Effect of Sesamin in *Acanthopanax senticosus* Harms on Behavioral Dysfunction in Rotenone-Induced Parkinsonian Rats

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The aim of this study was to determine whether sesamin, a component from *Acanthopanax senticosus* Harms (ASH) pharmacologically offers protection against Parkinson’s disease (PD) and its related depressive behavior in rats administered rotenone. We also examined how sesamin affected the rotenone-induced loss of tyrosine hydroxylase (TH) or glial cell line-derived neurotrophic factor (GDNF)-positive neurons in the midbrain of rats. Rats were orally administered sesamin (3, 30 mg/kg) once a day for 2 weeks before an intraperitoneal injection of rotenone (2.5 mg/kg). The pole test and catalepsy test were used to evaluate the effects of sesamin administration on bradykinesia and depressive behaviors in the PD model of rats given rotenone for 5 weeks. Those effects were compared with the ASH administrated group (250 mg/kg). Treatment with sesamin for seven weeks resulted in prophylactic effects on rotenone-induced parkinsonian bradykinesia and catalepsy, and the effects were equivalent to ASH effects. Immunohistochemical analysis using TH or GDNF antibody showed that sesamin provided cytoprotective effects against rotenone-induced loss of DA cells. The results suggest that it may be possible to use the ASH and sesamin for the prevention of nigral degenerative disorders, e.g., PD with depression, caused by exposure to pesticide or environmental neurotoxins in general.

Key words sesamin; *Acanthopanax senticosus*; rotenone; Parkinson’s disease; bradykinesia; catalepsy

*Acanthopanax senticosus* Harms is known as an adaptogenic medicine, and has been used as a crude drug to treat stress-induced physiological changes, various allergic conditions, inflammation and cancer. An aqueous extract of the stem bark of *A. senticosus* Harms (ASH) from Japan and its components, chlorogenic acid (CHA) and syringaresinol di-o-β-D-glucoside (SYG), markedly prevent the ulcerogenesis in rats subjected to restraint stress in water for 7 h. Among CHA, SYG, syringin and sesamin, components of the n-butanol extract prepared from ASH, sesamin suppressed the growth and induced apoptosis in KATO III cells. A study demonstrates that ASH has a specific activity in the nigrostriatal dopaminergic system and it results in protection against the development of parkinsonian bradykinesia and catalepsy reaction caused by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). However, it is unclear if the direct action of the ASH component, sesamin on the nigrostriatal DAergic system actually contributes to the prevention of Parkinson’s disease (PD) caused by exposure to rotenone. Selective nigral degeneration with inclusion formation with α-synuclein and ubiquitin was provoked by systemic administration of the herbicide rotenone through inhibition of mitochondrial complex I. The result raises the question of pesticide exposure and environmental neurotoxins in general, as a cause of PD. In such much dangerous information, it is equal to there being no means in which the agricultural chemical user protects the their own body as a category of the prevention medical treatment. The administration of exogenous glial cell line-derived neurotrophic factor (GDNF) or the delivery of GDNF via gene therapy can increase the apparent capacity of DA neurons to release their transmitter and protect and even rescue adult DA neuron from injury caused by 6-hydroxydopamine (6-OHDA) and MPTP. However, though this research is good news to the human who becomes PD, it is far from the feasibility still. In short, it is necessary to carry out the effort which does not become the PD even in now.

In this study, we examined whether the ASH and sesamin have a preventive effect on bradykinesia, depressive behavior and the loss of tyrosine hydroxylase (TH) or GDNF-positive neurons in the midbrain of the rats using a PD model induced by rotenone. We herein report that ASH and sesamin act on the midbrain to suppress rotenone-induced depletion in DA cells and consequently prevents the parkinsonian bradykinesia and depressive behavior.

MATERIALS AND METHODS

**Animals** Male rats of the Lewis strain, 5 weeks of age, were purchased from SLC, Inc. (Shizuoka, Japan). Rats were placed in preliminary group-housing (3 to 4 per cage) and were handled daily for 2 weeks; food (CE-2 rat chow, CLEA, Tokyo, Japan) and water were available *ad libitum*. The animals were kept in a room maintained at 23−25°C and 50–60% humidity under a 12-h light/12-h dark cycle of artificial lighting (lights on at 07:10).

The animal facilities and protocols were approved by the Institutional Animal Care and Use Committee, Mie University Faculty of Medicine. All procedures were in accordance with the National Institute of Health’s guidelines regarding the principles of animal care (1996).

**Sample Preparations** The stem bark of ASH from the eastern part of Hokkaido, Japan, was extracted with 100% ethanol, 50% ethanol and hot water. All obtained extracts were combined and evaporated to dryness. The resulting residues were dissolved in distilled water and used as ASH.
extract.

Powdered sesamin which was extracted by Suntory Co., Ltd., (Osaka, Japan) was dissolved with distilled water/olive oil (1 : 3).

Administration of Samples In order to evaluate the effects of ASH and sesamin on PD-related behaviors such as bradykinesia and catalepsy, we dissolved ASH in 0.3 ml of distilled water and orally administered it through a probe once daily to rats at a dose of 250 mg/kg 2 weeks before an intraperitoneal (i.p.) injection of rotenone (2.5 mg/kg). Sesamin (3, 30 mg/kg/d) in 0.3 ml of distilled water/olive oil (1 : 3) and orally administered it once daily to rats 2 weeks before an i.p. injection of rotenone (2.5 mg/kg). Rotenone was administered once daily for 5 weeks. The doses of ASH and sesamin were determined based on results from a recent study.11) The same volume, 0.3 ml, of distilled water/olive oil (1 : 3) was orally administered once daily to rats in the H2O group and it was made to be control for the ASH or sesamin group. Tests on behavior were carried out every morning 30 min after administration of ASH, sesamin, H2O/olive oil or/and rotenone. After the administration of rotenone once daily for 2 weeks, animals were decapitated and their brains were quickly removed, immediately frozen on powdered dry ice, and stored at −80°C until sectioning.

Immunohistochemical Analysis Immunohistochemistry (IHC) was done on 4% paraformaldehyde-fixed, 16-μm-thick frozen sections of the brain from adult male rats of the Lewis strain; procedures described using monoclonal mouse antibody to tyrosine hydroxylase (TH) or GDNF were then performed (TH (1 : 2000): Chemicon, Temecula, CA, U.S.A., GDNF (1 : 100): Santa Cruz Biotechnology Inc., CA, U.S.A.). Sections were sequentially incubated in primary antibody to TH or GDNF diluted in PBS for 24 h, followed by 1 h incubation with biotinylated secondary antibody. Incubations with PBS only were used as negative controls. The avidin biotinylated horseradish peroxidase complex method was used to detect the antigen signal (ABC, Vector Laboratories, Burlingame, CA, U.S.A.); 3,3’-diaminobenzidine tetra-chloride (DAB: Sigma Fast DAB, Sigma, U.S.A.) in the presence of hydrogen peroxide was used to stain the product light brown. Densitometric units per μm2 area and positive cells were measured using image analysis software (NIH Image, 1.61).

Pole and Catalepsy Tests The degree of bradykinesia in the rats was determined using the pole test as described in detail previously.11) The animal was placed head upward near the top of a rough-surfaced wood pole (5 cm in diameter and 100 cm in height), and the time(s) the animal took to turn completely downward and climb down to the floor was recorded. Triplicate pole tests were carried out 30 min after ASH administration and the average values were calculated. The degree of bradykinesia of the rat was also determined. After the pole test, the cataleptic response of the rat was studied by placing the rat in an observation box of 10 cm in height. The duration of abnormal postures was measured as described previously.12) The catalepsy test was ended when the animal’s forelimbs touched the bottom or wall of the box or when the animal climbed onto the bar.

Statistical Analysis Macintosh Super ANOVA was used in the data analysis. Data are shown as mean±S.E.M. The significance of differences between the values was determined by Scheffe/Fisher’s PLSD post-hoc procedure test after evaluating differences among treatment groups by one-way ANOVA; probability (p) values less than 0.05 were taken as significant.

RESULTS

Effect of ASH/Sesamin on Rotenone-Induced Parkinsonian Bradykinesia The times it took to turn downward and to reach the floor in the H2O/olive oil-treated rats with five-week administration of rotenone were about 4.9 and 3.1 times greater, respectively, than those of the H2O/olive oil-treated rats. The seven-week oral administration of ASH at 250 mg/kg to rats suppressed the rotenone-induced bradykinesia. The seven-week oral administration of sesamin at 3 or 30 mg/kg to rats significantly suppressed the parkinsonian bradykinesia caused by rotenone (Figs. 1, 2).

Effect of ASH/Sesamin on Rotenone-Induced Parkinsonian Catalepsy The catalepsy reaction was increased by about 48 times compared to H2O/olive oil-treated rats when rotenone was administered for 4 weeks to the H2O-treatment rat (Fig. 3). The oral administration of ASH at 250 mg/kg for 7 weeks markedly suppressed the rotenone-induced catalepsy reaction (Fig. 3). The oral administration of sesamin at 3 or 30 mg/kg for 7 weeks significantly suppressed the parkinsonian catalepsy caused by rotenone (Fig. 3).

![Fig. 1. Effect of ASH and Sesamin on Rotenone-Induced Bradykinesia 1](image1)

The pole test was conducted 3 times 30 min after ASH administration and the average value was plotted. Each column and vertical line show the mean value and S.E.M. of 8—12 independent determinations. *p<0.05 vs. H2O/olive oil group.

![Fig. 2. Effect of ASH and Sesamin on Rotenone-Induced Bradykinesia 2](image2)

Pole test was conducted on animals as shown in Fig. 1 and the average value was plotted. Each column and vertical line show the mean value and S.E.M. of 8—12 independent determinations. *p<0.05 vs. H2O/olive oil group.
Effect of ASH/Sesamin on Rotenone-Induced Dopaminergic Degeneration in the Substantia Nigra
IHC of TH, the phenotypic marker for dopaminergic neurons, indicated dopaminergic degeneration in the substantia nigra pars lateral (SNl), substantia nigra pars compacta (SNc), substantia nigra pars reticulata (SNr) and ventral tegmental area (VTA) after the five-week administration of rotenone (Fig. 4A). TH-positive cells in the SN and VTA in rotenone-treated rats were decreased by 50% compared to H2O/olive oil-treated rats (Fig. 4B). The prophylactic administration of ASH for 7 weeks prominently suppressed the rotenone-induced dopaminergic degeneration in the SN and VTA (Fig. 4B). Seven-week oral administration of sesamin dose-dependently suppressed the dopaminergic degeneration in the SN and VTA (Fig. 4B). Though in normal rats administered sesamin, the loss tendency of the DA cells was shown in the SN and VTA, the statistically significant difference did not be recognized, while in ASH administration, such declining tendency did not be observed (Fig. 4B).

Effect of ASH/Sesamin on Loss of GDNF-Positive Neurons in the SN in the Rotenone-Treated Rats
IHC of GDNF showed loss of GDNF-positive neurons in the SN and VTA after the five-week administration of rotenone (Fig. 5A).

GDNF-positive cells in the SN and VTA in rotenone-treated rats were decreased by 50% compared to H2O/olive oil-treated rats (Fig. 5B). The oral administration of ASH for 7 weeks prominently suppressed the rotenone-induced loss of GDNF-positive neurons in the SN and VTA in the rotenone-treated rats (Fig. 5B). Dose-dependently prevention against the loss of GDNF in the SN and VTA was observed after the seven-week oral administration of sesamin (Fig. 5B). Though in sesamin administration, the loss tendency of the GDNF-positive cells were shown in the SN, the statistically significant difference did not be recognized (Fig. 5B).

DISCUSSION

We herein report that ASH and its component, sesamin have a specific effect in suppressing the behavioral features associated with PD induced by rotenone, and that the rotenone-induced depletion of DA- or GDNF-positive cells in the SN and VTA after the five-week administration of rotenone (Fig. 5A).

GDNF-positive cells in the SN and VTA in rotenone-treated rats were decreased by 50% compared to H2O/olive oil-treated rats (Fig. 5B). The oral administration of ASH for 7 weeks prominently suppressed the rotenone-induced loss of GDNF-positive neurons in the SN and VTA in the rotenone-treated rats (Fig. 5B). Dose-dependently prevention against the loss of GDNF in the SN and VTA was observed after the seven-week oral administration of sesamin (Fig. 5B). Though in sesamin administration, the loss tendency of the GDNF-positive cells were shown in the SN, the statistically significant difference did not be recognized (Fig. 5B).

Effect of ASH/Sesamin on Rotenone-Induced Loss of DA Cells in the SN
(A) Distribution of TH-positive neurons in SN and VTA. The loss of DA cells in the SNc, SNl, SNr and VTA of rats administered rotenone was shown in H2O/olive oil group. Scale bar=1 mm. (B) TH-positive cell number in the SN. Each column and vertical line show the mean value and S.E.M. of eight independent determinations. *p<0.005 vs. H2O/olive oil group. **p<0.0005 vs. H2O/olive oil group. ***p<0.0001 vs. H2O/olive oil group.

Fig. 4. Effect of ASH and Sesamin on Rotenone-Induced Loss of DA Cells in the SN

Effect of ASH/Sesamin on Rotenone-Induced Dopaminergic Degeneration in the Substantia Nigra
IHC of TH, the phenotypic marker for dopaminergic neurons, indicated dopaminergic degeneration in the substantia nigra pars lateral (SNl), substantia nigra pars compacta (SNc), substantia nigra pars reticulata (SNr) and ventral tegmental area (VTA) after the five-week administration of rotenone (Fig. 4A). TH-positive cells in the SN and VTA in rotenone-treated rats were decreased by 50% compared to H2O/olive oil-treated rats (Fig. 4B). The prophylactic administration of ASH for 7 weeks prominently suppressed the rotenone-induced dopaminergic degeneration in the SN and VTA (Fig. 4B). Seven-week oral administration of sesamin dose-dependently suppressed the dopaminergic degeneration in the SN and VTA (Fig. 4B). Though in normal rats administered sesamin, the loss tendency of the DA cells was shown in the SN and VTA, the statistically significant difference did not be recognized, while in ASH administration, such declining tendency did not be observed (Fig. 4B).

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DISCUSSION

We herein report that ASH and its component, sesamin have a specific effect in suppressing the behavioral features associated with PD induced by rotenone, and that the rotenone-induced depletion of DA- or GDNF-positive cells in the SN and VTA after the five-week administration of rotenone (Fig. 5A).

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rons from MPTP, and improves the cardinal symptoms of parkinsonism: bradykinesia, rigidity, akinesia and postural instability.(14)

In summary, our studies demonstrate that ASH prevents bradykinesia and the catalepsy reaction in the rotenone-induced model of PD. Furthermore, rotenone-induced depletion of TH or GDNF-positive neurons in the SN and VTA was strikingly inhibited by ASH administration to the PD model rats. These results suggest that cytoprotection by sesamin in the SN and VTA during long exposure to a neurotoxin is effective and the sesamin-driven action in the midbrain is important for preventing behavioral dysfunction. This indicates that sesamin which is a component of ASH in the n-butanol extract may be partially related on the preventive effect of ASH on the rotenone-induced parkinsonian behaviors and depletion of DA or GDNF-positive cells and may be useful for the critical prevention of PD in a hostile environment. The mechanism by which ASH and sesamin exhibits an inhibition in the midbrain against rotenone-induced inclusion formation with α-synuclein and ubiquitin is unknown and this intriguing and important question remains unanswered.

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REFERENCES AND NOTES

6) Quantitative determination of the components of the ASH extract by HPLC (condition: apparatus L-6200 (Hitachi); column, TOSO ODS-80Ts (5 μm) 4.6×250 mm; eluent, CH3CN–H2O–HCOOH (50 : 50 : 0.1); flow rate, 0.5 ml/min; detection, UV at 285 nm; room temperature) was carried out and the results are as follows: CHA, 1632.8 μg; SYG, 1370.8 μg; syringin, 531.3 μg; sesamin, 9.6 μg in ASH extract (100 mg).