Chapter II

Taurine ameliorates intracerebroventricular streptozocin induced cognitive impairment and oxidative stress in rats: A relevant model of sporadic dementia of Alzheimer’s type
Introduction:

Alzheimer's disease (AD) was first described by Alois Alzheimer in 1907. AD is the main cause of dementia in the elderly people and mainly characterized by the progressive loss of memory and impaired cognitive functions (Alzheimer et al., 1995; Selkoe, 2002). This disease starts with mild cognitive impairment (MCI), and later affects long-term memory, consequently leads to severe dementia (Walsh and Selkoe, 2004). The major risk factor for the developing AD is the advanced age, it is reported that the incidence is around 1-2% in people aged around 60-65 years, rising to about 30% in those aged 85 years or more (Blennow et al., 2006). The main neuropathological hallmarks of AD are extracellular deposits of amyloid beta (Aβ) peptide, intracellular deposits of neurofibrillary tangles that consist of hyperphosphorylated tau protein and progressive loss of synapses, dendrites and neurons (Braak and Braak, 1995). One of the most important pathological hallmarks of AD is β-amyloid peptide that contributes to progressive loss of neurons and neuronal cell death in various brain regions, particularly in hippocampus, that is responsible for memory and cognition. Oxidative stress is associated with excessive production of free radicals and loss of antioxidant enzymes that plays crucial role in the pathogenesis of AD. Antioxidants supplementation is used for the treatment of various neurodegenerative diseases including AD (Gary et al., 2005; Butterfield, 2004).

It is well documented that intracerebroventricular injection of streptozotocin (ICV-STZ) in rats causes desensitization of neuronal insulin receptor that may further lead to prolonged impairment of brain glucose and energy metabolism (Lannert and Hoyer, 1998). Impaired glucose and energy metabolisms accompanied with loss of learning and memory (Jee et al., 2008; Hoyer and Lannert 2008; Ishrat et al., 2006), additionally, decreased to choline acetyl transferase level and increased oxidative stress in hippocampus (Ishrat et al., 2006; Kumar et al., 2003; Sharma and Gupta, 2001) have contributed in the dementia in rats. It is reported that ICV-STZ has no effect on blood glucose level (Mayer et al., 1990; Lester-Coll et al., 2006), indicating that its role is independent of producing hyperglycemia. Experimental ICV-STZ in rats has been shown to induce biochemical changes and neuropathological changes similar to those found in sporadic Alzheimer's disease and therefore considered to be a valid model to study the experimental dementia of Alzheimer's type (Salkovic-Petrisic and Hoyer, 2007; Grunblatt et al., 2007; Salkovic-Petrisic, 2008).
Memory impairment can be prevented by the treatment of antioxidants, melatonin and resveratrol (Sharma and Gupta, 2001; Blokland and Jolles, 1993) indicating key role played by the reactive oxygen species in the etiology of AD. Taurine (2-aminoethanesulfonic acid) is present at high concentrations in the mammalian brain (Guidotti et al., 1972) and plays important role in intracellular calcium ion homeostasis, transmembrane Ca" flux and membrane integrity in the brain (Satoh et al., 1998; Chen et al., 2001). It is also reported that taurine has antioxidant and osmoregulatory property (Militante et al., 2004). Additionally it has been shown that taurine is related to neuroprotection against several neurological diseases (Paula-Lima et al., 2005; Tadros et al., 2005). Taurine has a modulated action against neurotoxicity and protects the neurons against glutamate neurotoxicity (Wu et al., 2009).

Taurine may play a role in attenuation of cognitive deficits induced by neurodegenerative diseases, such as AD via its antioxidant and neuroprotective properties. These properties of taurine have stimulated us to study its protective effects on cognitive impairment and oxidative stress in hippocampus in a rat model of sporadic dementia of Alzheimer's type.

Materials and methods: As described in section III.

Results

Behavioural observation

Effect of taurine (T) on performance in Morris water maze task

Latency:

The animals of all groups have improved Morris water maze acquisition performance. S and T+S group animal's shows decreased latency to find the platform from the second to fifth day of experiment. However, L group animals presented significantly higher latency (p<0.001) to find the platform than S, but T+L group animals has shown a significant (p<0.001) improvement as compared to L group animals (Fig. 1).

Path length:

The path length was improved in the animals of all groups. S and T+S group animals shows decreased path length to find the platform from the first to last day of experiment.
However, L group animals presented significantly (p< 0.001) higher path length to find the platform than S but T+L group animals has shown a significant (p<0.001) improvement as compared to L group animals (Fig. 2).

![Fig 1](image1.png)

**Fig 1:** Effect of taurine administration on escape latency to find the platform in Morris water maze test in ICV-STZ infused rats. Values are expressed as mean±S.E.M (n=10). Swimming times of four trials per day for 5 days to each group animals are shown. Average escape latency to find the submerged platform was significantly (***(p<0.001)** prolonged in the L group animals when compared to the S group animals. Pre-treatment with taurine has **decreased** it significantly (###p<0.001) in T+L group animals as compared with the L group animals.

![Fig 2](image2.png)

**Fig. 2:** Effect of taurine administration on path length to find the platform in Morris water maze test in ICV-STZ infused rats. Values are expressed as mean±S.E.M (n=10). Swimming times of four trials per day for 5 days to each group animals are shown. Average distance travelled to find the submerged platform was significantly (***p<0.001) prolonged in the L group animals when compared to the S group animals. Pre-treatment with taurine has **decreased** it significantly (###p<0.001) the learning deficits in T+L group animals as compared with L group animals.
Biochemical Observations

Effect of taurine on TBARS content:

The effect of taurine on TBARS content was measured to demonstrate the oxidative damage on lipids in hippocampus of ICV-STZ rats. There was no significant alteration in TBARS content in T+S group animals as compared to S group animals, while it was elevated significantly ($p < 0.001$) in L group animals as compared to S group animals and significantly ($p < 0.05$) decreased by T+L group animals (Fig. 3).

Effect of taurine on GSH:

Protective effect of taurine on GSH level was observed. The level of GSH was not elevated significantly in T+S group as compared to S group animals but it was depleted significantly ($p < 0.01$) in L group animals as compared to S group animals, and significantly ($p < 0.05$) protected by the pre-treatment of taurine in T+L group animals (Fig. 4).

Effect of taurine on activity of antioxidant enzymes:

The activity of antioxidant enzymes (GPx, GR, GST, SOD and CAT) was decreased significantly in L group animals as compared to S group animals and was protected significantly by the pre-treatment of taurine in T+L group animals as compared to L group animals. No significantly change was observed in T+S group as compared to S group animals (Table 1).

Effect of taurine on AchE activity in hippocampus:

The activity of AchE was increased significantly ($p<0.001$) in L group as compared to S group animals. The pre-treatment with taurine has protected its activity significantly ($p<0.05$) in T+L group as compared to L group animals (Fig. 5).

Effect of taurine on the cresyl violet staining:

Normal neuronal cell bodies with distinct nucleus and nucleoli along with Nissl substance were observed in CA1 region of hippocampus of S group animals. L group animals showed degenerated cell bodies with prominent pyknotic nuclei while pre-treatment of taurine has protected the normal morphological feature of the neurons (Fig. 6).
Fig. 3: Effect of taurine pre-treatment on TBARS content in the hippocampus of ICV-STZ infused rats. Values are expressed as mean±SEM. TBARS content was significantly increased in the L group as compared to S group (***p<0.001 L vs. S group). Taurine pre-treatment significantly decreased TBARS content in the T+L group animals as compared with L group animals (#p<0.05 L vs. T+L group).

Fig. 4: Effect of taurine pre-treatment on GSH level in the hippocampus of ICV-STZ infused rats. Values are expressed as mean±SEM. GSH content was significantly decreased in the L group as compared to S group (**p<0.01 L vs. S group). Taurine pre-treatment significantly increased GSH content in the T+L group animals as compared with L group animals (#p<0.05 L vs. T+L group).
Effect of taurine on the histopathological evaluation:

High power photomicrograph of the brain showing a portion of CA1 region. In S group, all the neurons have large round vesicular nuclei with prominent nucleoli and amphophilic cytoplasm. The neurons of lesion group has shown dense and hyper chromatic nuclei and condensed cytoplasm (arrow). T+L group neurons have normal size and shape with partial neuronal loss (Fig. 7).

Effect of taurine on the expression of choline acetyl transferase (ChAT):

Cholinergic deficiency is supported by the reduced expression of ChAT in CA1 hippocampal region of the brain. A reduced expression of ChAT in L group was observed as compared to S group animals. Taurine pre-treated group animals have increased the expression of ChAT in T+L group animals as compared to L group animals (Fig. 8).
Table 1: Values are expressed as mean±SEM. STZ infusion leads to significant alterations on the activities of antioxidant enzymes (GPx, GR, CAT, SOD, and GST) in hippocampus in L group animals as compared to S group animals. Administration of taurine has significantly attenuated the activity of these enzymes in T+L group animals as compared to L group animals. Values in parenthesis are showing the percentage increase or decrease with respect to their control. (*p<0.05, **p<0.01, ***p<0.001 L Vs S, #p<0.05, ##p<0.01, ###p<0.001 L Vs T+L).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>S</th>
<th>L</th>
<th>T+L</th>
<th>T+S</th>
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<tr>
<td>GPx (nmol NADPH oxidized min⁻¹ mg⁻¹ protein)</td>
<td>447.88±18.23</td>
<td>225.89±13.22*** (-49.56%)</td>
<td>397.99±25.49### (43.24%)</td>
<td>466.20±23.20 (4.09%)</td>
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<tr>
<td>GR (nmol NADPH oxidized min⁻¹ mg⁻¹ protein)</td>
<td>754.62±33.89</td>
<td>365.01±24.05*** (-51.62%)</td>
<td>611.33±24.65### (40.29%)</td>
<td>790.18±33.54 (4.71%)</td>
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<td>Catalase (nmol of H₂O₂ consumed/mg protein)</td>
<td>25.03±2.11</td>
<td>11.88±1.86*** (-52.53%)</td>
<td>22.15±2.50# (46.36%)</td>
<td>25.36±2.20 (1.31%)</td>
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<tr>
<td>SOD (nmole of epinephrine protected from oxidation/min/mg protein)</td>
<td>735.89±64.13</td>
<td>499.33±28.06** (-32.14%)</td>
<td>645.51±36.50# (22.64%)</td>
<td>777.83±58.66 (5.69%)</td>
</tr>
<tr>
<td>GST (nmole of CDNB conjugate formed/min/mg protein)</td>
<td>1205.34±20.99</td>
<td>649.33±56.60*** (-46.12%)</td>
<td>923±40.01### (29.65%)</td>
<td>1233.80±25.80 (2.36%)</td>
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Fig 6: Section through CA1 hippocampal sub region showing cresyl violet staining of ICV-STZ infused rats. Normal neuron cell bodies with distinct nucleus, nucleoli and cytoplasm is evenly filled with Nissl substance were seen in S group animal (arrow black) while in L group animals, degenerating cell bodies with pyknotic nuclei were observed (white arrow). Taurine pre-treated group animals shows normal Nissl staining as observed in S group with few pyknotic nuclei (Magnification 40X).
Fig 7: Histopathological changes in the CA1 region of hippocampus. Sections were stained with hematoxylin and eosin. Black arrows with neuron indicate the normal pyramidal neuron in sham group (B) and black arrow indicates the degenerated pyramidal neuron in L group (D) while L+T group shows normal pyramidal neuron staining (F). Magnification 10X (A,C,E) and 40X (B,D,F).
**Discussion:**

The present study was undertaken to investigate the neuroprotective effect of taurine on cognitive dysfunctions and biochemical alterations in hippocampus. In neurodegenerative disorders, the targets of oxidative alterations induced by reactive oxygen species (ROS) are lipids and proteins which are the main structural and functional components of cell membrane (Liu et al., 2003). It is well known that lipid peroxidation and protein oxidation play an important role in the aging and other neurodegenerative diseases.

Morris water maze test suggested that a cholinergic function is important for the learning and memory and it is well understood that its alteration is one of the main causes of cognitive impairment in AD. Our Morris water maze data showed that lesioned group rats took longer time and travelled long distance (path length) to find the submerged platform, indicating the impairment in learning and memory process, which is consistent with the earlier findings (Blokland, 1993; Prickaerts, 1995; Ishrat et al., 2006). Increased latencies and path length were protected significantly in taurine pre-treated group animals. The improvement in the learning and memory with the supplementation of taurine is possibly due to its important action in improving memory and long term potentiation (Rivas-Arancibia et al., 2003).

Oxidative stress is defined as imbalance between the reactive oxygen species (ROS) and antioxidant system which may originate from an overproduction of ROS or from a reduction in antioxidant defences (Halliwell, 2001). Brain has multiple sources of ROS (Faraci et al., 2006) and a large oxidative ability, but its capacity to fight against oxidative stress is limited.
Oxidative stress caused the loss of antioxidant enzymes that alter the cellular redox status of the neuron, and treatment with antioxidants boost up the immune system and combat with the variety of neurodegenerative diseases (Gary et al., 2005; Butterfield, 2004). The hippocampal cells are primarily responsible for spatial learning and memory (Poucet et al., 2000) and more vulnerable to oxidative damage. ICV-STZ infusion in sub-diabetogenic dose leads to failure of cellular energetic due to oxidative stress, and has been used as a model of sporadic dementia.

ICV-STZ infusion leads to ROS production increasing lipid peroxidation, which leads to cellular disintegrity and progressive dementia. Lipid peroxides and hydroperoxides cause secondary injury by further generating relatively more stable and diffusible cytotoxic agents like malondialdehyde (MDA) and 4-hydroxy-trans-2-nonenal (4-HNE), respectively, and amplify oxidative cascade. Increased content of TBARS has been reported in AD brains (Arlt et al., 2002). The present study showed that the lipid peroxidation level (in terms of TBARS content) was significantly increased with remarkable impairments in learning and memory in L group, which is consistent with the earlier reports (Ishrat et al., 2006; Kumar et al., 2003; Sharma and Gupta, 2001). Taurine administration significantly reduced the level of lipid peroxidation. There are several reports about the protective effect of taurine on lipid peroxidation, glutathione and antioxidant enzymes following brain injury (Tadros et al., 2005; Rosenberg et al., 2010). In agreement with this finding, we also found that taurine significantly reduced the TBARS level along with increase in level of glutathione and antioxidant enzymes activity.

Glutathione (GSH) is a well-known antioxidant that is synthesized in the cytoplasm and is present in higher concentrations in the mitochondrial matrix. The low levels of GSH may be directly related to increased ROS, lipid peroxides, and highly reactive hydroxyl radicals (Ansari et al., 2006; Ahmad et al., 2005). The lipid peroxidation reacts avidly with cellular nucleophiles such as glutathione (GSH), and causes continuous decrease in its level through increased oxidant content or protein modification. To eliminate the peroxides, GSH works in conjunction with GPx to form glutathione disulfide (GSSG), which is reduced to GSH by GR at the consumption of one molecule of NADPH. A reduction in GSH may hamper $\text{H}_2\text{O}_2$.
clearance and promote 'OH formation, thus increasing the free radical formation, which triggers oxidative stress (Ansari et al., 2006, 2008) associated pathological pathways in AD (Perez-De La Cruz et al., 2005).

GST catalyse the conjugation of GSH via sulphhydryl group to the electrophilic centre of peroxides which can alleviate damage from lipid peroxidation. Thus, GPx, GR and GST are secondary antioxidant enzymes that play an important role in detoxifying ROS by maintaining a ready supply of GSH (Shah et al., 2002; Ansari et al., 2008). Decreased activity of GPx and GR would directly affect GSH level and reduction in the activity of GST, resulting in overall low levels of antioxidant system, thus predicting the uncontrolled influences of ROS in AD. The enzyme superoxide dismutase (SOD) catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide (H₂O₂), which is one of the most toxic molecules in the brain. Moreover, CAT participates in the detoxification of H₂O₂ into O₂ and H₂O. The depletion of CAT activity represents the loss of one of the major defence against ROS.

Cholinergic neuronal systems play an important role in the cognitive deficits associated with aging and neurodegenerative diseases (Quirion et al., 1986). Pivotal role of cholinergic system in memory is further underlined by use of acetylcholine esterase inhibitors in AD to prevent memory decline. Acetylcholine esterase is present within the synaptic cleft that hydrolyses acetylcholine a neurotransmitter associated with learning and memory, to choline and acetic acid, thus preparing the synapse for the passage of new impulse. In AD patients the activity of acetylcholine esterase is increased which is closely associated with reduced amount of acetylcholine available for the brain. It is reported that acetylcholine esterase activity increased following ICV-STZ administration in rats (Pathan et al., 2006; Plaschke et al., 1993; Ishrat et al., 2006). Increased acetyl cholinesterase activity may lead to diminished cholinergic transmission due to decrease in acetylcholine level. In the current study, we observed significant increase in acetyl cholinesterase activity in L group, which is decreased by the taurine pre-treatment. It is reported that taurine increases the brain levels of acetylcholine in experimental animals, and decreased levels of taurine have been found in Alzheimer's patients (Birdsall, 1998).

Synthesis of acetylcholine is carried out by the presence of acetyl Co-A provided by the breakdown of glucose and insulin, which control the activity of choline acetyl transferase a synthesizing enzyme for acetylcholine (Ishrat et al., 2006). It has been suggested that ICV-
STZ injection markedly reduces the expression of ChAT in the hippocampus (Biokland and Jolles, 1993; Ishrat et al., 2006). Our results showed that ICV-STZ infused animals had deficits in spatial learning and memory as analysed by Morris water maze test, also decreased expression of ChAT. Moreover, taurine supplementation significantly increased ChAT expression and improved the deficits in learning and memory in ICV-STZ infused rats.

Histopathological analysis shows that neuronal layers of CA1 region of the hippocampus did not reveal any evidence of neuronal loss and neuronal densities in significant manner between the groups. However, distinct differences in neuronal structure were seen. The neurons of the S group were large, conical shaped cells with well delineated amphophilic cytoplasm and round vesicular nuclei with prominent nucleoli. In the L group, the CA1 layer neurons showed pronounced shrinkage of the neuronal bodies with the nuclei losing their regular outlines and becoming hyper chromatic. This feature was seen in all the neurons in the CA1 region in a scattered fashion in L group animals. Taurine pre-treatment has protected the neurons and reduced the shrinkage.

Nissl staining showed, pyramidal neurons of the CA1 region of hippocampus of S group animals demonstrated round or oval nuclei and ribosome were equally dispersed within the cytoplasm while in L group animals showed reduced pyramidal neurons in the CA1 region and pyramidal neurons in the CA1 region demonstrated pathological changes in the shape of a condensation of the nucleus and the cytoplasm. Moreover, neurons were dark and irregular in shape and they possessed dark, irregular nuclei. Taurine supplementation in T+L group animals has improved the morphological feature of pyramidal neurons of CA1 region.