Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder of the aged brain and has become a major medical and social trouble for industrialized and developing countries. It is the most important cause of senile dementia and is characterized by memory and cognitive loss, the formation of beta-amyloid plaques, neurofibrillary tangles and degeneration of the cholinergic neurons (Weiss et al., 2008; Johnson et al., 2008). A safe and sound effective therapy for AD that addresses the source of the damage found in brain is urgently needed. None of the several hypotheses proposed to explain AD etiology has been confirmed, but free radical generation is often cited as an important factor. A large body of evidence suggests that free radicals and oxidative stress have been considered as the main candidates mediating the behavioral impairment and memory deficits in age related neurodegenerative disorders.

Oxidative stress is an imbalance between free radicals and the antioxidant system and is an important outset feature in the pathogenesis of AD (Johnson et al., 2008; Verri et al., 2012; Zhang et al., 2012). The brain is at higher risk to the damage caused by oxidative stress due to high content of polyunsaturated fatty acid, high consumption of oxygen, elevated metabolic activity and relatively limited ability to combat with oxidative stress (Cassarino and Bennett, 1999; Halliwell et al., 2001; Ishrat et al., 2009). Oxidative damage to the lipid leads to the disruption of cell membrane and its integrity, inactivation of antioxidant enzymes, and finally cell death. It has been reported that supplementation with antioxidant treatment may help the system to stay normal against the oxidative stress. Earlier findings from our research group has investigated and reported protective effect of certain antioxidant against different experimental model of neurodegenerative disease. (Ishrat et al., 2009; Khan et al., 2010; Javed et al., 2011, 2012).

ICV injection of STZ, in a sub diabetic dose causes prolonged impairment of brain glucose and energy metabolism and oxidative damage and leads to cognitive dysfunctions by inhibiting the synthesis of adenosine triphosphate (ATP) and acetyl-Co A. This ultimately results in elevated level of AChE in hippocampus and reduced level of ChAT in the hippocampus (Ishrat et al., 2006; Shoham et al., 2007; Hoyer and Lannert 2007, 2008) and provides a relevant model for sporadic dementia of Alzheimer's type (SDAT).

Catechin hydrate (CH) is a polyphenolic substance present in beverages, plant fruits vegetables such as olive oil, red wine and tea. CH possesses antioxidant, anticancer and anti-inflammatory properties (Alshatwi, 2010). Pharmacological property of CH have been studied including protection against coronary heart disease (Vinson et al., 1995) various types of cancer (Ogata et al., 1995; Middleton et al., 2000) and in inflammatory diseases (Middleton
et al., 2000). CH also shows a protective effect in case of neurodegenerative disorder/neuroinflammatory disease such as cerebral ischemia (Jullian et al., 2007; Hady, 2007) Parkinson’s disease [PD] (Choi et al., 2002; Nie et al., 2002;) and Alzheimer’s disease [AD] (Mandel et al., 2004; Bastianetto and Quirion, 2002). Recently our research group has investigated and reported the neuroprotective effect of CH on rat model of cerebral ischemia/reperfusion injury (Ashafaq et al., 2012). This study investigates the pretreatment effect of CH on behavioral, biochemical and histochemical alterations in ICV-STZ infused rats.

**Material and method**

**Chemicals and reagents**
As described in materials and methods, chapter III

**Animals and treatments:**
As described in materials and methods, chapter III

**Experimental design**
Rats (400 ± 10 g) were divided into six groups of 8 animals each

**Group I:** Sham operated vehicle treated control (S) group.

**Group II:** ICV-STZ infused and vehicle treated lesion (L) group

**Group III:** ICV-STZ infused and pre-treated with catechin hydrate 10 mg/kg body weight (CH10 + L).

**Group IV:** ICV-STZ infused and pre-treated with catechin hydrate 20 mg/kg body weight (CH20 + L).

**Group V:** Sham operated and pre-treated with catechin hydrate 10 mg/kg body weight (CH10 + S).

**Group VI:** Sham operated and pre-treated with catechin hydrate 20 mg/kg body weight (CH20 + S).
Chapter V

Experimental design

Fig.1: Experimental design for catechin hydrate

Surgery

Intracerebroventricular injection of streptozotocin
As described in materials and methods, chapter III

Post-operative care
As described in materials and methods, chapter III

Behavioral testing
As described in materials and methods, chapter III

Biochemical analysis:

Tissue preparation
After 21 days of ICV-STZ infusion, the rats were sacrificed and their brains were taken out quickly to dissect the hippocampus and frontal cortex as described in materials and methods section III.

Biochemical analysis:
The biochemical assays (TBARS, GSH, GPx, GR, Catalase, AChE and Protein) are described in materials and methods chapter III.

Hematoxylin and Eosin (H & E) and cresyl violet (CV) stain
The histological studies were done as described in materials and methods chapter III.

Immunohistochemistry
As described in materials and methods chapter III.
Statistical analysis
As described in materials and methods chapter III

Results

Behavioral observations

Effect of CH on performance in Morris water maze task

Latency

Decreased latency showed by all the groups to find the platform from the second to fifth day of experiment. However, L group animals presented a significantly (*$p < 0.05$, **$p < 0.01$) higher latency to find the platform than S group animals, but pretreatment of CH has shown a significant (#$p < 0.05$, ##$p < 0.01$) and dose dependently improvement in latency as compared to L group. Slightly decreased in escape latency was observed dose dependently in CH + S group as compared to sham group. No significant change was observed between the CH10 +S.CH20+S pretreated groups and sham group (Fig. 2).

![Fig. 2. Effect of CH supplementation on escape latency in MWM test in ICV–STZ rats. Values are expressed as mean ± S.E.M *$p < 0.05$, **$p < 0.01$ Lesion vs. Sham, # $p < 0.05$, ## $p < 0.01$ CH 10 + L , CH 20 + Lvs. Lesion group.](image)

Path length

Decreased path length showed by all the groups to find the platform from the second to fifth day of experiment. However, L group animals took significantly (*$p < 0.05$ **$p < 0.01$) longer distance (path length) to find the platform than sham group animals, but pretreatment of Catechin hydrate has shown a significant (#$p < 0.05$, # $p < 0.01$) and dose dependently improvement in path length as compared to L group. Slightly improve in path length was observed dose dependently in CH + S group as compared to sham group. No significant change was observed between the CH10 +S.CH20+S pretreated groups and sham group (Fig. 3).
Biochemical observations

Effect of CH on TBARS content in hippocampus and frontal cortex

The effect of CH on TBARS content was measured to demonstrate the oxidative damage to the membrane in hippocampus and cerebral cortex. The content of TBARS was significantly elevated \(p < 0.05\) in L group as compared to S group and its content was significantly and dose dependently protected by the pretreatment with CH \(p < 0.05, L \text{ vs } CH_{10} + L; p < 0.01 L \text{ vs } CH_{20} + L\). The level of TBARS was slightly decrease dose dependently in sham + drug group as compared to sham group. No significant change was observed between the CH pretreated sham \((CH_{10} + S; CH_{20} + S)\) groups and sham group (Fig.4).

Effect of CH on GSH content in hippocampus and frontal cortex

The content of GSH was significantly \(p < 0.05\) decreased in hippocampus and cerebral cortex of L group as compared to S group. The level of GSH was protected significantly and dose dependently by CH \((p < 0.05, L \text{ vs } CH_{10} + L; p < 0.05, L \text{ vs } CH_{20} + L\). The content of GSH was slightly increased dose dependently in S + CH group as compared to sham group. No significant change was observed between the CH pretreated sham \((CH_{10} + S; CH_{20} + S)\) groups and sham group (Fig.5)
Fig. 4. Effects of CH on TBARS levels in the hippocampus and frontal cortex of ICV–STZ rats. Values are expressed as mean ± S.E.M *p < 0.01 Lesion vs. Sham, # p < 0.05, ## p < 0.01 CH + L vs. Lesion group (L).

Fig. 5. Effect of CH supplementation on GSH levels in the hippocampus and frontal cortex of ICV–STZ rats. Values are expressed as mean ± S.E.M *p < 0.05 Lesion vs. Sham, # p < 0.05, ## p < 0.01 CH + L vs. Lesion group (L).

**Effect of CH on the activity of Acetylcholinesterase (AChE) in hippocampus**

The activity of acetylcholinesterase (AChE) was increased significantly in L group as compare to S group and pretreatment with CH has significantly and dose dependently attenuate the activity of AChE in the hippocampus. No significant change was observed between the CH pretreated sham (CH10 + S; CH20 + S) groups and sham group. (Fig 6)
Fig. 6 Effect of CH on AChE activity in hippocampus of ICV–STZ rats. Values are expressed as mean ± S.E.M *p < 0.05 Lesion vs. Sham, # p < 0.05, ## p < 0.01 CH+L vs. Lesion group (L).

Effect of CH on the activity of antioxidant enzymes in hippocampus and frontal cortex

The activities of antioxidant enzymes (GPx, GR, and Catalase) were decreased significantly in L group as compare to S group and pre-treatment with CH has significantly protected the activity of these enzymes dose dependently in the hippocampus and frontal cortex (Tables 1 & 2). The values of these enzymes were slightly increased dose dependently in CH+S groups as compared to sham group. No significant change was observed between the CH pre-treated sham groups and S group.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>GPx (nmoles NADPH oxidized/min/mg protein)</th>
<th>GR (nmoles NADPH oxidized/min/mg protein)</th>
<th>Catalase (nmoles of (H_2O_2) consumed/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>262.13 ± 20.38</td>
<td>323.37 ± 51.45</td>
<td>51.79 ± 5.45</td>
</tr>
<tr>
<td>L</td>
<td>150.88 ± 6.65 * (-42.43%)</td>
<td>179.56 ± 12.06 * (-44.47%)</td>
<td>22.78 ± 1.72 (-56.01%)</td>
</tr>
<tr>
<td>CH10+L</td>
<td>185.80 ± 11.34 # (23.14%)</td>
<td>237.92 ± 8.88## (32.50%)</td>
<td>33.95 ± 2.78# (49.03%)</td>
</tr>
<tr>
<td>CH20+L</td>
<td>198.93 ± 12.22## (32.42%)</td>
<td>259.10 ± 10.33# (44.29%)</td>
<td>35.73 ± 2.91## (56.84%)</td>
</tr>
<tr>
<td>CH10+S</td>
<td>264.41 ± 28.66 (-0.87%)</td>
<td>326.07 ± 37.91 (-0.83%)</td>
<td>51.78 ± 3.28 (0.019%)</td>
</tr>
<tr>
<td>CH20+S</td>
<td>268.81 ± 12.37 (-2.54%)</td>
<td>325.33 ± 21.99 (-0.60%)</td>
<td>51.60 ± 1.84 (0.36%)</td>
</tr>
</tbody>
</table>
Table 1: Values are expressed as mean ± SEM of ten animals. Values in parenthesis show the percentage increase or decrease with respect to their control \(^* p < 0.05\), L vs. S group. \(^\# p < 0.05\), \(^{##} p < 0.01\) CH+L vs. L group.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>GPx (nmoles NADPH oxidized/min/mg protein)</th>
<th>GR (nmoles NADPH oxidized/min/ mg protein)</th>
<th>Catalase (nmoles of H(_2)O(_2) consumed/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>203.02 ± 21.32</td>
<td>293.50 ± 11.57</td>
<td>49.51 ± 3.58</td>
</tr>
<tr>
<td>L</td>
<td>113.47 ± 8.29*</td>
<td>140.79± 7.69*</td>
<td>24.78 ± 1.76*</td>
</tr>
<tr>
<td></td>
<td>(-44.10%)</td>
<td>(-52.03%)</td>
<td>(-49.94%)</td>
</tr>
<tr>
<td>CH10+L</td>
<td>150.92 ± 9.57#</td>
<td>182.67 ± 9.32##</td>
<td>36.06 ± 2.02##</td>
</tr>
<tr>
<td></td>
<td>(33.03%)</td>
<td>(29.74%)</td>
<td>(45.52%)</td>
</tr>
<tr>
<td>CH20+L</td>
<td>164.51 ± 14.07##</td>
<td>234.00 ± 35.85#</td>
<td>42.20 ±4.79#</td>
</tr>
<tr>
<td></td>
<td>(44.98%)</td>
<td>(66.20%)</td>
<td>(70.29%)</td>
</tr>
<tr>
<td>CH10+S</td>
<td>213.77 ± 15.95</td>
<td>297.15 ± 16.68</td>
<td>49.56 ± 3.67</td>
</tr>
<tr>
<td></td>
<td>(-5.2%)</td>
<td>(-1.24%)</td>
<td>(-0.10%)</td>
</tr>
<tr>
<td>CH20+S</td>
<td>215.6 ± 19.26</td>
<td>294.6 ± 27.33</td>
<td>49.25 ± 3.58</td>
</tr>
<tr>
<td></td>
<td>(-6.20%)</td>
<td>(-0.39%)</td>
<td>(0.52%)</td>
</tr>
</tbody>
</table>

Table 2: Values are expressed as mean ± SEM of ten animals. Values in parenthesis show the percentage increase or decrease with respect to their control \(^* p < 0.05\), L vs. S group. \(^\# p < 0.05\), \(^{##} p < 0.01\) CH+L vs. L group.

Effects of CH on TNF-α and IL-1β

Quantification of TNF-α and IL-1β by ELISA in the hippocampus of STZ-infused rats showed significant increased level of TNF-α (\(^* p < 0.05\)) and IL-1β (\(^* p < 0.05\)) as compared to the sham group. While CH pretreatment significantly and dose dependently ameliorate the level of TNF-α (\(^{##} p < 0.01\) for CH20 vs. lesion) and IL-1β (\(^{##} p < 0.01\) for CH20 vs. lesion) as compared to lesion group animals (Fig.7&8).
Fig. 7. Effect of CH on the level of TNF-α in hippocampus. Result showed significantly increased level of TNF-α and IL-1β and its level was attenuated with CH supplementation in CH10 + L, CH20 + L groups as compare to the L group animals (#p < 0.05; ##p < 0.01). Values are expressed as Mean ± SEM (n = 6).

Fig. 8. Effect of CH on the level of IL-1β in hippocampus. Result showed significantly increased level of IL-1β and its level was attenuated with CH supplementation in CH10 + L, CH20 + L groups as compare to the L group animals (#p < 0.05; ## p < 0.01). Values are expressed as Mean ± SEM (n = 6).

Effect of CH on histological changes in hippocampus

High dose showed remarkable protection in biochemical and behavioural analysis. Therefore, high dose of CH was taken for histological evaluation. The sections were stained with hematoxylin-eosin. The hematoxylin and eosin stained the nuclear structure in dark blue and all cytoplasmic and inter sub cellular substances with varying shades of pink. The loss of pyramidal neurons was not detected in the sham group. On the other hand, lesion group has shown degenerated and abnormal neurons. CH pre-treatment ameliorated the hippocampal neuronal abnormalities in (CH20 + L) group animals as compared to L group animals (Fig. 9A).
Effect of CH on cresyl violet staining

The Section of ICV-STZ group showed significant neuronal loss in CA1 region of hippocampus. The intact neurons were almost absent in this area. Cresyl violet staining of section of CH20 + L group animals showed partial loss and shrinkage of neurons in this region. The sham group did not show any pathological changes in this area (Fig. 9B).

Effect of CH on iNOS and ChAT expression

Neuroprotective effect of CH on the expression of iNOS and ChAT was assessed by Immunohistochemistry. Higher expressions of iNOS were seen in lesion group as compared to sham group animals in CA1 region of hippocampus. CH pretreatment attenuated higher expressions of iNOS in CH20 + L group animals as compared to the lesion group animals (Fig 10A). Expression of ChAT was observed extremely low in CA1 region of hippocampus in lesion (L) group as compare to sham (S) group animals. Pretreatment with CH attenuated the ChAT expression in CH20 + L group animals as compare to L group animals (Fig 10 B).

Fig.9 (A) Representative photomicrographs show the H & E staining in the CA1 region of the hippocampus. White arrows indicate the normal pyramidal neuron in S group and Black arrows indicate the degenerated pyramidal neuron in L group while L group pretreated with CH shows normal pyramidal neuron staining in CH20+L group (9B) represent cresyl violet staining shows the neuronal alterations in the hippocampus region of ICV-STZ treated rats. Black arrow in L group show shrinkage
Chapter V

of nucleus and cytoplasm in neurons present in lesion groups (black arrow showing damage cells), whereas white arrow in sham (S) vehicle treated group show normal pyramidal neurons. Administration of CH clearly ameliorates in ICV-STZ infused neuronal damaged in CH20 + L groups. Magnification = 40X.

Fig.10A. Representative Photomicrograph showing the representation of CA1 region of the hippocampus. Profound expression of the iNOS was observed in the L group animals as compared to S group animals. While CH supplementation decreases the expression of iNOS in CH20 + L group animals shown in (10B) ChAT expression showed that neuronal alterations in the hippocampus region of ICV-STZ treated rats. Profound expression of ChAT was observed in S group animal and its expression was found to be decreased in L group animals as compared to S group animals. CH pre-treatment has shown the moderate staining of ChAT in CH20 + L group animals shown in (10B). Magnifications=40X (n=4).

Discussion

The present study was undertaken to evaluate the neuroprotective effect of the most abundant occurring natural polyphenolic compound CH on ICV-STZ induced dementia of Alzheimer’s type in rat. It is well known that ICV-STZ infused rodent model is an appropriate animal model used for the study of sporadic dementia of Alzheimer’s type (Nitsch and Hoyer, 1991; Lannert and Hoyer 1998; Agrawal et al., 2009; Ishrat et al., 2009; Javed et al., 2011). ICV-STZ induced dementia is associated with the biochemical, behavioral and histopathological alterations in brain which is seen to be well ameliorated with the pretreatment with CH. Here,
we have observed that CH prevents ICV-STZ induced changes in hippocampus and frontal cortex by ameliorating oxidative damage.

Cholinergic function is important for the learning and memory and its alteration play a crucial role in the development of cognitive impairment. Morris water maze test was employed in the present study to test the spatial learning and memory of animals. In this behavioral test the animal’s escape from the water reinforces its desire to find the submerged platform quickly, and on subsequent trails the animal would be able to locate the platform more rapidly. Therefore, such improvement in performance occurs because the animals have learned where the hidden platform is located in the water pool relative to the conspicuous visual cues. Our Morris water maze data presented that ICV-STZ infused rats travelled long distance (path length) and took longer time (escape latency) to reach the submerged platform, indicating poor learning and memory process which is consistent with the previous reports (Khan et al., 2012; Ishrat et al., 2009). Moreover, CH administration significantly ameliorated the deficits in learning and memory in ICV-STZ rats as showed by reduced path length and escape latency to reach the hidden platform. Our findings are in accordance with others findings carried out by others where CH supplementation is effective in retarding memory dysfunction resulting from hippocampal neuron loss such as in Alzheimer’s disease (Rezaie-Zadeh et al., 2008). The data showed the consistency of memory impairment indicating the beneficial effect of CH in enhancing these behavior test induced by ICV-STZ.

It has been well documented that oxidative stress plays a significant role in the pathogenesis of Alzheimer’s disease (Verri et al., 2012; Zhang et al., 2012). Cells have evolved complex system including enzymatic and non-enzymatic system to manage with various form of oxidative stress. Excess amount of free radical generation is thought to be the key role of neuronal damage because of their high reactivity and capacity to produce cellular impairments. Lipid peroxides and hydroperoxides cause secondary injury by further generating relatively more stable and diffusible cytotoxic agents such as malondialdehyde and 4-hydroxy-trans-2-nonenal, respectively, and amplify oxidative cascade. These free radicals induce damage to membrane and macro molecule of cells (lipid, sugar, protein and nucleic acids) plays major role in development of aging and age related neurodegenerative disorder including Alzheimer’s disease (Liu et al., 2010; Wickens, 2001; Zhang et al., 2012). GSH is a tripeptide an essential antioxidant, which is responsible for detoxification of hydroxyl and superoxide free radicals in the brain (Dringen et al., 2000). It eliminates H$_2$O$_2$ and organic peroxide by GPx (Meister 1988). During free radicals clearance, oxy radicals are reduced by GPx at the cost of reduced to form glutathione disulfide (GSSG). GSH is again formed by
redox recycling, in which GSSG is reduced to GSH by GR with an expenditure of one NADPH molecule. Reduced levels of GSH impair H₂O₂ clearance and endorse formation of OH radicals the most toxic molecule to the brain leading to more free radical level and oxidative stress (Sun, 1990; Dringen et al., 2000). Results from the biochemical estimation showed significant increase in TBARS content and decrease in the level of reduced glutathione (GSH) and its dependent enzyme GPx and GR in the brain of ICV–STZ infused rats compare to sham group rats. Previous studies confirm that CH is a powerful antioxidant, which scavenges free radical–induced damages (Ruch et al., 1989; Guo et al., 1996; Kashima 1999; Nanjo et al., 1999; Zhao et al., 2001). The present data encompass the role of oxidative radicals in the depletion of glutathione and its dependent enzymes, which were protected significantly by the treatment of CH, reflecting its potential antioxidant potential. Besides defending against oxidant stress, another existing and encouraging finding is that CH significantly attenuated histologic changes, that is, it caused minimal less neural damage along with presence of intact neuron in hippocampus.

There was change in the control value of chapter IV, chapter V, chapter VI& chapter VII. In chapter VI&VII we used glass homogenizing tube with Teflon which has maximum rpm of 5000 and the working rpm between 3,000-4,000. At higher rpm there were chances of breaking down of the glass homogenizing tube. In chapter IV&V, Ultra Turex T-25 was used which has rpm 25,000 and the used rpm is between 20,000-25,000, which gives a superb homogenate as compared to glass teflon homogenizer and less cells debris than in glass and teflon homogenizer. Our lab don’t have a fixed temperature, it varies according to the seasons. So the biochemical values were high in summer and low in winter (Chapter IV&V).

There is growing evidence that the brain cholinergic system, which plays an important role in learning and memory, may be closely linked to human aging diseases, like AD and it seems to be more prone to oxidative damage and pathogenesis of AD (Francis et al., 1999; Khan et al., 2012). Choline acetyltransferase (ChAT) is one of the specific cholinergic marker proteins for the functional state of cholinergic neurons, which can play a key role in the maintenance of acetylcholine (ACh) levels, in these neurons. Its synthesis depends on acetyl CoA, provided by the breakdown of glucose and insulin, which regulates the activity of ChAT. It has been demonstrated that ICV-STZ injection disturbs glucose energy metabolism by inhibiting the insulin receptor system (Hoyer and Lannert, 1999; Hoyer and Lannert, 2007) and decreasing the activity of ChAT in the hippocampus (Ishrat et al., 2006; Khan et al., 2012). Hence, the expression of ChAT was investigated in the present study to demonstrate the role of the cholinergic system in ICV-STZ induced rats had deficits in spatial learning and
memory as indicated by MWM. The hippocampus has been shown to be required for various
types of learning and memory formation in rats and other mammals. Therefore, ChAT
expression was expected in the hippocampus in our present study, and decreases in ChAT
protein that we observed in ICV-STZ induced rats hippocampus are similar in magnitude to
those seen at autopsy in human individuals who had AD (Perry et al., 1985). Furthermore, CH
pretreatment significantly protected ChAT expression and ameliorated the deficits in learning
and memory in ICV-STZ induced rats. This suggests that CH may serve as an antioxidant to
reduce oxidative damage by enhancing the level of antioxidant defense system to the brain
and thereby improves learning and memory deficits.

Neuroinflammation is well known to participate in development of pathology of many
neurodegenerative disorder including AD (McGeer and McGeer, 2003; Rojo et al., 2008).
Any sort of injury in brain results generation of glutamate and proinflammatory cytokines by
activating the glial cells i.e. microglia and astrocytes (Tuppo and Arias, 2005). Tumor
necrosis factor-α (TNF-α) is a key pro-inflammatory cytokine, which commonly manifests
synergistic effects with IL-1β to produce inflammatory processes in neurodegenerative
disorders (Dinarello, 2003). The elevated level of TNF-α and IL-1β were found in ICV-STZ
infused rats and CH pretreatment significantly and dose dependently decreases the level of
these inflammatory markers in CH pretreated groups. It is well documented that inducible
nitric oxide synthase (iNOS) produces nitric oxide (NO) and NO-derived reactive nitrogen
species such as peroxynitrite. In healthy neuronal tissue iNOS is not commonly present, but it
can be expressed by astrocyte, neurons and endothelial cells after brain offence, where it can
initiate the production of high amounts of NO (Murphy et al., 1993; Brosnan et al., 1997).
Overproduction of NO may lead to neuronal damage and death. The reaction between NO and
super-oxide anion generate the cytotoxic compound, peroxynitrite that leads to neuronal
toxicity (Javed et al., 2012). Under normal physiological conditions, antioxidant enzymes are
responsible to eliminate the highly reactive molecules (Javed et al., 2011). However, under
unphysiological conditions, the excessive accumulation of reactive species induces several
cellular dysfunctions. The present data revealed that ICV-STZ injection induced a marked
iNOS expression, which served as indicator of NO production in lesion group. Interestingly,
CH supplementation decreased iNOS expression in CH pretreated group.

The present finding indicates that ICV-STZ infusion cause behavioral, biochemical and
histopathological changes due to generation of free radical. CH pretreatment significantly and
dose dependently attenuated these changes, which is supported by increased endogenous
antioxidant defense system and decreased inflammatory responses. These beneficial effects of
CH may be attributed partly to its antioxidant and anti-inflammatory potential. This study shows that CH could be used as a useful probe for studying the clinical pharmacology of neuronal damage and thus might be beneficial in reducing oxidative stress and inflammation in neurodegenerative diseases including AD.