Benefits of betanin in rotenone-induced Parkinson mice

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Abstract
The present study aimed to investigate betanin’s neuroprotective effect in mice with rotenone-induced Parkinson-like motor dysfunction and neurodegeneration. Forty male ICR mice were divided into 4 groups: Sham-veh, Rot-veh, Rot-Bet100 and Rot-Bet200. Rotenone at 2.5 mg/kg/48 h was subcutaneous injected in Rot groups, and betanin at 100 and 200 mg/kg/48 h were given alternately with the rotenone injections in Bet groups for 6 weeks. Motor dysfunctions were evaluated weekly using hanging wire and rotarod tests. Brain oxidative status including malondialdehyde, reduced glutathione, catalase, superoxide dismutase, with neuronal degeneration in the motor cortex, striatum and substantia nigra par compacta were evaluated. The immunohistochemical densities of tyrosine hydroxylase in striatum and in substantia nigra par compacta were also measured. We found that rotenone significantly decreased the time to fall in a hanging wire test after the 4th week and after the rotarod test at the 6th week (p < 0.05). The percentage of neuronal degeneration in substantia nigra par compacta, striatum and motor cortex significantly increased (p < 0.05), and the tyrosine hydroxylase density in substantia nigra par compacta and in striatum significantly decreased (p < 0.05). Betanin at 100 and 200 mg/kg significantly prevented substantia nigra par compacta, striatum and motor cortex neuronal degeneration (p < 0.05) and maintained tyrosine hydroxylase density in substantia nigra par compacta and in striatum (p < 0.05). These findings appeared concurrently with improved effects on the time to fall in hanging wire and rotarod tests (p < 0.05). Treatment with betanin significantly prevented increased malondialdehyde levels and boosted reduced glutathione, catalase and superoxide dismutase activities (p < 0.05). Betanin exhibits neuroprotective effects against rotenone-induced Parkinson in mice regarding both motor dysfunction and neurodegeneration. Betanin’s neurohealth benefit relates to its powerful antioxidative property. Therefore, betanin use in neurodegenerative disease is interesting to study.

Keywords Betanin · Motor cortex · Motor dysfunction · Parkinson’s disease · Striatum · Substantia nigra par compacta

Introduction

Parkinson’s disease (PD) is the most common neurodegenerative disorders associated with degeneration of dopaminergic neuron in substantia nigra pars compacta (SNc), and loss of dopaminergic projection sending to striatum cause imbalance of motor control (Davie 2008). Loss of dopamine lead to motor dysfunction including rest tremor, bradykinesia, postural instability and rigidity (Guo et al. 2018). Several risk factors such as age, head trauma, heredity and exposure to toxins contribute to the progressive of PD (Zeng et al. 2018). Neurotoxin, rotenone derived from the roots and leaves of Lonchocarpus and Derris is used to reproduce symptoms and neurodegeneration of PD-like. It caused damage specific to the nigrostriatal pathway, especially the dopaminergic neurons in the SNc (Terron et al. 2018). Rotenone also induced striatum damage with the alteration of dopaminergic function (Crutchfield and Dluzen 2006). Because it is highly hydrophobic, rotenone can easily cross the blood brain barrier (BBB). Once it reaches the inside of a neuron, its alteration effect on oxidative mechanism can cause neurodegeneration. This includes the selective inhibition of mitochondria complex I, resulting in mitochondrial dysfunction and rising reactive oxygen species (Terron et al. 2018), therefore, oxidative stress is proposed major pathological mechanism (Inden et al. 2011).
Oxidative stress plays a major role in neurodegenerative disease, and antioxidative substances are part of therapeutic intervention. Betanin, a powerful antioxidant, has been used as natural red food colorant that can prevent lipid oxidation in meats. Betanin has various health benefits, including inhibitory effects on low-density lipoprotein oxidation, inducible nitric oxide synthase, cyclooxygenase and lysosome protease activities (Ahmadi et al. 2020; Allegra et al. 2007; Esatbeyoglu et al. 2015; Indumathi et al. 2018). The powerful antioxidation depends on how it affects the erythroid 2-related factor 2 antioxidant response element, which activates the mRNA and protein expression of antioxidative enzymes, glutathione S-transferase, heamoxygenase-1 and NAD(P)H quinone dehydrogenase 1 (Krajka-Kuzniak et al. 2013). An abrogate effect on the lipid peroxidation process of reducing the malondialdehyde (MDA) level and the myeloperoxidase activity has been reported (Tural et al. 2020). Betanin’s anti-cancer effect as an angiogenesis inhibitor and apoptotic inducer via caspase 3, 7 and 9 activations in human lung cancer cell lines were also reported (Zhang et al. 2013). A multifunctional molecule with powerful antioxidative properties, betanin is interesting as therapeutic intervention for neurodegenerative diseases. Our recent study indicates betanin’s neuroprotective effect against trimethyltin-induce neurodegeneration in mice, which involves various antioxidative properties (Thong-Asa et al. 2020). Interestingly, betanin’s ameliorative effect on oxidative stress-induced apoptotic death in PC 12 cells with 6-OHDA exposed (in vitro PD model) has recently appeared with precise mechanism including SAPK/JNK and PI3 K partial inhibition (Hadipour et al. 2020). It likely that betanin exhibit neuroprotective effect against neurotoxic in in vitro PD model, however, in in vivo PD model is not study yet. Whether betanin exhibit neuroprotection both in in vitro and in vivo for PD model? To elucidate in vivo betanin’s effect on neuronal pathology and behavioral correlation, therefore the present study investigates betanin’s effect in mice with rotenone-induced neurodegeneration and motor dysfunctions.

**Materials and Methods**

**Chemicals and reagents**

Betanin, rotenone and other analytic chemicals and reagents were purchased from Chemical Express Co., Ltd., Merck, Millipore, Germany and Agilent, USA.

**Animals**

Forty male ICR mice, 8 weeks old and weighing 30–50 g, were purchased from the National Laboratory Animal Center at Mahidol University, Salaya, Nakorn Pathom. They were housed in a room with controlled humidity (55%) and temperature (25 °C). They had free access to standard food (No. 082G) and reverse osmosis water.

**Experimental protocol**

The experimental protocol was approved by the Animal Ethics Committee in the Faculty of Science at Kasetsart University (ID#ACKU63-SCI-002). Mice were divided to 4 groups: Sham-veh, Rot-veh, Rot-Bet100 and Rot-Bet200. Mice in the Rot groups received subcutaneous injections of rotenone (Rot) at 2.5 mg/kg/48 h (Rahimmi et al. 2015). Betanin (Bet) at 100 and 200 mg/kg was dissolved into normal saline was given to Rot-Bet100 and Rot-Bet200, respectively. Normal saline also used as vehicle (veh) given to Sham-veh and Rot-veh. Betanin and/or vehicle were given via intra-gastric gavage every 48 h alternately with rotenone injections (24 h after rotenone injection). All treatments were given continuously for 6 weeks. Body weight of each animal was monitored every week.

**Hanging wire test**

Hanging wire test was perform after 24 h of rotenone injection and after 30 min of vehicle or betanin administration. A four-limbs hanging test was used to evaluate muscle strength and balance. A cage lid was used as a hanging grid and positioned 25 cm above soft bedding to protect the mouse when it falls. Each mouse was placed on the grid. When it grabbed the grid with four paws, the grid was inverted and the hanging time began. Each mouse had 120 s maximum with two more tries (three tries total), and the time to fall (sec) was recorded. All mice received training (for baseline) before starting the experiment and were tested once a week for 6 weeks (Aartsma-Rus and van Putten 2014).

**Rotarod test**

Forty-eight hours after hanging wire test, we used a rotarod test to evaluate fore and hind limb motor coordination and balance impairments (Aartsma-Rus and van Putten 2014). Before the rotenone injection, mice were briefly trained in the rotarod at 11–15 rpm until all mice reach a stable performance (for baseline). A weekly rotarod test was delivered until the end of the experiment. For the test, after 30 min of vehicle or betanin administrations, each mouse was placed on a rotating tube at a steady speed of 5 rpm. The speed was increased from 5 to 15 rpm in 15 s, and this speed was maintained for at least 180 s, with two more tries. The running time was collected and expressed as time to fall (sec) for each mouse.
Biochemical analysis

After behavioral tests, mice were euthanized with 180 mg/kg sodium pentothal. They were quickly decapitated and the fresh brains were collected for biochemical analyses. Brain of each animal including cortex, striatum and brain stem were washed in cold normal saline and homogenized as a whole in a 10% w/v phosphate buffer saline (PBS). Half of the homogenate was separated for a malondialdehyde (MDA) assay, and the rest was further centrifuged (10,000 g, 4 °C). Supernatant was collected for total protein evaluation using Lowry’s protein assay (1951), and reduced glutathione (GSH), catalase (CAT) and superoxide dismutase (SOD) were also evaluated (Sakamula and Thong-Asa 2018).

Histological analysis

Brains were processed and embedded in paraffin blocks. Five-micrometer sections were created serially using microtomes. The motor cortex and striatum sections were collected at bregma -0.34 mm, and SNc sections were collected at bregma -3.52 mm (Paxinos and Franklin 2008). Five sections with 125 µM space intervals were collected from each mouse and stained with 0.1% cresyl violet (Thong-Asa et al. 2020). Brain sections were deparaffined and rehydrated via serial changes of xylene and 100%, 95%, 80% and 70% ethanol. They were dipped in distilled water before staining with 0.1% cresyl violet for 30 s. They were dehydrated and cleared in reverse via serial changes of ethanol, xylene and finished by sealing with cover glasses. Brain area of SNc, striatum and motor cortex were capture at 200× magnification via 3 non-overlapping images in each hemisphere. Two investigators counted viable and degenerating cells in a blind fashion using NIH image J. The data were interpreted as the percentage of degeneration using formula % degeneration = 100 x degenerating/[viable + degenerating] (Somrednegan and Thong-Asa 2018).

Tyrosine hydroxylase (TH) immunohistochemistry

Brains were fixed in cold 4% paraformaldehyde for 24 h (4 °C) and then transferred to PBS containing 20% sucrose. When the brains sunk, they were frozen as 20-µm thick sections with cryostat. Five brain sections covering the striatum and SNc area were selected from each mouse with a minimum 100-µm space interval. Brain sections were rinsed in PBS for three 5-min then incubated in 3% H2O2/10% methanol 5–10 min at room temperature (RT). They were washed in PBS for two 5-min intervals and then in the blocking solution (PBS containing 5% goat serum) for 60 min at RT. After a brief rinse in PBS, they were covered by anti-tyrosine hydroxylase (AB152) diluted at 1:500 in carrier solutions (PBS-T containing 2% goat serum). Brain sections were incubated at RT for 3 h before being transferred to a 4 °C environment for a 24 h incubation. The next day, brain sections were rinsed in PBS-T for three 5-min intervals and incubated with biotinylated goat anti-rabbit antibodies diluted at 1:200 (PBS-T containing 2% goat serum) for 60 min at RT. They were washed in PBS-T for 10 min, washed in PBS for two 10-min intervals and then incubated in an ABC solution for 60 min. They were washed in PBS for two 5-min intervals and covered with diaminobenzide for 15 min. After washing in PBS for two 5-min intervals, brain sections were mounted onto cover glasses. Three non-overlapping images of striatum and SNc were capture in each hemisphere. At 100× magnification, TH density was analyzed using NIH Image J and represented as the % TH density related to the % of control (Javed et al. 2016).

Statistical analysis

Animal weights, latency to falls in hanging wire and rotarod tests, biochemical data and the % of neuron degeneration in
motor cortex, striatum, SNc and TH optical density related to % of control were analyzed via a one-way variance analysis, followed by a Fisher’s PLSD post hoc test. Statistical significance was accepted for p-values under 0.05.

Results

Body weights and mortality rate

To verify the unspecific effects of rotenone that may reduce body weight, we measured the mice’s weight from baseline till the experiment’s end. Mice with rotenone injections showed gradual weight loss with no statistical significance (p > 0.05, Fig. 1a). None of the Sham-veh or Rot-Bet100 mice died during the experimental period. The mortality rate of Rot groups stayed below 20%. Two Rot-veh mice died in the 2nd and 3rd weeks, and 1 Rot-Bet200 mouse died in the 4th week of the experiment. All three animals died within 60 min after rotenone injection with seizure-like symptom before death. No sign of infection or other pathology on animal body. Only one sign presented that their weights lower than other animal in their group and without statistic significant.

Motor dysfunctions

Behavioral tests indicated that motor coordination, balance and strength gradually decreased in rotenone-treated mice. Figure 1b indicated early signs of muscle strength and balance impairment for Rot-veh mice in the hanging wire test from the 4th to 6th weeks (p = 0.0016, 0.0077 and 0.025, respectively) compared to Sham-veh mice. The motor coordination and balance in rotarod mice gradually decreased, with a significant difference only in the experiment’s 6th week (p = 0.0042, Fig. 1c). Treatment with betanin at 100 and 200 mg/kg significantly prevented the decline of muscle strength in hanging wire tests from the 4th to 6th weeks (Rot-Bet100 compared to Rot-veh, p = 0.0063, 0.0052 and 0.0071; Rot-Bet200 compared to Rot-veh, p = 0.0399, 0.0471 and 0.0046, respectively; Fig. 1b). Betanin also prevented the decline of motor coordination in rotarod at the 6th week (Rot-Bet100 compared to Rot-veh, p = 0.0105; Rot-Bet200 compared to Rot-veh, p = 0.0037; Fig. 1c).

Brain oxidative status

Rotenone-induced lipid peroxidation was indicated by significantly increased MDA levels (p = 0.0041, Fig. 2a). It significantly decreased CAT and SOD activities (p = 0.0037 and 0.0012, respectively, Fig. 2b, 2c) but not the GSH level (p > 0.05, Fig. 2d) when comparing Rot-veh to Sham-veh. Betanin at 100 and 200 mg/kg significantly decreased MDA levels (p = 0.0013 and 0.0057, respectively; Fig. 2a) and significantly increased CAT activity (p = 0.0427 and 0.0255, respectively, Fig. 2b) and SOD activity (p = 0.0035 and 0.0168, respectively, Fig. 2c) when comparing Rot-Bet100
and Rot-Bet200 to Rot-veh. Rotenone did not change GSH levels, but treatments with betanin significantly increased GSH levels at both 100 and 200 mg/kg (p = 0.0089 and 0.0127, respectively; Fig. 2d).

**Brain histology**

We demonstrated significant neuronal degeneration in SNc, striatum and motor cortex induced via rotenone injection. The % of degeneration of SNc, striatum and motor cortex significantly increased in Rot-veh compared to Sham-veh (p = 0.0006, < 0.0001 and < 0.0001, respectively). Betanin treatment significantly reduced the % of degeneration of SNc, striatum and motor cortex when comparing Rot-Bet100 (p = 0.0044, < 0.0001 and < 0.0001, respectively) and Rot-Bet200 (p = 0.0400, < 0.0001 and 0.0003, respectively) to Rot-veh (Figs. 3, 4, 5). Oddly, Sham-veh presented almost 50% degeneration. This may involve the sensitivity of SNc neuron to vehicle such as 10% DMSO or may be the effect of other factors.

**TH density**

Tyrosine hydroxylase immunological staining density appeared in SNc and in striatum, revealing that rotenone significantly reduced the TH staining density in SNc and in

![Image](https://example.com/image.png)

*Fig. 3* Photomicrographs of substantia nigra par compacta at 200× magnification, including staining with 0.1% cresyl violet for Sham-veh (n = 6), Rot-veh (n = 4), Rot-Bet100 (n = 6) and Rot-Bet200 (n = 5), respectively, and with a 50-µm scale bar. Histograms show the % of neuronal degeneration in SNc. Red arrowhead indicates degenerating cell, green arrowhead indicates viable cell, *indicates a significant difference compared to Sham-veh; #indicates a significant difference compared to Rot-veh.
striatum (p < 0.001 and < 0.0001, respectively; Fig. 6 and 7) compared to Sham-veh. In the group that receive only rotenone injections for 6 weeks found the decrease of TH density about 38 – 40% in both regions. In the groups of rotenone with betanin treatment TH density reduced only about 7 – 12% in striatum and about 20 – 22% in SNc. In SNc, betanin significantly prevented TH density reduction when comparing Rot-Bet100 (p = 0.0041) and Rot-Bet200 (p = 0.0026) to Rot-veh (Fig. 6). It also significantly differs from Sham-veh (Rot-Bet100, p = 0.0010 and Rot-Bet200, p = 0.0034; Fig. 6). In striatum, both betanin doses significantly prevented a decrease of TH density when comparing Rot-Bet100 (p = 0.0003) and Rot-Bet200 (p = 0.0001) to Rot-veh (Fig. 7).

**Discussion**

Our study demonstrated the neurohealth benefits of betanin in ameliorating rotenone-induced neurodegeneration concurrent with Parkinson's-like symptoms in mice. Using 2.5 mg/kg/48 h of rotenone for 6 weeks to reproduce Parkinson's in animal models clearly induced some Parkinson's symptoms in ICR mice. Rotenone significantly reduced muscle strength, motor coordination, and balance, with significant neuronal degeneration in SNc, striatum and motor cortex and reduced TH density in SNc and striatum. Moreover, rotenone significantly changed the brain oxidative status, significantly increased MDA levels, and reduced CAT and SOD.

**Fig. 4** Photomicrographs of striatum at 200× magnification, including staining with 0.1% cresyl violet for Sham-veh (n = 6), Rot-veh (n = 4), Rot-Bet100 (n = 6) and Rot-Bet200 (n = 5), respectively, and with a 50-µm scale bar. Histograms show the % of neuronal degeneration in striatum. Red arrowhead indicates degenerating cell, green arrowhead indicates viable cell, *indicates a significant difference compared to Sham-veh; #indicates a significant difference compared to Rot-veh.
activities. These results confirm rotenone’s involvement via the oxidative stress neurodegenerative pathomechanism.

The PD pathomechanism not fully understood, but is correlated to alteration in the nigrostriatal pathway (Cannon et al. 2009). One major pathogenesis was an increase reactive oxygen species (ROS), which contributed to oxidative damage. Attacking cell macromolecules caused the alteration of mitochondrial function, so ROS subsequently led to neuronal damage (Guo et al. 2018). Rotenone’s alteration of oxidative mechanisms, resulting in neurodegeneration, was relevant to PD regarding the oxidative pathomechanism. It induced mitochondrial dysfunction, including the inhibition of Complex I in the mitochondrial respiratory chain, which led to ATP depletion and ROS leakage. Via the rising ROS, various cascades were activated that led to apoptosis, necroptosis, and necrotic neuronal death (Caliizot et al. 2019). Rotenone also induced neuronal oxidation by increasing MDA and NO levels, which alternated with decreased antioxidants such as CAT, SOD, and GSH (Hasan et al. 2020). When using rotenone-induced PD in rodents (low or high dose), the frequency of rotenone exposure led to differences in timelines and in behavioral and neurological deficits in rats and mice (Richter et al. 2007; Zhang et al. 2017). Rotenone caused weight loss and high mortality rates for doses up to 2.5 mg/kg via daily injection (Zhang et al. 2017). Rahimmi et al. (2015) used 2.5 mg/kg/48 h in rats and confirmed no unspecific effects on body weight, with the benefit of reproducing the motor and neurological deficits within 3 weeks. The ICR mice with rotenone 2.5 mg/kg/48 h used in our present study did not show unspecific

**Fig. 5** Photomicrographs of motor cortex at 200× magnification, including staining with 0.1% cresyl violet for Sham-veh (n = 6), Rot-veh (n = 4), Rot-Bet100 (n = 6) and Rot-Bet200 (n = 5), respectively, and with a 50-µm scale bar. Histograms show the % of neuronal degeneration in motor cortex. Red arrowhead indicates degenerating cell, green arrowhead indicates viable cell, *indicates a significant difference compared to Sham-veh; #indicates a significant difference compared to Rot-veh.
effects that occurred on body weight. This was indicated by our result that animal weights in the Rot-veh group did not significantly differ from those in the Sham-veh group. It also revealed a low mortality rate, unlike in Zhang et al. (2017). Motor deficits appeared from 4 to 6 weeks depending on the tests. Muscle weakness represented via hanging wire tests appeared in the 4th week, and decreased motor coordination and balance in rotarod testing appeared during the 6th week of rotenone injection. The expression of motor deficits in rotarod testing appeared later compared to a rat model with the same frequency and dosage of rotenone exposure (Rahimmi et al. 2015). Differences of animal species in rotenone endurance and behavioral test intensity must be considered (Aartsma-Rus and van Putten 2014).

Rotenone-induced behavioral deficits associated with brain tissue oxidation were indicated via the significant increase of MDA levels and the decrease of CAT and SOD found in our study. In addition, we found significantly increased neuronal degeneration, with TH density reduction in SNc and striatum. These results confirmed rotenone-induced motor and neurological deficits, including oxidative stress, neuronal damage, and alteration of TH density. The degeneration of neuronal cells appeared specifically on the nigrostriatal structure in SNc dopaminergic neurons and in striatum neurons. We also found neurodegeneration beyond the nigrostriatal pathway (e.g., in the motor cortex) that may involve motor dysfunction. This resembles Abdel-Salam’s (2014) report that

**Fig. 6** TH immunohistochemistry in SNc. Photomicrographs of TH staining in SNc captured at 100 × magnification of Sham-veh (n = 6), Rot-veh (n = 4), Rot-Bet100 (n = 6) and Rot-Bet200 (n = 5), respectively, and with a 100-µm scale bar. A histogram shows TH density in SNc related to the % of control (j). *indicates a significant difference compared to Sham-veh; #indicates a significant difference compared to Rot-veh.
rotenone’s effect is not limited to the nigrostriatal pathway but involves various brain areas, including the cortex, hippocampus, striatum, substantia nigra, medulla, and cerebellum. These brain areas may contribute to behavioral deficits as well.

Our study indicated that betanin has a neuroprotective effect against rotenone-induced neuronal degeneration and motor dysfunction. We found betanin’s ameliorative effect is associated with antioxidative properties. Its preventive effect against oxidative stress was indicated by a significant reduction of MDA levels and a boosting effect on CAT, SOD, and GSH. This relates to Tural et al.’s (2020) indication that betanin abrogates the lipid peroxidation process via both the MDA level and via myeloperoxidase activity. It also relates to betanin’s effect on mRNA and on the protein expression of antioxidative enzymes (Krajka-Kuzniak et al. 2013). Hadipour et al. (2020) also recently reported protection against oxidative stress-induced apoptotic death in PC 12 cells exposed to 6-OHDA used as an in vitro PD model associated with SAPK/JNK and partial PI3 K inhibition. We indicated betanin’s protective effect in an in vivo PD model and found the benefit of betanin against neurodegeneration in nigrostriatal structures such as SNC and the striatum, along with dopaminergic neuron preservation (indicated by TH density) in these two areas. In addition to the nigrostriatal structure, motor cortex neurons damaged by rotenone were also protected by betanin administration. Betalain pigment was previously reported as able to cross BBB and to

**Fig. 7** TH immunohistochemistry in striatum. Photomicrographs of TH staining in striatum were captured at 100× magnification of Sham-veh (n=6), Rot-veh (n=4), Rot-Bet100 (n=6) and Rot-Bet200 (n=5), respectively, and with a 100-µm scale bar. A histogram shows TH density in striatum related to the % of control. *indicates a significant difference compared to Sham-veh; #indicates a significant difference compared to Rot-veh.
modulate the bioelectric activity of hippocampal neurons (Gambino et al. 2018; Rahimi et al. 2019). As a chief betanain pigment, this supports the possibility that betanin may also reach and act directly on the brain. Therefore, it can protect against neurotoxins in the brain area; for instance, in the SNc, striatum, and motor cortex. Our recent study (Thong-Asa et al. 2020) also supports this by indicating betanin’s neuroprotective effect against trimethyltin-induced neurodegeneration in mice. We determined betanin’s effects on other brain areas, including the hippocampus, and these correlated with the antioxidative properties of betanin.

Oddly, TH density in SNc was significantly lower than that of Sham-veh in the Rot-Bet100 and Rot-Bet200 groups. It did not rise back to or equal normal levels after exposure to rotenone and betanin. However, it seems sufficient to maintain dopaminergic projection to the striatum and contribute to motor deficit mitigation. TH is a rate-limiting step in the biosynthetic pathway of catecholamines including dopamine, noradrenaline, and adrenaline (Weihe et al. 2006), and the striatum may have a more conjugating circuit of catecholamine neuromodulators. We find that betanin exhibits neuroprotection against cell death in SNc, but has no such effect on TH activation. Therefore, our study can only clarify precise mechanisms to a limited degree—for instance, the effect of betanin on TH levels, especially in the SNc, is unknown. Does betanin benefit against oxidative stress (leading to neuroprotection without TH activation) in the SNc? Further investigation is needed to clarify these questions.

Conclusion

Betanin exhibits neuroprotective effects against rotenone-induced Parkinson’s disease in mice regarding both motor dysfunction and neurodegeneration. Betanin’s neurohealth benefit is related to its powerful antioxidative property.

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Author contributions Wachirayah Thong-asa conceived and designed research, analyzed data and wrote the manuscript. Sujira Jedsadavitaya-kol and Suchawalee Jutarattananon conducted experiments. All authors read and approved the manuscript for publication.

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Data availability Available upon request.

Declarations

Ethics approval “All applicable international and national guidelines for the care and use of animals were followed”.

Consent to participate All authors agree to participate.

Consent for publication All authors agree to publish.

Conflict of interest The authors declare that they have no conflict of interest.

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