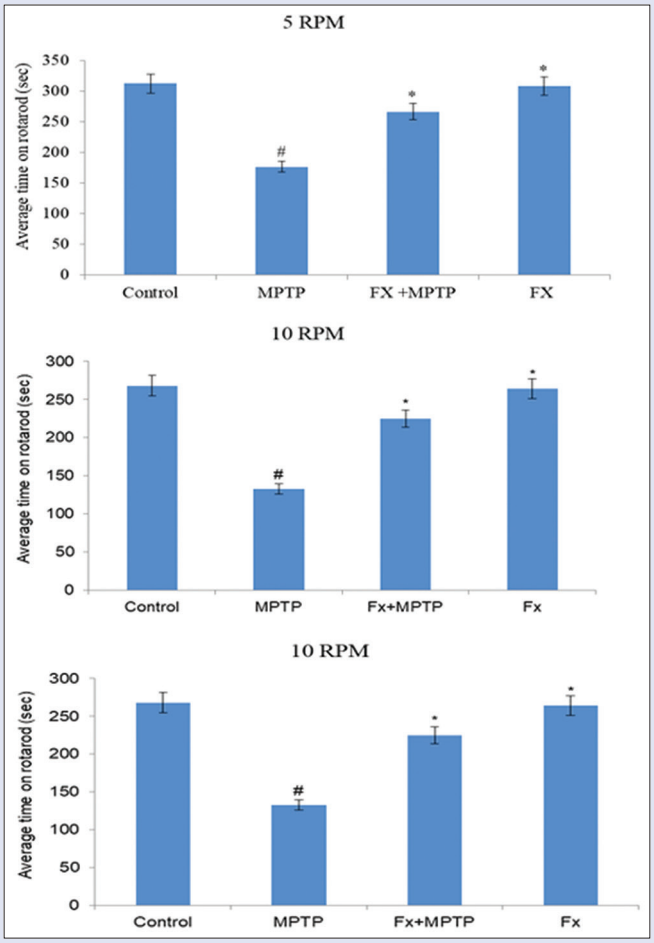

Figure 1: Effect of fucoxanthin on retention time on the rotarod apparatus at 5, 10, 15 rpm after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine administration. #,*differ significantly at $P < 0.05$

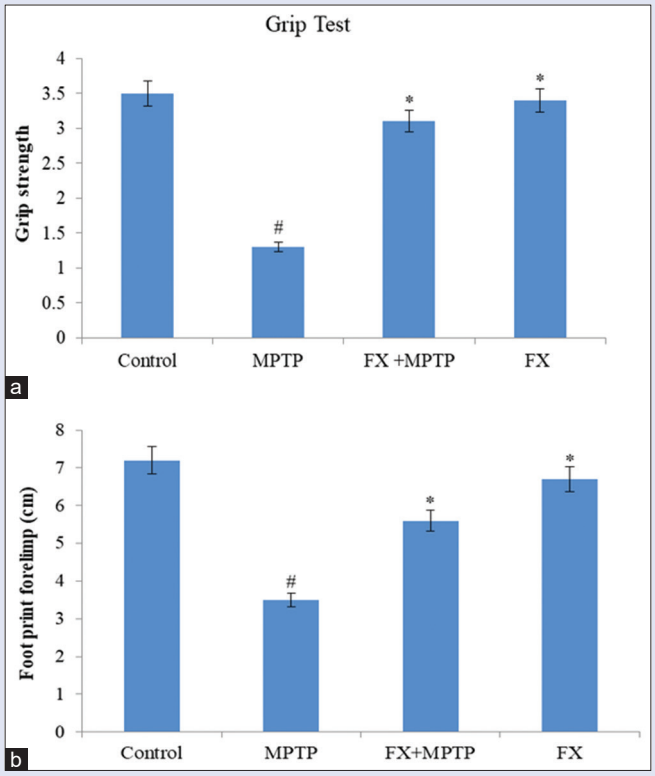

Figure 2: (a) Grip test performance in experimental mice: Values are given as mean ± standard deviation for six mice in each group. Error bars sharing common symbol #,*differ significantly at $P < 0.05$. (b) Footprint examination of fucoxanthin and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine -treated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

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 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

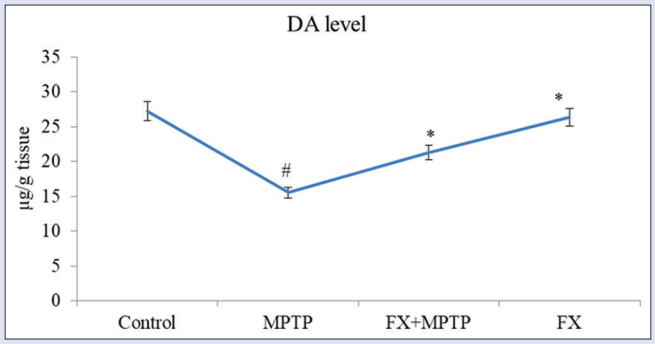

Figure 3: 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced depletion of dopamine conserved by fucoxanthin treatment. Administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine depleted the levels of dopamine attenuated by treatment of fucoxanthin. Results given are mean ± standard deviation,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

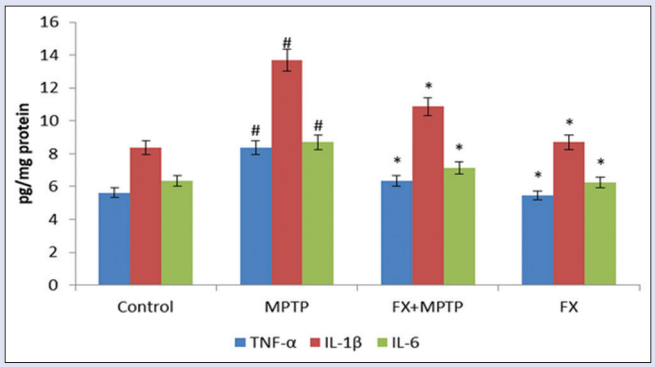

Figure 4: Effect of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and fucoxanthin on tumor necrosis factor -α, interleukin-1β, interleukin-6 in the mice brain. The bar graph shows the levels of interleukin-1β, interleukin-6, tumor necrosis factor-α. Data are shown as mean ± standard error of mean. #,*differ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

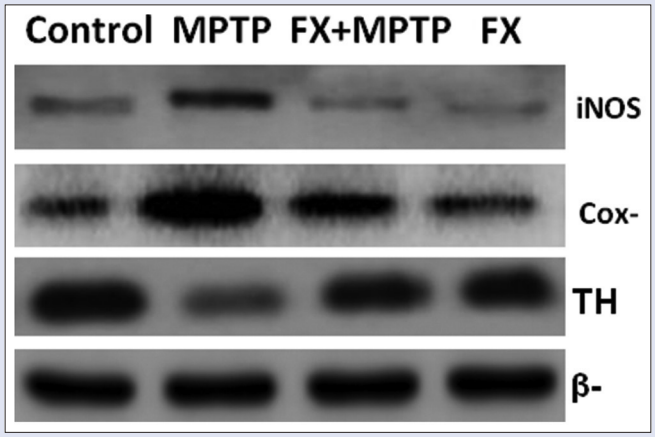

Figure 5: Effect of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and Fucoxanthin on inducible nitric oxide synthase, Cox-2 and TH in the mice brain. The bar graph shows the density normalized to the β-actin loading control for inducible nitric oxide synthase, Cox-2 and TH

strength, time on the rod, climbing speed, in addition to gait aberration comprises increased stride width, reduced stride length.[29] Consistent 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-kB 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-κB (NF-κB) which exerts a significant role in the various proinflammatory mediator regulation which are mainly implicated in the development of neuroinflammation.[45] NF-κB (inactive form) is located in the cytosol in relationship with IkB (an inhibitory protein), thereby it averts the nuclear translocation essential for transcriptional action.[46] Followed by proteolytic degradation of IkB, phosphorylation resultant in NF-κB translocation into the nucleus, then it attached to the target DNA which in turn regulates transcription of various proinflammatory cytokines genes include TNF-a, Cox-2, IL-1b, 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-κB 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-kB 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

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-kB 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 Aβ1 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.

Financial support and sponsorship

The authors would like to thank the Shandong Provincial Hospital for the research funding and facitities to conduct the research work.

Conflicts of interest

There are no conflicts of interest.

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