CHAPTER-5

SUMMARY AND CONCLUSION

Alzheimer’s disease (AD), the most common form of dementia afflicts elderly people worldwide (Tanzi and Bertram, 2001). About 25 million people are affected worldwide, and the incidence is expected to quadruple by 2050 to approximately 80 million cases owing mainly to increasing life expectancy (Giannakopoulos et al., 2009). AD is the predominant form of senile dementia and is characterized by the presence of extracellular amyloid plaques and intracellular neurofibrillary tangles. The AD brain is marked by severe neurodegeneration such as synaptic loss, atrophy, neuronal loss and depletion of neurotransmitter systems in the hippocampus and cerebral cortex (Blacker et al., 1994; Govaerts et al., 2007). These facts underline the role of AD as a major health burden and emphasize the need for identification of risk factors and targets for diagnosis, prevention, and treatment.

Several hypotheses have been proposed to understand AD etiology, but free radical generation is often cited as an important factor. Evidence suggests that oxidative stress and free radicals are the important factor regulating the behavioral impairment and memory deficits in age related neurodegenerative disorders (Pratico and Delanty, 2000). The brain is at higher risk to the oxidative damage due to high content of polyunsaturated fatty acids, high consumption of oxygen, elevated metabolic activity and relatively limited concentration of antioxidants, making it more susceptible to oxidative damage in comparison to the other organs (Black, 1991; Cassarino and Bennett, 1999; Kamboj et al., 2009). Oxidative attack to the lipids leads to the perturbation of cell membrane and its integrity and inactivation of antioxidant enzymes resulting in cell death (Ejaz Ahmed et al., 2013). Antioxidants may prevent the onset of AD as high dietary intake of vitamin C and E has been reported to be associated with lower risk of the disease (von Arnim et al., 2012). Therefore, the present study was designed to evaluate the neuroprotective potential of Bacopa monnieri (BM), an Indian traditional medicinal plant effective against cognitive impairment, in colchicine-induced dementia. Keeping in view the above objective, male wistar rats weighing about 200-250 g were randomly segregated into four groups viz. control group, Bacopa group, Colchicine group, Colchicine + Bacopa group. Animals were intracerebroventricularly (i.c.v.) infused with drug solution (15 µg colchicine dissolved in 5 µl artificial cerebrospinal fluid). BM was given orally in the form of water
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Suspension, starting a day after surgery at a dosage of 50 mg/kg body weight daily for a period of 15 days.

Important observations of this study are given below:

- Cognitive deficits were assessed by elevated plus maze test, passive avoidance test and morris water maze test whereas the motor dysfunctions were assessed using rota-rod treadmill and actophotometer. Colchicine administration showed a significant decline in memory retention which was restored by the BM supplementation. However, there was found no alteration in the motor activity following colchicine administration. BM also lacked any effect on the motor function in control and colchicine treated animals.

- Colchicine administration caused significant increase in oxidative stress as shown by significant increase in ROS, LPO and protein carbonyl levels in both cortex and hippocampus regions studied as compared to controls. However, treatment with BM attenuated the colchicine-induced increase in ROS, LPO and protein carbonyls comparable to normal control values.

- In case of colchicine treated animals, there was a significant reduction in the GSH content in cerebral cortex and hippocampus. BM treatment protected against colchicine induced reduction in GSH levels in the rat brain.

- The activities of superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glutathione-s-transferase were significantly decreased in the cerebral cortex and hippocampus, whereas BM administration to colchicine treated animals restored the enzyme activities to control levels.

- A significant decrease in the levels of total lipids, phospholipids and gangliosides were observed in cortex and hippocampus of colchicine treated rats, which were reversed following BM supplementation.

- Cholesterol levels were found to be significantly increased, whereas triglycerides levels were unaffected in both the brain regions. BM supplementation significantly restored the cholesterol levels to control values.

- Cholesterol to phospholipid ratio indicative of membrane fluidity was also found to be decreased. This was further confirmed by increased fluorescence polarization, anisotropy and order parameter assessed using DPH. Annular fluidity assessed by pyrene was also
found to be decreased in colchicine treated rats. BM supplementation on the otherhand was effective in restoring the membrane fluidity to control values.

- Alterations in membrane fluidity were accompanied by significant alterations in the activities of membrane bound enzymes (Na\(^{+}\)K\(^{-}\)-ATPase and Acetylcholinesterase) in colchicine treated animals and BM supplementation was able to restore the activities of these enzymes to comparable values observed in control animals.

- Activity of Ca\(^{2+}\)-ATPase, a membrane bound enzyme was found to be decreased alongwith elevated intrasynaptosomal calcium levels in colchicine treated animals. BM supplementation restored the Ca\(^{2+}\)-ATPase activity and thus attenuated the intrasynaptosomal calcium levels to near control levels.

- In this study, the levels of neurotransmitters assessed by HPLC method were observed to be impaired in colchicine treated animals and BM supplementation restored them to near control values.

- Inflammation has been shown to play a critical role in the pathogenesis of AD, which is characterized by the activation of glial cells and release of pro-inflammatory cytokines and chemokines. In this study, Cytokines (IL-6, TNF\(\alpha\)) and chemokine (MCP-1) levels measured using Elisa assay were found to be elevated in colchicine treated animals. Furthermore, Real Time PCR analysis also revealed significant increase in mRNA expression of IL-6, TNF\(\alpha\) and MCP-1 in colchicine treated animals in comparison to control which was normalized by BM supplementation.

- Pro-inflammatory mediators (Cox-2, iNos) were also examined by western blotting and Real Time-PCR analysis. The mRNA expression and protein levels were found to be markedly increased in colchicine treated animals which were attenuated by BM supplementation.

- BACE-1 activity and A\(\beta\) production were also examined in this study by using FRET assay and thioflavin-T staining respectively. BACE-1 activity was found to be increased in both regions in colchicine treated animals which was decreased by the BM supplementation. In consequence of BACE-1 activity A\(\beta\) production was found to be increased in colchicine treated, while BM supplementation attenuated this enhancement.
In the histopathological studies, hematoxylin and eosin staining revealed that the colchicine treated group showed complete degeneration, shrunken, pyknotic and darkly stained morphology. Furthermore, colchicine induced demyelination was analyzed by luxol fast blue staining. BM supplementation to colchicine treated animals resulted in normalization of brain architecture.

These findings demonstrated the biochemical alterations produced by colchicine administration and the beneficial effects of BM supplementation. It is evident that oxidative stress plays a crucial role in colchicine induced neurotoxicity. A consequence of oxidative stress, perturbation of lipid composition and membrane integrity, further lead to dysfunctioning of the membrane bound enzymes resulting in increased synaptosomal calcium levels. Moreover, elevated intracellular calcium levels in colchicine treated animals were found to be associated with activation of pro-inflammatory mediators resulting in neuronal death. BM supplementation restored the changes following colchicine administration. Impaired neurotransmitters in colchicine treated animals were restored by BM supplementation via preventing the oxidative injury, enhancing synthesis of biogenic amines and by modulating of metabolism of neurotransmitters in AD brain.

It can be suggested that BM exerted multi targeted pharmacological actions by preventing the Aβ accumulation, modulating the metabolism of monoaminergic neurotransmitters, enhancing the acetylcholine levels, inhibiting oxidative stress and inflammation in AD brain. The findings from the study may, at least in part, explain the nootropic action of BM demonstrated in experimental and clinical studies. Therefore, BM can have the therapeutic potential of being an effective and safe treatment for AD.
Graphical summary