HISTOPATHOLOGICAL REPORT

The brain section of control animals injected with PBS, shows normal configuration and architecture of cells in hippocampus (Figure: 74; a). In Aβ negative control, the hippocampal sections showed degenerated nerve cells and enlarged swollen nuclei as well as spongiform architecture. This swollen and enlargement indicates the presence of diffused plaques which is neurotoxicity of Aβ in the hippocampal cells (Figure: 74; b). The brain sections of standard drug treated animals shows the normal nuclei and architecture of cells (Figure: 74; c). In the drug treated group, DAEA, all the three different doses such as 12.5, 25 and 50mg/kg shown normal cells of hippocampus and cortex without any spongiform cells, indicates the protective effect of DAEA (Figure: d, e, f, I, j, k and l). Even though all the brain samples of DAEA treated animals show the protective effect in all doses, the low dose treated animals brain sections indicated a slight edematous nature of the neuronal architecture

VI. DISCUSSION

The neuroprotective effect on ethanolic extract of A. galanga, P. viscida, various fractions obtained from A. galanga and the bioactive compound isolated from the chloroform fraction of A. galanga improved the
cognition on Aβ induced neurotoxicity in mice. In this present investigation, preliminary studies with crude ethanolic extracts indicated the improvement in cognitive function both in behavioral and biochemical aspects. In behavioral aspect the study with open field, step down inhibitory avoidance and water maze indicated the cognitive function. After the i.c.v administration of Aβ, the animals exhibited cognitive dysfunction and was examined by open field exploratory behavior, passive avoidance (step down inhibitory) and Morris water-maze tests.

Aβ_{25-35} is a potent neurotoxic peptide present in AD patients.Earlier studies revealed that the deposition of Aβ causes the impairment of habituation reminiscence during exploration in open field (Muralidharan et al., 2010). Open field exploration involves the habituation memory of rodents such as head dips, line crossings and rearings. It is also well recognized that the injection of Aβ_{25-35} in rodents through i.c.v administration show evidence of marked cognitive impairment in STM as well as in LTM, additionally impairs the hippocampal memory indicates the loss of spatial learning in water maze (Maurice et al., 1996).
In rodents the training on passive avoidance with step down inhibitory type activates two separate types of memory (STM and LTM) (Izquierdo et al., 1998). On the succession of interval, STM was set to last from a short duration of seconds to hours, and was sensitive for any disruption (easily lost). The aquistation of STM do not require mRNA or novel protein. In contrast to STM, LTM last for hours from days and weeks to number of years. This LTM was not disturbed by any aspects of changes in the brain and this aquistation of LTM requires synthesis of novel protein.

The spatial learning memory which indicates the hippocampal activity was determined by water maze. In various neurodegenerative diseases and other age related dementia such as AD, the hippocampus and its afferent neuronal system were disrupted. In these pathological conditions the hippocampal memory fails to execute and are linked through failure of integrity which involves in the cholinergic innervations of hippocampus and cortex (Lleo et al., 2006). Grafting of septal cholinergic neuronal cells enhanced the spatial learning of hippocampal memory and retention. This also evidenced by the upregulation of muscarnic receptors which influences on the learning and behavior (Bjorklund et al., 1983; Bjorklund and Stenevi, 1977; Dunnett et al., 1982). Injection of cholinergic muscarnic blockers disturbs learning and
retention where as inhibition of AChE enhances the cognitive function and was proved by prenatal injection of muscarnic blockers as well as AChE inhibitors respectively. Learning and retention of hippocampal activity in water maze was found to be dependent with the level of AChE in various discrete regions of brain such as parasympathetic nuclei, nucleus basalis of hippocampus, globus pallidum, and dentate gyrus of the hippocampus. Higher levels of AChE envisaged the reduced the retrieval memory of spatial learning in water maze (Menahem et al., 1988).

The treatment with EAG, EEPV, different fractions of EAG and DAEA decreased the Aβ induced memory dysfunction by enhancing the exploratory behavior, extended step down latency and decreased the time in escape latency of water maze. All the test extracts and fractions exhibited increased memory retention in both STM and LTM and also revealed an increased open field habituation memory with general locomotion by increasing head dipings, line crossings and rearings. The effect of EAG, EEPV, different fractions of EAG and DAEA on hippocampal learning in water maze test had shown decreased escape latency. These studies in regards with anti-amnesiac effect related to behavioral parameters indicate the escalation of cognition.
In concern with the biochemical aspect, the neurotransmitter metabolic enzyme (AChE and MAO) were attenuated in the brain which indicate the enhancement of learning and memory process.

In AD type of dementia, one of the distinguishable changes occurs was increased level of AChE. The AChE is an enzyme which hydrolyzes the acetylcholine molecule in cholinergic neurons and non-cholinergic neurons in brain. In AD brain, AChE enhances the conversion of Aβ peptide into the form of fibrils, which are highly neurotoxic than that of Aβ peptides (Alvarez et al., 1998). Evidence implicates the over expression of AChE was initiated during the generation of free radicals in neurodegeneration elicited by Aβ peptide. The free radicals generated in neuronal membrane, initiates the peroxidation of unsaturated fatty acids to release malondialdehyde (MDA) and 4-hydroxy-2,3-neonal compound (4-HNE). These by products produced by lipid peroxidation impair the membrane bound ATPase and calcium voltage sensitive channels to disturb homeostasis of ion and reduce the neuronal conduction. (Mark et al., 1997).

Reactive oxygen species are also thought to be produced by the modulation of biogenic amines and MAOs. The neurotransmitter biogenic
amine, such as nor-adrenaline, dopamine and serotonin undergoes oxidative deamination through MAOs. MAOs are flavoprotein exists in two forms such as MAO-A and MAO-B. The isoenzyme catalyze the biogenic amines and produces the corresponding aldehydes (Fernandes and Soares-da-Silva 1992). Both MAO-A and MAO-B are highly substrate specific. In serotonergic neurons and in glial cells MAO-B was preferentially distributed and MAO-A was distributed in dopaminergic system as well as in catecholaminergic system (Abell and Kwan, 2001). Both isoenzymes were elevated in neurodegenerative disorders. As previously discussed, oxidative stress plays important role in neurodegeneration (Richards et al., 1998) and the reactive oxygen species produced as a byproduct (NH₃ and H₂O₂) on oxidative deamination of biogenic amines by MAO also induces the neurodegeneration through lipid peroxidation (Zhang and Piantadosi, 1991). MAO is recognized to circuitously create cytotoxic reactive oxygen species throughout aging and in neurodegeneration such as AD. These free radicals produce abnormal cleavage of APP to triggers beta-amyloid, which initiate the chain reaction of free radicals.

In our present investigation, we found the escalated levels of neurotransmitter metabolic enzyme in neurodegeneration induced mice and the treatment with EAG, EEPV, different fractions of EAG and DAEA
reduced the expression of both enzymes, which indicates the favourable cognitive improvement. The histopathological studies on the treated animal’s brain sections indicated the protective effect of ethnolic extracts of EAG and EEPV and as well as the activity of fractions of EAG and isolated compound DAEA.

As Aβ has the potential activity to induce oxidative stress and inflammation during the pathological condition of AD and antioxidants can be used for the treatment. Additionally Aβ has been accounted to provoke the production of H₂O₂ and LPO in rat brain hippocampal neuronal cells (Yatin et al., 2000). Kim et al. (2003) established that constant i.c.v. infusion of Aβ (1-42) in rats produced a significant decline in expression of protein responsible for antioxidants such as superoxide dismutase, glutathione peroxidase and glutathione S-transferase. The treatments with antioxidants are implicated to act by hindering the configuration of aggregatory form of Aβ to Aβ fibrils (Yankner, 1996). The innate antioxidant present in the cells includes superoxide dismutase, which sequester the superoxide ion by accelerating the dismutation. Catalase is a haeme enzyme, which precisely removes H₂O₂ and GPx was a selenium holding enzyme, which forages the H₂O₂ and other peroxides (Blake et al., 1987). The reactive oxygen species scavenging property of SOD is efficient when it is preceded by the events of catalase and GPx, as
because the dismutase property of SOD spawns $H_2O_2$ from the superoxide radical, which is highly toxic than oxygen-derived free radicals. These are further required to be scavenged by catalase and GPx. One of the major non-enzymic regulators in all cells magnitudes the redox homeostasis was glutathione (Meister and Anderson, 1983). This is a cysteine-enclosed tripeptide exists in two different forms such as reduced (GSH) or oxidized (GSSG) and contributes in redox reactions through reversible oxidation in its active thiol group. Glutathione, scavenges free radicals directly, acts as substrate for GPx and GST on the detoxification process of $H_2O_2$, LPO and electrophilic complexes.

Various studies indicate that β-carotene, vitamin A, C and E may protect the oxidation of nucleic acid and protein oxidation leading to senescence of neuronal cells. Vitamin C and E are likely to reduce Aβ-plaque deposition in neurons. Ascorbic acid is essential in recycling alpha- tocopherol and recycles the oxidized transition compound to their corresponding reduced form.

In our study, antioxidant activity in the brain was improved which was depicted by the increased levels of SOD, GPx, CAT and vitamin C. We found the attenuation of neurotoxicity induced by Aβ in mice which can be well correlated with the reduction of oxidative stress by the
treatment of EAG, EEPV, different fractions of EAG and DAEA; these suggest the positive effects on cognition through antioxidant property.

In this anti-amnesic evaluations we found that the activity of crude extract obtained from *A. galanga* was much active than *P. viscida* extract. Our study was designed to identify a bioactive molecule from the potential fractions (AGH, AGC and AGE) of *A. galanga*. Evaluation of behavioral and biochemical effects of various fractions revealed the chloroform fraction (AGC) as the most bioactive fraction. In behavioral estimations, there was an improvement in open field exploration, STM, LTM and decreased escape latency in water maze. In biochemical evaluation the effect of fractions are indicated with decreased neurotransmitter metabolic enzyme levels, increased levels of antioxidants and enhanced the integrity of membrane bound ATPase (Na\(^+\)/K\(^+\)-ATPase). In our study we assessed the activity of Na\(^+\)/K\(^+\)-ATPase as because impaired levels of enzyme were depicted in Alzheimer’s brain. Na\(^+\)/K\(^+\)-ATPase participates in conduction and generation of neuronal impulse by consuming 40 to 50% of ATP (Mahendravarma and Surendrakumar, 1996; Erecinska and Silver, 1994). Na\(^+\)/K\(^+\)-ATPase also involve in the storage, uptake and metabolism of biogenicamines in CNS (Carageorgiou et al., 2007). Earlier studies revealed that the activities of Na\(^+\)/K\(^+\)-ATPase are decreased in frontal lobe of Alzheimer’s brain (Mark et
al., 1995) and sporadic amnesia of AD express peroxidation of membrane lipids and proteins, in turns disrupts the fluidity of membrane and inhibits Na\(^+\)/K\(^+\)-ATPase pump. Despaired activity of Na\(^+\)/K\(^+\)-ATPase pump are linked with escalated level of AChE and reduced the retrieval performance of memory in hippocampal neurons (Tauheed et al., 2009). It was recognized that Aβ\(_{25-35}\) hinders the Na\(^+\)/K\(^+\)-ATPase to arrest neuronal conduction (Bores et al., 1998). In our study the treatment with different fractions of EAG (AGH, AGC and AGE) increased the Na\(^+\)/K\(^+\)-ATPase activity in neurodegeneration induced mice.

Especially, of all the fractions, the potential neuroprotective effect of AGC was well interpretable with the enhanced cognitive function and the potential effect of AGC doesn’t vary significantly when compared to the standard (Donepezil) treated group, reveals that the action of AGC was nearly comparable to the standard drug. Further to isolate and identify a bioactive molecule from the fraction (AGC), it was charged in column chromatography. Fractionation by column chromatography we eluted one of the biopotential molecule and was recognized to be 1´δ-1´-acetoxyeugenol acetate, which acquire the anti-amnesic effect.

Inorder to evaluate the bidirectional involvement of neuroendocrine and neuroimmune system, as the A. galanga is also claimed to be
immunostimulant, the isolated bioactive compound was evaluated for the inhibition of immune function, the corticosteroid level in brain and proinflammatory cytokine (TNF-α) in the Aβ induced mice.

Recent studies indicates that the Hypothalamus-Pituitary-Adrenal axis (bidirectional communication between brain and immune system) was also controlled by parasympathetic nervous system and it monitor’s the status of immune function as well as inflammation through cholinergic pathway (Kawashima and Fujii, 2003; Pavlov and Tracey, 2004; Czura and Tracey, 2005). The immune function was concluded by gene expression and ligand binding studies in both muscarinic acetylcholine and nicotinic acetylcholine receptors, T lymphocytes of human and rodent (Kawashima and Fujii, 2000) and macrophages (Tracey, 2002). Activation of Ach secreting neurons in the CNS controls the inflammation through cholinergic anti-inflammatory pathway by efferent vagus nerve. This in turns communicates with the nicotinic acetylcholine receptors in the macrophages and deactivates the cytokine release (Czura et al., 2003; Pavlov et al., 2003).

In addition to neuroimmune modulation of cholinergic system, the immune system was altered by neuroendocrine coordination from HPA
axis. Higher levels of corticosteroids due to chronic stress modulated by adrenal gland leads to neuronal atrophy and cytotoxic effects especially in hippocampal cells; leaving other neuronal cells like dopaminergic, serotonergic neurons in stress is well predicted. Increased amounts of dopamine in prefrontal area of cortex impair the spatial learning. Single i.c.v administration of corticosterone activates the HPA axis and impairs the cognitive function by elevating the circulatory corticosteroid level (Do-Hoon et al., 1998)

In the evaluation of stress parameters in our study, corticosterone level in the plasma and TNF-α in the brain tissue were determined. These determinations on neurotoxicity induced mice after the treatment indicated the protective effect of the drug by inhibiting the pro-inflammatory cytokine (TNF-α) and also decreased the level of stress hormone (corticosterone). These are also well supported by our preliminary studies on leukocyte migration inhibition assay as well as neutrophil adherence test.

In the study with basic behavioral parameters the cognitive function of DAEA treated animals indicated increased open field exploration, increased retention of STM, LTM and decreased the time taken to escape on to the escape platform. In biochemical estimations the neurotransmitter metabolic enzymes were decreased in treatment groups
indicated the attenuation of neurotoxicity. The turnover of biogenic amines in the brain causes varied changes in learning and memory process. The influence of 5HT receptors was well established and it is renowned that 5HT1A, 2A, 2B, 5HT4 and 5HT6 improve the LTM upon blocking them while STM was improved by blocking 5HT1B. In dopaminergic system, the relation between striatal dopaminergic system and higher cognitive center indicates the important role of dopamine in attention and executive process of brain, particularly D2 receptors present in striatum significantly contributed in learning performances and episodic memory in AD. The utility of D2 agonist favourably enhance the cognition. (Meneses, 2007). In this study, the evaluation of biogenic amine in the brain tissue indicated improvement in their (serotonin and dopamine) turnover to increase the cognition.

Our study in mice of Alzheimer’s disease type indicated anti-amnesic effect may be due to the neuroimmune and neuroendocrine involvement of the bioactive principle. In our investigations, the decreased AChE activity suggest the cognitive role of A. galanga fractions can be well correlated with previous studies on in-vitro AChE inhibition (Khattak et al., 2005), and we found the renovated activity of Na+/K+-ATPase with antioxidants; also we predict the anti-amnesic effect may be mediated by cholinesterase inhibition and through inhibition of pro-
inflammatory cytokines (TNF-α with the reduction of stress hormone corticosterone).

These results suggest further studies has to carried out in the aspects of neurotransmitter levels and its metabolic enzymes as well as pro-inflammatory cytokine on mechanism based correlation to be carried out for the isolated compound in APP/Ps1 mice or other transgenic Alzheimer's disease model.

**VII. CONCLUSION**

- The pharmacological evaluations on ethanolic extracts of *A. galanga*, *P. viscida*, different fractions of *A. galanga* and 1'-δ-1'-acetoxyeugenol acetate indicated the anti-amnesic effect.
- The determination of behavioral and biochemical parameters after treatment on neurotoxicity induced mice promptly ruled out the potential effect on improving the cognitive function. In this study, concerned with behavioral aspect, the hippocampal memory and classical memory were improved. Furthermore the neuroprotective and anti-amnesic effect of these drugs were evidently supported by decrement of neurotransmitter metabolic enzyme (AChE and MAO) with escalation in antioxidants.
The activity of Na⁺/K⁺-ATPase was increased after treatment with various fractions of *A. galanga* which revealed the maintenance of Na⁺ and K⁺ gradients across the plasma membrane with renewed activity. This indicates that the fractions may be possess characteristic antioxidant property to enhance the membrane bound Na⁺/K⁺-ATPase activity and anti-amyloidogenic property.

The pharmacological evaluations with behavioral and biochemical determinations on the various fractions indicated the potential neuroprotective effect of AGC and was well interpretable with the enhanced cognitive function.

Moreover the bioactive molecule from the chloroform fraction expressed a prospective turnover of biogenic amine (serotonin and dopamine) for learning and memory process associated with neuroendocrine and neuroimmune bidirectional pathways.

From the results of the present study the decreased turnover of stress hormone (corticosterone) and inhibition of proinflammatory cytokines can be well correlated with neuroendocrine and neuroimmune effects.

Thus to conclude, ethanolic extracts of *A. galanga* & *P. viscida*, different fractions of *A. galanga* (AGH, AGC and AGE) and bioactive compound of AGC (1′δ-1′-acetoxyeugenol acetate) are endowed with anti-amnesic properties and it reveals the anti-amnesic effect may be