Introduction
Alzheimer’s disease (AD) is neurodegenerative disease of elderly, characterised by progressive cognitive decline, atrophication of the brain and neuronal loss (Gasparini et al., 2002). Late onset of sporadic form of Alzheimer’s disease (SAD) usually occurs after the age of 65 years. Several factors such as environmental factor, oxidative stress, mitochondrial dysfunction, inflammatory process and apoptosis are responsible for the induction of SAD. These factors act together by various mechanisms and leads to the neurodegeneration and finally cell death (Labak et al., 2010; Javed et al., 2012).

Oxidative stress plays major role in case of neurodegenerative disease because of high metabolic rate, limited amount of antioxidant enzymes and rich amount of polyunsaturated fatty acids (PUFA) in the brain (Butterfield et al., 2001; Moreira et al., 2005). Thus, it can be speculated that treatment with herbal supplements antioxidant may improve the neurons from degeneration against oxidative stress.

There is promising evidence that mitochondrial dysfunction and oxidative stress play major role in neurodegenerative disease (Lin and Beal, 2006). However, many other biological alterations like inflammation and apoptosis may occur in neurodegenerative disorders like AD. Evidences from previous studies indicate that these processes may causally be related to dysfunction and death of neurons in AD (Perry et al., 2002; Golden et al., 2002; Keller et al., 2005; Javed et al., 2012).

STZ, a glucosamine nitrosourea compound when injected intracerebroventricularly (ICV) as a sub-diabetogenic dose, causes prolonged impairment of the brain glucose and energy metabolism. This is accompanied by impairment in learning and memory in addition to oxidative stress, neuroinflammation, increased acetyl cholinesterase (AChE) and decreased choline acetyltransferase (ChAT) activities (Lannert and Hoyer, 1998; Sharma and Gupta, 2002; Javed et al, 2012).

Development of effective therapies for the treatment of AD is a major thrust area in geriatric world. Natural compounds have been reported to reduce ROS mediated reactions and to rescue neurons after the ICV-STZ infusion (Javed et al., 2011, 2012, Khan et al, 2012). Sesamin is most important lignin component of sesame seed oil (Fukuda et al, 1986). Sesamin has been known for antihypertensive, antioxidant, and anti-inflammatory properties (Hou et al., 2003; Fujikawa et al., 2005; Jeng et al., 2005; Miyawaki et al, 2009). Recently, Ahmad et al., 2006 and Khan et al., 2010 have reported the protective effects of sesame oil and sesamin for the treatment of cerebral
ischemia. Therefore, sesamin has created our interest to study its modulatory effects in ICV-STZ infused rats of sporadic dementia of Alzheimer’s type.

Materials and methods

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

Animals
As described in materials and methods, chapter III

Experimental design

![Experimental design for sesamin](image)

Rats (400 ± 10 g) were divided into four groups of 8 animals each

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

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

**Group III:** ICV-STZ infused and pre-treated with sesamin 30 mg/kg body weight (SN + L).

**Group IV:** Sham operated and pre-treated with sesamin 30 mg/kg body weight (SN + S)

**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 chapter III.

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

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

Immunohistochemistry
As described in materials and methods chapter III.

Statistical analysis

Protein estimation
As described in materials and methods chapter III

Statistical analysis
As described in materials and methods chapter III

Results

Behavioral observations

Effect of sesamin on performance in Morris water maze task
Escape latency

Escape latency was increased significantly (*p < 0.05, **p < 0.01) in L group from third day to fifth day compared with the S group, showing a poor learning performance due to ICV-STZ infusion. Pretreatment with sesamin in (SN + L) group has shown significantly (#p < 0.05, ##p < 0.01) improved latency from 3rd day to 5th day as compare to S group animals. Slightly decreased in escape latency was observed in SN + S group as compared to sham group. No significant change was observed in sesamin pretreated (SN + S) group animal as compare to S group (Fig. 2).

Path length

The S and (SN+S) group animal have shown decreased path length to find the platform from day third to day fifth of the experiment. However, the L group animals have shown a significantly (*p < 0.05, **p < 0.01) higher path length than the S group animals to find the platform which was improved significantly (#p < 0.05, ##p < 0.01) in SN + L group animals from third day to fifth day. Slightly improve in path length was observed in SN+ S group as compared to sham group. No significant change was observed in sesamin pretreated (SN + S) group animal as compare to S group (Fig. 3).

Fig. 2. Effect of SN 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 SN + L vs. Lesion group.
Biochemical observations

Effect of sesamin on TBARS content

The effect of sesamin on TBARS content was estimated to show the oxidative damage on membrane lipids in hippocampus and frontal cortex of ICV-STZ–infused rats. A significantly increased \((p < 0.05)\) TBARS content was observed in the L group animals as compared to S group animals. This increase in TBARS content was significantly \((p < 0.05)\) attenuated in the sesamin pretreated SN + L group animals as compared to L group. The level of TBARS was slightly decrease in SN+S group as compared to sham group. No significant change was observed between the SN+S pretreated sham groups and S group (Fig. 4).

Effect of sesamin on GSH content

The protective effect of sesamin on GSH content in the hippocampus and frontal cortex is shown in (Fig. 5). GSH content was depleted significantly \((p < 0.05)\) in L group as compared to S group. The decrease in GSH content was protected significantly \((p < 0.05)\) in SN+L group as compared to L group. The content of GSH
was slightly increased in SN+S group as compared to sham group. No significant change was observed between the SN+S pretreated sham group.

**Fig 4.** Effects of SN on TBARS 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, #$p < 0.01$ SN + L vs. Lesion group.

**Fig 5.** Effect of SN 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, #$p < 0.01$, SN + L vs. Lesion group.

**Effect of sesamin on AChE activity**

**Fig 6** shows the effect of sesamin on AChE activity in the hippocampus and frontal cortex. AChE activity was increased significantly ($p < 0.05$) in L group as compared to S group. The increased AChE activity was protected significantly ($p < 0.05$) in SN + L group as compared to L group. No significant change was observed in the sesamin pretreated sham (SN + S) group and sham group.
Fig 6. Effect of SN 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 SN+L vs. Lesion group.

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

Table 1 and 2 show the activities of antioxidant enzymes (GPX, GR, GST, CAT and SOD) in hippocampus and frontal cortex. The activity of these enzymes were decreased significantly in hippocampus (p < 0.01, GR and p < 0.05, GPx, catalase, SOD, GST) and frontal cortex (p < 0.01, GPx and p < 0.05, GR, catalase, SOD and GST) in Lesion group as compared with sham group. Pretreatment with sesamin (SN+L group) has significantly ameliorate the activities of these enzymes in hippocampus \([p < 0.05, \text{GPx and SOD and } p < 0.01, \text{GR, catalase, GST}]\) and frontal cortex \([p < 0.05, \text{catalase and } p < 0.01, \text{GPx, GR, SOD, GST}]\) as compared with lesion (L) group. The values of these enzymes were slightly increased dose dependently in SN+S groups as compared to sham group. No significant change was observed between the SN pre-treated sham groups and S group.
<table>
<thead>
<tr>
<th>Parameters</th>
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<th>$L$</th>
<th>$SN+L$</th>
<th>$SN+S$</th>
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<tbody>
<tr>
<td><strong>GPx (nmol NADPH oxidized/min/mg protein)</strong></td>
<td>159.63±17.54</td>
<td>83.56±2.43**</td>
<td>117.70±3.81##</td>
<td>159.77±11.69 (-0.08%)</td>
</tr>
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<td></td>
<td>(-47.65%)</td>
<td>(40.85%)</td>
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<tr>
<td><strong>GR (nmol NADPH oxidized/min/mg protein)</strong></td>
<td>143.32±15.47</td>
<td>77.66±6.60*</td>
<td>116.92±7.44##</td>
<td>145.88±13.35 (-1.78%)</td>
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<td></td>
<td>(-45.81%)</td>
<td>(50.55%)</td>
<td></td>
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<tr>
<td><strong>Catalase (nmol of H$_2$O$_2$ consumed/min/mg protein)</strong></td>
<td>33.63±3.38</td>
<td>19.92±2.87*</td>
<td>29.01±1.48#</td>
<td>33.75±4.01 (-0.35%)</td>
</tr>
<tr>
<td></td>
<td>(-40.76%)</td>
<td>(45.63%)</td>
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<tr>
<td><strong>SOD (nmol of epinephrine protected from oxidation/min/mg protein)</strong></td>
<td>102.32± 5.69</td>
<td>56.22± 6.17*</td>
<td>85.70± 2.84##</td>
<td>102.35± 8.04 (-0.029%)</td>
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<td></td>
<td>(-45.05%)</td>
<td>(52.45%)</td>
<td></td>
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<tr>
<td><strong>GST (nmole of CDNB conjugate formed/min/mg protein)</strong></td>
<td>491.47±44.84</td>
<td>295.39±29.51*</td>
<td>408.92±12.18##</td>
<td>490.88±55.99 (0.12%)</td>
</tr>
<tr>
<td></td>
<td>(-39.38%)</td>
<td>(38.43%)</td>
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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, ""p < 0.01 L vs. S group, "p < 0.05, ""p <0.01, SN+L vs. L group.
<table>
<thead>
<tr>
<th>Parameters</th>
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<th>L</th>
<th>SN+L</th>
<th>SN+S</th>
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<tbody>
<tr>
<td>GPx (nmoles NADPH oxidized/min/mg protein)</td>
<td>162.75 ±11.78</td>
<td>100.62 ±.94* (-38.17%)</td>
<td>133.59 ± 1.58# (32.76%)</td>
<td>168.22±11.24 (-3.36%)</td>
</tr>
<tr>
<td>GR (nmoles NADPH oxidized/min/mg protein)</td>
<td>128.54 ± 16.76</td>
<td>74.25 ± 3.90** (-42.23%)</td>
<td>105.14 ± .86## (41.60%)</td>
<td>134.02 ± .53 (-4.26%)</td>
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<tr>
<td><strong>Catalase</strong> (nmoles of H₂O₂ consumed/min/mg protein)</td>
<td>38.70 ± 2.40</td>
<td>21.29 ± 1.62* (-44.91%)</td>
<td>30.41 ± 2.05## (42.83%)</td>
<td>40.71 ± 2.36 (-5.19%)</td>
</tr>
<tr>
<td>SOD (nmoles of epinephrine protected from oxidation/min/mg protein)</td>
<td>141.11 ± 14.86</td>
<td>86.01 ± 7.33* (-39.01%)</td>
<td>118.75 ± 4.71# (38.06)</td>
<td>141.93 ± 6.06 (-0.58%)</td>
</tr>
<tr>
<td>GST (nmoles of CDNB conjugate formed/min/mg protein)</td>
<td>491.47± 44.84</td>
<td>295.39±29.51* (-39.89%)</td>
<td>408.92±2.18## (38.43%)</td>
<td>490.88±55.99 (-0.12%)</td>
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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, **p < 0.01 L vs. S group, *p < 0.01, ##p <0.001, SN +L vs. L group.
Effect of sesamin on the morphology of pyramidal neuron in the CA1 region of hippocampus

The sham group animals show normal histopathological architecture of pyramidal neurons in the CA1 region of the hippocampus. A large number of abnormal, as well as degenerated, neurons were seen in the lesion group animals as compared to sham group animals. However, pretreatment with sesamin has protected ICV-STZ-induced histological abnormalities in SN + L group animals as compare to L group animals (Fig. 7).

Fig 7. Histopathological changes in CA1 region of hippocampus. Sections were stained with H & E. White arrows indicate the normal pyramidal neuron in the sham group (B) and black arrows indicate the degenerated pyramidal neurons in lesion group (D). The lesion group pretreated with sesamin shows normal pyramidal neuron staining (F) with less degenerated neurons. Magnification 10x (A, C, E) and 40x (B, D, F).
Effect of sesamin on iNOS, NF-κB, Hsp 70, p53, Caspase-9, apaf-1, Bax, and Bcl-2 expressions

A normal immunohistochemical expressions of iNOS, Hsp70, caspase-9, Bax, Bcl-2 and Apaf-1 were observed in the sham group animal was seen normal. Expressions of iNOS, Hsp 70, caspase-9, Bax and Apaf-1 in lesion group animal was up regulated in CA1 region of hippocampus as compared to sham group animal. Up regulated expressions of NF-κB and p53 was also observed in CA1 region of hippocampus with suppressed expression of Bcl-2. However sesamin pretreatment has down regulated the expressions of NF-κB, iNOS, Hsp 70, caspase-9, Bax, apaf-1 and up regulated Bcl-2 as compared to L group animal (Fig. 8, 9A & 9B).

![Fig 8 Effect of sesamin pretreatment on iNOS (A-C), Hsp70 (G-I) and NF-κB (D-F) expression in CA1 region of hippocampus in ICV-STZ-infused rats. A high expression of iNOS in L group (B), Hsp70 (H) and NF-κB (E) was observed in STZ-infused hippocampus. Sesamin, however, successfully reduced the expressions of these proteins (C, F, I). The sham group has shown basal level of expressions (A, D, G). Magnification 40x (A-I).](image-url)
Discussion

The bilateral ICV-STZ infusion in rat brain causes learning and memory deficit oxidative stress, inflammation and apoptosis as reported earlier finding (Ishrat et al., 2009; Khan et al., 2006; Javed et al., 2011, 2012). The major finding of present study is that sesamin prevent the early accumulation of lipid peroxidation products and enhance the potential of antioxidant enzymes and suppressed the apoptosis response. The therapeutic effect of sesamin on oxidative damage after the ICV-STZ infusion describes the relationship between sesamin and apoptosis. Oxidative stress is caused due to an imbalance between the production of reactive oxygen species and a biological systems ability to easily detoxify the reactive intermediate or easily repair the resulting damage. This cellular redox environment is conserved with enzymes that maintain the reduced state through a constant utilization of metabolic energy. Any disturbances in the normal redox state can cause toxic effects through the production of peroxides and free radicals. In case of brain injury after the ICV-STZ infusion oxygen free radicals are thought to cause oxidative damage to a various type of molecular and cellular targets, which include proteins, DNA,
lipids, mitochondria, and membrane structures leading to subsequent necrosis or apoptosis (Jewen et al., 2000; Javed et al., 2012). Sesamin is a natural free radical scavenger with antioxidant property (Fujikawa et al., 2005; Y. Kiso 2004; Wu et al., 2006) has been proven to reduce neurobehavioral deficits significantly in sesamin pretreated animal by scavenging free radicals.

The biochemical mechanism involved in the development of ICV-STZ induced cognitive impairment is well studied (Ishrat et al., 2009; Javed et al., 2012). Reactive oxygen species (ROS) are important determinants in neurodegenerative disease. It is found that ICV-STZ infusion cause significant increase in lipid peroxides accompanied by significant depletion of GSH which is considered the most common and important intracellular non-protein thiol, has a crucial role as a reactive oxygen species scavenger. GSH along with GPx forms GSSG, which is reconverted to GSH by an enzyme GR thus maintain the pool. GPx uses GSH as proton donor, convert H$_2$O$_2$ to water and molecular oxygen. Decrease in GPx and GR activity could suggest inactivation by ROS (Tabassum et al., 2012; Haung, 1996). The SOD is another important enzyme in the brain which provides first line of defense. SOD with the help of GPx and catalase acting as a free radical scavengers which prevent tissue damage due to peroxide damage. Any decrease in the activity of antioxidant enzymes cause excessive presence of superoxide anion in the brain (O$_2^-$) and hydrogen peroxide
(H$_2$O$_2$), which generate hydroxyl radical (OH) leads to the initiation and propagation of lipid peroxidation. SOD can catalyze the dismutation of O$_2^-$ into H$_2$O$_2$, which then deactivate by GPx or catalase (Aebi H, 1984; Kumuhkekar, 1992). In the present study, sesamin significantly elevated the GSH and its related enzymes activities along with an increase in activities of SOD and catalase, which is in corroboration with the former reports where antioxidant used to mitigate the oxidative stress successfully (Islam, et al., 2002; Khan et al., 2011; Ishrat et al., 2009; Javed et al., 2011).

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).

AChE is one of the important enzymes for the metabolism of acetylcholine (Ach) which is necessary for memory and its retrieval. Its synthesis depends on the availability of acetyl Co-A, provided by the breakdown of glucose. Alzheimer’s disease has been linked to deficiency in the brain Ach (Isharat et al., 2006). On the other hand, the activity of AChE, a hydrolyzing enzyme of ACh has been considered as the best marker of cholinergic function. AChE regulates cholinergic nerve and neurotransmission. Increased activity of AChE leads to rapid degradation of ACh that in turn, results in cholinergic system abnormalities with cognitive impairment. In the present study, we have observed the elevated activity of AChE in hippocampus and frontal cortex which was successfully ameliorate to near normal values. Our results are in congruence to previous finding by (Ishrat et al., 2006).

ICV-STZ infusion also triggers the activation of inflammatory molecules. We have shown that ICV-STZ infusion readily upregulated the expressions of iNOS and NF-κB. We further found that expression of Hsp-70 has also been found to be increased. Hsp-70 is a ubiquitous heat shock chaperone protein and readily upregulated under the presence of toxic chemicals or stress. The NF-κB plays an important role in the pathogenesis of oxidative stress associated neurodegenerative disorder (Lezoualc’h et al., 1998). NF-κB usually remains inactive in the cytoplasm by its I-kB which
degrades under cellular stress leading to phosphorylation and nuclear translocation of p50-p65 which subsequently binds to DNA and induces the transcription of targeted genes such as iNOS which produce NO that may carry out the inflammatory reactions (Shen et al., 2010; javed et al., 2012). In the present study, we observed the higher expression of Hsp70, NF-κB and iNOS in the lesion group animals in CA1 region of hippocampus. However, the sesamin pretreatment (SN+L) successfully down regulated the expressions of these proteins.

Several protein families highly conserved during evolution are considered to be specifically involved in regulating apoptotic cell death such as apaf-1, Bax and Bcl-2. The relative expression of these two proteins determined the cell death or survival after STZ-infusion. When Bcl-2 homo-dimer predominates and protects cell survival. It has been reported that the over expression of Bcl-2 protects cells from apoptosis induced by diversified death stimuli, including chemotherapeutic agents, radiation, tumor necrosis factor and glutamate (Zhonge et al., 1993; Domen et al., 1998; Howard et al., 2002). Bcl-2 has been proposed to prevent apoptosis by regulating an antioxidant pathway (Hockenbery et al., 1993). In the agreement of above fact, we observed down expression of Bcl-2 protein in ICV-STZ infused rats which was protected by the pretreatment of sesamin in (SN+L) group in the CA1 region of hippocampus.

The tumor suppressor and nuclear transcription factor p53 is a tetramer phosphor-protein that can regulate several major cellular functions including gene transcription, DNA synthesis, DNA repair, cell cycle regulation, senescence, and cell death (Sherr et al., 2002; Hofseth et al., 2004). The up regulation of p53 in response to a diverse array of cellular insults ranging from ischemia/hypoxia and excitotoxicity to oxidative stress in neuronal cells suggests that p53 is a key factor involved in neuronal death in response to different forms of acute and chronic neurodegenerative conditions. The regulation of p53 in a variety of neurodegenerative disorder raised the possibility that inhibitors of p53 might be effective in suppressing the associated mechanism of neuronal cell death. The sesamin pretreatment used as prophylactic treatment successfully down regulated the expressions of p53 in the CA1 region of hippocampus.