

Fucoxanthin Attenuates Behavior Deficits and Neuroinflammatory Response in 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine-Induced Parkinson's Disease in Mice

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ABSTRACT

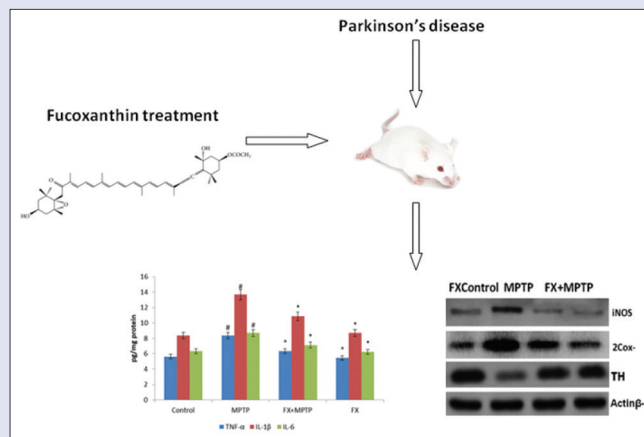
Background: Parkinson's disease (PD) is one of the foremost neurological disorders which is differentiated next to the progressive dopamine (DA) loss, especially in the area of substantia nigra pars compacta (SNpc). Aberrant neuroinflammation, as well as excessive reactive oxygen species (ROS) generation, have exposed to stimulate neuronal defeat in the gradual developing PD. The current study investigated whether fucoxanthin could attenuate the pathophysiology seen in an 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-mediated PD model.

Materials and Methods: C57BL/6 mice received 30 mg/kg of MPTP (i.p.) every day for 5 consecutive days to establish PD, subsequently everyday treatment with fucoxanthin intended for 7 days. Intraperitoneal injection of MPTP, resulted in impaired motor functions, dopaminergic neuronal loss, decreased DA, Tyrosine hydroxylase (TH) levels, and microglial activation-mediated neuroinflammation. **Results:** Fucoxanthin administration ameliorated α -synuclein abnormal accumulation and motor impairment in MPTP-induced chronic PD mouse model. Our observed findings revealed that fucoxanthin administration reverses the MPTP-mediated decline of DA neuron, TH along with microglial activation predominantly in the SNpc region. The Western blot analysis exposed that fucoxanthin suppressed the expression patterns of proinflammatory cytokines following MPTP administration. **Conclusion:** In conclusion, Fucoxanthin exerts neuroprotective potential in opposition to MPTP-mediated PD mice through repressing α -synuclein expression, oxidative stress and gliosis, recommended that fucoxanthin might perform as a beneficial remedy toward PD amelioration.

Key words: Dopamine, fucoxanthin, inflammation, neuroprotective, Parkinson disease

SUMMARY

- Fucoxanthin administration ameliorated α -synuclein abnormal accumulation and motor impairment
- Fucoxanthin suppressed the expression patterns of proinflammatory cytokines following MPTP administration.



Abbreviations used: PD: Parkinson's disease; DA: Dopamine; SNpc: Substantia nigra pars compacta; MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; ROS: Reactive oxygen species; ELISA: Enzyme linked immuno-sorbant assay.

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INTRODUCTION

Parkinson's disease (PD), a second leading long-term motor disease which affects about 3% population >65 years of age, differentiated by means of discriminating dopamine (DA) neuronal loss accompanied by a motor function associated disorders, including bradykinesia, tremors, rigidity, postural instability, and akinesia.^[1] Besides the impairment of motor function, cardiovascular dysfunction, vision problems, emotional, and cognitive disorders were observed in the later stage.^[2] It is predictable to affect the people around 8.7 million globally next to 2030 and nearly 1.2 million American peoples. A diverse range of factors are implicated within the beginning and upgrading of PD pathogenesis.^[3] However, it is exactly that pathological process has been not entirely elucidated till now. The primary characteristic feature of PD is a typical DA neuronal defeat (over 80%) within the region of substantia nigra pars compacta

(SNpc).^[4] There are enormous mechanisms claimed to be caused PD pathophysiology includes enhanced glutamatergic transmission, neuroinflammation, DA neuronal differentiation, alpha-synucleinopathy, and ubiquitin-proteasome degradation.^[5] The neuronal deterioration

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process resultant to worsening of non-motor indication such as sleep disturbances, cognitive decline, and depression.^[6]

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is used toward initiate PD symptoms, which acquire an elevated resemblance to PD affected human patients.^[7] MPTP-mediated neurological intoxication is the frequently designed model for PD.^[8] MPTP administration injured the DA neuronal systems in the area of striatum and SNpc.^[9] MPTP administration in *in vivo* model recapitulates abundant characteristics of PD, together with neuronal cell fatality, neuroinflammation as well as oxidative stress, act as an ultimate screening model for treatment.^[10] At the current instance, there is no ideal therapy for PD pathogenesis.^[11] For PD, current therapies offered symptomatic treatment alone, but do not reverse/prevent the progressive DA neuronal loss in PD patients and concomitant decline.^[12] Although, this method could not arrest or delay the underlying process in PD pathology. Consequently, it is extremely suggested to find beneficial drugs that efficiently alleviate PD pathogenesis.^[13] Thus, novel invention of beneficial therapeutic agents, center of attention on neuroprotection to inhibit the progression of PD pathogenesis is urgently needed.

Fucoxanthin, abundant carotenoids, predictably about 10% of the entire production of carotenoids from natural sources. In the algae, it acts as the most significant light-harvesting molecule which transmits energy to chlorophyll protein complex. It exerts efficient high energy transfer that is considered to be associated with the inimitable carotenoid structure.^[14] In recent times, fucoxanthin, along with its derivatives exerts enormous advantageous health effects includes cardioprotective activity,^[15] anticancer,^[16] antihypertensive, anti-inflammatory, antioxidant,^[17] antiobesity,^[18] and antidiabetic effects.^[19,20] Previously, it was found that fucoxanthin reverses the neurotoxicity mediated through A β 1-42 in the region of cerebral cortex.^[21] However, whether fucoxanthin has any protective effects on PD is still remains unidentified. Here, in this investigation, researchers intended to examine therapeutic efficiency of fucoxanthin for the treatment of MPTP-mediated chronic PD model with the possible underlying mechanisms.

MATERIALS AND METHODS

Animals

These experiments were carried out on male C57BL/6 mice (8–9 weeks old, weighing 25–30 g) were acquired from the breeding center (NCTR). They were maintained under typical environmental circumstances includes temperature (22°C \pm 1°C), light (between 06:00 and 18:00 h), *ad libitum* admittance to water as well as irradiated NIH-41chow. This experimental procedure was permitted through the ethical committee. A chronic PD model with rigorous neurodegeneration on 5 days schedule pattern was employed as previously reported investigation. C57BL/6 mice were segregated at random into four different groups ($n = 6$). The Group I mice served as control. Group II mice received MPTP (i.p injections) at 30 mg/kg b.wt everyday over 5 successive days. Group III mice received fucoxanthin (10 mg/kg b.wt.) everyday over 2 weeks followed by MPTP induction 9 days later/from the 10th day onward. Group III C57BL/6 mice received fucoxanthin (10 mg/kg b.wt.)^[22] alone daily for 2 weeks. After 14 days, C57BL/6 mice were humanly anesthetized and then were evaluated for the affirmation of PD by behavioral test.

Rotarod test

Rotarod test is used for the evaluation of motor action in C57BL/6 mice.^[23] This test was employed on revolving rod, in which experimental mice were positioned contrary direction for rotating. Earlier than this test, all the C57BL/6 mice undertaken 2 days series to attain a steady presentation. Later, this test was performed by initiate acceleration lying

on the day previous to scarification. The revolving speed ranging from 10 mph, enhanced up to 50 mph. Then, chronometer initiated while the mice were situated at the rod. Finally, the declining times of rolling rod of mice were measured.

Footprint analysis

Footprint studies were carried out as previously illustrated.^[24] The day before, mice were skilled in a dark tunnel. To conduct the test, mice's forelimbs were immersed in blue ink as well the hindlimbs in red ink. Further the mice were placed at the entrance of a dark tunnel. The footprints were determined on a clear white paper positioned on the tunnel floor. The two preliminary steps were expelled from the determinations, and only steps carried out in a straight line were measured. To avoid diversity in the stride length as a consequence of velocity variations, footprints were alone determined while the mice walked by the side of the tunnel with a standard velocity, exclude the mice that carried out the test with detectable velocity alteration. The length of the stride was evaluated by quantifying the distance between every step on the equal side of the body. In experiments, mice were treated with MPTP and fucoxanthin (10 mg/kg) earlier than carrying out the footprint test. The upright grid test was carried out as earlier described report,^[25] determining both the time to turn and entire time.

Grip test

This test was carried out to assess the muscle's strength (skeletal) at C57BL/6 mice.^[26] The grip strength's apparatus is consist of wire grid linked into an isometric pressure transducer. C57BL/6 mice were detained through tails, which is permitted to clutch grid with the help of forepaws. Then, C57BL/6 mice were softly pulled toward the back through tail till the grid was free. The mean force was exerted by mouse earlier than grip lost was measured. The mean of 10 dimensions for every mouse was recorded, and then mean force was calculated. The muscle strengths were recorded and also expressed as grams force.

Western blot

Brain Tissues of C57BL/6 mice were isolated followed by homogenized by using ice-cold RIPA buffer. The supernatants were collected, thereby protein concentrations were evaluated by Nanodrop (Thermo Scientific). A total of 50 μ g proteins were separated with the help of SDS-PAGE electrophoresis (10%) and transferred to nitrocellulose membrane (0.45 mm). Membranes were incubated with tumor necrosis factor-alpha (TNF- α), interleukin (IL)-6, inducible nitric oxide synthase (iNOS) IL-1 β , and Cox-2, β -actin primary antibodies. Further the membranes were incubated with 2°C antibody. Membranes were quantitatively measured using chemiluminescent detecting system (Biorad).

RESULTS

Fucoxanthin improved the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine induced behavior abnormalities

To asses MPTP-mediated motor coordination impairment, mice were subjected to RRT performance at the range of 5, 10, and 15 rpm (time spent on rotating rod). The average mean time taken from the mice toward drop in each group is plotted in opposition to different rpm as shown in Figure 1 showed decreased retention time in MPTP alone received mice when compared with control mice. Fucoxanthin administered with MPTP induced mice exhibited drastically enhanced retention time, which is further pronounced by 10 mg/kg b.wt.

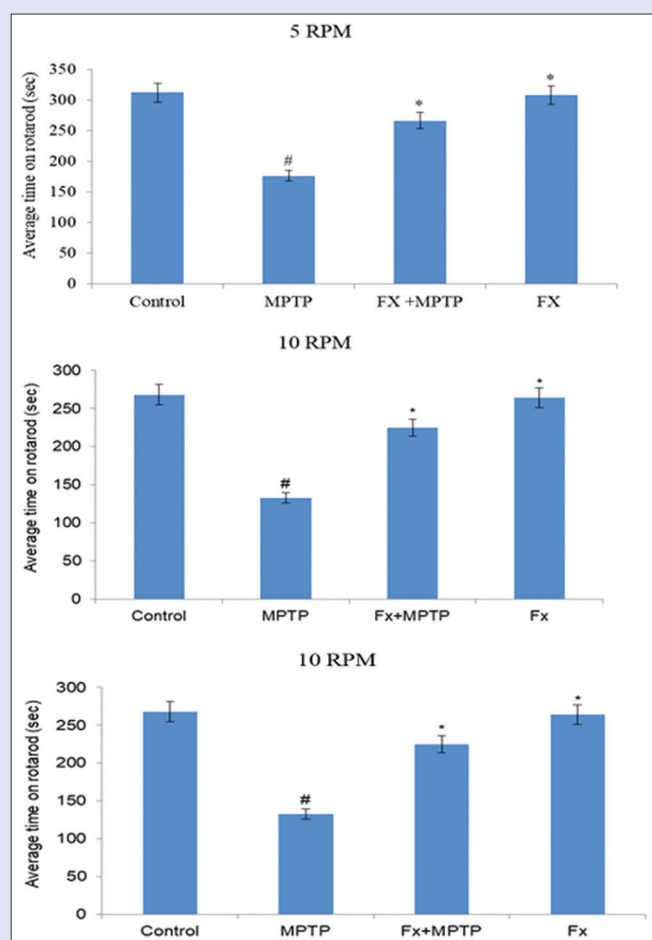


Figure 1: Effect of fucoxanthin on retention time on the rotarod apparatus at 5, 10, 15 rpm after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine administration. [#],^{*}differ significantly at $P < 0.05$

Grip test was used to determine the highest strength of muscle's forelimbs, jointed forelimbs as well as hind limbs as a 1°C phenotypic screening. As revealed in figure, the mean average score of grip test in MPTP induced mice exhibited considerable decrease in motor strength when compared with untreated control mice. Fucoxanthin coadministration considerably enhanced the motor strength as confirmed through acquired average score. Fucoxanthin coadministration (10 mg/kg b.wt.) drastically scored the maximum preventive effect [Figure 2]. MPTP injected mice exhibited considerable attenuation of DA. Fucoxanthin treatment enhanced the striatal DA level in MPTP-induced PD mice than fucoxanthin untreated PD mice.[Figure 3].

Our analysis showed that MPTP treatment significantly increases expressions of TNF- α , IL-1 β , and IL-6 mRNA as shown in Figure 4. Further, fucoxanthin prevents the expression levels of TNF- α , IL-1 β , and IL-6 increased by MPTP. We did not observe any changes among control and fucoxanthin alone treated mice.

Fucoxanthin restored the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine induced protein expressions

After finishing the treatment, we observed the protein expression of TH was examined via immune-blotting, and then it was also measured quantitatively by means of β -actin as an interior level of protein control. MPTP induction noticeably reduced the nigral TH protein levels than that of control mice. The standardized results exhibited as fucoxanthin

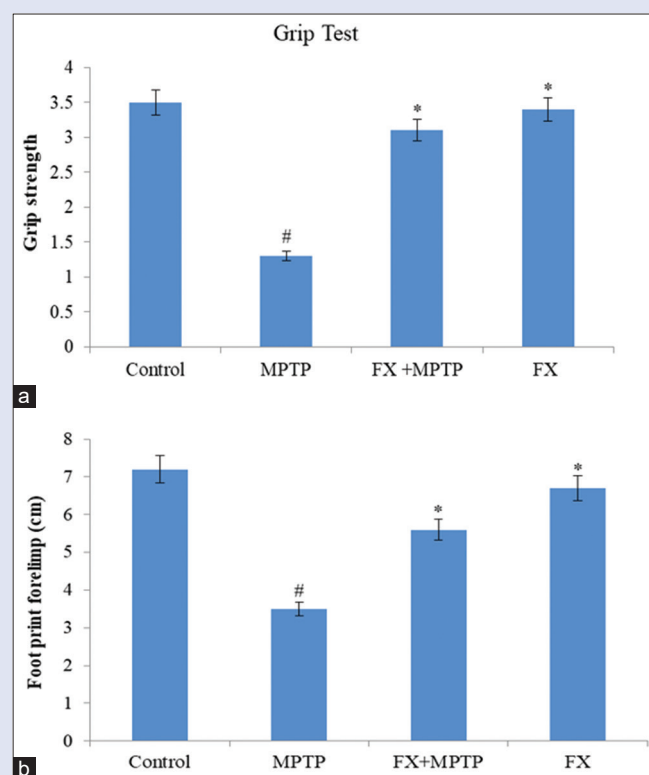


Figure 2: (a) Grip test performance in experimental mice: Values are given as mean \pm standard deviation for six mice in each group. Error bars sharing common symbol [#],^{*}differ significantly at $P < 0.05$. (b) Footprint examination of fucoxanthin and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated mice ($n = 6$) showing the higher stride variability for the forelimbs in the parkinsonian 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mice

coadministration reinstated the level of TH in MPTP-induced mice. Fucoxanthin (10 mg/kg b.wt.) alone administration exhibited no considerable changes than control mice [Figure 5].

In addition, Western blot analysis showed that MPTP treatment significantly increases in Cox-2 and iNOS protein expressions as shown in Figure 5. Fucoxanthin prevents Cox-2 and iNOS protein expression levels. No significant changes were observed between control and fucoxanthin alone treated mice.

DISCUSSION

MPTP actively metabolized in the BBB, here it gets converted to MPP⁺ through monoamine oxidase-B, after that, it released in the extracellular space.^[27] DAT draft MPP⁺ in DA neurons and gets settled down in mitochondrial space and damage ATP (adenosine triphosphate) generation process by the way of blocking complex I and flow of electrons into the respiratory chain.^[28] Our research revealed that the neuropreventive potential of fucoxanthin in opposition to MPTP intoxication mediated Parkinsonism in a mouse model. The application of a broad series of motor assessment permitted for collecting information on a range of motor associated parameters in MPTP-induced C57BL/6 mice includes akinesia and muscle's strength, bradykinesia, gait patterns as well as synchronized motor actions in exercise-driven or liberally moving circumstances.

This motor associated test exhibited that MPTP-mediated bi-lateral partial striatal DA terminal lesions were related through enhancement of reaction time and immobility, a decline of stepping action, muscle

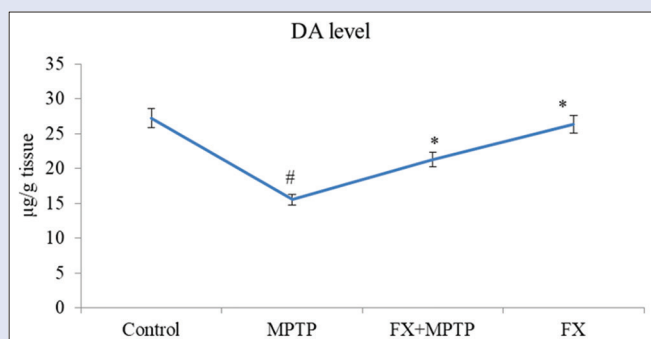


Figure 3: 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced depletion of dopamine conserved by fucoxanthin treatment. Administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine depleted the levels of dopamine attenuated by treatment of fucoxanthin. Results given are mean \pm standard deviation, ($n = 6$), data are shown as mean \pm standard error of mean. [#],^{*}differ significantly at $P < 0.05$

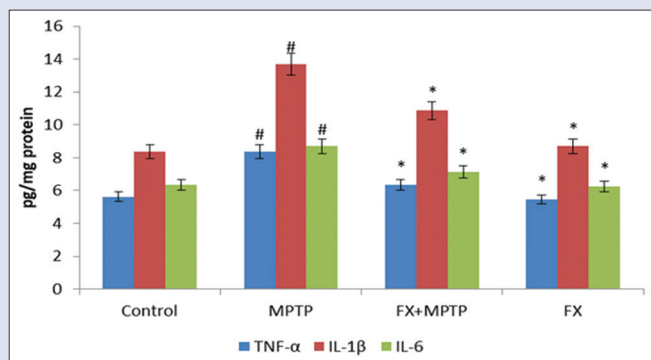


Figure 4: Effect of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and fucoxanthin on tumor necrosis factor α , interleukin-1 β , interleukin-6 in the mice brain. The bar graph shows the levels of interleukin-1 β , interleukin-6, tumor necrosis factor α . Data are shown as mean \pm standard error of mean. [#],^{*}differ significantly at $P < 0.05$

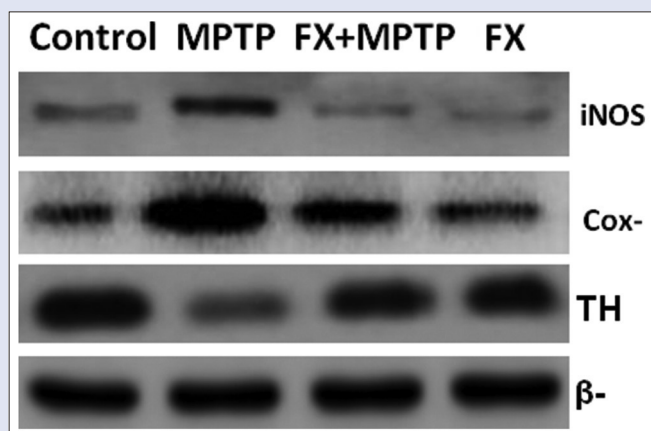


Figure 5: Effect of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and Fucoxanthin on inducible nitric oxide synthase, Cox-2 and TH in the mice brain. The bar graph shows the density normalized to the β -actin loading control for inducible nitric oxide synthase, Cox-2 and TH

with the previous reports stated that loss of DA cells is maximal at 2–7 days subsequent to the induction of MPTP, motor decline was optimum the day followed by MPTP induction and subside 3–4 days afterward, being still detectable a week afterward.^[30,31] The facts of a Parkinsonian related phenotype at 1 week subsequent to the intoxication was confirmed by the positive reaction toward DA agonists.^[32] In our results, MPTP caused altered variations drastically in the motor function were confirmed by the behavioral tests.

DA neuronal loss in the SN is the most significant hallmark of PD.^[33] In the brain, the Nigro-striatal pathway is mainly implicated in the DA neuronal production, an essential neurotransmitter in charge of movement and balance.^[34] In addition, TH is a fundamental rate-limiting enzyme, plays a main function in the rate of catecholamines synthesis and release.^[35] Here, in this investigation, MPTP administered mice show a remarkable decline in the DA neuronal cell number as well as TH expression levels. However, fucoxanthin treatment diminished the loss of DA neurons and also it rescues the decrease in TH expression mediated by MPTP. The above findings mentioned that fucoxanthin prevents MPTP-mediated decreased DA levels and also their metabolites defending the DA neurons and retrieved the declined TH expression in MPTP-mediated PD mice.

Neuroinflammation is principally mediated by microglial cell activation, and the accumulation of stimulated microglial cells has investigated in preceding reports of PD model.^[36] MPTP, a well-recognized DA neurotoxin which can be perceptively damage the DA neurons.^[37] MPTP can go through the BBB where it's transformed into MPP⁺, then selectively converted into DA neurons via DA transporter.^[38,39] In of electron transport chain, MPTP induction blocks the mitochondrial complex I, leads to excessive reactive oxygen species (ROS) production.^[40] Earlier research findings suggested that MPTP-mediated neuroinflammation by which it causes the production of inflammatory cytokine mediators by ROS, ultimately resultant to cellular death.^[41] Hence, inhibition of ROS overproduction might exist essential for protection of the cascade dependent neuroinflammation as well as neurodegeneration.^[42]

Recently, activated microglial induced neuroinflammation appeared as the main role in PD pathogenesis.^[43] Microglial activation resultant into NF- κ B nuclear translocation increases the proinflammatory cytokine marker release in PD.^[44] During the process of neuroinflammation, reactive nitric oxide (NO) and ROS could stimulate the redox susceptible transcription factors, nuclear factor- κ B (NF- κ B) which exerts a significant role in the various proinflammatory mediator regulation which are mainly implicated in the development of neuroinflammation.^[45] NF- κ B (inactive form) is located in the cytosol in relationship with I κ B (an inhibitory protein), thereby it averts the nuclear translocation essential for transcriptional action.^[46] Followed by proteolytic degradation of I κ B, phosphorylation resultant in NF- κ B translocation into the nucleus, then it attached to the target DNA which in turn regulates transcription of various proinflammatory cytokines genes include TNF- α , Cox-2, IL-1 β , IL-6, iNOS, and adhesion molecules.^[47] Moreover, mitochondrial function is potentially inhibited by MPTP in PD pathogenesis.^[48] Due to the inhibition of mitochondrial enzyme activities, where enhanced superoxide anions generations which resultant into upregulate the NF- κ B activation and its related inflammatory cascade.

Depending on these findings, it is possible that the therapeutic drug has the capability to avert ROS production that might have protected the DA neurons from neurotoxin. Current investigations stated that fucoxanthin administration significantly inhibited the NF- κ B activation through attenuation of ROS production, in turn, activates the releasing proinflammatory cytokines from the activated microglial, also induces iNOS expression in response to MPTP-mediated neuroinflammation. Microglial activation is accompanied by the iNOS upregulation, might

strength, time on the rod, climbing speed, in addition to gait aberration comprises increased stride width, reduced stride length.^[29] Consistent

have an important role in PD. Especially, neuroinflammatory processes allied with enhanced expression of COX-2 and prostaglandin has been concerned in the cascade of deadly events consequentially leads to neurodegeneration.^[49] Previous investigational reports exhibited that COX-2 inhibitors and nonsteroid anti-inflammatory drugs may diminish the prevalence of PD.^[50] Our current findings demonstrated that fucoxanthin exercises its anti-inflammatory action by suppressing the expression of NF- κ B and the consequent release of iNOS, Cox-2, TNF- α , IL-6, and IL-1 β .

Therefore, effective drugs allied with antioxidative and anti-inflammatory properties are recommended to be a potential therapeutic molecule for the neurodegenerative disorder.^[51] Naturally, derivative compounds include flavonoids are exceptionally scavenges free radical, anti-inflammatory, neuroprotective in demyelinating, and neurodegenerative disorder.^[52] Yu *et al.* (2017)^[53] confirmed that fucoxanthin exhibits plentiful neuroprotective properties, but with diverse effectiveness in a variety of bioassay testing various defense mechanisms. At less concentration of fucoxanthin established potential neuroprotective and neurite-enhancing against A β 1 than astaxanthin.^[54]

CONCLUSION

MPTP, an exceedingly lipid-soluble molecule which can be readily cross the BBB, that leads to a sensitive dopaminergic neuronal cell damage in the SNpc region cause PD-like neuroinflammation and pathophysiological alteration in experimental mice. MPTP administration resulted in impaired motor functions, dopaminergic neuronal loss, decreased DA, TH levels, and neuroinflammation. Hence, fucoxanthin has the potential to counteract MPTP-induced DA neuronal loss through the downregulation of neuroinflammation and oxidative stress. In conclusion, fucoxanthin exerts neuroprotective effects in opposition to MPTP-mediated PD mice through repressing oxidative stress and inflammatory molecules. Finally, the present study recommended that fucoxanthin could perform as a potential therapeutic drug to improve PD.

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Conflicts of interest

There are no conflicts of interest.

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