Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by deterioration of memory and cognitive function. It affects millions of people and has become a major medical and social burden for developing countries. AD is linked with progressive and irreversible loss of neurons, mainly in the cortex and hippocampus. The neuropathological hallmarks of AD include amyloid beta plaques and neurofibrillary tangles composed of the hyper phosphorylated tau protein (Tundo et al., 2012; Thompson and Vinters., 2012).

Oxidative stress, an imbalance between prooxidant and antioxidant level, is known to contribute the pathogenesis of neurodegenerative disorders including AD (Butterfield, 2004; Zhu et al., 2005). This imbalance may originate from an overproduction of free radicals or from a reduction in antioxidant defenses due to the high consumption of oxygen and polyunsaturated fatty acids (Halliwell and Gutteridge, 1985; Halliwell, 2001; Khan et al., 2009). Oxidative damage to lipid and protein (protein carbonyl formation) can lead to structural and functional disruption of the cell membrane, inactivation of enzymes, and, finally, cell death. Thus, it can be speculated that supplemental antioxidant treatment may boost the system to stay normal against the oxidative stress.

ICV injection of STZ in rats impairs brain glucose, energy metabolism, cholinergic transmission, and increases generation of free radicals, leading to cognitive impairment (Hoyer and Lannert, 1999; Ishrat et al., 2009; Javed et al., 2011). Streptozotocin when administered ICV damages the septohippocampal system (Prickaerts et al., 1999) whereby memory impairment in rat could occur due to direct damage to the system. This is supported by reduced ChAT activity in the hippocampus (Blokkland and Jolles, 1994). Experimental intracerebroventricular administration of streptozotocin in rats has been shown to produce biochemical alterations similar to those observed in sporadic AD and therefore considered to be a valid experimental model. Since oxidative damage is concerned in the etiology of neurological complications including AD and treatment with antioxidants has been used as a therapeutic approach in neurodegenerative disease.

WS (commonly known as ashwagandha) is used in many indigenous systems of medicine, mainly Ayurveda in India (Ahmad et al., 2005; Sehgal et al., 2012). The known biological active chemical constituents of WS are withaferin A and saponins.
(Mishra et al., 2000). WS has been reported to be a potent enhancer of cellular antioxidants and possess a significant free radical scavenging activity in various disease models (Davis and Kuttan, 2001; Bhattacharya et al., 2001). Withaferin-A, is the principal withanolide in Indian WS and highly oxygenated steroidal lactone, related to Solanaceae species. Its pharmacological properties includes anti-inflammatory, antitumor and antioxidant (Sharada et al., 1996; Bhattacharya et al., 1997; Koner et al., 2011; Sehgal et al., 2012). Therefore, the present study was executed to test the antioxidant potential of characterized WS to ameliorate the cognitive impairment in ICV-STZ model of 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 eight 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 WS 100 mg/kg body weight (WS100 + L).
Group IV: ICV-STZ infused and pre-treated with WS 200 mg/kg body weight (WS200 + L).
Group V: ICV-STZ infused and pre-treated with WS 300 mg/kg body weight (WS300 + L).
Group VI: Sham operated and pre-treated with WS 100 mg/kg body weight (WS100 + S).
Group VII: Sham operated and pre-treated with WS 200 mg/kg body weight (WS200 + S).
Group VIII: Sham operated and pre-treated with WS 300 mg/k body weight (WS300 + 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, GPx, GR, Catalase, AChE and Protein) are described in materials and methods chapter III.

**Hematoxylin and Eosin (H & E) stain**
The histological studies were done as described in materials and methods chapter III.
Immunohistochemistry of ChAT
As described in materials and methods chapter III.

Statistical analysis
As described in materials and methods chapter III

Results

Quantification of withaferine A from *W. somnifera* extract

The standard chromatograph shows peaks of Withaferin A at R_f 0.37 ± 0.02 (Fig 1). The peak area thus obtained with different concentration of standards were treated with linear least square regression to get the regression equation \( y = 554.503 + 14.202x \) (\( r^2 = 0.9964 \)), which was used for quantification of Withaferin A in sample (in duplicate). The average of duplicate samples were taken and reported as concentration of Withaferin A. It is found that withania extract contains 0.0232% (w/w) of Withaferin A in the sample.

![A and B](image)

Fig 2. HPTLC chromatogram of sample at 214 nm showing presence of Withaferin A at R_f 0.37 (B). Developed TLC plate showed the presence of Withaferin A in sample S2(A). The Withaferin A was quantified as 0.0232 % (w/w) in extract.

Behavioral observations

Effect of WS on performance in Morris water maze task
Latency
A decreased latency was observed in all groups of animals 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. The pretreatment with WS in WS100+L, WS200+L and WS300+L has shown a significant (#$p < 0.05$, ##$p < 0.01$) and dose dependently improvement in latency as compared to the L group. Slightly decreased in escape latency was observed in the sham groups pre-treated with WS (WS100 +S, WS200+S, WS300+S) as compared to the sham group animals but the change was not significant as compared to sham group animals (Fig. 3).

Path length
Decreased path length was shown by all groups of animals to find the platform from the second to fifth day of experiment. However, L group animals took significantly ($p < 0.01$) longer distance (path length) to find the platform than S group animal. The pretreatment with WS in WS100+L, WS200+L and WS300+L has significant ($p < 0.01$) and dose dependently improvement the path length as compared to L group. Slightly improve in path length was observed dose dependently in WS + S group as compared to sham group. No significant change was observed between the WS100 +S, WS100 +S, WS200+S, WS300+S WS200+S, WS300+S pretreated groups and sham group (Fig.4).

Fig 3. Effect of WS on escape latency in MWM test in ICV–STZ infused rats. Values are expressed as mean ± S.E.M *$p < 0.05$, **$p < 0.01$ Lesion vs. Sham, #$p < 0.05$, ##$p < 0.01$ WS 100 + L, WS 200 + L, WS 300 + L vs. Lesion group.
Biochemical observation

Effect of WSOn TBARS content in hippocampus and frontal cortex

The effect of WS on TBARS content was measured to demonstrate the oxidative damage to the membrane in hippocampus and frontal cortex (Fig 5). 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 various dose of WS (#p < 0.05, L vs WS100 + L; ###p < 0.01 L vs WS200 + L and WS300 + L). The level of TBARS was slightly decreased in WS100 +S, WS200+S, WS300+S sham + drug treated groups as compared to sham group. No significant change was observed between the WS pre-treated sham group and S group.

Effect of WS on GSH in hippocampus and frontal cortex

The content of GSH was significantly (*p < 0.05) decreased in hippocampus and frontal cortex of L group as compared to S group. The level of GSH was protected significantly (#p < 0.05, L vs WS100 + L; ###p < 0.01, L vs WS 200 + L; ###p < 0.01 L vs WS 300 + L) and dose dependently by WS (Fig. 6). The content of TBARS was slightly increased dose dependently in sham + drug group as compared to sham group. No significant change was observed between the WS pretreated sham group as compared to S group.
Fig 5. Effect of WS pre-treatment on TBARS content in the hippocampus and in Frontal cortex of ICV-STZ infused rats. Values are expressed as Mean±SEM. TBARS content was significantly increased in L group as compared to S group (*p < 0.05 L Vs. S group). WS pre-treatment significantly attenuated TBARS content in the hippocampus and Frontal cortex in, WS100+L, WS200+L, WS300+L, group animals as compared with L group animals (# p < 0.05 L Vs. WS100 + L, ## p < 0.01 L Vs WS200 + L and WS300 + L).

Fig 6. Effect of WS pre-treatment on the content of GSH in the hippocampus and Frontal cortex of ICV-STZ infused rats. GSH level was significantly decreased in L group as compared to S group (*p < 0.05 L vs. S group). WS pre-treatment has significantly ameliorated the level of GSH in hippocampus and Frontal cortex in WS100 + L, WS200+L, WS300 + L group animals as compared to L group animals (# p < 0.05 L vs. WS100 + L; ##p < 0.01, L vs WS200 + L and WS300 + L). Values are expressed as mean ± S.E.M. (n=8).

**Table 1. Effect of WS on the activity of antioxidant enzymes in hippocampus of ICV-STZ treated rats**

The activities of antioxidant enzymes (GPx, GR, and catalase) were decreased significantly in the L group as compared to the S group animals, and pretreatment with
WS has significantly protected the activities of these enzymes dose dependently in the hippocampus (Tables 1). The values of these enzymes were slightly increased dose dependently in WS+S groups as compared to sham group. No significant change was observed between the WS pre-treated sham groups and S group.

### Table 1. Administration of WS has significantly attenuated the activity of these enzymes in WS100+L, WS200+L, WS300+L group animals as compared to L group animals. Value are expressed as Mean ± S.E.M. *p < 0.05, L vs. S group; #p < 0.05, ##p < 0.01 WS100 + L , WS200 + L, WS300 + 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 H2O2 consumed/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>338.92 ± 13.8</td>
<td>356.5 ± 13.85</td>
<td>52.6 ± 3.8</td>
</tr>
<tr>
<td>L</td>
<td>146.6 ± 21.48*</td>
<td>144.5 ± 6.8*</td>
<td>17.2 ± 0.8*</td>
</tr>
<tr>
<td></td>
<td>(-56.74%)</td>
<td>(-59.46%)</td>
<td>(-67.3%)</td>
</tr>
<tr>
<td>WS100+L</td>
<td>195.26 ± 19.59#</td>
<td>185.7 ± 4.8*</td>
<td>21.3 ± .87*</td>
</tr>
<tr>
<td></td>
<td>(33.58%)</td>
<td>(28.5%)</td>
<td>(23.8%)</td>
</tr>
<tr>
<td>WS200+L</td>
<td>214.24 ± 7.56#</td>
<td>201.2 ± 3##</td>
<td>26.1 ± 0.83##</td>
</tr>
<tr>
<td></td>
<td>(46.1%)</td>
<td>(39.2%)</td>
<td>(51.74%)</td>
</tr>
<tr>
<td>WS300+L</td>
<td>259.46 ± 9.09##</td>
<td>212.4 ± 8.4##</td>
<td>33.1 ± 0.53##</td>
</tr>
<tr>
<td></td>
<td>(32.0%)</td>
<td>(46.98%)</td>
<td>(92.4%)</td>
</tr>
<tr>
<td>WS100+S</td>
<td>341.2 ± 7.05</td>
<td>338.268 ± 14.68</td>
<td>52.8 ± 3.72</td>
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<tr>
<td></td>
<td>(-6.72%)</td>
<td>(5.11%)</td>
<td>(-0.38%)</td>
</tr>
<tr>
<td>WS200+S</td>
<td>349.78 ± 14.50</td>
<td>340.7 ± 20.06</td>
<td>53.0 ± 2.72</td>
</tr>
<tr>
<td></td>
<td>(-3.2%)</td>
<td>(4.43%)</td>
<td>(-0.76%)</td>
</tr>
<tr>
<td>WS300+S</td>
<td>356.5 ± 13.85</td>
<td>341.52 ± 8.7</td>
<td>56.4 ± 2.70</td>
</tr>
<tr>
<td></td>
<td>(-5.18)</td>
<td>(4.20%)</td>
<td>(-7.22)</td>
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</table>

Table 2. Effect of WS on the activity of antioxidant enzymes in frontal cortex of ICV-STZ treated rats

The activities of antioxidant enzymes in frontal cortex of ICV-STZ treated rats (GPx, GR, and catalase) were decreased significantly in the L group as compared to the S group animals, and pretreatment with WS has significantly protected the activities of these enzymes significantly and dose dependently (Tables 2).
The values of these enzymes were slightly increased dose dependently in WS+S groups as compared to sham group. No significant change was observed between the WS pre-treated sham groups and S group.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>GPx (nmole NADPH oxidized/min/mg protein)</th>
<th>GR (nmole NADPH oxidized/min/mg protein)</th>
<th>Catalase (nmole of H₂O₂ consumed/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>340.34 ± 11.39</td>
<td>478.23 ± 28.5</td>
<td>38.25 ± 2.7</td>
</tr>
<tr>
<td>L</td>
<td>152.57 ± 5.0* (-55.17%)</td>
<td>282.9 ± 16.2* (-40.8%)</td>
<td>18.57 ± 2.5* (-51.45%)</td>
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<tr>
<td>WS100+L</td>
<td>198.33 ± 11.7# (29.99%)</td>
<td>319.9 ± 13.2# (13.0%)</td>
<td>23.98 ± 2.6# (29.13%)</td>
</tr>
<tr>
<td>WS200+L</td>
<td>216.24 ± 7.1# (41.73%)</td>
<td>352.7 ± 12.1## (24.6%)</td>
<td>26.288 ± 1.1# (41.51%)</td>
</tr>
<tr>
<td>WS300+L</td>
<td>231.13 ± 3.9## (51.49%)</td>
<td>388.05 ± 20.7## (37.16%)</td>
<td>29.84 ± 2.1## (60.06%)</td>
</tr>
<tr>
<td>WS100+S</td>
<td>340.98 ± 4.5 (-0.18%)</td>
<td>479.67 ± 8.1 (-0.30%)</td>
<td>39.62 ± 3.6 (-3.58)</td>
</tr>
<tr>
<td>WS200+S</td>
<td>340.91 ± 4.08 (-0.16%)</td>
<td>478.31 ± 49.93 (-0.016)</td>
<td>38.16 ± 1.7 (-0.23%)</td>
</tr>
<tr>
<td>WS300+S</td>
<td>342.35 ± 5.07 (-0.59%)</td>
<td>486.31 ± 52.0 (-1.6%)</td>
<td>39.94 ± 4.6 (-4.41%)</td>
</tr>
</tbody>
</table>

Table 2. Administration of WS has significantly attenuated the activity of these enzymes in WS100 + L, WS200 + L, WS300 + L group animals as compared to L group animals. Values are expressed as Mean ± S.E.M. *p < 0.05, L vs. S group; #p < 0.05, ##p < 0.01 WS100 + L, WS200 + L, WS300 + L vs. L group.

**Effect of WS 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 *W. somnifera* has protected significantly the activity of these enzymes dose dependently in the hippocampus (Fig.7).
Effect of WS pre-treatment on the activity of AChE in the hippocampus of ICV-STZ infused rats. Values are expressed as mean ± S.E.M. AChE activity was significantly increased in L group as compared to S group (*p < 0.05 L vs. S group). WS pre-treatment significantly ameliorated the activity of AChE in WS100 + L, WS200 + L, WS300 + L group animals as compared with L group animals (#p < 0.05 L Vs. WS100 + L, ##p < 0.01 L vs WS200 + L and WS300 + L).

Effect of WS on histochemical changes in hippocampus

Normal neuronal morphology with distinct cytoplasm and prominent nucleoli were observed in the sham (S) group animals. In lesion (L) group, a photomicrograph shows vacuolation as the degenerative changes in the CA1 region of the hippocampus. The black arrow shows the degenerative changes in the neurons in the hippocampus of the CA1 region. WS pretreatment ameliorated the hippocampal neuronal abnormalities in the WS300 + L group animals as compared to L group animals (Fig. 8A).

Effect of WS on ChAT expression

To show the neuroprotective effect of WS the expression ChAT was assessed by immuno histochemistry. In lesion group animal (L) expression of ChAT immuno positive cells in CA1 region of hippocampus decreased as compared to the sham (S) group animals. Pretreatment with WS restore the ChAT expression in W300 + L group animals as compare to L group animal (Fig. 8 B).
Fig. 8. (A) Representative photomicrograph showing H & E staining in the CA1 region of the hippocampus. Black arrows indicate the normal pyramidal neuron in S group (A) and white arrows indicate the degenerated pyramidal neuron in L group (B) while L group pretreated with WS extract (300 mg/kg) has shown normal pyramidal neuronal staining(C). × 40 magnification. (B) The immunohistochemical expression of ChAT in the CA1 region of the hippocampus. Prominent ChAT expression was observed in the S group animals (a); however, the L group animals showed a lower expression of ChAT (b) compared to the S group. Considerably more ChAT expressions were found in the WS pretreated group animals (C) as compared to the L group animals (×40 magnification).

Discussion

The present study examined the pre-treatment effect of standardised W. somnifera on cognitive deficits, oxidative stress, and histopathological changes in ICV-STZ induced model of memory impairment in rats. It is well documented that ICV-STZ rodent model is an appropriate animal model used for the study of cognitive impairment (Hoyer 1991; Lannert and Hoyer 1998; Agrawal et al., 2009; Ishrat et al., 2006; Javed et al., 2011). In our study, WS pre-treatment significantly alleviated the cognitive deficits, biochemical and histopathological alterations in ICV-STZ infused rats. Neuroprotective potential of WS suggests that it is a powerful antioxidant, corroborating previous reports (Ahmad et al., 2005; Konar et al., 2011; Sehgal et al., 2012). Our result showed a moderate amount of Withaferine A (0.0232% w/w) is found to be present in the extract. Withaferine A is reported to have potential
antioxidant property in different type of oxidative stress associated disease (Grover et al., 2010).

The Morris water maze test was used to evaluate the spatial learning and memory deficit in rats. A decreased escape latency and path length in Morris water maze task in repeated trials demonstrate intact learning and memory function by the pre-treatment with WS. ICV-STZ infused rats has shown a significant elevated escape latency and path length as compared to sham group. While a significantly decreased time and distance travelled to reach the hidden platform was observed dose dependently in WS pretreated group. The data has shown the conformity of memory impairment indicating the beneficial effect of WS in ameliorating the cognitive deficits induced by STZ. Our findings are in agreement with other finding where WS attenuated the behavioural deficits in animal model of cognitive impairment (Naidu et al., 2006; Soman et al., 2012).

Oxidative stress refers to the cytological consequences of imbalance between the production of free radicals and the ability of the cell to defend against them. This imbalance results in a buildup of oxidatively modified molecules that can cause cellular dysfunction and neuronal death. Under normal conditions, an array of endogenous cellular defense system exists to counter balance reactive oxygen species (ROS) (Halliwell and Gutteridge, 1985; Liu et al., 2001). The antioxidant system requires reduced glutathione (GSH), a tripeptide and an essential antioxidant, which is responsible to buffer the free radicals in the brain tissue (Meister, 1988). It eliminates H₂O₂ and organic peroxides by glutathione peroxidase and catalase (Sun, 1990). During free radical clearance, oxy radicals are reduced by glutathione peroxidase at the cost of reduced glutathione to form glutathione disulphide (GSSG). GSH is further produced by redox recycling, in which GSSG is reduced to GSH by glutathione reductase with an expenditure of one NADPH molecule. Reduced level of GSH impairs H₂O₂ clearance and endorse the formation of •OH radical, the most toxic molecule to the brain, leading to more free radical level and oxidative stress (Freeman and Crapo, 1982; Meister, 1988; Sun, 1990). Lipid peroxidation indicates neuronal membrane degeneration and reported early AD brain (Blokland and Jolles, 1994). There are several reports about modulatory effect of WS on lipid peroxidation and antioxidant enzymes (Mishra, 2000; Ahmad et al., 2005; Koner et al., 2011). In agreement with these findings, we also found that WS significantly reduced the
TBARS content and increased the activities of antioxidant enzymes along with GSH level in hippocampus and in frontal cortex following STZ infusion. The response of WS could be attributed to its potential antioxidant effect suggesting that STZ induced learning and memory impairment is associated with oxidative stress.

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).

The histological examination provides for evaluating neural damage and the treatment effect. In the present study, we observed marked morphological changes in the hippocampus of ICV-STZ-induced rats. It is interesting that degree of suppression of histopathologic lesions in the brain by WS roughly parallels the degree of suppression of behavioral and biochemical parameters by WS and this finding corroborate the efficacy of WS in this model. Moreover, the effects of WS on ICV-STZ induced rats are almost similar to those of curcumin, naringenin and s-allyl cystein (Ishrat et al., 2009; Javed et al., 2011; Khan et al., 2012). Thus, these results indicate that WS could be an effective approach for attenuating the STZ-induced neurotoxicity via modulation of oxidative damage.

Beside oxidative stress, there is decreased activity of glycolytic enzymes in the STZ model of memory deficit results the reduction in acetylcholine level (Racchi et al., 2004; Ishrat et al., 2006) which is intricately associated with cognition. It is mainly found at neuromuscular junctions and cholinergic neurons, where its activity serves to terminate synaptic transmission. Acetylcholine required for proper function of cholinergic transmission to regulate the learning and memory deficit. Synthesis of AChE depends on the presence of acetyl-CoA (formed by breakdown of glucose during glycolysis). It is degraded by the inhibitors of AChE which are the most effective pharmacological approach for the symptomatic treatment of AD. The enhancement of cholinergic transmission by inhibition of AChE is the basis of
symptomatic treatment of dementia. In the present study, the decrease AChE activity in hippocampus leads to increase cholinergic transmission to facilitate the learning and memory deficit as supported by the other findings (Sonkusare et al., 2005; Ishrat et al., 2006). STZ treatment has shown significantly increased AChE activity in hippocampus which is in consistent with earlier studies (Racchi et al., 2004; Sonkusare et al., 2005; Ishrat et al., 2006). Moreover, ChAT plays critical role by recycling of acetylcholine neurotransmitter which is responsible for the memory and cognition. STZ treatment has shown significantly increased AChE activity and decreased ChAT expression in hippocampus which is in consistent with earlier studies (Racchi et al., 2004; Ishrat et al., 2006; Jin et al., 2009). WS pretreatment decreased AChE activity and increased ChAT expression by ameliorating oxidative loads in hippocampus of STZ treated rats.

There are few limitations of this study. First, we have studied only pretreatment effect of WS on STZ-induced cognitive impairment and neurodegeneration. Second, our study is acute that includes 21 days after ICV-STZ injection to the brain which is much smaller than the reported time (almost 6-9 months) to develop pathological hallmark of Alzheimer’s disease like amyloid beta production and hyperphosphorylation of Tau. Further investigation into the therapeutic effect of WS on taupathy, beta-amyloid deposits and cytoskeletal abnormalities is needed at different time points after ICV-STZ administration.

Our present investigations indicate that ICV-STZ cause behavioral deficits and oxidative stress due to free radical generation and downregulation of the antioxidant defense systems. WS offered significant neuroprotection in ICV-STZ infused rats, which may attribute the improvement of behavioral deficit, inhibition of oxidative stress, apoptosis response and upregulation of endogenous antioxidant status. Most of the neuroprotective effect afforded by the extract can be attributed to its main component withaferine-A, present in the moderate quantity. The findings add to a growing body of research demonstrating the power of phytochemicals as broad spectrum neuroprotective agents. Further investigation into the role and mechanisms of antioxidant action of WS is needed to determine whether it can be an effective remedy for neurodegenerative disease including AD.