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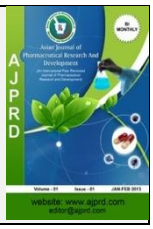

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Research Article

# Neuroprotective Effect of *Withania Somnifera* Root Extract In Type 2 Diabetes Mellitus Induced Cognitive Impairment And Neurodegeneration In Rats

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## A B S T R A C T

The present study was conducted to assess the effects of *Withania somnifera* root extract (WS) on cognitive deficit and neurodegeneration induced by type 2 diabetes mellitus in rats. Type 2 diabetes mellitus (T2DM) was induced in rats by feeding them high fat diet (HFD) for 8 weeks followed by intraperitoneal administration of low dose Streptozotocin (STZ; 35 mg/kg). After four weeks of STZ administration the levels of metabolic parameters (fasting blood glucose, serum triglycerides and serum total cholesterol) were measured to confirm the development of diabetes. WS and Pioglitazone (PZ) were administered daily for 8 weeks to the diabetic rats. At the end of the treatment, behavioural analysis was done to observe the effects of diabetes on memory by Morris water maze (MWM) test. Thereafter, animals were sacrificed; blood and brain samples were collected for biochemical analysis. Metabolic parameters (fasting blood glucose, serum triglycerides and total cholesterol) were found to be significantly elevated in diabetic rats as compared to normal control animals; treatment with WS and PZ for 8 weeks significantly lowered these levels. In MWM test, mean escape latency time (ELT) on day 4 was found to be increased and time spent in the target quadrant (TSTQ) on day 5 was reduced significantly in DC rats as compared to NC. However, TSTQ increased significantly and mean ELT was found to be decreased in the rats treated with WS at the dose of 100 and 300 mg/kg for 8 weeks as compared to DC rats. The level of Aβ1-42, a main component of amyloid plaques, was found to be increased significantly in hippocampus and prefrontal cortex of DC rats as compared to NC and treatment with WS lowered the levels of Aβ1-42. Further, the level of brain-derived neurotrophic factor (BDNF) was found to be reduced significantly in diabetic rats whereas WS treated groups showed a significant increase in BDNF. The data suggests that treatment with standardized root extract of *Withania somnifera* normalized the elevated metabolic parameters in diabetic animals when compared with untreated diabetic animals and the results were comparable with pioglitazone. Also it was found that T2DM adversely affects memory and cause neurodegeneration. WS treatment showed neuroprotective effects and improved memory retention in diabetic animals. Thus root extract of *Withania Somnifera* may have neuroprotective effects for the management of T2DM induced cognitive deficit.

**Keywords:** Metabolic syndrome, Type 2 diabetes, Cognition, Neurobehavioral studies, High-fat diet, Neurodegeneration, *Withania somnifera.*

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## INTRODUCTION

Type 2 diabetes mellitus (T2DM) or non-insulin dependent diabetes mellitus (NIDDM) is the most common and fastest growing metabolic disorder of the world that is characterized by chronic hyperglycemia i.e., abnormally high concentration of glucose in blood and glucose intolerance. International Diabetes Federation (IDF) stated that in 2019, 463 million adults between 20 to 79 years of age were living with diabetes in 2019, and this number may rise up to 578 million by 2030 and 700 million by 2045[1]. T2DM is much more prevalent form of diabetes which is characterized by increase in insulin secretion as a result of compensatory mechanism to insulin resistance (IR) and finally leads to β-cells dysfunction. T2DM is an extremely common disease nowadays and is generally associated with obesity. Chronic hyperglycemia and dyslipidemia are the major hallmarks of the disease[2]. Consumption of foods rich in saturated fats and sugar accompanied by physical inactivity causes insulin resistance and obesity leading to a variety of metabolic disorders like T2DM. Rather than type 1, most of the diabetic cases are

being diagnosed for T2DM which is due to both insufficient insulin action (Insulin resistance) and impaired insulin secretion by pancreatic β-cells, which results in hyperglycemia[3].

Aging is the risk factor for both T2DM and Alzheimer's disease (AD). Individuals with type 2 diabetes mellitus, obesity and other metabolic syndromes are at high risk to develop AD that may be due to mitochondrial dysfunction[4]. T2DM leads to structural as well as physiological changes in the brain that cause cognitive deficit and the condition is known as diabetic encephalopathy[5]. Persistent high blood glucose levels in diabetics, damage small blood vessels which leads to microvascular complications like diabetic neuropathy, nephropathy and retinopathy whereas damage to large blood vessels leads to macrovascular complications like cerebrovascular disease, cardiovascular disease and stroke[6]. Over a course of time, due to insulin resistance, high blood glucose level starts affecting brain functions which is responsible mild cognitive impairment (MCI) in early stage and AD in later stage. Hence, AD has been considered as brain type diabetes[7].

Amyloid β (Aβ) peptides have 39-43 residues of amino acids and are present in the brain in diffused state. Aβ1-40 and Aβ1-42 are the two most common forms of amyloid β-peptides and between these two; Aβ1-42 is more amyloidogenic which tends to aggregate rapidly than Aβ1-40 and most importantly, it is hydrophobic in nature. The formation of Aβ aggregates and their extracellular deposition activates astrocytes and microglia that subsequently promotes neuroinflammation which plays a major role in AD pathogenesis[8]. Extracellular deposition of β-amyloid fibrils and their compaction into senile plaques is the major hallmark of AD. Amyloid plaques in the brain are mainly composed of Aβ peptides that are derived from a larger protein known as amyloid precursor protein (APP). Aβ peptides are formed by an enzymatic reaction in which beta-site amyloid precursor protein-cleaving enzyme 1 (BACE-1), β-secretase and γ-secretase proteolyse APP. The production of Aβ peptides is increased in AD whereas the clearance rate is slowed down. This causes the deposition of small oligomers or fibrils of Aβ which further contribute to synaptotoxicity and neurodegeneration and hence in progression of AD. The process of formation and deposition of amyloid beta peptides in different body tissues is common in both T2DM and AD[9]. Moreover, type 2 diabetes mellitus (T2DM) is directly correlated with the development of symptoms of AD; therefore AD is also considered as a type 3 diabetes. Insulin resistance (IR) which is a major hallmark of T2DM is linked with the excessive production and deposition of Aβ peptides in the brain[10].

Insulin is an anabolic peptide hormone secreted by pancreatic β-cells that plays a key role in glucose, fat and protein metabolism in the human body. According to recent studies, insulin along with other growth factors like brain-derived neurotrophic factor (BDNF) transmits the signals to regulate cognitive functions mediated by hippocampus. BDNF is an important neurotrophin that is produced in periphery as well as in brain tissues and in CNS it acts prominently via TrkB receptors present on neurons[11]. Although CNS is the major site of action for BDNF, it also plays important role in controlling glucose metabolism, energy homeostasis and lipid levels and hence it is also known as "metabokine". It has been reported that variation in the levels of BDNF in serum and CNS is associated with T2DM, atherosclerosis, obesity, Alzheimer's diasease (AD), depression and Parkinson's disease[12]. Various animal studies have revealed beneficial effects of BDNF in metabolic profile as evidenced by increased insulin resistance (IR) in response to reduced levels of BDNF and vice versa. Also, calorie restrictions in the diet reduce blood glucose levels by stimulating brain cells to produce BDNF[13].

Currently, there is no drug therapy that provides definite solution for prevention or management of cognitive deficit and neurodegeneration induced by T2DM. However, IR is the major therapeutic target for the development of new drugs for T2DM. In traditional system of medicine, the roots of *Withania somnifera* (WS) are categorised as rasayanas which literally means the path of juice (Rasa: Juice, Ayana: Path). They are phytochemicals that are reputed to promote health and longevity by augmenting defence against disease, arresting the aging process, revitalising the body in debilitated conditions, increasing the capability of the individual to resist adverse environmental factors and creating a sense of mental well-being[14]. In traditional Indian system of medicine (Ayurveda), WS is sub-categorized as Medhya rasayana which means nootropic herb or memory enhancing drug. Several pre-clinical and clinical studies have reported the role of WS as nootropic agent. Leaf and root extracts and compounds isolated from WS have been proved to be efficacious in the experimental model of Alzheimer's disease and Parkinson's disease[15]. Hence, the present study was designed to investigate the neuroprotective effect of aqueous root extract of *Withania Somnifera* extract on experimental model of type 2 diabetes mellitus induced cognitive deficit and possible mechanisms mediating these effects.

## MATERIAL AND METHODS:

### Subjects:

Sprague Dawley (SD) rats (200-250 g) were used for the study and each experimental group was comprised of 5 animals. The rats were housed under standard laboratory conditions (22 ± 2 $^{o}$C, 12 hour light/dark cycle – lights on at 0800 hours). Care of animals has been taken as per guidelines of CPCSEA for use of animals in Scientific Research with approval of Institutional Animals Ethics Committee (IAEC).

### High fat diet- streptozotocin (HFD-STZ) Alzheimer Model

After one week of acclimatization period, rats were divided into two groups. Controls: rats (n=10) were given normal pellet diet for 8 weeks and Diabetic controls (DC): rats (n=20) were fed with in-house prepared high fat diet (HFD) (60% calories as fat) the diet composition includes powdered (Normal Pellet Diet) NPD (425g/kg), lard (310 g/kg), casein (250g/kg), cholesterol (10 g/kg), DL-

methionine (3g/kg), yeast powder (1g/kg) and sodium chloride (1g/kg) for a period of 8 weeks. After 8 weeks of dietary manipulation, rats were injected intraperitoneally (i.p) with a low dose of streptozotocin (STZ) 35 mg/kg dissolved in 0.1 M citrate buffer pH 4.5, while the respective control rats were treated with vehicle citrate buffer in a dose volume of 1 ml/kg, i.p. [16, 17]. The STZ induced hypoglycemic mortality was avoided by feeding the DC rats with 20% glucose solution for 24 hrs[18].

After 4 weeks of STZ administration, the fasting blood glucose (FBG) level was determined from the tail vein by glucometer. Rats with FBG level ≥200 mg/dL were considered as diabetic and selected for further pharmacological screening. Blood (1.0 ml) was collected using capillary from retroorbital plexus of the rats under mild anesthesia. The serum was separated by centrifugation and the levels of triglycerides (TG) and total cholesterol (TC) were measured by semi-automatic analyzer. After biochemical analysis, control animals were divided into following two groups consisted of 5 animals per group (n=5):

**Normal Control (NC):** Animals were treated with vehicle orally for 8 weeks of treatment period.

**NC animals treated with *Withania somnifera* (NC+WS):** NC animals were treated with standardized aqueous root extract of *Withania Somnifera* 300 mg/kg/day orally for 8 weeks.

Diabetic animals (n=20) were segregated into four different groups containing 5 animals each (n=5), 8 weeks treatment was given as follows:

**Diabetic control (DC):** Diabetic animals were kept on high-fat diet and administered orally with Na-CMC suspension for 8 weeks.

**DC+WS-100:** Diabetic animals were kept on high fat diet and given extract of *Withania somnifera* 100 mg/kg/day, orally for 8 weeks.

**DC+WS-300:** Diabetic animals were kept on high fat diet and given extract of *Withania somnifera* 300 mg/kg/day, orally, for 8 weeks.

**DC+PZ-20:** Diabetic animals were kept on high fat diet and treated with standard antidiabetic drug Pioglitazone 20 mg/kg/day, orally for 8 weeks.

### Morris water maze (MWM) test:

After 8 weeks of treatment animals were subjected to the MWM test to analyze cognitive function. In brief, a circular tank (160 cm diameter and 50 cm height) was filled with warm water (22-24 °C) to a height of 30 cm. The pool area was divided into four quadrants (NE, SE, SW and NW) by four points (E, S, W and N), which were equally spaced along the circumference of the pool. A circular escape platform (12 cm diameter and 28 cm height) was fixed in the middle of quadrant NE, 2 cm below the surface of water for the non-visible trial. Each animal was subjected to four consecutive trials per day (one from each starting point) for the four consecutive days to locate the hidden platform in the target quadrant. In the non-visible platform trials, if the animal was unable to reach the platform in the given time interval (90 s), it was guided gently to the platform and allowed to remain there for 30 seconds. The time taken by the animal to reach the platform (escape latency time, ELT) was recorded. A probe trial was performed on the 5th day by removing the platform and the time spent in the target quadrant (TSTQ) was recorded. The average ELT and the mean TSTQ was recorded as an index learning and retention of memory respectively[19].

### Tissue preparation

After the completion of neurobehavioral studies, fasting blood glucose (FBG) was estimated by tail vein method using glucometer. Then rats were sacrificed with an overdose of ketamine and blood samples were taken by cardiac puncture and serum was separated. Then, the animals were perfused intracardially with 0.9% saline solution. Brains of the rats were taken out quickly on ice to dissect the hippocampus and prefrontal cortex. The dissected brain parts were homogenized at 4$^{o}$C in 0.1 M phosphate buffer pH 7.4. The homogenates were centrifuged at 10500×g for 30 minutes at 4$^{o}$C and supernatants were stored at -80°C for further estimations of Aβ1-42 and BDNF by ELISA.

Serum TG and TC were estimated by semi-automatic analyzer using commercial kits according to the manufacturer's protocol.

### Statistical Analysis

The data is expressed as Mean ± SEM. Comparisons among different groups is analyzed by one way ANOVA followed by Tuckey's post hoc analysis. Non-parametric statistical tests (Mann-Whitney U test) is used for analyzing data wherever appropriate. A 'P' value of less than 0.05 is considered as the level of significance for all statistical tests.

## RESULTS

### Effect of HFD-STZ and *Withania Somnifera* (WS) on fasting blood glucose (FBG) levels:

After 12 weeks, i.e. after four weeks of STZ administration as well as at 20 weeks, i.e. after 8 weeks of vehicle treatment, FBG levels were found to be significantly high in DC rats when compared with NC animals as shown in Table 1 and 2. Interestingly, in DC+WS-100, DC+WS-300 and DC+PZ-20 animals, a significant reduction in FBG levels was found as compared to DC rats after 8 weeks of treatment with respective drugs as summarized in Table 2.

### Effect of HFD-STZ and *Withania Somnifera* (WS) on serum triglycerides (TG) level:

At the end of 12th week of the study, diabetic control animals showed a significant increase in serum triglycerides level as compared to normal control group as described in Table 1. After 8 weeks of treatment (vehicle), animals of DC group continued to have a significant increased TG levels as compared to NC group. Animals treated with *W somnifera* (100 and 300 mg/kg) showed a significant decrease in the serum TG level when compared with the DC group and the results were comparable with pioglitazone. The results are described in Table 2.

### Effect of HFD-STZ and *Withania Somnifera* (WS) on serum total cholesterol (TC) level:

After 4 weeks of STZ administration (i.e. after 12 weeks of study) as well as at 20 weeks of study, DC animals showed a significant increase in serum TC level as compared to NC group as described in Table 1 and 2. Animals of test (*W somnifera* 100 and 300 mg/kg) and standard (Pioglitazone 20 mg/kg) groups showed a significant decrease in the TC levels after the treatment when compared with the DC group. The results are depicted in Table 2.

### Effect of HFD-STZ and *Withania Somnifera* (WS) on body weight of animals:

Animals of control group were found to gain weight in first 12 weeks period. The body weight of diabetic control animals was decreased slightly but not significantly as compared to normal control animals as shown in Table 1. After 20 weeks, i.e. after 8 weeks of treatment with respective drugs, it was found that the body weight of diabetic control animals was slightly reduced as compared to control group ($p>0.05$). Also, there were no significant changes in the body weight of animals treated with WS-100, WS-300 and PZ-20 when compared with diabetic control animals. The results are summarized in Table 2.

**Table 1:** Effect of HFD-STZ treatment on metabolic parameters after four weeks of STZ administration:

| Group | Parameters (After 12 weeks) | | | |
|---|---|---|---|---|
| | Body weight (gm) | FBG (mg/dl) | TG (mg/dl) | TC (mg/dl) |
| NC | 262.0±2.72 | 92.21±2.25 | 86.14±2.76 | 73.23±3.24 |
| DC | 244.9±2.20 | 228.20±3.26*** | 129.6±3.13** | 104.30±4.12** |

All data are expressed as Mean ± SEM; NC: Normal control animals, DC: Diabetic control (HFD-STZ treated) animals. ***P<0.001, **P<0.01 Compared to NC group.

**Table 2:** Effect of *Withania somnifera* (WS) treatment on metabolic parameters in HFD-STZ induced diabetic rats after 8 weeks of treatment:

| **Group** | **Parameters (At the end of 20 weeks)** | | | |
|---|---|---|---|---|
| | **Body weight (gm)** | **FBG (mg/dl)** | **Serum TG (mg/dl)** | **Serum TC (mg/dl)** |
| NC | 263.80±3.37 | 98.30±5.92 | 96.24±2.77 | 73.54±3.66 |
| NC+WS 300 | 265.20±3.39 | 103.60±6.80 | 88.94±3.91 | 70.17±2.57 |
| DC | 216.50±4.51* | 256.0±4.92*** | 168.60±4.44** | 113.50±2.71*** |
| DC+WS-100 | 233.80±4.97 | 206.40±6.01$^{\#}$ | 156.20±2.29 | 99.18±2.59$^{\#}$ |
| DC+WS-300 | 240.8±4.77 | 164.30±6.95$^{\#\#}$ | 140.0±3.23$^{\#}$ | 88.49±3.42$^{\#\#}$ |
| DC+PZ-20 | 253.3±2.92 | 135.70±3.53$^{\#\#\#}$ | 147.30±2.24$^{\#}$ | 97.12±2.23$^{\#}$ |

All data are expressed as Mean ± SEM; NC: Normal control animals, NC+WS-300: Normal control animals treated with WS 300 mg/kg,p.o. DC: Diabetic control (HFD-STZ treated) animals, DC+WS-100 and-300 : Diabetic animals treated with WS 100 mg/kg or WS 300 mg/kg respectively. DC+PZ-20: Diabetic animals treated with Pioglitazone 20 mg/kg, p.o, ***P<0.001, **P<0.01 Compared to NC group; #P<0.05, ##P<0.01, ###P<0.001 as compared to diabetic control group.

### Effects of *Withania Somnifera* (WS) on spatial learning in HFD-STZ induced diabetic rats:

In all the rats, mean time to find the hidden platform, i.e. mean Escape latency time (ELT) on day 4 (Fig. 1) and Time spent in target quadrant (TSTQ) on day 5 of Morris water maze (MWM) test were measured. Time to reach the submerged platform, i.e. ELT, decreased ($p<0.01$) in DC rats on day 4 of training in water maze as compared to NC rats (Fig. 1). After 8 weeks of treatment with WS, the mean ELT on day 4 was increased significantly ($p<0.05$, for each group) in DC+WS-100 and DC+WS-300 groups. In the probe trial, i.e. on day 5, the time spent in target quadrant (TSTQ) was decreased significantly ($p<0.01$) in diabetic group as compared to NC group. Administration of *Withania somnifera* at the dose of 100 and 300 mg/kg showed significant ($p<0.5$, $p<0.01$ respectively) increase in TSTQ as compared to the DC group. Pioglitazone treated group also showed a significant ($p<0.5$) increase in TSTQ as compared to the diabetic control group. Results are shown in Fig. 1 and 2.

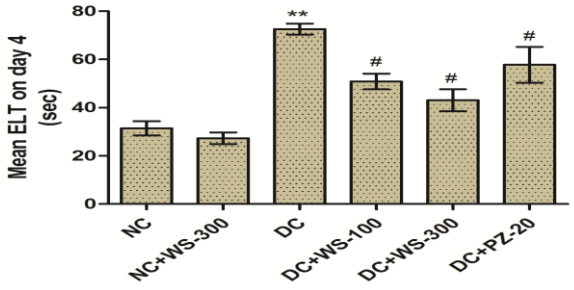


**Fig 1:** Effects of *Withania somnifera* (WS) on mean Escape latency time (ELT) on day 4 during Morris water maze (MWM) test, in HFD-STZ induced diabetic rats.

All data are expressed as Mean ± SEM; NC: Normal control, NC+WS-300: Normal control animals treated with *Withania somnifera* 300 mg/kg,p.o. DC: Diabetic control, DC+WS-100: Diabetic animals treated with *Withania somnifera* 100 mg/kg, p.o. DC+WS-300: Diabetic animals treated with *Withania somnifera* 300 mg/kg, p.o. DC+PZ-20: Diabetic animals treated with Pioglitazone 20 mg/kg, p.o.,

**P<0.01 Compared to NC group; #P<0.05 as compared to DC group.

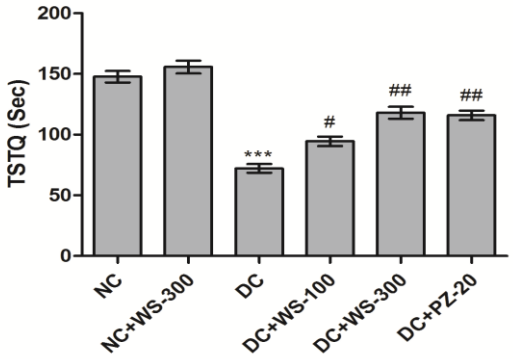


**Fig 2:** Effect of *Withania somnifera* (WS) on time spent in target quadrant (TSTQ) during probe trial in Morris water maze test, in HFD-STZ induced diabetic rats.

All data are expressed as Mean ± SEM; NC: Normal control, NC+WS-300: Normal control animals treated with *Withania somnifera* 300 mg/kg,p.o. DC: Diabetic control, DC+WS-100: Diabetic animals treated with *Withania somnifera* 100 mg/kg, p.o. DC+WS-300: Diabetic animals treated with *Withania somnifera* 300 mg/kg, p.o. DC+PZ-20: Diabetic animals treated with Pioglitazone 20 mg/kg, p.o., ***P<0.001 Compared to NC group; ##P<0.01, #P<0.05 as compared to DC group.

## Effects of *Withania Somnifera* (WS) on Aβ1-42 levels in HFD-STZ induced diabetic rats:

After 8 weeks of WS treatment period, i.e. at the end of 20 weeks of study, the levels Aβ1-42 were estimated in prefrontal cortex and hippocampus of rats. It was found that the animals of DC group have significantly increased levels of Aβ1-42 when compared with NC group. The level of Aβ1-42 was found to be reduced significantly in prefrontal cortex and hippocampus of DC+WS-300 rats when compared with the DC group. However no significant change was found in both of the tissues in DC+WS-100 group. The results obtained were compared with anti-diabetic drug pioglitazone. The results are depicted in Table 4.

**Table 3:** Effects of *Withania somnifera* (WS) treatment on Aβ1-42 levels in prefrontal cortex and hippocampus of HFD-STZ induced diabetic rats

| Group | Aβ 1-42 (pg/ml) | |
|---|---|---|
| | Prefrontal cortex | Hippocampus |
| NC | 2.96±0.33 | 3.31±0.29 |
| NC+WS-300 | 3.28±0.26 | 3.18±0.30 |
| DC | 5.95±0.90* | 6.66±0.50** |
| DC+WS-100 | 5.01±0.31 | 6.07±0.32 |
| DC+WS-300 | 4.10±0.36 | 4.77±0.29# |
| DC+PZ-20 | 3.94±0.46 | 4.20±0.47# |

All data are expressed as Mean ± SEM; NC: Normal control, NC+WS-300: Normal control animals treated with *Withania somnifera* 300 mg/kg,p.o. DC: Diabetic control, DC+WS-100: Diabetic animals treated with *Withania somnifera* 100 mg/kg, p.o. DC+WS-300: Diabetic animals treated with *Withania somnifera* 300 mg/kg, p.o. DC+PZ-20: Diabetic animals treated with Pioglitazone 20 mg/kg, p.o., **P<0.01, *P<0.05 compared to NC group; #P<0.05 as compared to DC group.

**Table 4:** Effects of *Withania somnifera* (WS) treatment on brain-derived neurotrophic factor (BDNF) levels in prefrontal cortex and hippocampus of HFD-STZ induced diabetic rats.

| Group | BDNF (pg/ml) | |
|---|---|---|
| | Prefrontal cortex | Hippocampus |
| NC | 32.27±1.31 | 34.02±1.37 |
| NC+WS-300 | 33.79±1.69 | 35.13±1.02 |
| DC | 19.44±1.22** | 21.01±1.29** |
| DC+WS-100 | 21.58±1.02 | 23.87±1.10 |
| DC+WS-300 | 26.82±1.27# | 27.13±1.05# |
| DC+PZ-20 | 29.25±1.20## | 29.29±1.23# |

All data are expressed as Mean ± SEM; NC: Normal control, NC+WS-300: Normal control animals treated with *Withania somnifera* 300 mg/kg,p.o. DC: Diabetic control, DC+WS-100: Diabetic animals treated with *Withania somnifera* 100 mg/kg, p.o. DC+WS-300: Diabetic animals treated with *Withania*

*somnifera* 300 mg/kg, p.o. DC+PZ-20: Diabetic animals treated with Pioglitazone 20 mg/kg, p.o., **P<0.01, *P<0.05 compared to NC group; #P<0.05 as compared to DC group.

## DISCUSSION

There are several studies to indicate that type 2 diabetes mellitus (T2DM) plays an important role in the pathogenesis of AD. Epidemiological data have revealed that there is a high prevalence of AD in diabetic patients which results from metabolic abnormalities and insulin resistance (IR). High-fat diet and low dose of STZ (35mg/kg, ip) (HFD-STZ) is a well established model to induce T2DM that mimics the natural progression and represents the metabolic state of diabetes in SD rats[16, 20]. Hence, in this study, diabetes was induced by feeding rats with high-fat diet for 8 weeks followed by low dose of STZ to cause a mild destruction of pancreatic β-cells. It was found that after treatment with HFD-STZ, hyperglycemia and hyperlipidemia was developed in the rats which are important characteristic of T2DM. This finding is corroborated with the earlier studies where HFD-STZ treated rats showed hyperglycemia, hypertriglyceridemia and hypercholesterolemia[2, 16]. In our study, the development of insulin resistance in diabetes might be responsible for hyperglycemia observed in rats. It has been reported earlier that high-fat diet fed animals are more prone to develop hyperlipidemia after STZ administration as compared to the rats on normal diet. HFD-STZ treated (diabetic) rats have shown increased levels of triglycerides which may be due to increased absorption and formation of chylomicrons[16] or may be due to increased secretion of triglycerides from liver cells[2]. In a similar manner, hypercholesterolemia was observed in HFD-STZ treated animals and that may be due to increased absorption of dietary cholesterol from small intestine after the intake of HFD[16]. In this study, treatment with standardized aqueous root extract of *Withania somnifera* (WS) significantly reduced FBG level which may be due to reduced insulin resistance after treatment. WS also improved the lipid profile of diabetic animals by decreasing the levels of serum triglycerides (TG) and total cholesterol (TC) as compared to untreated diabetic animals.

In the present study we evaluated the effects of WS on cognitive dysfunction in HFD-STZ induced diabetic rats. Cognitive deficits were assessed by evaluating escape latency time (ELT) and time spent in target quadrant (TSTQ) in Morris water maze (MWM) test. ELT and TSTQ are the parameters to test learning (acquisition) and retention in the animals. In MWM test, DC rats showed maximum ELT and minimum TSTQ when compared with normal control animals. These observations suggested that HFD-STZ treated diabetic animals had impaired acquisition (learning) and retention. This finding is supported by previous study conducted by Yang et al, which showed that feeding the SD rats with high-fat diet followed by administration of streptozotocin (STZ) resulted in depressive behavior and deficit in cognition[21]. Also, it has been reported that impaired insulin signalling in diabetes is a main factor that connects Alzheimer's disease (AD) with diabetes[22]. Hippocampus and prefrontal cortex are the memory centres of the brain and are the main regions that control cognitive functions and emotional behaviour as well. In brain, insulin receptors are mainly concentrated in hippocampus, frontal cortex and hypothalamus and play a key role in the regulation of glucose uptake, learning and memory, food intake, weight control etc[23]. Studies have also reported that aberrant insulin signaling in hippocampus results in the development of cognitive dysfunction in diabetes and PI3K/Akt pathway also has an important role in the regulation of emotional behavior[24]. The cognitive deficit caused by diabetes in this study may be due to impairment in insulin signalling in hippocampal tissue.

Treatment with WS showed ameliorative effect on HFD-STZ induced cognitive deficit as evidenced by significant reduction of ELT during acquisition trial and increase of TSTQ in probe trial. The role of WS as cognition enhancer has been supported by earlier study by Bhattacharya et al.[25] who reported that administration of WS reversed the cognitive dysfunction in the animals with NBM lesion by increasing the cholinergic functions in the neurons of hippocampus and frontal cortex. Withanolide A has been shown to reverse the memory loss and prevent neurodegeneration in hippocampus by increasing the biosynthesis of glutathione in hippocampal neurons of SD rats[26]. Thus, it may be concluded that WS enhanced the cognitive functions in HFD-STZ induced diabetic rats which may be due to the effect of WS on hippocampal insulin signalling or cholinergic function.

In the present study, diabetic animals (HFD-STZ treated) showed high levels of Aβ1-42 in both prefrontal cortex and hippocampus. Treatment with WS for 8 weeks, significantly reduced Aβ levels in both areas of the brain. This showed that diabetes may increase the formation and deposition of Aβ1-42 in brain. The finding is supported by previous studies in which it has been reported that insulin resistance in T2DM increases the amyloid deposition in brain. A study by Ho et al.[27] showed that diet induced insulin resistance increased the deposition of amyloid beta peptide due to increased activity of γ-secretase and reduced activity of insulin degrading enzyme (IDE) in rats. It has been reported earlier that Aβ peptides in brain have a high ability to form oligomeric aggregates which disrupt the synaptic plasticity and interneuronal communications that eventually leads neurodegeneration. It has been reported earlier that the presence of Aβ peptides in the brain produces hindrance in binding of insulin and IGF-1 to their receptors and this in turn results in insulin resistance and secretion of pro-inflammatory cytokines[28].

Brain-derived neurotrophic factor (BDNF) is an important neurotrophin that is produced peripherally as well as centrally and acts prominently via TrkB receptors present on neurons. BDNF is mainly responsible for neuroplasticity, neuronal development and stimulation of nerve regeneration. BDNF and its mRNA are mainly found in specific areas of CNS such as hippocampus, limbic system and hypothalamus[11]. In the present study, HFD-STZ treated diabetic rats showed decreased levels of BDNF whereas 8 weeks treatment with WS increased the levels in prefrontal

cortex and hippocampus. This finding demonstrated that T2DM reduced the levels of BDNF in brain and this is corroborated by earlier studies. It has been shown in earlier studies that high-fat and sugar diet reduced the levels of BDNF in hippocampus which negatively affects neuronal plasticity and learning in animals[29] and calorie restrictions in the diet reduced blood glucose levels by stimulating brain cells to produce BDNF[13]. Previous reports suggested that variation in the levels of BDNF in serum and CNS is associated with T2DM, atherosclerosis, obesity, Alzheimer's disease (AD), depression and Parkinson's disease (PD). BDNF is also reported to affect the metabolic profile as evidenced by its positive impact on the effects of insulin resistance (IR) in diabetes[11].

The major finding in the current study is that streptozotocin along with chronic consumption of high-fat diet induced metabolic syndrome which eventually lead to memory impairments and reduced synaptic plasticity in prefrontal cortex and hippocampal neurons. The results obtained in this study showed that WS extract improved the metabolic dysfunction by reducing the levels of cholesterol, triglycerides and fasting blood glucose. WS also significantly improved the memory and spatial learning in diabetic rats with cognitive deficit. This may be due to neuroprotective action of WS treatment, as evidenced by reduced levels of Aβ1-42 and increase in BDNF levels in prefrontal cortex and hippocampus of diabetic rats. Hence, it can be concluded that WS has neuroprotective effects against T2DM induced cognitive impairment and neurodegeneration in rats. The study has translational value as WS may provide a potential lead during the drug development for the management of T2DM associated cognition deficits.

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## Conflict of Interest

Authors have declared that they have no conflicts of interest to disclose.

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