefore, in the present study AF64A was used to study role of RAS in memory impairment based on cholinergic hypofunction.

It is well known that AF64A induced memory impairment is mainly due to cholinergic dysfunction in brain. However, few studies have also shown elevated oxidative stress in brain following AF64A administration (Gulyaeva et al. 1996; Lautenschlager et al. 2000) and neuroprotective effect of vitamin E one of most important endogenous lipid-soluble antioxidants (Wortwein et al., 1994). This suggests that oxidative stress contributed to the cholinotoxicity of AF64A. Though exact mechanism of AF64A induced oxidative stress is not known, but indirect evidence suggests that the final phase of AF64A toxicity (i.e., cell death) might relate to the induction of oxidative stress (Johnson et al., 1988). In the present study we found a significant elevation of oxidative stress parameters such as malondialdehyde (MDA), a marker of lipid peroxidation, and nitrite, an indicator of NO generation, and significant decrease in glutathione (endogenous antioxidant) in brain following AF64A treatment which is in well agreement with previous reports (Gulyaeva et al. 1996).

Inhibition of central RAS by perindopril and candesartan was effective against AF64A induced oxidative stress in mice brain. The reduced oxidative stress, as indicated by depleted MDA level, nitrite level and increased glutathione by perindopril and candesartan in this study is an expected observation as several studies reported antioxidative effect of these agents in several pathological conditions (Wright and Harding, 2004).

### 6.3. References


32. Tota S, Kamat PK, Saxena G, Hanif K, Najmi AK, Nath C. 'Central angiotensin converting enzyme facilitates memory impairment in
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