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Original article

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

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ABSTRACT

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 imbalance 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 α-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 α-synuclein.

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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, Lahaie-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 α-synuclein

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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 0.9% 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 constructed 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. To 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 apopain 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) H_2O_2 in PBS for 3–8 min in the darkness. Slides

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