Summary and Conclusion
Alzheimer’s disease was first identified more than 100 years ago by Dr. Alois Alzheimer, who in 1907 published an account of rapidly deteriorating mental illness in a 51 year old woman but research into its symptoms, causes, risk factors and treatment has only gained momentum in the last 30 years. Alzheimer’s disease is the most common form of dementia which mainly affects the elderly population. Early onset of Alzheimer’s disease is also found but to a less extent. The most common symptom pattern begins with gradually worsening difficulty in remembering new information. This is because disruption of brain cell function usually begins in regions involved in forming new memories like hippocampus.

The etiology of AD is still unknown but several factors have been suggested that appear to reduce the incidence of the disease, or for which a hypothesis has been put forward based on scientific investigations. None of the proposed theories have been completely accepted since they are largely based on epidemiological studies and other factors might be responsible for the differences observed. Nevertheless these factors have been exploited in the search of drugs to treat AD. It is characterized histopathologically by the presence of two hallmarks in the brain lesions, extracellular deposition of beta-amyloid in neuritic plaques and intracellular neurofibrillary tangles (NFTs), oxidative stress, inflammatory process, apoptotic cell death and neurotransmitter disturbances. Many theories were put forward to describe AD but none of them described AD as a whole. The free radical theory gained much attention and somehow described the pathophysiology related to AD. Thus, various antioxidants were used to prevent the onset of the disease and to some extent there was success too. Other theory which fascinated the whole world was neuroinflammation. Earlier, brain was treated as immune deprived organ but recent studies have shown that inflammation is the key mechanism in most of the neurodegenerative disease including AD. Both oxidative stress and inflammation ultimately lead to apoptosis in neurodegenerative disorders.

At present there is no definite treatment or cure of AD. Researchers are in progress in the search of some novel drugs either from herbal origin or synthetic base for the cure of AD. We have attempted to search some
drugs for the prevention of AD. The drugs have shown a better protection of AD.

**Chapter 4:** Beta-amyloid (Aβ) peptides are considered to play a major role in the pathogenesis of Alzheimer's disease (AD) and compounds that can prevent pathways of Aβ induced neurotoxicity may be potential therapeutic agents for the treatment of AD. The present study examined the hypothesis that thymoquinone (TQ) would reduce oxidative stress and mitochondrial dysfunction in differentiated pheochromocytoma (PC12) cells exposed to Aβ fragment 25-35 (Aβ25-35). To test this hypothesis, Aβ was used to induce an in vitro model of AD in differentiated PC12 cell line of rat. After 24 h of exposure with Aβ25-35, a significant reduction in cell viability and mitochondrial membrane potential was observed. In addition, a significant elevation in the contents of TBARS and nitric oxide (NO) and activity of acetylcholine esterase was observed which was restored significantly by TQ pretreatment. Furthermore, TQ also ameliorated glutathione and its dependent enzymes (glutathione peroxidase, glutathione reductase) which were depleted by Aβ25-35 in PC12 cells. These results were supported by the immunocytochemical finding that has shown protection of cells by TQ from noxious effects of Aβ25-35. These results indicate that TQ holds potential for neuroprotection and may be a promising approach for the treatment of neurodegenerative disorders including AD.

**Chapter 5:** The objective of this study was to examine the protective role of Thymoquinone (TQ) on sporadic dementia of Alzheimer’s type. Aged rats were supplemented with Thymoquinone (5 mg/kg b. wt., i.p.) for 15 days and then infused with streptozotocin i.c.v. (ICV-STZ) bilaterally (5 mg/kg b. wt.) whereas sham rats received the same volume of vehicle. Thereafter, cognitive performance was tested by Morris water maze after 2 weeks followed by euthanization for biochemical, histopathological and immunohistochemical studies. TQ pretreatment significantly ameliorated the cognitive performance, thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH) and activities of its dependent enzymes (glutathione peroxidase [GPx] and glutathione reductase [GR]), catalase and acetylcholine esterase (AChE) in the hippocampus and the frontal cortex of
ICV-STZ rats. The histopathological findings have shown edematous morphology with vacuolated architecture and pyknotic nuclei in H & E staining in the STZ group brain which was successfully attenuated by TQ administration. Moreover, TQ also succeeded in increasing the expression of ChAT and lowering the expression of Hsp-70. The study thereby suggests the effectiveness of TQ in preventing cognitive deficits and might be beneficial for the treatment of sporadic dementia of Alzheimer type (SDAT).

Chapter 6: Alzheimer’s disease (AD) is the most common neurodegenerative disorder to date and the inflammatory process has a fundamental role in the pathogenesis of AD. Neuroinflammation includes the brain cells such as microglia and astrocytes, as well as cytokines and chemokines. 1,8-Cineole (Cin), a terpenoid oxide present in the oil of many plants displays an inhibitory effect on inflammation i.e. paw oedema induced by carrageenan and cotton pellet-induced granuloma in rats. In the present study, Cin has been found to restore the generation of reactive oxygen species (ROS) by amyloid beta (Aβ25-35) administration and disruption of mitochondrial membrane potential (MMP). The anti-inflammatory effect of Cin on Aβ25,35 toxicated PC12 cells was studied and it was found to be effective in inhibiting the different inflammatory markers. It was also confirmed by the immunohistochemical analysis of nitric oxide synthase (NOS-2), cyclooxygenase-2 (COX-2) and NF-κB. The present results when taken together with the recent reports describe the inhibitory effects of Cin on the formation of prostaglandins and cytokines by stimulated monocytes in vitro, thus provide additional evidence for its potential beneficial use in therapy as an antiinflammatory agent.

Chapter 7: Increasing evidence demonstrates that oxidative stress and inflammation play an important role in pathogenesis of AD. Cineole is reported to possess strong anti-oxidative and anti-inflammatory properties. Hence, the present study was undertaken to evaluate the neuroprotective effect of cineole against sporadic dementia of alzheimer’s type (SDAT)-induced neuronal damage. SDAT was induced by intracerebroventricular-streptozotocin (ICV-STZ) injection in rats with the help of double manipulator stereotaxic apparatus. Cineole was administered at
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(50, 100 and 200 mg/kg/day p.o.) for 7 days before surgery. From 16th to 20th day of surgery, the Morris Water maze test was performed to analyze the cognitive impairment in rats. Animals were sacrificed on 21st day of surgery and the brain tissues were isolated for histopathological examination. Hippocampal and frontal cortex were also used for the determination of TBARS, GSH levels and the activities of the antioxidant enzymes (GPx, GR, and catalase) and cytokines profile. Immunohistochemistry of NOS-2, COX-2, NF-κB and ChAT were also performed. Hippocampal neural injury in rats was demonstrated by histopathological observation, which revealed significant neural cell death in the hippocampus CA1 area 21 days post surgery (49 % cell loss). Additionally, oxidative injury in rats was demonstrated along with increase levels of cytokines. Pretreatment of cineole attenuated ICV-STZ-induced neuronal damage manifested by significantly decreasing the number of dead hippocampal neurons (30.06 % in cineole-treated), which confirm the protective role of cineole in ICV-STZ injury. Also, pretreatment of lesion rats with cineole decreased the elevated levels of TBARS and increased GSH contents, GPx, GR, catalase activities and cytokines to normal levels. These results were supported by the immunohistochemical findings. This suggests that the protection of cineole increased resistance to oxidative stress.

Chapter 8: This study demonstrated that nanoformulation of D609 is effective against the toxicity caused by Aβ25-35 on PC12 cells. D609 is a know antioxidant, anti-inflammatory and anti-apoptotic agent but is expensive. It was found that 100 nM nD609 was effective in combating the toxic effects of Aβ25-35. nD609 has decreased the ROS generation, NO production by Aβ25-35. Membrane integrity was lost by Aβ25-35 as depicted by MMP and lactates dehydrogenate activity and nD609 has prevented these effects too. Immunohistochemical analysis of Apaf-1, Bax, Bak and Bcl2 revealed that nD609 has anti-apoptotic activity also. Different inflammatory markers like NOS-2, COX-2 and NF-κB were also restored by this nanoformulation. The expression of p53 and Hsp-70 was also decreased by nD609. All these results conclude that D609 is a potent antioxidant, anti-inflammatory and anti-apoptotic agent and that nanoformulation also exhibited the same results as the crude drug. So, further studies are needed to promote this pilot
study for suggesting nD609 as a potent drug for the prevention of AD.

**Chapter 9:** In order to check the efficacy of nanoparticles of D609 on *in vivo* model, we used about 1000 times lower doses as reported in literature for D609. nD609 decreased the oxidative stress along with the neuroinflammation in rats probably by its antioxidant and antiinflammatory property. Our results also indicated that nD609 prevented neuronal loss by apoptosis, making it a potent drug of interest in AD therapy. Overall study indicates the potential of nD609 for its clinical efficacy in the prevention of AD. Further studies in this direction are needed to confirm whether nD609 is effective in AD patients or not.

In a conclusion, present findings indicate that treatment with Aβ25-35 for 24 h will decrease the cell viability, biochemical alteration and immunocytological changes in differentiated PC12 cells. Pretreatment with thymoquinone, cineole and nD609 prevented these alterations probably due to their antioxidant and antiinflammatory properties.

The ICV-STZ model caused cognitive impairment, biochemical and histological alterations in hippocampus and frontal cortex of rats probably by its ability to generate free radicals and decrease in acetylcholine synthesis. Supplementation with thymoquinone, cineole and nD609 prevented these alterations suggesting them as favored medicine in AD pathogenesis.

TQ was found to have anti-oxidative effect on both *in vitro* and *in vivo* models of AD preventive the deleterious effects of the neurotoxins (Aβ25-35 and STZ). Cineole was also found to be effective in regulating the oxidative damage and neuroinflammation as a result of Aβ25-35 treatment in differentiated PC12 cells and STZ model of sporadic dementia of Alzheimer’s type. Nanoformulated D609 was effective in preventing the cell loss, oxidative stress, inflammation and apoptosis in differentiated PC12 cells and behavioral, biochemical and apoptotic parameters in ICV-STZ rats.

So the selected neuroprotective agents, thymoquinone, cineole and nD609 can be used as favored remedies pending elucidation of proper molecular mechanism and deciphering appropriate signaling pathway.