Nanostructure Lipid Carriers Enhance Alpha-Mangostin Neuroprotective Efficacy In Mice With Rotenone-Induced Neurodegeneration

Romkase Sakamula

Department of Zoology, Faculty of Science, Kasetsart University

Teerapong Yata

Chulalongkorn University Faculty of Veterinary Science

Wachiriah Thong-asa (✉ fsciwyth@ku.ac.th)

Animal Toxicology and Physiology Specialty Research Unit <https://orcid.org/0000-0003-0065-7339>

Research Article

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Abstract

Neurodegenerative disease, for instance, Parkinson's disease (PD), is associated with substantia nigra dopaminergic neuronal loss with subsequent striatal dopamine reduction, leading to motor deficits. Currently, there is no available effective therapy for PD; thus, novel therapeutic agents such as natural antioxidants with neuroprotective effects are emerging. Alpha-mangostin (α M) is a xanthone derivative compound from mangosteen peel with a cytoprotective effect depicted in neurodegenerative disease models. However, α M has low aqueous solubility and low biodistribution in the brain. Nanostructured lipid carriers (NLC) have been used to encapsulate bioactive compounds delivered to target organs to improve the oral bioavailability and effectiveness. This study aimed to investigate the effect of α M and α M encapsulated in NLC (α M-NLC) in mice with rotenone-induced PD-like neurodegeneration. Forty male ICR mice were divided into normal, PD, PD+ α M, and PD+ α M-NLC groups. Vehicle, α M (25 mg/kg/48 h), and α M-NLC (25 mg/kg/48 h) were orally administered, along with PD induction by intraperitoneal injection of rotenone (2.5 mg/kg/48 h) for 4 consecutive weeks. Motor abilities were assessed once a week using rotarod and hanging wire tests. Biochemical analysis of brain oxidative status was conducted, and neuronal populations in substantia nigra pars compacta (SNc), striatum, and motor cortex were evaluated using Nissl staining. Tyrosine hydroxylase (TH) immunostaining of SNc and striatum was also evaluated. Results showed that rotenone significantly induced motor deficits concurrent with significant SNc, striatum, and motor cortex neuronal reduction and significantly decreased TH intensity in SNc ($p < 0.05$). The significant reduction of brain superoxide dismutase activity ($p < 0.05$) was also detected. Administrations of α M and α M-NLC significantly reduced motor deficits, prevented the reduction of TH intensity in SNc and striatum, and prevented the reduction of neurons in SNc ($p < 0.05$). Only α M-NLC significantly prevented the reduction of neurons in both striatum and motor cortex ($p < 0.05$). These were found concurrent with significantly reduced malondialdehyde level and increased catalase and superoxide dismutase activities ($p < 0.05$). Therefore, this study depicted the neuroprotective effect of α M and α M-NLC against rotenone-induced PD-like neurodegeneration in mice. We indicated an involvement of NLC, emphasizing the protective effect of α M against oxidative stress. Moreover, α M-NLC exhibited broad protection against rotenone-induced neurodegeneration that was not limited to nigrostriatal structures and emphasized the benefit of NLC in enhancing α M neuroprotective effects.

Introduction

Parkinson's disease (PD) is the second most common age-related neurodegenerative disease that constitutes much of the health burden. Not only are genetic factors involved in PD, but exposure to environmental toxins such as pesticides, herbicides, and heavy metals has contributed to the disease development (Ball et al. 2019). The pathology of PD seems to affect mainly the nigrostriatal pathway by the loss of dopaminergic neurons in substantia nigra pars compacta (SNc) and subsequent imbalance of dopamine content in striatum. This leads to motor and non-motor symptoms appearance characterized by tremors, rigidity, bradykinesia, anxiety, and depression (Dauer and Przedborski 2003). The pathomechanisms of PD include excessive production of reactive oxygen species (ROS), oxidative stress,

mitochondrial dysfunction, inflammation, and alpha-synuclein aggregation (DeMaagd and Philip 2015; Parkhe et al. 2019). At present, an effective PD treatment is not available; several treatments help delay the disease's progression or relieve symptoms. Moreover, some drugs have side effects and do not exhibit neuroprotection in patients (Maiti et al. 2017; Mythri and Bharath 2012). As oxidative stress plays a major role in PD progression, novel therapeutic agents with a natural antioxidant base are in focus.

Alpha-mangostin (αM), a xanthone derivative compound isolated from mangosteen pericarp, has a variety of pharmacological benefits, including antioxidant, anticancer, anti-inflammation, antimicrobial, and chemopreventive activities (Ibrahim et al. 2016). According to the pharmacological activities, αM has been used in various neurodegenerative disease models (Do and Cho 2020). In *in vitro* studies, it exhibits a cytoprotective effect in MPP⁺-induced apoptotic cell death and rotenone-induced PD in SH-SY5Y cells, which involve the decrease of ROS formation and mitochondrial membrane potential (MMP), modulation and balance of pro- and anti-apoptotic genes, and caspase-3 suppression (Hao et al. 2017; Janhom and Dharmasaroja 2015). In *in vivo* studies, oral administration of αM was capable of reducing brain proinflammatory cytokines in mice with peripheral LPS-induced neuroinflammation (Nava Catorce et al. 2016). Intraperitoneal injection of αM exhibited a protective effect against rotenone-induced toxicity in rats by protection against neuronal death concurrent with improvement of locomotor activity and memory deficits (Parkhe et al. 2019). Some inconveniences of *in vivo* use of αM were also presented. This is due to its cytotoxicity when accumulated in organs, poor aqueous solubility, low stability, and low biodistribution (Yao et al. 2016).

Many drugs and bioactive substances have some inconveniences in use; therefore, nanoparticle delivery systems were developed to improve the bioavailability and effectiveness of these substances (Wathoni et al. 2020). Nanostructured lipid carriers (NLC) are lipid nanoparticles composed of a solid lipid matrix incorporated with liquid lipid or oil (E Eleraky et al. 2020). The NLC were developed in a millennium and prepared by hot and high-pressure homogenization techniques. Indeed, NLC have advantages over other lipid-based nanoparticles including high drug loading, encapsulation efficiency, and stability (Fang et al. 2012; Yostawonkul et al. 2017). Recently, NLC-encapsulated bioactive compounds used in neurodegenerative disease animal models indicated the prolonging of the drug in the target organ, enhanced drug transport across the blood-brain barrier (BBB), and improved pharmacological profile of bioactive compounds without any adverse effects (Hassanzadeh et al. 2017; Hernando et al. 2020). It is very interesting to use NLC for drug delivery, especially in neurodegenerative diseases. Therefore, the present study aimed to investigate the effect of αM and αM encapsulated in NLC (αM-NLC) in mice with rotenone-induced PD-like neurodegeneration.

Materials And Methods

Chemicals and reagents

Rotenone, dimethyl sulfoxide (DMSO), tween80, hydrogen peroxide (H₂O₂), methanol, phosphate buffered saline (PBS), paraformaldehyde, sucrose, Nissl dye, normal goat serum (NGS), TritonX-100, anti-tyrosine

hydroxylase antibody, anti-rabbit IgG (Goat) biotin conjugate, streptavidin, alkaline phosphatase conjugate, and 3-3'-diaminobenzidine were purchased from Chemical Express Co., Ltd., Merck, Millipore, Germany and Agilent, USA. α M and α M-NLC were provided from the National Nanotechnology Centre (NANOTEC) (Pathum Thani, Thailand).

Animals

Forty male ICR mice (*Mus musculus*) eight weeks old, weighing 40 ± 2 grams, were obtained from the National Laboratory Center, Mahidol University (Nakhon Pathom, Thailand). Mice were housed one per cage at a constant room temperature of 25°C with a 12-hour light/dark cycle. They were fed a standard diet (Mouse diet food NO. 082G) and reverse osmosis (RO) water. The experimental protocol was approved by the Animal Ethics Committee, Faculty of Science, Kasetsart University (ID#ACKU61-Sci-011).

Experimental protocol

Mice were divided into 4 groups as follows: Normal (oral administration of 10% tween80 [vehicle], intraperitoneal injection of 10% DMSO in sunflower oil), PD (oral administration of 10% tween80, intraperitoneal injection of rotenone 2.5 mg/kg dissolved in 10% DMSO in sunflower oil), PD+ α M (oral administration α M 25 mg/kg, intraperitoneal injection of rotenone 2.5 mg/kg dissolved in 10% DMSO in sunflower oil), and PD+ α M-NLC (oral administration α M-NLC 25 mg/kg, intraperitoneal injection of rotenone 2.5 mg/kg dissolved in 10% DMSO in sunflower oil). All administrations were given every 48 h and continuously for 4 weeks. The dose of α M was selected due to being the lowest effective dose provided from a previous study (Kumar et al. 2016), and the dose of rotenone with exposure time followed the previous protocol that claimed not to have an unspecific effect on body weight and mortality, with benefits for induced PD-like neurodegeneration within 3 weeks (Rahimmi et al. 2015).

Hanging wire test

Muscle strength was assessed by a hanging wire test using a standard wire cage lid. All mice were trained for baseline hanging on the cage lid with the maximum time reached of 120 sec or each mouse have equal hanging ability confirmed by statistical insignificance before the start of the experiment. For testing, the mouse was placed on the top of the lid and allowed to grab the lid with all 4 paws and then turned upside down to start the time of the hanging test. The hanging wire test was performed once a week and consisted of 3 trials with 120 sec, and latency to fall was recorded for muscle strength indication (Yeung et al. 2017).

Rotarod test

Motor coordination was evaluated using a rotarod test (rotary rod diameter 5 cm, length 15 cm, raised 15 cm above the bottom of the rotarod enclosure). The rotarod test was performed once a week. Before the actual test, all mice were trained for 5 consecutive days using a rotation speed starting from 10 rounds per minute (rpm) on the first day and reaching 15 rpm at the end of the training, with 300 sec of maximum time. The rotarod test consisted of 3 trials with a 30-min intertrial time for resting. During the

actual test, the animal was placed on a rotating rod, and the speed was gradually increased until it reached 20 rpm. At a fixed speed of 20 rpm, a maximum time of 300 sec was allowed, and the latency to fall was recorded (Zhang et al. 2017).

Biochemical analysis of brain oxidative status

Mice were euthanized with an intraperitoneal injection of sodium pentothal (>60 mg/kg), and quick decapitation was performed. The fresh brains were collected and washed in cold normal saline. They were homogenized in 10% w/v PBS. The homogenates were separated into 2 parts. The 1st part was kept at -20 °C for further evaluation of malondialdehyde (MDA) and reduced glutathione (GSH). The 2nd part was centrifuged at 10,000 g, 4 °C, for 10 min and kept supernatant for further evaluation of total protein, catalase (CAT), and superoxide dismutase (SOD) activities (Sakamula and Thong-Asa 2018).

Nissl staining

Mice were euthanized via intraperitoneal injection of sodium pentothal (>60 mg/kg). Cardiac perfusion with PBS followed by 4% paraformaldehyde was done. Brains were collected and stored in 4% paraformaldehyde at 4 °C for 24 h. After that, brains were processed and embedded in paraffin and sectioned to 5 µm thick with a rotary microtome. Brain sections were cleaned and rehydrated via a serial change of xylene and ethanol 100%, 95%, and 70%, respectively. After 5 min sunk in distilled water, they were stained with Nissl dye (0.1% Cresyl violet) for 30 sec and washed in distilled water, followed by a dehydrating process and finished with cover glass mounted. The images were captured with a light microscope at 200x magnification. The neuronal populations with Nissl staining in SNc, striatum, and motor cortex were evaluated and represented as the percent area of Nissl positive by using NIH Image J (Zhang et al. 2019). The data were presented as the percentage of Nissl positive relative to percentage of control (normal group).

Tyrosine hydroxylase (TH) immunohistochemistry

After being stored at 4 °C in 4% paraformaldehyde for 24 h, brains were moved into 20% sucrose for 48 h at 4 °C. Serial coronal sections (25 µm thick) of striatum (bregma +1.2 mm to +0.25 mm) and SNc (bregma -2.50 mm to -4.24 mm) were cut with cryostat (Churchill et al., 2019). Brain sections were washed in PBS and incubated with 3% H₂O₂ in 10% methanol followed by incubation with 5% NGS in 0.1% TritonX-100 in PBS. The sections were incubated in 1:200 anti-tyrosine hydroxylase antibody in 2% NGS at 4 °C. After 24 h of incubation, the sections were washed with 0.1% TritonX-100 in PBS and incubated with 1:200 anti-rabbit IgG (Goat) biotin conjugate in 2% NGS for 2 h at room temperature. The sections were washed with 0.1% TritonX-100 in PBS and PBS, respectively, followed by incubation with streptavidin, alkaline phosphatase conjugate in PBS for 2 h, and washing with PBS; then, 3-3'-diaminobenzidine was added for 10-15 min. After washing with PBS, brain sections were mounted with cover glass. The images were captured with a light microscope at 100x magnification, and the percentage of TH intensity was analyzed by using NIH Image J and presented as the percentage of TH intensity relative to percentage of control (normal group).

Statistical analysis

Statistical analyses were carried out using the Statistical Package for Social Sciences version 16.0 (SPSS Inc., Chicago, IL). Data were analyzed by one-way analysis of variance (ANOVA) with Fisher's PLSD post hoc test. The data were expressed as mean $\pm$ standard error of mean (SEM). A value of $p < 0.05$ was considered statistically significant.

Results

Body weights

The body weights of all groups were not significantly difference at baseline, the 1st week, the 2nd week, the 3rd week, and the 4th week of the experiment ($p > 0.05$, Fig. 1a). This indicated no unspecific effects of rotenone that could reduce body weight when given every 48 h and continued for 4 weeks.

Rotarod test

At baseline, all mice had equal running ability on the rotarod; after running the experiment, we found that the latency time of the PD group was significantly decreased at the 1st and 4th weeks of the experiment ($p = 0.0024$ and 0.0065 , respectively), but at the 2nd and 3rd weeks, there was no significant difference compared with the normal group. This indicated the reverse of motor deficit in the early weeks of rotenone exposure. Although there was no significant difference between the normal and PD groups at the 2nd and 3rd weeks, the latency time of the PD- α M group was significantly longer than that of the PD group ($p = 0.0136$, 0.0002 , 0.0339 , and < 0.0001 , respectively), and also significantly longer than that of the normal group at the 2nd week ($p = 0.0364$). This was similar to the latency time of the PD- α M-NLC group, which was significantly longer than that of the PD group from the 1st to the 4th week ($p = 0.0065$, 0.0016 , and 0.0087 , respectively), except the 3rd week. Moreover, the effect of α M and α M-NLC showed no significant difference when compared with each other (Fig. 1b). This indicated a preventive effect of α M and α M-NLC on motor coordination deficits in the rotarod test both in early and long-term rotenone exposure.

Hanging wire test

Only during the 4th week did we find that the hanging time of the PD group was significantly decreased compared with the normal group ($p = 0.0044$), and the hanging times of the PD- α M and PD- α M-NLC groups were also significantly longer than that of PD group ($p = 0.0290$ and 0.0108 , respectively). There was no significant difference between the PD- α M and PD- α M-NLC groups compared with each other and with the normal group (Fig. 1c). This indicated that rotenone exposure every 48 h for 4 weeks significantly induced muscle weakness, and both α M and α M-NLC help prevent this weakness.

Brain oxidative status

Biochemical analysis of brain oxidative status is presented in Table 1. Only SOD showed a significant reduction by rotenone exposure compared with the normal group ($p = 0.0078$). Although MDA did not

significantly increase in the PD group, in the PD- α M-NLC group, a significant reduction of MDA level was found when compared with the PD and PD- α M groups ($p = 0.0055$ and 0.0029 , respectively). This indicates a superior benefit of α M-NLC superior than α M in lipid peroxidation mitigation. Moreover, α M-NLC showed a significant effect on antioxidant enzymatic activity boosting, indicated by significantly increased CAT and SOD activities compared with the PD group ($p = 0.0254$ and 0.0158 , respectively).

Table 1
Biochemical analysis of brain oxidative status

Groups	Biochemical analysis				
	Protein (mg/ml)	MDA (μ M/mg protein)	GSH (mM/mg protein)	CAT (U/mg protein)	SOD (U/mg protein)
Normal	0.472 $\pm$ 0.150	4.326	0.988	25.649	18.150
PD	0.497 $\pm$ 0.004	4.845	0.766	11.837	10.193 *
PD + α M	0.498 $\pm$ 0.009	4.942	0.835	18.095	15.106
PD + α M NLC	0.494 $\pm$ 0.010	3.886 #@	0.859	28.471 #	16.854 #
The results are expressed as mean $\pm$ SEM. * indicates $p < 0.05$ compared with the normal group, # indicates $p < 0.05$ compared with the PD group, and @ indicates $p < 0.05$ compared with the PD + α M group.					

Nissl-stained neuronal population

The Nissl-stained neuronal population in SNc (Fig. 2a) revealed that the percentage of Nissl-positive cells relative to the percentage of control in the PD group was significantly less than in the normal, PD- α M, and PD- α M-NLC groups ($p = 0.0138$, < 0.0001 , and < 0.0001 , respectively), and the percentage of Nissl-positive cells in the PD- α M and PD- α M-NLC groups was significantly higher than in the normal group ($p = 0.0373$ and 0.0022 , respectively). There was no significant difference between the PD- α M and PD- α M-NLC groups. These results indicated that both α M and α M-NLC significantly prevented the reduction of the SNc neuronal population.

The Nissl-stained neuronal population in striatum (Fig. 2b) showed that the percentage of Nissl-positive cells in the PD and PD- α M groups was significantly lower than in the normal group ($p = 0.0007$ and 0.0098 , respectively). In the PD- α M-NLC group, the percentage of Nissl-positive cells was significantly higher than in the PD and PD- α M groups ($p = 0.0023$ and 0.0277 , respectively). These results indicated only α M-NLC exhibited a protective effect against striatal neuronal population reduction.

The Nissl-stained neuronal population in motor cortex (Fig. 2c) showed that the percentage of Nissl-positive cells in the PD and PD- α M groups was significantly lower than in the normal group ($p < 0.0001$ and 0.0003 , respectively). In the PD- α M-NLC group, the percentage of Nissl-positive cells was significantly

higher than in the PD and PD- α M groups ($p < 0.0001$ and 0.0004 , respectively). These results were similar to the striatum results that only α M-NLC exhibited a protective effect against motor cortex neuronal population reduction.

TH intensity

In SNc (Fig. 3), the percentage of TH intensity relative to the percentage of control in the PD group was significantly lower than in the normal group ($p = 0.0009$), and the percentage of TH intensity of the PD- α M and PD- α M-NLC groups was significantly higher than in the PD group ($p = 0.0005$ and 0.0081 , respectively) and showed no significant difference compared with the normal group. As TH intensity indicated the dopaminergic neuronal population in SNc, the result indicated that 4 weeks of rotenone exposure significantly reduced dopaminergic neurons in SNc, and α M and α M-NLC significantly prevented rotenone-induced dopaminergic neuronal loss in SNc. In striatum (Fig. 4), although the PD group did not show a significant difference from the normal group, the percentage of TH intensity of the PD- α M and PD- α M-NLC groups was significantly higher than in the PD group ($p = 0.0057$ and 0.0335 , respectively). This result indicated that α M and α M-NLC maintain dopaminergic innervation to striatum.

Discussion

We evaluated the neuroprotective effect of α M and α M-NLC against rotenone-induced PD-like neurodegeneration in mice and indicated the emphasized effect of NLC on α M capacity. The present study used a rotenone-induced neurodegeneration protocol by using a rotenone injection with a dose of 2.5 mg/kg/48 h , which has been claimed to reduce the unspecific effect on body weight, mortality rate, and induced motor deficits within 3 weeks (Rahimmi et al. 2015). Due to the potential neurotoxicity of rotenone, it can cross the BBB, enter the glia cell into the dopaminergic neuron through the dopamine transporter, induce neuronal damage via inhibiting of mitochondrial complex I, and interfere with the mitochondrial electron transport chain, resulting in mitochondria dysfunction. This is followed by excessive ROS production, oxidative stress, and apoptotic cell death (Soderstrom et al. 2010). Previous studies reported that chronic administration of rotenone at low doses in rodents can induce dopaminergic neuronal degeneration, and this was found along with motor deficits (Cannon et al. 2009; Ferris et al. 2013; Sonia Angeline et al. 2012).

In animal models, there were reports that rotenone exposure had variable effects on motor deficits. This is because the sensitivity to rotenone is different in each strain, and differences in the reverse effect were also reported (Fleming et al. 2004; Johnson et al. 2015). Intravenous injection of rotenone (2.5 mg/kg/day) in Lewis rats showed a rearing behavior deficit in the 1st week and a reverse effect a week later (Fleming et al. 2004). Intraperitoneal injection with rotenone (2.5 mg/kg/day) in SD rats significantly decreased motor coordination and muscle strength within 1 day after injection and did not reverse (von Wrangel et al. 2015), and C57BL/6 mice with systemic rotenone exposure (2.5 mg/kg/day) showed significantly decreased motor coordination in the 1st week of the experiment, which reversed in the 2nd week and became significantly decreased in the 3rd and 4th weeks of the experiment (Miyazaki et al.

2020). The present study was the first to apply a rotenone dose of 2.5 mg/kg/48 h in ICR mice and found benefits similar to those in the rat model that was initiated by Rahimmi and colleagues (Rahimmi et al. 2015). Regarding motor deficit in the aspect of muscle strength evaluated by the hanging wire test, the deficit was found at the 4th week of rotenone injection, while the motor coordination in the rotarod test was detected since the 1st week, subsequently reversed at the 2nd and 3rd weeks, and significantly decreased at the 4th week. Although motor coordination was reversed in the middle week of the experiment, at the end of the experiment, it was significantly decreased and correlated with histological results.

Motor impairments are the primary manifestation of PD and associated with SNc dopaminergic neuronal loss (Lee et al. 1996; Mazzoni et al. 2012). This includes the TH loss in SNc, which affects movement frequency and contributes to motor deficits (Salvatore et al. 2019). Neuronal degeneration of SNc led to depletion of dopamine, such as in the projecting terminal at striatum; this further led to alteration of striatal dopaminergic modulation and caused an imbalance of direct and indirect pathways of striatum, which contributes to motor deficits (Balakrishnan et al. 2021). Neuronal population and TH intensity that were observed in the nigrostriatal structure at 4 weeks of rotenone exposure showed significantly reduced SNc and striatum Nissl-stained neurons. Regarding TH intensity, only SNc was found to be significantly reduced, but not the striatum. TH is a rate-limiting step in the biosynthetic pathway of catecholamines including dopamine, noradrenaline, and adrenaline (Weihe et al. 2006); the projecting terminal, such as in the striatum, may have a conjugating circuit of catecholamine neuromodulators and is gradually affected by rotenone injection. The present study did not find significant striatal TH intensity reduction compared with normal, but it also exhibited motor deficits. According to previous studies, chronic exposure to rotenone may affect the expression and activity of striatal TH by increasing TH production in residual dopaminergic neurons (Fleming et al. 2004). In addition, rotenone can damage nondopaminergic striatal neurons and contribute to motor impairment as well (Ferrante et al. 1997; Höglinger et al. 2003). The present study also observed the neuronal population in motor cortex and found clearly reduced Nissl-stained neurons, which indicated that the deterioration effect of rotenone was not limited to the nigrostriatal structure. A report indicated a broad effect of rotenone on various brain areas, including substantia nigra, striatum, medulla, cerebellum, hippocampus, and cerebral cortex (Abdel-Salam O 2014). Therefore, the deterioration effect of rotenone on motor cortex neurons may contribute to motor deficits as well.

The present study found benefits of αM and αM-NLC administration in ICR mice with rotenone-induced PD-like neurodegeneration. Both αM and αM-NLC could prevent the reduction of Nissl-stained neurons in SNc by rotenone. A superior benefit of αM-NLC beyond that of αM was also indicated, and it was a preventive effect on neuronal reduction in striatum and motor cortex. In addition, both αM and αM-NLC equally maintained TH intensity in SNc and striatum concurrently with improvement of motor deficits. A precise difference was in the brain oxidative status in that rotenone induced a change only in SOD activity but not MDA, GSH, or CAT activities, and αM-NLC significantly reduced MDA level with significantly increased CAT and SOD activities, whereas αM exhibited no change in these brain oxidative parameters, compared with neither the normal nor the PD group. SOD plays an important role in

conversion of superoxide anion to hydrogen peroxide, together with CAT, which further converts hydrogen peroxide into water and oxygen that protect against oxidative damage. Excessive ROS production can lead to a variety of deteriorative cascades, one of which lipid peroxidation (LPO), resulting in MDA products. The present study found a significant reduction of MDA level by α M-NLC, concurrent with the increase of SOD and CAT activities. An antioxidative effect of α M in MDA reduction and increased GSH, SOD, and CAT were also reported (Fang et al. 2016; Kumar et al. 2016). However, comparing the efficacy of the lowest effective dose that we used, it is likely that NLC emphasize α M efficacy. Regarding α M and α M-NLC increasing TH intensity, this is related to a previous study indicating that α M upregulated TH protein expression in rotenone-treated SH-SY5Y cells (Hao et al. 2017). We found that both α M and α M-NLC improved motor deficits and correlated with the increase of TH intensity in both SNc and striatum, even though the neuronal population in striatum of α M was not different from that of PD. Our results indicated some amount left of neuronal populations was enough for some functions, and maintenance of TH level had therapeutic importance in PD (Rangasamy et al. 2019). Moreover, NLC helped α M to protect against neuronal death by activation of antioxidative processes contributing to motor deficit mitigation. Therefore, the present study confirmed the effect of NCL on α M efficacy in neuroprotection against rotenone-induced PD-like neurodegeneration in ICR mice.

Conclusion

This study depicted the neuroprotective effect of α M and α M-NLC against rotenone-induced PD-like neurodegeneration in ICR mice. NLC emphasized the protective effect of α M against oxidative stress, with broad protection against rotenone-induced neurodegeneration that was not limited to nigrostriatal structures. We indicated a benefit of NLC in enhancing α M neuroprotective efficacy.

Declarations

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Data availability

Available upon request.

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Author information

Affiliations

Animal Toxicology and Physiology Specialty Research Unit (ATPSRU)

Physiology Division, Department of Zoology, Faculty of

Science, Kasetsart University,

50 Ngamwongwan road, Jatuchak,

Bangkok Bangkok, Thailand, 10900

Contributions

Wachiryah Thong-asa and Romkase Sakamula are equally conceived and designed research, analyzed data and wrote the manuscript. All authors read and approved the manuscript for publication.

Corresponding author

Correspondence to Wachiryah Thong-asa.

Compliance with ethical standards

Ethics 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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Figures

Figure 1

Histogram of body weights (a), the latency time on rotarod (b), and the latency time on hanging wire (c) were recorded at baseline, the 1st week, the 2nd week, the 3rd week, and the 4th week of the experiment. Data are presented as mean $\pm$ SEM. * indicates $p < 0.05$ compared with the normal group, and # indicates $p < 0.05$ compared with the PD group.

Figure 2

Nissl-stained neuronal population in SNc (a), striatum (b), and motor cortex (c). Photomicrographs were captured at 200x magnification with scale bar = 50 μ m. Histograms represent the percentage of Nissl-positive cells related to the percentage of control in each area. Data are presented as mean $\pm$ SEM. * indicates $p < 0.05$ compared with the normal group, # indicates $p < 0.05$ compared with the PD group, and @ indicates $p < 0.05$ compared with the PD+ α M group.

Figure 3

Representative binary photomicrograph of SNc after manual adjusting of threshold in NIH Image J (a). Histogram of the percentage of TH intensity in SNc is presented as area percentage relative to percentage of control, and data are presented as mean $\pm$ SEM (b). * indicates $p < 0.05$ compared with the normal group, and # indicates $p < 0.05$ compared with the PD group.

Figure 4

Representative binary photomicrograph of striatum after manual adjusting of threshold in NIH Image J (a). Histogram of the percentage of TH intensity in striatum is presented as area percentage relative to percentage of control, and data are presented as mean $\pm$ SEM (b). # indicates $p < 0.05$ compared with the PD group.