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Ameliorative Effect of Ashwagandha (*Withania somnifera*) Root Extract on Brain Oxidative Stress and Depression of Diabetic Rats

Heba A. Hashem[1*], Zohour I. Nabil[2], Heba N. Gad EL-Hak[3]

Zoology Department, Faculty of Science, Suez Canal University, Ismailia, Egypt.

***Correspondence**
Corresponding author: Heba A. Hashem
E-mail address: hebahashemm@gmail.com

Abstract

Evaluation of the efficacy of ashwagandha root extract to ameliorate the oxidative stress and depression resulting from diabetes in male rats was conducted in the current study. Thirty-six male albino rats were randomly grouped into two main groups: normal (n=18) and diabetic (n=18), diabetes was induced by a single dose of 150 mg/kg BW alloxan injected intraperitoneally. After six weeks, both normal and diabetic groups were further subdivided into six sub-groups; normal control, 100 & 200 mg/kg BW ashwagandha treated normal, diabetic control, 100 and 200 mg/kg BW ashwagandha treated diabetic groups, for another six weeks. The forced swim test was used to assess depression, and serum serotonin levels were measured. In brain tissue homogenates, the glutathione reduced content, superoxide dismutase, and catalase activity were measured, as well as the total antioxidant capacity, total oxidative capacity, and malondialdehyde levels. Moreover, histopathological examination of the brain (cerebral cortex and cerebellum) were conducted. The obtained results revealed that the administration of ashwagandha extract to diabetic rats reduced immobility time during the forced swim test while increasing the serotonin levels significantly when compared with the diabetic group. Similar to this, brain total antioxidant capacity, glutathione reduced content, superoxide dismutase, and catalase activity increased significantly, while brain total oxidative capacity, oxidative stress index, and malondialdehyde levels decreased significantly when compared with the diabetic group. Furthermore, the histopathological changes in brain sections were reversed by ashwagandha root extract. In conclusion, ashwagandha root extract can be used to ameliorate the brain oxidative stress and depression brought on by diabetes mellitus at doses of 100 and 200 mg/kg BW.

KEYWORDS
Ashwagandha, Depression, Oxidative stress, Brain, Diabetes

INTRODUCTION

Diabetes mellitus (DM) is defined by elevated blood glucose levels (hyperglycemia) caused by deficiencies in insulin secretion, action, or both (Ozougwu *et al*., 2013). As hyperglycemia becomes chronic with time, it leads to serious consequences in several tissues, especially those that are insulin-insensitive (retina, neurons, kidneys) (Giri *et al*., 2018). Diabetes-induced neuronal damage is most likely caused by oxidative stress (OS) (Vincent *et al*., 2004). Reactive oxygen species (ROS) play an essential role in signal transduction cascades under physiologically typical circumstances, but when they are present in excess, they become neurotoxic and cause depression and neurodegeneration (Wink *et al*., 2011; Pierzchala *et al*., 2022). The brain is one of many organ systems affected by DM, and it is thought to be more vulnerable to OS because It uses a lot of oxygen, has a lot of polyunsaturated fatty acids, and has a lot of protective enzymes (Singh *et al*., 2019). When there is an increased demand for oxygen, more ROS are produced (Halliwell, 1991). The increased free radicals increase neuronal death in several brain regions, and cause DNA damage, oxidized proteins, and peroxide lipids in membranes (Pop-Busui *et al*., 2006). The effects of DM on brain complications are becoming more commonly recognized (Sima, 2010). Aleem *et al*. (2022) observed that DM brought on by alloxan is known to promote depression. According to Hozayen *et al*. (2012), DM has a higher incidence of nervous manifestation due to insulin-induced brain OS that causes disturbances in brain neurotransmitters. Martínez-Tellez *et al*. (2005) also suggested a connection between DM and changes in the brain's structure and function.

In recent years, the medical system has struggled to manage DM without causing negative side effects. In addition to insulin, a range of oral synthetic hypoglycemic medications with their side effects are available for the treatment of DM (Tran *et al*., 2015). The demand for natural products that are less expensive and have antidiabetic activity with fewer side effects is rising as a result. In India's Ayurvedic medical system, *Withania somnifera*, also known as ashwagandha, is a shrub in the Solanaceae family (Visweswari *et al*., 2013). Numerous studies on this plant have revealed that it has anti-inflammatory, antitumor, antistress, antioxidant, immunomodulatory, and hematopoietic properties in addition to its beneficial effects on the endocrine system, vascular system, and nervous system. Several animal studies have

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demonstrated that Ashwagandha is an anxiolytic, antidepressant, and neuroprotective agent because it improves memory and other brain and nervous system functions (Singh *et al*., 2011; Durg *et al*., 2015). However, there are only a few studies in the literature focusing on how ashwagandha can prevent brain damage caused by DM. Subsequently, the objective of the current study was to test ashwagandha's ability to reduce depression and the OS induced by DM in the brain tissues of diabetic rats.

MATERIALS AND METHODS

Plant material

Ashwagandha root extract available as a commercial dietary supplement capsule (450 mg ashwagandha root extract) was purchased from Now Foods Company, USA. According to the manufacturer's details, each capsule contained min. 2.5% total withanolides 11 mg, rice flour, stearic acid (vegetable source), and hypromellose (cellulose capsule) found by high-performance liquid chromatography (HPLC) as per the information provided by the manufacturer. This product was registered in India as a traditional herbal medicine. The contents of an ashwagandha capsule were dissolved in distilled water and administered orally via an intragastric tube, 100 and 200 mg/kg BW of ashwagandha were the dosages chosen for this study according to Khan *et al*. (2015).

Animals

Thirty-six healthy male albino rats weighing between 140 and 200g were obtained and randomly grouped into two main groups: normal (n=18), without any treatments, and diabetic (n=18), which were induced to be diabetic through intraperitoneal injection of a single dose (150 mg/kg BW) of alloxan (Oxford Lab Fine Chem LLP, Maharashtra, India). After six weeks, the normal group was subdivided into three sub-groups; (1) NC, normal control group without any treatments, (2) ASH100, and (3) ASH200, normal rats belong to the 2nd and 3rd groups received 100 and 200 mg/kg BW ashwagandha respectively, which was dissolved in distilled water and administered orally on daily basis for 6 weeks. The diabetic group was subdivided into three groups, (4) DC, a diabetic control group without any additional treatments, (5) DC + ASH100 and (6) DC + ASH200. Rats belong to the 5th and 6th groups of diabetic rats were treated with 100 and 200 mg/kg BW ashwagandha respectively, which was dissolved in distilled water orally on daily manner for 6 weeks.

Ethical statement

Suez Canal University's research committee gave the go-ahead for all experimental procedures following the international standards for the care and use of laboratory animals code (Rec 68/2020).

Depression testing

One of the most popular assays for examining depressive-like behavior in rodents is the forced swim test (FST). Rats were put in an impenetrable cylindrical container (Figure 1) filled with tap water (25±1 °C). Two sessions of the test were held. Rats were first made to swim for 15 minutes before the test during the pre-test session. The process was repeated 24 hours later for a 6-minute FST session that was recorded on a mobile device. The animal was initially attempted to flee but eventually became immobile, which can be seen as a sign of behavioral despair. The total amount of time spent motionless (measured in seconds) after the first two minutes was over four minutes. The rats were deemed to be immobile when they continued to float with no other movements than those required to keep their noses above the water. Water was changed after the sessions and animals were removed and placed in separate cages to dry before being returned to their home cages (Yankelevitch-Yahav *et al*., 2015).

Blood serum collection and brain sampling

Before the beginning of behavioral tests, a suitable amount of blood was collected into a test tube without any additives using the retro-orbital technique for serotonin analysis. The blood was further centrifuged at 3,000 rpm for 10 minutes. After that, serum was collected and separated with a clean dropper in sterilized tubes, properly labeled, and then frozen at -20°C. At the end of the experiment, rats were weighed, then sacrificed, and the brain was dissected and washed several times in saline (0.9 % NaCl). Half of each brain was isolated and stored at -20°C for homogenization. Homogenization was carried out at 20 % (w/v) in phosphate-buffered saline (0.01 M, pH=7.4). Whole homogenates were used for the estimation of brain total antioxidant capacity (TAC), total oxidative capacity (TOC), malondialdehyde (MDA) level, superoxide dismutase (SOD), catalase (CAT) activity, and glutathione reduced (GSH) content.

Determination of serum serotonin activity

Determination of Serotonin activity assay using Rat serotonin ELISA kit was purchased from My Biosource, Inc., P.O. Box 153308, San Diego, CA 92195-3308, USA using Sánchez *et al*. (2008) method.

Estimation of brain TOC, TAC, MDA, SOD, CAT, and GSH in brain homogenate

The brain TOC assay (Ma *et al*., 2010) and brain TAC assay (Navaie *et al*., 2018), their kits were obtained from Labor Diagnostika Nord GmbH & Co. KG, Nordhorn, Germany; the oxidative stress index (OSI) value was calculated as follows: TOC ÷ TAC (Gunbatar *et al*., 2020); the brain MDA (Kowalczuk and Stryjecka-Zimmer, 2002); brain SOD (Bordet *et al*., 2000); brain CAT (Hamby-Mason *et al*., 1997), their kits were purchased from Cell Biolabs, Inc., 7758 Arjons Drive San Diego; and brain GSH (Terpstra *et al*., 2003) used GSSG/GSH quantification kit that was obtained from Kamiya Biomedical Co., 12779 Gateway Drive, Seattle WA 98168.

Histopathological studies of the brain (Cerebral cortex and cerebellum)

The second half of each brain from rats was fixed in 10% formalin, cleaned with distilled water, dried in ethyl alcohol with increasing concentrations, cleared with xylene, and finally embedded in paraffin wax. For routine histological analysis under a light microscope, coronal sections were cut using a rotary microtome and then stained with hematoxylin and counterstained with eosin (H&E).

Statistical analysis

The statistical package SPSS 20.0 was used for data analysis (SPSS Inc., Chicago, IL, USA). The data were analyzed using

descriptive statistics., and the results were shown as the mean and standard error of the mean (Mean±SE). One-way analysis of variance (ANOVA) was used to analyze the data, and P < 0.05 was accepted as statistically significant. Duncan's multiple comparisons were then used as post hoc multiple comparisons.

RESULTS

Effect of ashwagandha on immobility duration during FST

The DC group showed a significant increase (P < 0.05 vs. NC) in immobility duration during FST when compared with the NC group. On the other hand, both D+ASH100 and D+ASH200 groups exhibited a significant decrease (P < 0.05 vs. DC) in immobility duration during FST when compared with the DC group. No significant change (P < 0.05 vs. NC) in immobility duration during FST was observed in both ASH100 and ASH200 groups during FST when compared with the NC group (Table 1).

Effect of ashwagandha on serotonin levels in serum

The present DC group manifested a significant decrease (P < 0.05 vs. NC) in serotonin levels when compared with the NC group, while both D+ASH100 and D+ASH200 groups displayed a significant increase (P< 0.05 vs. DC) in serum serotonin levels when compared with the DC group. A significant increase (P < 0.05 vs. NC) in serum serotonin levels was observed in both ASH100 and ASH200 groups when compared with the NC group (Table 1).

Effect of ashwagandha on oxidative / anti-oxidative parameters in the brain

The present DC group exhibited a significant increase (P < 0.05 vs. NC) in brain TOC, OSI, and MDA levels while there was a significant decrease in brain TAC, GSH content, SOD, and CAT activity when compared with the NC group. Conversely, both D+ASH100 and D+ASH200 groups displayed a significant decrease (P < 0.05 vs. DC) in brain TOC, OSI, and MDA levels, while there was a significant increase (P < 0.05 vs. DC) in brain TAC, GSH content, SOD, and CAT activity when compared with the DC group. The ASH200 group showed a significant decrease (P < 0.05 vs. NC) in brain TOC and MDA levels while there was a significant increase (P < 0.05 vs. NC) in brain TAC, GSH content, SOD, and CAT activity when compared with the NC group. The ASH100 group showed a significant increase (P < 0.05 vs. NC) in brain SOD and GSH levels while there was no significant change (P < 0.05 vs. NC) in brain TOC, TAC, MDA levels, and CAT activity when compared with the NC group (Tables 2 and 3).

Table 1. The immobility duration during forced swimming test (FST) and serum serotonin level in normal control and different treated groups.

Groups	Immobility Duration (s) FST	(%)	Serum Serotonin (mmol/L)	(%)
NC	120±3.7^{d}	-------	295±2.74^{b}	-------
ASH100	117±3.1^{d}	-3%	301±1.32^{a,b}	2%
ASH200	114±4.1^{d}	-5%	304±1.67^{a}	3%
DC	181±3.9^{a}	51%	153±4.49^{e}	-48%
D+ASH 100	151±2.6^{b}	26%	202±2.01^{d}	-32%
D+ASH 200	140±4.0^{c}	17%	253±2.03^{c}	-14%

Values are expressed as Mean ± (SE), (n= 6/group). Values with different letters differ, P < 0.05 using Duncan's test; one way ANOVA. (NC: normal control, ASH100 & ASH200: 100 & 200 mg/kg ashwagandha, DC: diabetic control, D+ASH100 & D+ASH200: 100 & 200 mg/kg ashwagandha-treated diabetic groups).

Table 2. The brain total oxidative capacity (TOC), brain total anti-oxidative capacity (TAC), and brain OSI values in normal control and different treated groups.

Groups	Brain TOC (μmol H_2O_2 Eq/g p)	(%)	Brain TAC (μmol Trolox Eq/g p)	(%)	Brain OSI (arbitrary unit)	(%)
NC	0.36±0.007^{d}	-------	2.40±0.01^{b}	-------	0.15±0.004^{d}	-------
ASH100	0.35±0.004^{d,e}	-3%	2.41±0.004^{a,b}	0%	0.15±0.002^{d}	0%
ASH200	0.34±0.004^{e}	-6%	2.43±0.002^{a}	1%	0.14±0.000^{d}	-7%
DC	0.70±0.002^{a}	94%	0.93±0.02^{e}	-61%	0.76±0.01^{a}	407%
D+ASH 100	0.55±0.002^{b}	53%	1.42±0.01^{d}	-41%	0.39±0.004^{b}	160%
D+ASH 200	0.40±0.004^{c}	11%	1.88±0.02^{c}	-22%	0.21±0.004^{c}	40%

Values are expressed as Mean ± (SE), (n= 6/group). Values with different letters differ, P < 0.05 using Duncan's test; one way ANOVA. (NC: normal control, ASH100 & ASH200: 100 & 200 mg/kg ashwagandha, DC: diabetic control, D+ASH100 & D+ASH200: 100 & 200 mg/kg ashwagandha-treated diabetic groups).

Table 3. The brain malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH) in control and different treated groups.

Groups	Brain MDA (nmol/g p)	(%)	Brain SOD (U/mg p)	(%)	Brain CAT (U/mg p)	(%)	Brain GSH (μmol/g p)	(%)
NC	0.12±0.004^{d}	-------	7.17±0.05^{c}	-------	5.38±0.05^{b}	-------	12.46±0.07^{b}	-------
ASH100	0.13±0.002^{d}	8%	7.32±0.02^{b}	2%	5.40±0.03^{a,b}	0%	12.60±0.03^{a}	1%
ASH200	0.11±0.002^{e}	-8%	7.42±0.01^{a}	4%	5.48±0.04^{a}	2%	12.70±0.02^{a}	2%
DC	0.29±0.002^{a}	142%	4.66±0.03^{f}	-35%	3.94±0.03^{e}	-27%	8.09±0.06^{e}	-35%
D+ASH 100	0.24±0.002^{b}	100%	5.41±0.03^{e}	-25%	4.60±0.03^{d}	-15%	9.38±0.03^{d}	-25%
D+ASH 200	0.17±0.004^{c}	42%	6.64±0.03^{d}	-7%	4.91±0.02^{c}	-9%	10.53±0.05^{c}	-16%

Values are expressed as Mean ± (SE), (n= 6/group). Values with different letters differ, P < 0.05 using Duncan's test; one way ANOVA. (NC: normal control, ASH100 & ASH200: 100 & 200 mg/kg ashwagandha, DC: diabetic control, D+ASH100 & D+ASH200: 100 & 200 mg/kg ashwagandha-treated diabetic groups).

Histopathological studies

Light microscopic examination of the cerebral cortex sections of NC, ASH100, and ASH200 groups was the same. Six layers of gray matter were found to be ordered and well-organized from outside to inside as follows: outer molecular layer, external granular layer, external pyramidal cell layer, internal granular layer, internal pyramidal, and polymorphic cell layer (Figure 2a, b & c). The DC group's cerebral cortex sections revealed loss of organization of layers, vacuolation of nerve cells, and aggregation of inflammatory cells (Figure 2d). Examination of cerebral cortex sections from D+ASH100 and D+ASH200 groups revealed organized and regularly arranged six layers of gray matter (Figure 2e & f). Light microscopic examination of the cerebellum of NC, ASH100, and ASH200 groups showed the same results. Sections revealed organized and regularly arranged three layers. These layers were the Molecular layer (outer), Purkinje layer (middle), and Granular layer (inner) as shown in Figure 3a, b & c. Cerebellum sections from the DC group revealed degeneration and vacuoles in the molecular layer (Figure 3d). Examination of cerebellum sections of D+ASH100 and D+ASH200 groups revealed organized and regularly arranged three layers (Figure 3e & f).

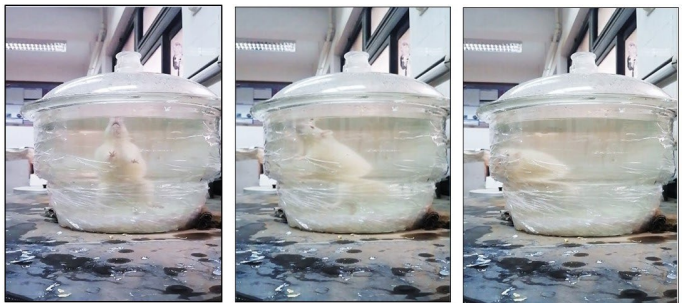

Figure 1. Rat was placed in a glass container filled with tap water during the forced swim test.

DISCUSSION

The current research shows that ashwagandha consumption in rats protected their brains from the oxidative damage brought on by DM. The current investigation examined the depression tests, lipid peroxidation and antioxidant defense mechanisms, and the histopathological organization of the cerebral cortex and cerebellum. In the current study, a modified FST model of depression is used to characterize the effects of ashwagandha in diabetic rats. When forced to swim, the diabetic rats treated with 100 & 200 mg/kg BW ashwagandha performed significantly better than the normal control group. The antidepressant effect of ashwagandha agreed with Zahiruddin *et al*. (2020). The presence of the active constituent, withanolides, has been linked to ashwagandha's antidepressant action (Mk *et al*., 2017; Speers *et al*., 2021). Depression and other neuropsychological complications are frequently linked to DM because of altered neurotransmitter function. Due to altered physiological processes, such as increased glucose oxidation and insulin deficiency, DM has a depression prevalence rate of 24–30% (Roriz-Filho *et al*., 2009). To confirm the FST results, the levels of serotonin, a neurotransmitter involved in the pathophysiology of depression, were measured (Abomosallam *et al*., 2023). In the current study, serum serotonin levels of the DC group significantly decreased when compared with the NC group. This agreed with Gupta *et al*. (2014) who observed that serotonin levels were found to be lower in diabetic animal models. Manjarrez-Gutiérrez and Hernández-Rodríguez (2016) demonstrated a specific change in the serotonergic system during DM, consisting of a decrease in serotonin biosynthesis owing to a decrease in free fractions of L-tryptophan in plasma and the brain. On the other hand, diabetic groups that received 100 & 200 mg/kg BW of ashwagandha demonstrated a significant rise in serum serotonin levels when compared with the DC group. This is in line with an earlier study by Bansal and Banerjee (2016) who found that chronic ashwagandha administration significantly increased serotonin levels. Also, Priyanka *et al*. (2020) pointed to the ability of ashwagandha in increasing serotonin concentrations. This suggests that the serotonergic system may play a role in the antidepressant effects of ashwagandha (Jahanbakhsh *et al*., 2016).

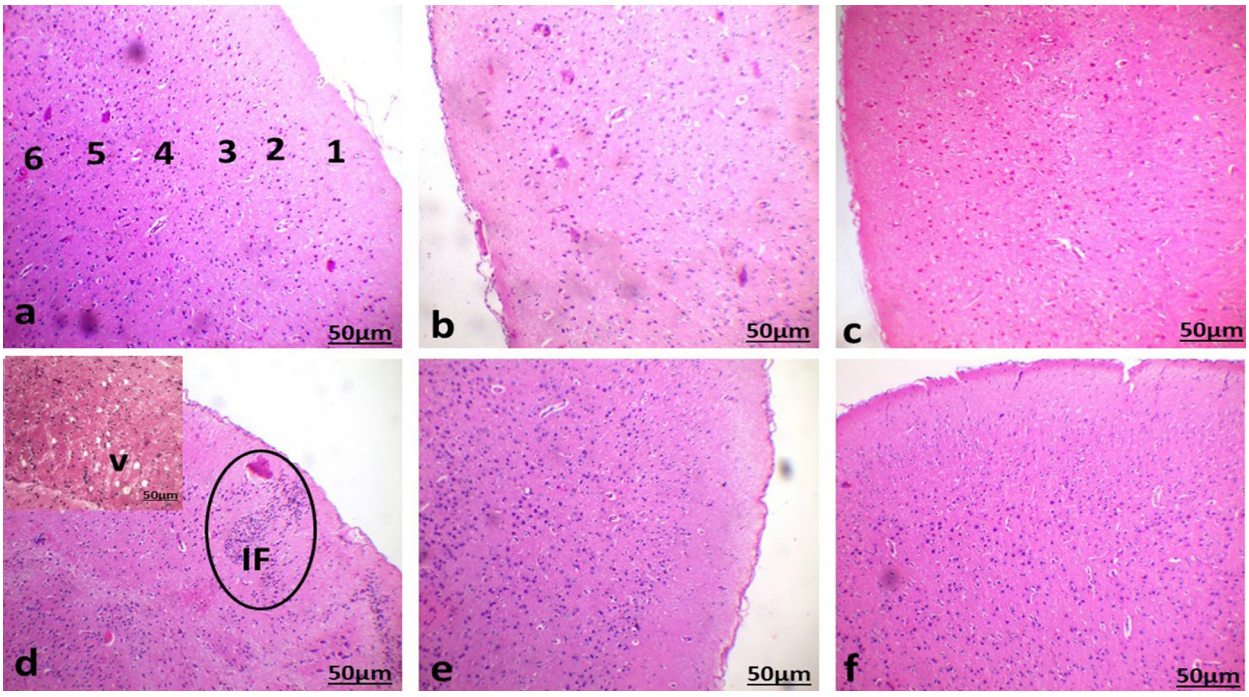

Figure 2. Photomicrographs of sections in the cerebral cortex of the frontal lobe of adult albino rats from (a) the control group showed well organized regularly arranged six layers from outer to the inner surface: (1) Molecular layer, (2) external granular, (3) external pyramidal, (4) internal granular, (5) internal pyramidal and (6) polymorphic layer. (b &C) ASH100 and ASH200 showed well-organized regularly arranged six layers. (d) DC group revealed loss of organization of layers, vacuoles of nerve cells (V), and aggregation of inflammatory cells (IF) (circle). (e&f) D+ASH 100 and D+ASH 200 groups revealed organized and regularly arranged six layers of gray matter (H.&E., × 100).

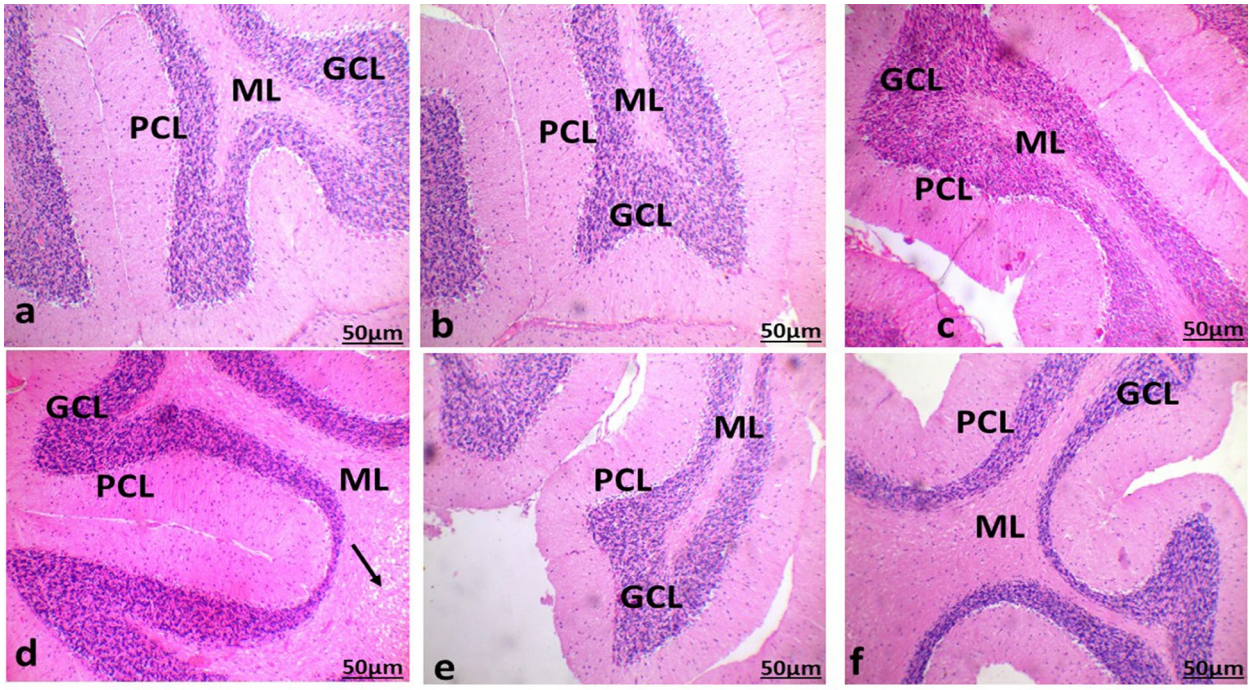

Figure 3. Photomicrographs of sections in the cerebellum of adult albino rats from (a) the control group showed well-organized regularly arranged three layers (b &C) ASH100 and ASH200 showed well organized regularly arranged three layers. (d) DC group revealed degeneration and vacuoles (arrow) in the molecular layer. (e&f) D+ASH 100 and D+ASH 200 groups revealed organized and regularly arranged three layers. Molecular layer (ML), Purkinje layer (PCL), and Granular layer (GCL) (H.&E., × 100).

Diabetic-induced neuronal damage is likely caused by OS (Tan *et al*., 2022). The levels of oxidant/antioxidant parameters were investigated to better evaluate OS in brain tissues. In the current study, the rats in the DC group showed a significant increase in brain TOC, OSI, and MDA levels along with a significant decrease in brain TAC, GSH content, SOD, and CAT activity when compared with the NC group, indicating that they were experiencing OS, which can lead to neurological disorders. DM is frequently accompanied by an increase in MDA production, which raises the levels of MDA in diabetic tissues and blood (Tiwari *et al*., 2013). The elevated MDA levels in DC rats imply that lipid peroxidation (LPO) is being boosted because of an increase in ROS generation and that this increase tended to the OS produced by hyperglycemia. A decrease in enzymatic and non-enzymatic antioxidants of the defense system in diabetic rats may also be reflected in the rise in LPO (De M. Bandeira *et al*., 2013). The present results agreed with Uzar *et al*. (2012) who reported that after 21 days of treatment, diabetic rats' brain tissues had significantly higher TOC levels and OSI values, but significantly lower TAC levels when compared to control rats. Okutan *et al*. (2005) indicated that in the hippocampus of diabetic rats, LPO levels significantly increased while GSH content, SOD, and CAT activity significantly decreased. Similar to this, a prior study found that after 15 days of treatment, the cerebrum, cerebellum, and midbrain of diabetic rats compared to non-diabetic rats showed a significant increase in MDA levels and a significant decrease in GSH levels, CAT, and GPx activities (Kapoor *et al*., 2009). Sudhakara *et al*. (2012) and Samarghandian *et al*. (2015) also reported that STZ-induced DM in rats increased brain MDA levels while GSH content, SOD, and CAT activity had decreased.

On the other hand, diabetic groups treated with 100 and 200 mg/kg BW of ashwagandha showed a significant decline in brain TOC, OSI, and MDA levels, whereas brain TAC, GSH levels, SOD, and CAT activity significantly increased when compared with DC group. According to Singh *et al*. (2011), ashwagandha has strong antioxidant properties, which aid in the prevention of cellular damage caused by free radicals. The antioxidant capacity of ashwagandha is directly correlated with the total phenolic content (Alam *et al*., 2011). The administration of 200 mg/kg BW ashwagandha was the most effective in enhancing the overall state of antioxidants in the brains of normal rats in the present study, as it was able to significantly increase the brain TAC, GSH levels, SOD, and CAT activity whereas the administration of 100 mg/kg ashwagandha showed a significant increase in the brain GSH content and SOD activity only. This is following Parihar *et al*. (2016) who reported that giving ashwagandha to STZ-treated rats via oral administration resulted in a significant drop in MDA and a significant rise in GSH levels. Also, Ahmed *et al*. (2013) reported that administering ashwagandha extract orally after receiving STZ infusion resulted in a decrease in LPO level and an increase in antioxidant enzyme activities as well as a rise in GSH level in various regions of brain tissue. In like manner, John (2014) found that ashwagandha administration for 28 days tends to bring the MDA, SOD, and CAT values in a parkinsonism-induced mice model to near-normal levels and the ashwagandha is known to modulate brain OS markers, such as LPO, SOD, CAT, GPx, and GSH in another context. Conversely, Hosny *et al*. (2021) reported that a 30-day ashwagandha treatment did not affect the decrease in CAT and SOD activity in the hippocampus caused by induced hypothyroidism.

The current investigation looked at the histological changes caused by ashwagandha in the cerebral cortex and cerebellum of the brain of adult male albino rats with DM. In the current study, inflammatory cells were agglomerated, and the cortical layers of diabetic rats' cerebral cortex showed marked disorganization where they had lost their normal shape. The molecular layer of the cerebellum displayed vacuoles. These findings are consistent with those of Malik *et al*. (2011) who noted neuronal damage brought on by DM. These results coincide with Sharma *et al*. (2005) and Gad El-Hak and Mobarak (2019) who claimed that exposure to free radicals changed the histology of their brain. A disturbance in the balance of ROS production and antioxidant protection caused by excessive exposure to free radicals is known as OS (Birben *et al*., 2012). Because of its high oxygen intake and limited antioxidant content, the brain is highly susceptible to OS damage (Floyd, 1999). Protein modifications brought on by OS can result in a drop in enzymatic activity and a loss of function (Burton and Jauniaux, 2011). In this study, after the ashwagandha treatment, the cortical layer and cerebellum of the diabetic rats significantly improved. Further research can be carried out to define the active constituents responsible for the mechanism of action of ashwagandha root plants. Practical information on the

safety of ashwagandha root plants should be made in combination with chemical diabetic drugs.

CONCLUSION

Based on the available information, it is possible to draw the conclusion that ashwagandha supplementation protects against alloxan-induced diabetic neuropathy. By reducing the oxidative stress that diabetes causes, this effect was mediated. Considering the findings, it has been hypothesized that ashwagandha supplements have strong anti-depressant effects by enhancing FST behavior.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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