8. SUMMARY/CONCLUSIONS AND FUTURE PERSPECTIVES

The present thesis comprehensively analyzed the efficacy of bacosides and curcuminoids on ageing biomarker, neurotransmission system, behavioral paradigms, oxidative stress markers, mitochondrial functioning and inflammatory responses in the middle aged and aged rat brain in order to identify the diverse mechanism of action of putative drug candidates for promotion of healthy brain ageing and preventing and/or delaying the SDAT progression. In the current study both bacosides and curcuminoids exhibited significant anti ageing effect as depicted by the marked reduction in lipofuscin content, also referred as the “ageing garbage” in middle aged and aged rat brain cortex.

The present study also demonstrated the significant activity of bacosides and curcuminoids over age associated reduction of neurotransmitters viz., acetylcholine, serotonin, dopamine and nor epinephrine and associated behavioural deficits i.e cognitive dysfunctioning, depression and gross behavioural activity. Based upon the findings, it was hypothesized that bacosides exerted neuroprotective effect over cholinergic neurotransmission by modulating its biosynthesis and uptake whereas curcuminoids by suppressing its metabolism by inhibiting enzyme AChE. Among the monoaminergic neurotransmission chronic administration of curcuminoids significantly elevated the serotonin, dopamine and norepinephrine content whereas bacosides exhibited profound activity over serotonin and dopamine alone. Further, both the drugs exerted potent anti depressant activity among the middle aged and aged rats.

Long term treatment of bacosides and curcuminoids significantly attenuated the age dependant alterations of oxidative stress markers in the middle aged and aged rat brain cortex. Bacosides on one side specifically prevented the age dependant elevation of lipid peroxidation and nitrite content with concomitant reduction of glutathione system and GPx content whereas curcuminoids on the other hand exerted marked effect on almost all the oxidative stress markers except for SOD and protein carbonyl content in the middle aged and aged treated group.

Bacosides treatment selectively prevented the loss of mitochondrial enzymes and complexes viz., isocitrate dehydrogenase, NADH dehydrogenase, cytochrome c oxidase, Complex I and II – III activity in the middle aged and aged treated group. However, no significant alterations were observed in the 39kDa subunit of complex I
and COX I subunit of Complex IV protein expression in aged rat brain. Among the nNOS protein expression which indicated the status of NO generation, bacosides was found to down regulate the age associated up regulation of nNOS expression. Further, long term treatment of bacosides potentially prevented the age associated disruption of mitochondrial structural integrity in middle aged and aged rats brain mitochondria. This study reports for the first time the efficacy of bacosides over mitochondrial structure however only qualititatively due to restricted sample size. Despite of this, the results provides an insight for the remarkable influence of bacosides over energy transduction system. Finally the cumulative effect of bacosides over mitochondrial enzymes and complexes, nNOS expression and prevention of structural loss further leads to the sufficient ATP synthesis and thus availability to the brain, which otherwise gets compromised with ageing consequently leading to the functional deficits and increased vulnerability for the development of SDAT.

Similar to bacosides, curcuminoids also exerted significant activity over entire energy transduction system in the middle aged and aged rat brain cortex. Curcuminoids treatment maintained the mitochondrial enzymes and complexes activity viz., isocitrate dehydrogenase, NADH dehydrogenase, cytochrome c oxidase, Complex I activity in middle aged and aged group. In the protein expression studies curcuminoids exhibited significant down regulation of 39kDa subunit of Complex I and nNOS expression which was elevated in aged brain. However, no significant alterations in the Complex IV activity as well as COX I subunit was observed in the present research findings. In addition, curcuminoids administration significantly ameliorate the age dependant alterations in the mitochondrial ultrastructure and thus ATP content in the middle aged and aged rat brain cortex.

Finally in the present study both bacosides and curcuminoids exhibited potent anti- inflammatory activity by preventing the exacerbated generation of pro inflammatory cytokines viz., IL - 1β, TNF – α and IFN – Y in middle aged and aged rat brain cortex. In addition, the pro inflammatory cytokines mediated up regulation of iNOS expression which in turn accounts for the excessive NO synthesis was also down regulated on both bacosides and curcuminoids treatment in middle aged and aged rat brain cortex.

Based upon the series of experimentation the significant effect of bacosides and curcuminoids in attenuation of oxidative stress, perturbed mitochondrial functioning and inflammation has been depicted. However, it is worth to emphasize
that although bacosides and curcuminoids exerted pleiotropic activity in the investigated pathways their cumulative impact over the anti-aging effect and improved brain functioning as assessed by neurochemical and neurobehavioral responses may involve diverse mechanism of action as hypothesized through the correlation studies.

In the present study, the anti-ageing effect of bacosides was significantly associated with monoaminergic neurotransmission, particularly 5-HT and DA, mitochondrial enzyme cytochrome c oxidase and pro-inflammatory cytokine IL-1β. It was suggested that the improved mitochondrial enzymes functioning including cytochrome c oxidase on bacosides treatment will lead to the reduced generation of ROS, thus inhibiting the vicious cycle of ROS mediated mitochondrial dysfunctioning as well as reduced formation of lipofuscin. It is evident that lipofuscin accumulation is directly associated with oxidative stress and senescent, energetically inefficient mitochondrion are one of the major components of lipofuscin loaded lysosomal granules. In addition, the reduced accumulation of lipofuscin in the aged microglial cells on long term bacosides administration may reduce the age dependant aberrations in microglial cells including the reduced secretion of pro-inflammatory cytokines like IL-1β and thus preventing age associated neurodegeneration and SDAT. Moreover, the reduction of lipofuscin on bacosides treatment further leads to the enhanced brain functions, including the improved monoaminergic neurotransmission in part via reducing oxidative stress and neuroinflammation. Similarly, the anti-ageing effect of curcuminoids was also attributed to its effect of mitochondrial enzymes activity, including cytochrome c oxidase.

The present study demonstrated the cholinergic potentiating and memory enhancing activity of bacosides due to its anti lipid peroxidation activity. It was suggested that bacosides administration might have prevented the phospholipids damage due to excessive lipid peroxidation in aged brain and therefore enhanced functioning of membrane spanned receptor molecules and membrane bound enzymes essential for cholinergic neurotransmission and cognitive functioning. However, the remarkable activity of bacosides over the cognitive dysfunctioning and reduced neuronal loss of CA1 and CA3 neurons of hippocampus further suggested the additional mechanism of action of bacosides over cholinergic neurotransmission.

Alike bacosides whose direct influence over cholinergic neurotransmission is reported earlier, curcuminoids was not extensively explored. In the present study,
Curcuminoids was observed to exhibit pleiotropic activity by attenuating several pathways responsible for disrupted cholinergic neurotransmission with ageing. As far as oxidative stress mediated cholinergic degeneration was concerned, curcuminoids was found to alleviate the acetylcholine content by inhibiting excessive lipid peroxidation. In addition, the efficacy of curcuminoids over cholinergic neurotransmission and cognitive functioning was significantly associated with its preventive effect over depletion of mitochondrial enzymes including α-ketoglutarate dehydrogenase (KDGH) and overall elevation of ATP content. It was suggested that the aggregated effect of curcuminoids over oxidative phosphorylation and prevention of accumulation of inefficient mitochondria might have fulfilled the large bioenergetics demands of cholinergic neurons and thus promoting their effective transmission and reducing the susceptibility of SDAT development. Moreover, the modulation of cholinergic neurotransmission and hence enhanced memory function by curcuminoids was attributed in part to its potent anti-inflammatory activity. It was hypothesized that curcuminoids might have suppressed the NF-κB mediated IL-1β signalling cascade which otherwise was found to significantly inhibit the muscarinic cholinergic receptors and activation of enzyme AChE activity. Apart from this curcuminoids was also observed to inhibit the enzyme AChE activity in both in vitro and in vivo studies. However, the less effect of curcuminoids in maintaining the acetylcholine content as well as memory ability when compared with the bacosides also indicated no direct action of curcuminoids in acetylcholine biosynthesis pathway.

In addition to cholinergic degeneration, the present study also investigated the influence of bacosides and curcuminoids over monoaminergic neurotransmission and depression, the second problem after cognitive impairment among elderly and SDAT. Chronic administration of curcuminoids was found to alleviate the monoaminergic neurotransmission and thus anti-depressant activity by maintaining the endogenous antioxidant enzymes activity, inhibiting free radicals generation and mitochondrial dysfunctioning and modulating the altered metabolism of catecholamines. It was suggested that curcuminoids might have prevented the decline of oxidative phosphorylation and thus ATP content, which otherwise triggers the excessive metabolism of monoaminergic neurotransmitters through exacerbated oxidative stress and glutamate excitotoxicity in the aged brain. The potent anti-depressant activity of curcuminoids has been attributed in part due to its prevention of Complex I activity in the aged brain. In addition, the enhancement of monoaminergic neurotransmission
was also significantly associated with TNF–α on curcuminoids treatment, suggesting the involvement of potential anti–inflammatory activity of both the tested drugs in the amelioration of monoaminergic neurotransmitter system and depression. It was suggested that curcuminoids might have prevented the age associated excessive 5–HT metabolism by suppressing the TNF–α mediated Indoleamine 2, 3–dioxygenase induced tryptophan catabolism, the precursor of 5 HT and thus modulating the depressive behavior among the aged rats.

In the present study, bacosides was suggested to inhibit the excessive dopamine metabolism and depressive behaviour among the aged rats via inhibition of IL - 1β and lipid peroxidation. However, the modulatory effect of bacosides over monoaminergic neurotransmission and depressive activity was also due to its independant activity, perhaps by the direct action over their metabolism.

Brain ageing and its manifestations form the most important component of ageing process as the characteristic molecular and cellular changes may lead to more crippling impact than gross somatic ageing. Age-related alterations in oxidative, metabolic, inflammatory or other type of homeostatic stress, resulted in the accumulation of damaged subcellular structures, which in turn culminates into increased incidence of disease pathologies. Several neurons are particularly more vulnerable to age associated neurodegeneration and the determinants of vulnerability of specific neurons mainly include their metabolic or structural demand, existence of potentially toxic metabolic intermediates, repertoire of signal transduction pathways and stress protection mechanisms. There is evidence that both age-associated cognitive impairments and SDAT reflects vulnerability of similar neuronal circuits which suggest SDAT to be an integral part of the ageing. Regardless to their similarities, it appears that age-related changes increase the vulnerability of the nervous system for the SDAT progression and additional neuropathologic injurious stimuli must exist for SDAT development. Since, treatment of SDAT still remains the challenge, the use of neuroprotective interventions with safe and long term use offers much scope. In the present study, both bacosides and curcuminoids were observed to overall modulate the neurochemical and neurobehavioral deficits, which are used as indices of age associated neurodegeneration through their pleiotropic neuroprotective mechanism of action. Based upon the present results it was suggested that the early intervention right at the stage where the disease proximity begins would be highly beneficial to prevent age associated neurodegeneration and SDAT, since many of the
changes were found to be unaltered in the highly aged rats. Simultaneously, in view of the multiple etiologies behind the pathophysiology of age associated neurodegeneration and SDAT, and the pleiotropic mechanism of action of bacosides and curcuminoids, the present study advocates the administration of combination drugs/formulations as indicated in Indian System of Medicine for the promotion of successful ageing and prevention of SDAT and opens the challenging perspectives like synergistic mechanism to discover. In addition, the present comprehensive study further suggested that the pursuit of reductionist or single molecule approach should not be a paradigm barrier in clinical translation, particularly if herbal interventions promised therapeutic solutions, especially in chronic ailments where decade long prodromal stage persists before the disease appearance. Therefore, the present study demonstrated the neuroprotective effect of bacosides and curcuminoids which further opens the future perspectives for the development of combination plant based drugs/formulations for the promotion of healthy brain ageing and prevention of age associated neurodegenerative pathologies including SDAT.