Original article

Sesamin imparts neuroprotection against intrastriatal 6-hydroxydopamine toxicity by inhibition of astroglial activation, apoptosis, and oxidative stress

Tourandokht Baluchnejadmoojarad\textsuperscript{a,b}, Monireh Mansouri\textsuperscript{a}, Jamileh Ghalami\textsuperscript{a}, Zahra Mokhtari\textsuperscript{b}, Mehrdad Roghani\textsuperscript{c,*}

\textsuperscript{a} Dept. Physiology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
\textsuperscript{b} ENT Research Center, Tehran University of Medical Sciences, Tehran, Iran
\textsuperscript{c} Neurophysiology Research Center, Shahed University, Tehran, Iran

\textbf{A R T I C L E  I N F O}

Article history:
Received 11 September 2016
Received in revised form 20 January 2017
Accepted 20 January 2017

Keywords:
Sesamin
Parkinson's disease
6-hydroxydopamine
Apoptosis
Astroglisis
Oxidative stress

\textbf{A B S T R A C T}

Parkinson's disease (PD) is one of the most prevalent neurodegenerative disorders in elders. Sesamin is a lignan compound and the active constituent of sesame oil with antioxidant and anti-inflammatory properties. This study was carried out to explore the mechanisms underlying sesamin effect against unilateral striatal 6-hydroxydopamine (6-OHDA) model of PD. Intrastriatal 6-OHDA-lesioned rats were pretreated with sesamin at doses of 10 or 20 mg/kg/day for one week. Sesamin at a dose of 20 mg/kg attenuated motor impairment in narrow beam test, lowered striatal level of malondialdehyde (MDA) and reactive oxygen species (ROS), improved superoxide dismutase (SOD) activity, lowered striatal caspase 3 activity and \(\alpha\)-synuclein expression, attenuated glial fibrillary acidic protein (GFAP) immunoreactivity, depressed nigral neuronal apoptosis, and prevented damage of dopaminergic neurons using tyrosine hydroxylase (TH) immunohistochemistry. These findings reveal the reversal effect of sesamin in 6-OHDA model of PD via attenuation of apoptosis, astrogliosis, oxidative stress, and down-regulation of \(\alpha\)-synuclein.

\(\text{© 2017 Elsevier Masson SAS. All rights reserved.}\)

1. Introduction

Parkinson's disease (PD) is one of the most prevalent neurodegenerative disorders which affects about 1% of the population over 60 years [1]. PD patients display both motor (muscle rigidity, resting tremor, bradykinesia, and postural imbalance) and non-motor (cognitive dysfuction, depression, pain, and sleep disorders) symptoms [2]. Current therapies for patients with PD include dopamine replacement agents like levodopa and concurrent use of carbidopa to inhibit peripheral degradation of levodopa [3,4] and to apply deep brain stimulation techniques [4]. Although such treatments are rather effective in relieving some PD symptoms, but they are themselves associated with some further complications and they could not slow down PD progression process [5]. In recent years, much attention has been paid to evaluate the efficacy of natural products to develop potent agents with lower side effects as novel neuroprotective agents which could be used at early stages of PD development in order to prevent or delay its later complications [6].

Sesame seeds and oil have long been used as healthy foods to delay aging-associated changes. Sesamin is the principal lignan in sesame oil [7,8]. It is capable to inhibit inflammation [9], counteract oxidative stress [10,11] and to exert neuroprotective effect in various neurotoxic conditions [11–14]. In addition, Lahaië-Collins et al. showed protective effect of sesamin in PC12 dopaminergic cells under 1-methyl-4-phenyl-pyridine-induced oxidative stress [15]. Recently, the effect of post-lesion treatment with sesamin for 28 days on intranigral 6-hydroxydopamine (6-OHDA)-induced dopaminergic neuronal loss was evaluated and it was shown that sesamin could significantly increase the number of tyrosine hydroxylase-immunopositive neurons and enhance the level of neurotransmitters like dopamine and norepinephrine in the substantia nigra and neostriatum of 6-OHDA-lesioned rats [16]. We recently showed that sesamin could attenuate apomorphine-induced rotational asymmetry in 6-OHDA-induced model of PD in rats [17]. However, no studies have yet been done on its effects on astrogliosis, apoptosis, oxidative stress, and \(\alpha\)-synuclein.

\textsuperscript{*} Corresponding authors.

\textit{E-mail addresses: tmojarad@yahoo.com (T. Baluchnejadmoojarad), mroghani@shahed.ac.ir, mehjou@yahoo.com (M. Roghani).}
expression in 6-OHDA model of PD. Therefore, we tried to elucidate the involvement of these processes in the beneficial effect of sesamin in a model of PD in the rat.

2. Material and methods

2.1. Animals

Male adult Wistar rats (205–250 g; n = 65; Pasteur’s Institute, Tehran, Iran) were kept in a temperature-controlled animal house with 12/12 light-dark cycle. All protocols for the use and care of animals were approved by Ethics Committee of Iran University of Medical Sciences (Tehran, Iran) in 2013 as stipulated by NIH. Rats were randomly assigned to 5 groups, i.e. sham, sesamin-treated sham (at a dose of 20 mg/kg), lesion group (6-OHDA) and sesamin-treated 6-OHDA (at doses of 10 or 20 mg/kg). For induction of PD model, the neurotoxin 6-OHDA was microinjected into the left striatum of anesthetized rats (ketamine 80 mg/kg and xylazine 8 mg/kg, i.p.) in stereotaxic apparatus (Stoelting, USA) with coordinates: 3 mm lateral and 0.2 mm anterior to bregma and ventrally 5 mm below the dura [18]. The 6-OHDA group received 5 μl of 0.5% normal saline containing 2.5 μg/μl of 6-OHDA-HCl (SigmaAldrich, USA) and 0.2% ascorbate. The sham group received only ascorbate-saline solution. The sesamin-treated 6-OHDA group received 6-OHDA in addition to sesamin at doses of 10 or 20 mg/kg/day (p.o.) for 1 week until 1 h before the surgery. Dose of sesamin was selected with regard to our previous research on beneficial effect of sesamin in this model of PD via evaluating apomorphine-induced rotational behavior [17] and its attenuation of vascular dysfunction in diabetic rats [19].

2.2. Behavioral assessment

All behavioral experiments were done one week after the surgery (n = 11–12 for each group) and conducted between 10 a.m. and 15 p.m. by a trained observer whom was blind to procedures and treatments.

2.2.1. Elevated narrow beam test

The used protocol for this test has been reported before [20,21]. The narrow beam apparatus was a 105 cm long wooden beam with a width of 4 cm and a height of 3 cm. The beam was placed 80 cm above the ground. The beam at one end had the starting line and its other end was attached to rat home cage. At the start end of the beam, a line was drawn 20 cm from the end of the beam. During a test, the rat was placed entirely within this 20 cm starting zone facing its home cage and a stopwatch started immediately upon release of the animal. The time was recorded when the animal placed a weight bearing step entirely over the start line. This time represented the latency to begin the task. The stopwatch was then stopped when all four feet were placed entirely upon the finishing platform at the opposite end of the beam. The maximum time allowed for the task was 2 min. The start line must be crossed within 1 min from release or the test was cancelled and maximum time was recorded for that trial. A fall was also recorded as a maximum time. A testing session consisted of five trials on the beam, recording five latencies to begin the test, and five total times on the beam for each animal.

2.3. Oxidative stress assessment

Left dorsal striatal tissue (n = 6 for each group) was punched out and 10% homogenate was prepared in cold normal saline and in the presence of protease inhibitor cocktail (SigmaAldrich, USA) and the supernatant was aliquoted and stored at −70 °C until being assayed.

2.3.1. Determination of MDA, nitrite, ROS, SOD activity, GSH, caspase 3, and protein content

MDA concentration in the supernatant was measured as described before [22,23]. Briefly, trichloroacetic acid and thiobarbituric acid reactive substances (TBARS) reagent were added to supernatant, then mixed and incubated at boiling water for 90 min. After cooling on ice, samples were centrifuged at 1000 × g for 10 min and the absorbance of the supernatant was read at 532 nm. The results were obtained on tetraethoxypropane standard curve. For measurement of nitrite concentration, Griess reagent including sulfanilamide and N-naphthyl ethylenediamine was used and the absorbance was read at 540 nm and concentration was calculated on sodium nitrite standard curve [22,24].

ROS level was estimated with a nonfluorescent lipophilic dye, i.e. dichlorofluorescein diacetate, which is cleaved by intracellular esterase enzymes in the presence of ROS into 2,7-dichlorofluorescein that produces fluorescence [25,26]. The fluorescence is known to be directly proportional to the ROS level. Fluorescence was measured at 488 nm excitation and 525 nm emission. A standard curve was conducted using increasing concentrations of dichlorofluorescein incubated in parallel and results were expressed as μg of DCF (as ROS equivalent) formed/mg of protein.

GSH was measured as has been reported before [27–29]. Briefly, the supernatant was centrifuged with 5% trichloroacetic acid for 20 min. The 0.1 ml of homogenate, 2 ml of phosphate buffer (pH 8.4), 0.5 ml of 5′,5′′-dithiobis (2-nitrobenzoic acid) (DTNB) and 0.4 ml of double distilled water was added and the absorbance was read at 412 nm. SOD activity was measured according to earlier reports [30–32]. In this regard, supernatants were mixed with a solution of xanthine and xanthine oxidase in potassium phosphate buffer (pH 7.8, 37 °C) for 40 min, nitroblue tetrazolium was added and changes were monitored at a wavelength of 550 nm using a microplate reader (BioTek, USA). The amount of protein that inhibited NBT reduction to 50% of the maximum was considered as 1 nitrite unit (NU) of SOD activity.

Caspase 3 activity was determined as has been previously reported [33,34]. This assay is constructed on the hydrolysis of the p-nitroaniline (pNA) moiety by caspase-3. In brief, tissue supernatants were incubated in the presence of assay buffer consisting of Hepes, CHAPS, sucrose, EDTA, dithiothreitol, and a chromogenic pNA specific substrate (Caspase 3 apoptopain substrate). The amount of chromogenic pNA released was measured with a microplate reader (BioTek, USA) at 405 nm and the obtained values were expressed as optical density (OD).

The protein content of the samples was determined using Bradford method and bovine serum albumin as the standard [35].

2.4. Glial fibrillary acidic protein (GFAP) and tyrosine hydroxylase (TH) immunohistochemistry

Animals (n = 5 for each group) were anesthetized with ketamine, perfused with normal saline followed by 4% paraformaldehyde, brains were removed, kept at 30% sucrose for 2–3 days and 30 μm-thick midbrain sections prepared using a cryostat for TH and GFAP immunohistochemistry. Sections were washed with phosphate buffer saline (PBS), permeabilized with 0.4% Triton X-100/PBS for 15 min and non-specific staining was blocked by incubation with 10% normal goat serum in PBS for 1 h at room temperature. Then, sections were incubated with rabbit polyclonal anti-GFAP or anti-TH primary antibody (Abcam, USA) at a dilution of 1/500 in a moist atmosphere at room temperature overnight. Thereafter, slides were washed in PBS and incubated for 2 h with goat anti-rabbit antibody conjugated with HRP (Abcam, USA) at a dilution of 1/1000 in PBS. Following several rinses in PBS, slides were incubated with 3,3′-diaminobenzidine (SigmaAldrich, USA) and 0.01% (v/v) H2O2 in PBS for 3–8 min in the darkness. Slides
were then washed, mildly counterstained with 0.1% Cresyl violet for GFAP, dehydrated in a graded series of alcohol, cleared in xylene, coverslipped with Entellan and microscopically analyzed. Evaluation and counting was done blind to the treatments.

For TH immunohistochemistry, mesencephalic sections (interaural 2.9-4.2 mm) were examined by a method as described previously [36]. Briefly, TH-positive neurons of the SNC were manually counted (Light microscopy, ×400) on the left side using a superimposed grid to facilitate the procedure. Counting was done blind to the treatments received. For GFAP immunohistochemistry, density of GFAP immunoreactivity in midbrain sections was calculated using Image J software and reported relative to Sham.

2.5. Determination of DNA fragmentation by TUNEL histochemistry

In order to detect apoptotic cell death in left SNC, a TUNEL assay was performed using the In Situ Cell Death Detection Kit (Roche, Germany) according to a previous study [37]. Briefly, midbrain sections were post-fixed in ethanol-acetic acid (2:1), rinsed, incubated with proteinase K (100 μg/ml), rinsed, incubated in 3% H₂O₂, permeabilized with 0.5% Triton X-100, rinsed again, and incubated in the TUNEL reaction mixture. The sections were rinsed and visualized using a converter-POD and subsequent incubation with DAB (3,3′-diaminobenzidine tetrachloride) and hydrogen peroxide, counterstained with Haematoxylin, coverslipped and evaluated. A dark brown color indicating DNA breaks developed. Positive TUNEL-stained neurons were counted in an area of 0.1 mm² for three sections from each rat and counting was done at least two times for each section and its average was taken as the final value. Counting was done blind to the treatments received.

2.6. α-synuclein immunoblotting

For this purpose (n=4 for each experimental group), proteins were separated by a 10% sodium dodecylsulfate (SDS)-polyacrylamide (PAGE) gel and transferred to polyvinylidene difluoride (PVDF) membrane. The membrane was blocked with 5% skimmed milk in Tris-buffered saline (TBS) and 0.1% Tween 20 for 1 h (at room temperature) and incubated with primary antibody against GFAP (rabbit monoclonal to α-synuclein; Abcam, USA) overnight, then followed by horseradish peroxidase (HRP)-conjugated secondary antibody (goat anti-rabbit IgG coupled to HRP; Abcam, USA). The bands made visible after development with a chemiluminescence reagent (Amersham International, UK) and quantified by densitometry and normalized with respective β-actin as the loading control.

2.7. Statistical analysis

Data were reported as means ± standard error. For statistical evaluation of data, the parametric one-way ANOVA test followed by Tukey’s post-hoc test was used. In all analyses, the null hypothesis was rejected at a level of 0.05.

3. Results

One rat from the 6-OHDA group and one rat from sesamin-pretreated 6-OHDA group at a dose of 10 mg/kg were excluded from the study due to inappropriate injection site in the striatum.

Evaluation of animal performance in narrow beam test (Fig. 1) showed that the latency and the total crossing time were significantly higher in 6-OHDA group versus sham (5.02 and 5.8 fold increases, P<0.01 and P<0.001, respectively) and sesamin at a dose of 20 mg/kg (but not at a dose of 10 mg/kg) significantly reduced these times (decreases of 45.9% and 35.3%, respectively; P<0.05). Additionally, sesamin treatment of sham group at a dose of 20 mg/kg did not affect these behavioral parameters.

Measurement of oxidative stress-related markers (Fig. 2A–E) showed that 6-OHDA-lesioned group has a significantly elevated level of striatal MDA (a 2.04 fold increase; P<0.05) and ROS (a 2.54 fold increase; P<0.05) and lower level of GSH (a decrease of 32.3%; P<0.05) and SOD activity (a decrease of 30.3%; P<0.01) with no significant elevation of nitrite versus sham and sesamin treatment of 6-OHDA group at a dose of 20 mg/kg significantly reversed these changes for MDA (a decrease of 35.3%; P<0.05), ROS (a decrease of 46.4%; P<0.05), and SOD (a 1.32 fold increase; P<0.05) relative to 6-OHDA lesioned group.

Measurement of caspase 3 activity (Fig. 2F) as an important biomarker of apoptotic pathway showed that 6-OHDA lesioned group has a 2.37 fold increase of enzyme activity relative to sham group (P<0.005) and sesamin treatment of 6-OHDA group at a dose of 20 mg/kg significantly reduced this parameter by 32.8% (P<0.01).

Nigral measurement of density of GFAP immunoreactivity as a specific index of astrogliosis (Fig. 3A) revealed that 6-OHDA group has a prominent and higher density of GFAP immunoreactivity as compared to sham (an increase of 74.3%) and sesamin treatment of 6-OHDA group at both doses of 10 and 20 mg/kg significantly attenuated this activity (decreases of 35.6% and 74.3%, P<0.05 and P<0.005, respectively). Determination of apoptosis with counting of TUNEL-positive cells in SNC/unit area (Fig. 3B) indicated that 6-OHDA group has a high number of TUNEL-positive neurons and sesamin treatment of 6-OHDA group at both doses of 10 and 20 mg/kg significantly attenuated this (decreases of 40.5% and 50.1%, respectively, P<0.05).

The results of TH immunohistochemistry indicated a significant reduction (by 58.7%) in TH-positive neurons in SNC of 6-OHDA group versus sham (P<0.01) and sesamin treatment of 6-OHDA group only at a dose of 20 mg/kg was able to significantly prevent neuronal loss (a 1.74 fold increase, P<0.05) (Fig. 4A), Western blot analysis of striatal lysates from different groups showed that expression level of α-synuclein increases in 6-OHDA group relative to sham (a 1.69 fold increase) and treatment of 6-OHDA group with sesamin only at a dose of 20 mg/kg significantly mitigated expression level of this protein (a decrease of 34.3%, P<0.05) (Fig. 4B).
4. Discussion

In this study, sesamin treatment of 6-OHDA-lesioned group at a dose of 20 mg/kg reduced motor imbalance and hypokinesia in hemiparkinsonian rats and protected mesencephalic dopaminergic SNC neurons against 6-OHDA neurotoxicity via mitigation of oxidative stress, astrogliosis and apoptosis.

The specific neurotoxin 6-OHDA is routinely used for induction of experimental PD in rodents like rats through inducing selective degeneration of dopaminergic neurons [38]. This neurotoxin acts chiefly via augmentation of oxidative stress and through inducing mitochondrial respiratory dysfunction and some of its observed effects are the same that develop in brain of parkinsonian patients. Thus, its use for modeling PD has demonstrated high validity [39]. Following 6-OHDA microinjection, it mimics many of the biochemical attributes of PD like lower levels of striatal dopamine and tyrosine hydroxylase. Interestingly, since 6-OHDA could be injected at different locations of the dopaminergic nigrostriatal system (substantia nigra pars compacta, medial forebrain bundle, and/or striatum for mimicking PD, it can be used to induce late (total or subtotal damage of the nigrostriatal system) or early models of PD. In this respect, unilateral intrastratal injection of 6-OHDA generates early-stage model of PD [32,36,40–45], that this protocol was also exploited in our research study. Furthermore, when 6-OHDA is unilaterally injected into the striatum, it can induce dose-dependent damages of nigrostriatal system in the rat [46]. Meanwhile, 6-OHDA-induced model of PD accompanies distinct behavioral alterations that is usually assessed by gait assessment [47,48], apomorphine- or amphetamine-induced rotations [42], elevated body swing test [43,46], and/or narrow beam paradigm [41,49] tests. However, 6-OHDA model of PD does not mimic some of its clinical aspects and for this reason other toxins like 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) are preferred. In addition, other toxins for modeling PD are peripherally injected that are easier to be done than 6-OHDA and the latter warrants stereotactic surgery.

The unilateral damage of the nigrostriatal dopaminergic system following intrastratal injection of 6-OHDA is followed by motor asymmetry in apomorphine-induced rotational test [20,50] and development of imbalance and hypokinesia [20,21]. In our study, evaluation of performance of 6-OHDA-lesioned rats in narrow beam test showed an increase of the latency and the total time on the beam relative to sham, indicating that existing dopamine depletion in the striatum may have resulted in both an increased delay in initiating the task and a lower speed in crossing the beam, which would be indicating bradykinesia and/or akinesia [21] and sesamin treatment at a dose of 20 mg/kg was able to attenuate the observed dysfunction, indicating its potential in protection of dopaminergic neurons and in this way possibly to keep striatal dopamine level at a level that is not concomitant with marked
imbalance. In consistent with our finding, Park et al. have found out that post-lesion administration of sesamin (p.o.) at a dose of 30 mg/kg for 4 weeks could significantly increase the number of tyrosine hydroxylase-immunopositive neuronal cells and the levels of dopamine, norepinephrine, 3,4-dihydroxyphenylacetic acid, and homovanillic acid in the substantia nigra-striatum of 6-OHDA-lesioned rat model of PD with or without L-DOPA treatment [16]. In the latter study, model of PD was induced by injection of 6-OHDA into the SNC while microinjection into the neostriatum and sesamin treatment was started one week before the lesioning till 1 h pre-surgery that mimics earlier stages of PD. In our study, sesamin at a dose of 20 mg/kg significantly prevented reduction of TH-positive neurons of SNC area in 6-OHDA-lesioned rats, clearly indicating that this compound has protected neurons against destructive effect of the neurotoxin 6-OHDA.

Oxidative stress burden is an important and pivotal determinant for the survival and maintenance of dopaminergic neurons in PD. Excessive free radical generation leads to cell death through damaging cellular constituents [51]. On this foundation, free radical scavengers may be helpful in maintenance of dopaminergic neurons [52]. In our research study, sesamin treatment of 6-OHDA group at a dose of 20 mg/kg reversed enhanced level of MDA (as a reliable marker of lipid peroxidation) and ROS relative to 6-OHDA-lesioned group with no significant effect on nitrite level. Furthermore, sesamin at a dose of 20 mg/kg was capable to improve SOD activity in 6-OHDA-lesioned group, in this way augmenting the antioxidant defense system. In our study, sesamin could have attenuated 6-OHDA-induced neuronal insult through counteracting oxidative stress that is consistent with previous reports on its antioxidant ability as well as its capability to mitigate oxidative stress [19,53,54]. Neuroprotective property of sesamin in mouse model of ischemic brain stroke via attenuation of inflammatory mediators, enhancement of antioxidant defense system and lowering oxidative stress has also been reported [10]. In contrast to earlier studies that have shown a significant increase of nigral nitrite level following 6-OHDA [36,40,55–57], we did not have such a significant increase in striatal homogenate in 6-OHDA-lesioned group. One possible explanation is our different site of lysate preparation that was neostriatum instead of the nigral tissue with different cellular elements. In addition, a study by Singh et al. in 2005 has shown that augmented NO availability subsequent to nitric oxide synthase induction plays a significant role in the initial phase of neurodegeneration [58]. For this reason, we did not possibly had a significant enhancement of nitrite level one week after 6-OHDA microinjection.

Inflammation is also known as an important and contributing factor in the pathogenesis of PD [59,60]. Pro-inflammatory cytokines freed from glial cells could stimulate nitric oxide generation and exert a damaging effect on dopaminergic neurons by activating receptors that contain intra-cytoplasmic death domains involved in apoptotic pathway [61]. Sesamin could exert its anti-inflammatory effect [10,62,63] through blockade of the effect of pro-inflammatory mediators [62]. In this respect, there is growing evidence that some cases of PD are associated and related to microglia activation with concomitant elevation of inflammatory mediators and ensuing neuroinflammation [62]. It is possible that sesamin in our study has exerted a neuroprotective effect in 6-OHDA induced PD in rats through lowering the level of inflammatory mediators within the brain. In this regard, Hsieh et al. have shown that sesamin could ameliorate oxidative stress and mortality in kainic acid-induced status epilepticus via
inhibition of inflammatory related to mitogen-activated protein kinase (MAPK) and cyclooxygenase 2 (COX-2) [11]. In association with an inflammatory phenomenon in PD, enhanced GFAP expression has also been observed in 6-OHDA-lesioned rats that is suggestive of and is related to an inflammatory phenomenon in the injured striatum that has also been reported before [64] and sesamin treatment at a dose of 20 mg/kg was able to mitigate astrogliosis, as shown by a lower GFAP immunoreactivity. Thus, part of beneficial effect of sesamin in this respect could be attributed to its anti-inflammatory potential. In this study, although GFAP immunohistochemistry is somehow linked to inflammatory changes in the dopaminergic nigrostriatal system following striatal microinjection of 6-OHDA, however, measurement of some related inflammatory markers could have strengthened our findings and this is strongly recommended in future research works.

α-synuclein is the main component of Lewy bodies and a characteristic marker of PD [65]. Enhanced oxidative stress and concomitant depressed antioxidant system in PD results in excessive accumulation of α-synuclein, which contributes to the onset and progression of the disease [66,67]. In our study, 6-OHDA-lesioned group showed a higher expression level of α-synuclein and sesamin at a dose of 20 mg/kg was able to reduce its expression. Since there is still no report on the effect of sesamin on α-synuclein, it is possible that effect of sesamin may have directly (interaction with the protein) or indirectly (via attenuation of oxidative stress) exerted in this respect. However, more researches are required in this field.

Activation of apoptotic pathway is another important factor following exposure to neurotoxins like 6-OHDA [68]. In our study, sesamin at both doses of 10 and 20 mg/kg was able to reduce number of TUNEL-positive neurons in SNC, indicating a lower apoptosis and DNA fragmentation in 6-OHDA lesioned rats. The neurotoxic 6-OHDA induces cellular neurodegeneration through c-Jun N-terminal kinase (JNK) signaling and endoplasmic reticulum (ER) stress [69]. Sesamin could exert its protective effect via inhibition of oxidative stress-mediated apoptosis in mice via inhibition of caspase-3 and JNK pathway [70]. Additionally, sesamin at a dose of 20 mg/kg was capable to attenuate striatal caspase 3 activity in 6-OHDA-lesioned group that may partly be responsible for a lower apoptosis in our study.

Taken together, our results indicate the reversal beneficial effect of sesamin in 6-OHDA model of PD via attenuation of apoptosis, astrogliosis, and oxidative stress and this may be of potential advantage for neuroprotective therapy of PD at its early stages.

**Conflict of interest**

The authors declare no conflict of interest.

**Acknowledgement**

This study was part of a M.Sc. thesis project that financially supported by a research grant (No. 93-02-30-24693) from Iran University of Medical Sciences (Tehran, Iran).

**References**


