Protective effect of Allium jesdianum in an Alzheimer's disease induced rat model

Farzaneh Kamranfar  
Shahid Beheshti University of Medical Sciences

Razieh Pourahmad Jaktaji  
Shahrekord University of Medical Sciences

Kobra Shirani  
Tarbiat Modares University

Amirhossein Jamshidi  
Iran University of Medical Sciences

Fatemeh Samiei  
Shahid Beheshti University of Medical Sciences

Abdollah Arjmand  
Shahid Beheshti University of Medical Sciences

Mona Khoramjouy  
Shahid Beheshti University of Medical Sciences

Mehrdad Faizi  
Shahid Beheshti University of Medical Sciences

Jalal Pourahmad  (j.pourahmadjaktaji@utoronto.ca)  
Shahid Beheshti University of Medical Sciences

Research Article

Keywords: Alzheimer’s disease (AD), Allium jesdianum, Radial Arm Water Maze, Mitochondria, microRNA (miRNA), Bax, Bcl-2

Posted Date: April 6th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2678755/v1

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Abstract

**Aims:** Alzheimer is a multifactorial disease that is caused by several different etiopathogenic mechanisms. The aim of this study is to evaluate the protective effects of *Allium jesdianum* extract on cognitive dysfunction, mitochondrial/cellular, and genetic parameters in Streptozotocin-induced Alzheimer's disease (AD) Rat Model.

**Main methods:** A single dose of STZ (3 mg/kg, i.c.v.) was injected to male Wistar rats in order to establish a model of sporadic AD. *A. jesdianum* extract (100, 200, 400 mg/kg/day) and donepezil (5 mg/kg/day) were administered through oral gavage as treatment for 14 days after model induction. Cognitive function (radial arm water maze test), mitochondrial toxicity parameters consisting succinate dehydrogenase (SDH) activity, mitochondrial ROS formation, MMP decline, mitochondrial swelling and efflux of cytochrome c in various parts of the rat brain (whole brain, frontal cortex, hippocampus and cerebellum), and miR-330, miR-132, Bax and Bcl-2 genes expression in isolated rat brain neurons through RT-qPCR analysis were evaluated.

**Key findings:** *A. jesdianum* extract significantly attenuated i.c.v-STZ-induced cognitive dysfunction and mitochondrial upstream toxic events. As a result of STZ injection, Bax gene was highly expressed, whereas miR-330, miR-132 and Bcl-2 gene were poorly expressed and *A. jesdianum* reverses the expression of the above miRNAs and genes in favor of improving AD and reducing neuronal apoptosis.

**Significance:** *A. jesdianum* showed the neuroprotective capability against oxidative stress and cognitive impairment induced by STZ in rats shows its helpful therapeutic worth in AD.

1 Introduction

Alzheimer's disease (AD) is a complex multifactorial disorder with progressive neurodegenerative consequences that accounts for 60–65% of dementia cases. the exact etiology of AD is still not fully understood, and no curative treatments are available yet (Sonkusare et al. 2005; Wang et al. 2019). The pathological AD factors are amyloid beta plaque formation, neurofibrillar tangles (NFTs), dysfunction of the cholinergic system, oxidative stress, neuroinflammation, synaptic plasticity damage and eventually neuronal loss (Kamat et al. 2016). These hallmarks might be triggered by the mitochondrial disturbance and oxidative stress. Clinical studies have revealed a relationship between neuronal loss and mitochondrial dysfunctions, which finally leads to patients' memory impairment (Saini et al. 2021). Probably, mitochondrial dysfunctions cause synaptic transmission impairment in neurodegenerative conditions, such as Alzheimer's, Huntington's, and Parkinson's diseases (Beal 2005; Cenini et al. 2019).

Additionally, recent research has shown that some miRNAs may play a significant role in the pathological process of neurodegenerative disorders like AD (Pan et al. 2016). The functions of different miRNAs such as miR-1273g-3p, miR-330, miR-132, miR-592, miR-146a-5p and miR-743a have been discussed in detail in the pathogenesis of neurodegenerative disorders, especially AD. The possibility of miRNAs as an
alternative and more sensitive approach in AD detection, management and prevention has also been discussed (Silvestro et al. 2019).

Considering that AD is a multifactorial disease, single-target drugs that affect only one enzyme or one receptor are not suitable for its treatment. Most current Alzheimer’s medications, such as acetylcholine esterase (AChE) inhibitors, are only capable of alleviating cognitive symptoms, not stopping or delaying disease development. In addition, they have side effects, necessitating the quest for natural and secure therapeutic alternatives (Grutzendler and Morris 2001; Reisberg et al. 2003). In recent years, several compounds with therapeutic activity have been extracted from plants, animals and microorganisms, which have been found to have beneficial effects in controlling of AD by affecting several pathogenic mechanisms (Andrade et al. 2019; Cavalli et al. 2008). Thus, medicinal plants are an alternative to existing standard drugs for AD. Allium jesdianum Boiss & Bushe is a species of bulbous plant in the genus Allium and belongs to the Alliaceae family and is grown in the Zagros Mountains of Iran. According to research, A. jesdianum has some useful antioxidant, antipyretic, antibacterial, and antifungal properties (Alidadi et al. 2021; Sohrabinezhad et al. 2019).

The sub-diabetogenic dose of streptozotocin (STZ) injected intracerebroventricularly (i.c.v.) at a dose of 3 mg/kg provides a comparable model for the sporadic type of AD, which affects more than 90% of AD patients globally (Ramezani et al. 2016). It is reported that i.c.v.-STZ induces insulin resistance in the brain, brain glucose metabolism reduction, oxidative stress, cholinergic dysfunction, gliosis, learning and memory problems and accumulation of tau and Aβ proteins (Kamat 2015).

Despite the high nutritional and pharmacological values of A. jesdianum and its anti-oxidant properties, no study has evaluated its anti-Alzheimer’s potential in experimental models of AD. Thus, we aimed to evaluate the effects of A. jesdianum hydroalcoholic extract on learning and memory loss and mitochondrial/cellular and genetic parameters in STZ-induced AD rats for the first time.

2 Materials And Methods

2.1 | Preparation of the extract

The A. jesdianum was found and collected near in the Zagros Mountains of Shahrekord, Iran, in April 2021. The Iran’s Jondishapour University herbarium unit identified the plant and samples were stored there with the code number of A-0138. Extract of the whole plant of A. jesdianum was prepared by maceration method using ethanol: water (80:20 v/v) solution as a solvent and a solid-to-solvent ratio of 1:10 (w/v) for four days. A rotary vacuum was used to concentrate the extract until a crude solid was produced.

2.2 | Standardization of extract

Total phenolic components in the A. jesdianum hydroalcoholic extract were calculated using Folin Ciocalteu’s colorimetric assay (Leonelli et al. 2013). The calibration curve was established using gallic acid, and the total phenolic content (TPC) was reported as milligram gallic acid equivalent per milliliter
extract. The total flavonoid of *A. jesdianum* was evaluated by the aluminum chloride colorimetric method using a UV spectrophotometer (Waihenya et al. 2002). Through the quercetin calibration curve, the quantity of total flavonoids was expressed as milligram of quercetin equivalent per milliliter of the samples.

2.3 | Animals

All of the Wistar rats used in this study were male and weighed between 230 and 270 grams and were obtained from the Shahid Beheshti University of Medical Sciences. The rats were kept in an air-conditioned room with a controlled temperature of $25 \pm 2^\circ$C, a 12:12-hour light/dark cycle, 50 to 60 percent humidity, and free access to food and water. All experiments were conducted based on animal research international guidelines and were authorized by the Ethics Committee of SBMU (ethics code: IR.SBMU.PHARMACY.REC.1400.143).

2.4 | Study design

Randomly, the animals were separated into six groups ($n=8$). Group I was Sham (received normal saline i.c.v. + oral gavage of vehicle solution for two weeks); Group II received STZ (STZ 3 mg/kg i.c.v. once + oral gavage of vehicle solution for two weeks); Group III received STZ (STZ 3 mg/kg i.c.v. once) and treated with AJ (100 mg/kg/day, p.o. for two weeks); Group IV received STZ (STZ 3 mg/kg i.c.v. once) and treated with AJ (200 mg/kg/day, p.o. for two weeks); Group V received STZ (STZ 3 mg/kg i.c.v. once) and treated with AJ (400 mg/kg/day, p.o for two weeks). Group VI received STZ (STZ 3 mg/kg i.c.v. once) and treated with donepezil (5 mg/kg/day, p.o. for two weeks). The doses of AJ were selected based on previous studies (Jalili et al. 2020; Sohrabinezhad et al. 2019).

2.5 | Experimental induction of the AD model

In order to anesthetize the rats before the surgical operation, they were given a combination of ketamine (100 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.). Following this, the animal's head was positioned in a stereotaxic apparatus, and the scalp was shaved. The skull was exposed after a midline sagittal incision was made in the scalp. The stereotaxic coordinates for the lateral ventricles region were 0.8 mm posterior to bregma, 1.5 mm lateral to the sagittal suture and 3.6 mm beneath the cortical surface set according to the rat brain atlas (Paxinos and Watson 1986). Using a Hamilton syringe needle a burr hole created in the skull and 5 µl of STZ was slowly infused into each lateral ventricle. STZ solution was prepared in normal saline instantly before injection (Fanoudi et al. 2018). Postoperatively, in order to avoid infection, the animals were kept in an aseptic environment at a controlled temperature to avoid infection. Animals received daily oral gavage of *A. jesdianum* extract and donepezil 24 hours after the surgery for 14 days.

2.6 | Behavioral assessment

The behavioral test period began 15 days following the STZ injection. Radial arm water maze (RAWM) test was done on days 15–17. In addition to other studies, our preliminary experiments established learning and memory impairments two weeks post-STZ administration (Mehla et al. 2013; Mohebichamkhorami et al. 2022).
2.7 | Radial Arm Water Maze Test

The Radial Arm Water Maze (RAWM) test evaluated the animals' spatial learning and memory. The RAWM apparatus consists of a water tank and six (59×13 cm) arms. Visual cues were positioned at a certain distance from each arm. The water temperature was kept at 25 °C and the water depth was 50 cm. There were three consecutive days of testing for each animal. On the first two days, the animals were trained 15 times to learn the hidden platform location guided by the visual cues placed at the end of each arm. During each trial, the platform was placed in one of the six arms, 2 cm below the water surface (target arm). The animals were placed in one of the arms (starting arm) and allowed 60 s to reach the platform. The latency to find the platform and the number of entries to the non-target arm were recorded. On the last day, as the probe or test day, each rat was located in the starting arm and the hidden platform was removed from the target arm. The latency to enter in the target arm, the number of entries to the target arm and the time spent in the target arm were recorded. The Ethovision (Noldus, the Netherlands) tracking software was utilized for analyzing all data captured by digital cameras (Chaby et al. 2015; Khoramjouy et al. 2021).

2.8 | Biochemical measurements

Rats were sacrificed 24 hours after the probe test, and their brains were quickly taken out, cleaned with saline that was icy cold and three cerebral areas include the hippocampus, frontal cortex and cerebellum were dissected. Then, the mitochondria were isolated, and mitochondrial toxicity parameters were evaluated. Animal brain neurons were isolated for evaluation of miR-330, miR-132, Bax and Bcl-2 genes expression via real-time PCR.

2.9 | Isolation of Mitochondria

The brain tissue was immersed in an ice-cold isolation buffer containing 225 mM mannitol, 75 mM sucrose, 5 mM HEPES, 1 mg/ml fatty acid-free BSA, and 3 mM EGTA. The solution had a pH of 7.4 and was isotonized with KOH. Using a hand-held homogenizer, the tissues were minced and homogenized, and then, by performing differential centrifugation technique, mitochondria of the collected tissues were extracted. Before anything else, the wrecked cell debris and cores were centrifuged (2000 g, 10 min, 4°C) and deposited in sediment from the specimens. A second centrifugation (at 12000 g for 10 minutes at 4°C) was applied to the supernatant. The top layer was removed, and the mitochondrial pellet was washed before being put in the extraction medium and centrifuged again (12000 g, 10 min, 4°C). Then, the mitochondrial pellet was homogenized and kept cold by an ice shower until the tests. Coomassie blue protein-binding method, introduced as Bradford test, was used to estimate protein concentrations. For the normalization or unification of mitochondrial samples in every one of the accompanying tests, the mitochondrial samples were normalized based on protein concentration (0.5 mg mitochondrial protein per ml). All isolation procedures were carried out on ice to maintain mitochondrial quality during processing (Patel and Katyare 2006).

2.10 | Succinate dehydrogenase activity
Liu et al. used the reduction in MTT to evaluate the overall activity of the dehydrogenases in the mitochondrial preparation. All groups' mitochondrial suspensions were subjected to a 30-minute incubation at 37°C. After dissolving the formazan crystal product in 100 µl of DMSO, the absorbance at 570 nm was measured using an ELISA reader (Tecan, Rainbow Thermo, Austria) (Samiei et al. 2020).

2.11 | Mitochondrial ROS Level Measurement

Utilizing 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA), a fluorescence spectrophotometer was used to take measurements of the ROS levels in the mitochondria. In brief, isolated mitochondria were incubated in a respiration buffer (10 mM Tris, 0.32 mM sucrose, 20 mM Mops, 0.5 mM MgCl2, 50 mM EGTA, 5 mM sodium succinate, and 0.1 mM KH2PO4) before DCFH-DA was added (final concentration, 10 mM) and the samples were incubated for ten minutes. Upon entrance, hydrolysis of DCFH-DA generated non-fluorescent dichlorofluorescin (DCFH), which interacted with ROS to form highly fluorescent dichlorofluorescein (DCF). The emitted fluorescence wavelength, after being stimulated at 488 nm, was measured at 530 nm (Faizi et al. 2014).

2.12 | Mitochondrial Membrane Potential Measurement

To calculate the mitochondrial membrane potentials (MMP), the cationic fluorescent dye Rhodamine 123 (Rh123) was taken up by the mitochondria. Mitochondrial fractions were incubated with 10 mM of Rh123 in the MMP assay buffer (68 mM D-mannitol, 220 mM sucrose, 10 mM KCl, 5 mM KH2PO4, 2 mM MgCl2, 5 mM sodium succinate, 50 mM EGTA, 10 mM HEPES and 2 mM rotenone). Afterwards, a fluorescence spectrophotometer was used to measure the fluorescence at 490 and 535 nm for excitation and emission, respectively (Baracca et al. 2003).

2.13 | Determination of mitochondrial swelling

To determine mitochondrial swelling, light scattering changes at 540 nm were evaluated spectrophotometrically (Behzadfar et al. 2017). The absorbance was measured at 540 nm using an ELISA reader equipment after the isolated brain mitochondria were suspended in swelling buffer (3 mM HEPES, 70 mM sucrose, 230 mM mannitol, 5 mM succinate, 2 mM Tris-phosphate, and 1 mM of rotenone). The presence of swollen mitochondria was indicated by a decline in the absorbance.

2.14 | Release of cytochrome C

The Quantikine Rat/Mouse Cytochrome c Immunoassay kit from R&D Systems, Inc., Minneapolis, MN, USA, was used to measure cytochrome c release. This kit employs the quantitative sandwich enzyme immunoassay technique (Samiei et al. 2020).

2.15 | Rat neurons isolation

Brewer et al.'s method was used to isolate neurons from rats (Seydi et al. 2022). The cortex, hippocampus, and other parts were dissected briefly. After that, 0.5-mm slices were cut, and papain was
used to digest them for 30 minutes at 30°C. The cells were triturated for release in the subsequent step. Density gradients were utilized to purify the neurons. The cells were concentrated and resuspended in the desired medium in the next step. After that, the neurons were plated on a poly-Lys-coated glass substrate and placed in the Neurobasal/B27 with growth factors added. At 37 degrees Celsius, they were then incubated with 9 percent oxygen and 5 percent CO2.

2.16 | Quantitative real-time PCR

The expressions of miR-132, miR-330, Bcl-2 and Bax genes were determined using RT-qPCR. The RNeasy Mini Kit (QIAGEN, Hilden, Germany) and miRNA Isolation Kit (Yekta Tajhiz Co., Iran) were used to isolate total RNA from rat brain neurons according to the directions given by the manufacturer. miRNAs were transcribed into complementary DNA (cDNA) by using the stem-loop RT primer hybridization, based on the M-MLV Reverse Transcriptase Kit (Yekta Tajhiz Co., Iran). The stem-loop RT structures were designed according to the previous studies (Jia et al. 2019; Zhang et al. 2014). A Mic qPCR Cycler (Biomolecular Systems, Australia) and a SYBR Green kit (Yekta Tajhiz Co., Iran) were utilized for the real-time quantitative RT-PCR analysis and amplification of cDNA. The thermal cycle conditions for miRNAs were 15 s at 95°C and 30 s at 60°C for 40 cycles and for Bax and Bcl-2 were as follows: 10 s at 95°C, 20 s at 60°C, and 15 s at 72°C for 40 cycles. To normalize the cDNA variation, β-actin was used as a housekeeping gene for Bcl-2 and Bax genes and the relative amount of miRNAs were normalized by U6 small nuclear RNA. The Pfaffl method was utilized for relative quantification. Analysis using the Pfaffl method indicates that a ratio more than 2 indicates Overexpression, whereas a ratio less than 0.5 indicates Low Expression (Jalali et al. 2017). Table 1 shows the primer sequences.
### Table 1
PCR primer sequences for reverse transcription and qRT-PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sequence of primer 5′ 3′</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bax</td>
<td>F: CCCGAGAGGTCTTTTTTCCGAG R: CCAGCCCATGATGTTCTGAT</td>
</tr>
<tr>
<td>Bcl-2</td>
<td>F: GGTGGGGGTCATGTGTGTGG R: CGGTTCAGGTACTCACTCACTCC</td>
</tr>
<tr>
<td>ACTB</td>
<td>F: AACGGAAGGTGACAGCAGTCG R: GGCAAGGGACTCCCTGTAACAACG</td>
</tr>
<tr>
<td>miR-330</td>
<td>F: AAGCGCTCTCTGGGCTGTG R: CAGTGCGTGTCGTGTCGTGGAG</td>
</tr>
<tr>
<td>miR-132</td>
<td>F: CGCTAACAGTGCTACAGCC R: GCAGGGTCCGAGGTATTC</td>
</tr>
<tr>
<td>U6</td>
<td>F: GCTTCGGCAGCACATATACCTAAAT R: GCTTCACGAATTTGCGTGTCAT</td>
</tr>
<tr>
<td>RT-miR132</td>
<td>GTCGTATCCAGTGCTACAGCCACAGGACCAGCAT</td>
</tr>
<tr>
<td>RT-miR-330</td>
<td>GTCGTATCCAGTGCTACAGCCACAGGACCAGCAT</td>
</tr>
</tbody>
</table>

### 2.17 | Statistical Analysis

Graph Pad Prism was utilized for each and every statistical analysis (Graph Pad Prism software, version 8). Two-way ANOVA followed by Bonferroni’s post hoc test was used for parameters of the training days of RAWM and one-way ANOVA followed by Turkey’s post hoc test for parameters of the probe test. The one-way ANOVA was used to compare various mitochondrial parameters across the experimental groups, followed by Turkey's post hoc test. For RT-qPCR, the one-way ANOVA test and Turkey's post hoc test were performed. Data are reported as mean ± SEM. Statistical significance was set at P < 0.05.

### 3 Results

#### 3.1 | Characterization of *A. jesdianum* hydroalcoholic extract

The total phenolic compounds and total flavonoid of the *A. jesdianum* extract were 157.6 ± 13.4 mg of gallic acid equivalent per ml and 114.7 ± 10 mg of quercetin equivalent per ml, respectively.

#### 3.2 | RAWM results
In order to assess the *A. jesdianum*’s protective efficacy against STZ-induced memory loss in rats, the radial arm water maze assay (RAWM) were performed. Figure 1 displays the RAWM results of all the experimental groups in the first two days of the training experiment. In the majority of trials, animals in the STZ group required considerably longer time to locate the position of the platform, and the frequency of reference memory errors in the STZ group was significantly greater than the sham group throughout the training test days.

As shown in Fig. 1A, the animals that received the dose of 200 mg/kg *A. jesdianum* extract in trial 12 and the animals treated with donepezil in trials 6, 12 and 24 found the platform significantly faster than the STZ group. Figure 2B shows that the *A. jesdianum* at a dose of 200 mg/kg in trial 3, the dose of 400 mg/kg in trials 12 and 30 and donepezil in trials 27 and 30 were able to significantly reduce the number of entries into the non-target arm comparing to the STZ group.

To access memory recall, the platform was removed on probe day. The STZ group had a considerably significant latency to locate the target arm than the sham group (P < 0.0001). One-way analysis of variance showed a lower latency to locate the target arm in animals treated with *A. jesdianum* (400 mg/kg) and donepezil compared to the STZ group (P < 0.05 and P < 0.01 respectively; Fig. 2A).

Also, the STZ group notably had fewer entries to the target arm on probe day than the sham group, according to the results (P < 0.001). In comparison with the STZ group, medium and high dosages of *A. jesdianum* (200 and 400 mg/kg) and donepezil increased target arm entries significantly (P < 0.05, P < 0.01 and P < 0.01 respectively; Fig. 2B). Finally, the duration the target arm significantly decreased in the STZ group when compared to the sham group (P < 0.0001). Animals administered 400 mg/kg of *A. jesdianum* and donepezil spent more time in the target arm comparing to those in the STZ group (P < 0.01 and P < 0.001 respectively) (Fig. 3C).

### 3.3 Mitochondrial function

#### 3.3.1 Succinate dehydrogenase activity

The mitochondrial viability related to succinate dehydrogenase activity in the whole brain, frontal cortex, hippocampus and cerebellum was found to be significantly reduced in STZ-induced animals in comparison to sham animals (P < 0.0001). *A. jesdianum* at a low dose (100 mg/kg) showed no improvement in reduced viability. *A. Jesdianum* was able to dramatically enhance the lowered MTT levels in the whole brain, frontal cortex, and cerebellar area at a dosage of 200 mg/kg (whole brain and cerebellum: P < 0.05 and frontal cortex: P < 0.01). Supplementation at a high dosage (400 mg/kg) is more successful (P < 0.001) than STZ group in restoring the reduced MTT levels in the whole brain and frontal cortex, hippocampus, and cerebellum (P < 0.01). SDH activity was significantly higher in the donepezil group compared to the STZ group in the whole brain, frontal cortex and hippocampus (P < 0.001 for the whole brain and hippocampus and P < 0.0001 for the frontal cortex), but not in the cerebellum (Fig. 3).

#### 3.3.2 Mitochondrial ROS formation
The results of ROS generation test represented that the mitochondrial ROS levels, isolated from the whole brain, frontal cortex (P < 0.001), hippocampus and cerebellum (P < 0.0001) significantly increased by STZ injection in comparison to the sham group.

Additionally, the ROS level in the whole brain, frontal cortex, hippocampus and cerebellum regions was significantly reduced by all doses of *A. jesdianum* than the STZ group. *A jesdianum*, at dose of 100 mg/kg, significantly reduced the ROS level in the whole brain, frontal cortex, hippocampus and cerebellum regions compared to the STZ group (P < 0.05).

The ROS formation notably decreased by 200 and 400 mg/kg doses of *A. jesdianum* treatment in the mitochondria isolated from the whole brain (P < 0.01), cerebellum (P < 0.05 and P < 0.01 respectively), hippocampus (P < 0.01 and P < 0.001 respectively) and frontal cortex (P < 0.001). Treatment with donepezil tended to bring the ROS level towards normal values in the whole brain, frontal cortex (P < 0.001) and hippocampus (P < 0.01) regions compared to STZ-treated animals, while it was found non-effective in the cerebellum (Fig. 4).

### 3.3.3 | Mitochondrial membrane potential

We found the STZ groups reported significantly less MMP than the sham group (whole brain and frontal cortex, P < 0.0001; hippocampus and cerebellum, P < 0.001). A significant inhibition of MMP collapse, induced by STZ, is apparent in the mitochondria isolated from the whole brain, frontal cortex (P < 0.01); hippocampus and cerebellum (P < 0.05) in 200 mg/kg *A. jesdianum* treatment comparing the STZ-treated group. Moreover, high dose of *A. jesdianum* (400 mg/kg) was able to increase MMP in all the three brain regions and the whole brain (whole brain and hippocampus, P < 0.01; frontal cortex and cerebellum, P < 0.05). Analogous with previous findings, donepezil is showing promising results to restore mitochondrial membrane damage in the whole brain, frontal cortex and hippocampus (P < 0.001), while in the cerebellum region, it showed a non-significant effect (Fig. 5).

### 3.3.4 | Mitochondrial swelling

In order to evaluate the swelling of mitochondria, absorbance changes at 540 nm were selected, which is a sign of permeability in the membrane of mitochondria. STZ injection to the rats significantly increased mitochondrial swelling versus the sham group (Whole brain, P < 0.0001; all the three regions of the brain, P < 0.001). What stands out in the Fig. 6 is that the mitochondrial swelling in the whole brain, frontal cortex and cerebellum locales in the group receiving 200 mg/kg *A. jesdianum* was significantly reduced compared to the sham group but failed to have any effect in hippocampus region (whole brain, P < 0.01, frontal cortex and cerebellum, P < 0.05), as the post hoc examination uncovered 400 mg/kg *A. jesdianum* in STZ-treated animals, significantly decreased the swelling in the whole brain and different parts of the brain compared to the STZ group (whole brain, frontal cortex and cerebellum, (P < 0.01); hippocampus, P < 0.05). These results indicate that donepezil ameliorated the mitochondrial swelling in the whole brain.
(P < 0.001), frontal cortex and hippocampus (P < 0.01). However, donepezil showed an insignificant effect in cerebellum comparing to the STZ group.

3.4 | Cytochrome c release

As shown in Fig. 7, the i.c.v. injection of STZ significantly induced the release of cytochrome c in the mitochondria isolated from the whole brain, frontal cortex, cerebellum (P < 0.0001) and hippocampus (P < 0.001) compared to the sham group. Whereas, cytochrome c release was significantly inhibited during 200 mg/kg A. jesdianum treatment in whole brain, frontal cortex (P < 0.01) and cerebellum (P < 0.05). High dose (400 mg/kg) of A. jesdianum, as an MPT inhibitor, reduced STZ-induced cytochrome c release comparing to the STZ group in whole brain, hippocampus, frontal cortex and cerebellum (P < 0.01). We found that donepezil significantly inhibited cytochrome c release comparing to the STZ group in the whole brain, frontal cortex and hippocampus (P < 0.001); however, no significant difference in the level of cytochrome c in the cerebellum between the two groups was evident.

3.5 | Determination of miRNAs, Bax and Bcl-2 level

Isolated neurons from the rat brain showed significantly lower expression of miR-132 and miR-330 after STZ treatment compared to the sham group (P < 0.01, P < 0.05, respectively). Meanwhile, these miRNAs expression was significantly increased after treatment with higher doses of A. jesdianum and donepezil (Fig. 8).

Comparisons with the Sham group demonstrated a significant reduction of the anti-apoptotic Bcl2 gene in the STZ group (p < 0.001) (Fig. 9A). Additionally, the A. jesdianum supplementations (200 mg/kg and 400 mg/kg) and donepezil significantly increased the expression of this gene comparing to the STZ group (P < 0.001, P < 0.001 and P < 0.0001, respectively). According to Fig. 9B, the real-time PCR data demonstrated a significant increase expression of Bax gene in the STZ group as compared with the sham group (p < 0.0001). It was also revealed that 200 mg/kg and 400 mg/kg doses of A. jesdianum (P < 0.0001) and donepezil (P < 0.001) significantly reduced the overexpression of the Bax compared to the STZ group.

4 Discussion

Spices and medicinal herbs with antioxidants and anticholinesterase have gained a lot of attention as potential treatments for neurodegenerative disorders such as AD (Bahaeddin et al. 2018). The antioxidant properties and therapeutic effects of Allium. jesdianum against various diseases have already been widely studied (Khaksarian et al. 2017; Petropoulos et al. 2020) and According to previous studies, the protective effects of some members of the Allium genus have been proven in AD (Bahaeddin et al. 2018; Kaur and Shri 2018). However, no data exist on the effectiveness of A. jesdianum in AD. Considering the complexity of the disease, we assessed the effects of A. jesdianum hydroalcholohic extract on a few obsessive parts of AD, including learning and spatial memory, mitochondrial parameters and oxidative
stress in the whole brain and three brain regions (hippocampus, frontal cortex, cerebellum) and expression changes of miR-330, miR-132, Bax and Bcl-2 genes in brain neurons isolated from the rat model of AD.

I.C.V. STZ administration results in oxidative stress and a gradual loss of memory function, and is regarded as a suitable animal model of sporadic AD (Raheja et al. 2019). In accordance with earlier research, this study also found that STZ caused oxidative damage and cognitive impairment. To evaluate STZ-induced impairments in learning and memory, the RAWM assay, a reliable behavioral model in rats, was utilized in this study (Shukitt-Hale et al. 2004). Similar to earlier results, STZ administration during the first two days of the training trial dramatically enhanced both the latency to locate platform and the frequency of reference memory errors (Javed et al. 2012; Mansouri et al. 2013). Confirmation of STZ-induced cognitive decline was identified when the STZ groups, compared to the sham group, showed a marked increase in the latency to locate the platform, a significant decrease of the duration in target arms and the number of entries to the target arm in the probe test. While the oral administration of *A. jesdianum* extract had positive effects on enhancement learning ability and memory consolidation in the animals. In other words, *A. jesdianum* dramatically restored the STZ-induced impairment in learning and memory.

Many studies have shown that plants with antioxidants and mitochondria-targeting effects, including *Allium sativum*, *Allium cepa* and *Allium hirtifolium*, have potential impact on neurodegenerative diseases, including AD (Bahaeddin et al. 2018; Kaur and Shri 2018; Saini et al. 2021). As previously indicated, dysfunction of mitochondria and oxidative stress are known as central AD mechanisms (Wang et al. 2014). It has been demonstrated that i.c.v.-STZ induces mitochondrial dysfunction and oxidative stress through an increase in malondialdehyde (MDA) and a decrease in glutathione levels (Agrawal et al. 2009; Kamat et al. 2016). Farbood et al. showed that STZ exposure increased the production of reactive oxygen species (ROS) in the isolated mitochondria of the rat brain (Farbood et al. 2020). Similarly, our results indicated that STZ significantly increases the amount of ROS in the whole brain, hippocampus, frontal cortex and cerebellum and creates oxidative stress, indicating the role of ROS as one of the pathological factors of memory impairment. *A. jesdianum* extract at all three doses (100, 200 and 400 mg/kg) significantly decreased ROS production comparing to the STZ group and indicates the antioxidant properties of the extract. Sohrabinezhad Z et al. showed that the *A. jesdianum* extract improves oxidant/antioxidant balance in hepatic tissue in APAP-induced hepatic failure (Sohrabinezhad et al. 2019).

Researchers have found that complexes II, III, and IV in the mitochondria of the brains of AD models are directly inhibited by free radicals like ONOO- and NO (Saini et al. 2021). Consistent with previous research, STZ administration significantly decreased mitochondrial complex II enzymatic activities and mitochondrial viability. Medium and high doses of *A. jesdianum* in the whole brain, frontal cortex and cerebellum and High dose of *A. jesdianum* (400 mg/kg) in the hippocampus significantly increased the survival rate compared to the STZ group.
STZ intraventricular injection induces numerous bioenergetic defects in the rats' hippocampus and cerebral cortex. These defects include a decrease in oxygen uptake, complex respiratory activities, respiratory control ratio, ATP synthesis, and MMP (K Paidi et al. 2015). Increase in ROS production leads to the MMP collapse, the mitochondrial membrane integrity disruption, and ultimately mitochondrial swelling (Behzadfar et al. 2017). In this regard, we found MMP decline and mitochondrial swelling in the whole brain, hippocampus, frontal cortex and cerebellum after treatment with STZ. Our results indicated a significant reduction of STZ-induced mitochondrial swelling and MMP collapse after treatment with A. jesdianum.

Cytochrome c is a heme-containing protein in mitochondria's inner membrane space that is encoded by nuclear DNA. The cytochrome C released level is one of the most key determinants of mitochondrial dysfunction and cell apoptosis (Taghizadeh et al. 2016). A previous report demonstrated that i.c.v.-STZ led to a substantial rise in the levels of the amyloid beta peptide 1–42 (Aβ1–42) and the release of cytochrome c in cerebellar, prefrontal, and hippocampal neurons (C Correia et al. 2013). Altogether, our results demonstrated that STZ was accountable for the considerable removal of cytochrome c from mitochondria. A treatment with A. jesdianum extract was capable of preventing the STZ-induced cytochrome c release from isolated mitochondria in the whole brain, hippocampus, frontal cortex, and cerebellum. This lends credence to the hypothesis that STZ induces apoptosis via mitochondrial permeability transition pore (MPTP) opening and oxidative stress. Our results indicated that, for hippocampus, only the highest dose of A. jesdianum (400 mg/kg) was effective for degradation of STZ-induced cytochrome c release; however, the lower dose of A. jesdianum were also effective for the whole brain, frontal cortex and cerebellum. Therefore, we can conclude that the rate of degradation by STZ is higher in the hippocampus.

In the current investigation, donepezil, an acetylcholinesterase inhibitor, was utilized as a positive control group. According to Saxena et al., both tacrine and donepezil, in addition to inhibiting acetylcholinesterase, may also reduce oxidative stress and produce a considerable improvement in cognitive impairment caused by i.c.v.-STZ (Saxena et al. 2008). In mitochondria isolated from the brains of APP/PS1 transgenic rats deficient in the acetylcholinesterase enzyme, donepezil improves mitochondrial swelling and the decreases the ATP produced by Aβ1–42. It shows that in addition to inhibiting the acetylcholinesterase enzyme, donepezil may be involved in another non-cholinergic mechanism as a neuroprotective to alleviate mitochondria-related disorders (Ye et al. 2015).

We found that donepezil significantly attenuated STZ-induced increases in ROS generation, mitochondrial swelling, MMP collapse, and cytochrome c release in the whole brain, hippocampus, and frontal cortex, it had no such effect in the cerebellum. Donepezil is expected to be most effective on cholinergic neurons that are located in the basal nuclei of the forebrain (including the nuclei of the middle septum and Broca's area in the frontal lobe that extends to the hippocampus and the basalis nucleus of Meinert) and brainstem. Also, small amount of the cholinergic neurons are located in the cerebral cortex and olfactory bulb (McCorry 2007). Our findings support previous research that the cerebellum contains...
relatively low density cholinergic markers (Jaarsma et al. 1997), so it could be the reason for the weak effect of donepezil on the cerebellum in our study.

Various studies have reported that, in AD, miRNAs influence levels of amyloid beta (Aβ), phosphorylated tau, synaptic damage and also biological processes including affect cellular senescence, neuroinflammation and dysfunction of mitochondria (Silvestro et al. 2019). For instance, overexpression of miR-330 in AD decreases β-amyloid aggregation, oxidative stress, and mitochondrial dysfunction via targeting VAV1 gene through the MAPK signaling pathway (Zhou et al. 2018). Our results indicated that the expression level of miR-330 in STZ-induced AD rats is decreased significantly, and higher doses of *A. jesdianum* increases the expression of this miRNA which has beneficial properties for improving AD.

Also, The STZ and *A. jesdianum* effects on miR-132 expression were evaluated in the current research. It has been shown in an AD mouse model that miR-132-3p regulates histone deacetylase 3 (HDAC3), resulting in neuroprotection. Moreover, in the hippocampus, upregulation of miR-132 reduces spatial memory impairment in the MWM test, Aβ1–42 deposition and apoptosis (Zeng et al. 2022). Our results indicated a significant decrease of miR-132 in the AD group, and the higher doses of *A. jesdianum* significantly increased the expression of this miRNA.

Previous studies have shown that upregulation of bcl-2 results in reduction of the cleavage of amyloid precursor protein, tau protein and the amount of extracellular Aβ deposits (Paradis et al. 1996). Lannert et al. reported Bcl2 protein expression was decreased in rats that received STZ intraventricularly (Lannert and Hoyer 1998). Our results demonstrated that STZ significantly decreased Bcl-2 gene expression and *A. jesdianum* extract was able to increase Bcl-2 expression, increasing neuronal survival, and preventing programmed cell death.

Moreover, our study demonstrated that the expression of the Bax, as a proapoptotic gene, increased after STZ treatment, and *A. jesdianum* significantly reduced the expression of this gene and prevented the apoptosis of neurons due to STZ injection. In line with our results, SS Baek et al demonstrated that, in the hippocampus, STZ results in reduction of Bcl-2 expression and increment of Bax and caspase-3 expression, which leads to increase of Bax to Bcl-2 ratio (Baek and Kim 2016).

In conclusion, this study revealed that *A. jesdianum* may prevent STZ-induced cognitive dysfunction in the animal models of Alzheimer's disease through free radical scavenging activity, improvement of mitochondrial function and overexpression of miRNAs as effective agents for treatment of AD. These findings offer new opportunities for drug development against AD.

**Declarations**

**Ethical Approval**

All ethical themes of the studies on animals were considered carefully and the experimental protocol was approved by the Ethical Committee of Shahid Beheshti University of Medical Sciences with the code
number IR.SBMU.PHARMACY.REC.1400.143.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Jalal Pourahmad and Mehrdad Faizi contributed to this research in formulating the research question(s), designing the study, carrying it out as thesis supervisors, analyzing the data, and writing paper. Razieh Pourahmad Jaktaji, Kobra Shirani and Amirhossein Jamshidi contributed to this research in supervising some experiments and analyzing the data and giving scientific advice. Farzaneh Kamranfar contributed to this research in carrying out the experiments and performing statistical analysis and writing the paper as the PhD thesis student.

Funding

This research was financed with grant (4002362) from National Institute for Medical Research Development.

Availability of data and materials

Not applicable

References

biophysica acta (BBA)-bioenergetics 1606(1–3):137–146


Figures
Figure 1

Effect of *A. jesdianum* and donepezil on latency to locate platform (A) and reference memory errors number (B) on training days in the radial arm water maze test. Two-way ANOVA estimated differences. Mean ± SEM (n = 8) are the forms of data that presented; *p<0.05, **p<0.01, ***p<0.001 compared to the STZ group. #p<0.05, ##p<0.01, ###p<0.001, #### p<0.0001 compared to the sham group.
Figure 2

Effect of *A. jesdianum* and donepezil on latency to locate target arm (A), entry frequencies to target arm (B) and duration in target arm (C) in the probe day in the radial arm water maze test. One-way ANOVA estimated differences. Mean ± SEM (n = 8) are the forms of data that presented; *p<0.05, **p<0.01, ***p<0.001 compared to the STZ group. ###p<0.001, #### p<0.0001 compared to the sham group.
Figure 3

The mitochondrial SDH activity, isolated from the whole brain (A), hippocampus (B), frontal cortex (C) and cerebellum (D) of STZ-induced AD rats treated with *Ajedrianum* and donepezil. One-way ANOVA estimated differences. Mean ± SEM (n = 8) are the forms of data that presented; *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 in comparison with the STZ group. #### P<0.0001 in comparison with the sham group.
Figure 4

The mitochondrial ROS levels, isolated from the whole brain (A) hippocampus (B) frontal cortex (C) and cerebellum (D) of STZ-induced AD rats treated with A. jesdianum and donepezil. One-way ANOVA estimated differences. Mean ± SEM (n = 8) are the forms of data that presented; *P<0.05, **P<0.01 and ***P<0.001 compared to the STZ group. ### P<0.001, #### P<0.0001 compared to the sham group.
Figure 5

Mitochondrial membrane potential (MMP) collapse rate, isolated from the whole brain (A), hippocampus (B), frontal cortex (C) and cerebellum (D) of STZ-induced AD rats treated with *A. jesdianum* and donepezil. One-way ANOVA estimated differences. Mean ± SEM (n = 8) are the forms of data that presented; *P<0.05, **P<0.01 and ***P<0.001 compared to the STZ group. . ####P<0.0001 compared to the sham group.
**Figure 6**

The mitochondrial swelling level, isolated from the whole brain (A) hippocampus (B) frontal cortex (C) and cerebellum (D) of STZ-induced AD rats treated with *A. jesdianum* and donepezil. One-way ANOVA estimated differences. Mean ± SEM (n = 8) are the forms of data that presented; *P<0.05, **P<0.01 and ***P<0.001 compared to the STZ group. ###P<0.001, ####P<0.0001 compared to the sham group.
Figure 7

Mitochondrial cytochrome c release rates, isolated from the whole brain (A), hippocampus (B), frontal cortex (C) and cerebellum (D) of STZ-induced AD rats treated with A. jesdianum and donepezil. One-way ANOVA estimated differences. Mean ± SEM (n = 8) are the forms of data that presented; *P<0.05, **P<0.01 and ***P<0.001 compared to the STZ group. ######P<0.001 compared to the sham group.
Figure 8

MiR-132 (A) and miR-330 (B) relative expression in the neurons isolated from the brain that treated with A. Jesdianum and donepezil using qRT-PCR. One-way ANOVA estimated differences. Mean ± SD (n = 3) are the forms of data that presented; ***P<0.001 and ****P<0.0001 compared to the STZ group. #P<0.05, ##P<0.01 compared to the sham group.
Figure 9

Relative Bcl-2 (A) and Bax (B) genes expression in the neurons isolated from the brain that treated with *A. Jesdianum* and donepezil using qRT-PCR. One-way ANOVA estimated differences. Mean ± SD (n = 3) are the forms of data that presented; ***P<0.001 and ****P<0.0001 compared to the STZ group. ###P<0.001, ####P<0.0001 compared to the sham group.