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ORIGINAL ARTICLE

Garcinia morella extract confers dopaminergic neuroprotection by mitigating mitochondrial dysfunctions and inflammation in mouse model of Parkinson's disease

Ankumoni Dutta[1,2] · Banashree Chetia Phukan[1] · Rubina Roy[1] · Muhammed Khairujjaman Mazumder[3] · Rajib Paul[4] · Amarendranath Choudhury[5] · Diwakar Kumar[6] · Pallab Bhattacharya[7] · Joyobrato Nath[8] · Sanjeev Kumar[1] · Anupom Borah[1]

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Abstract

Dopaminergic neuroprotection is the main interest in designing novel therapeutics against Parkinson's disease (PD). In the process of dopaminergic degeneration, mitochondrial dysfunctions and inflammation are significant. While the existing drugs provide symptomatic relief against PD, a therapy conferring total neuroprotection by targeting multiple degenerative pathways is still lacking. *Garcinia morella* is a common constituent of Ayurvedic medication and has been used for the treatment of inflammatory disorders. The present study investigates whether administration of *G. morella* fruit extract (GME) in MPTP mouse model of PD protects against dopaminergic neurodegeneration, including the underlying pathophysiologies, and reverses the motor behavioural abnormalities. Administration of GME prevented the loss of dopaminergic cell bodies in the substantia nigra and its terminals in the corpus striatum of PD mice. Subsequently, reversal of parkinsonian behavioural abnormalities, viz. akinesia, catalepsy, and rearing, was observed along with the recovery of striatal dopamine and its metabolites in the experimental model. Furthermore, reduced activity of the mitochondrial complex II in the nigrostriatal pathway of brain of the mice was restored after the administration of GME. Also, MPTP-induced enhanced activation of Glial fibrillary acidic protein (GFAP) and neuronal nitric oxide synthase (nNOS) in the nigrostriatal pathway, which are the markers of inflammatory stress, were found to be ameliorated on GME treatment. Thus, our study presented a novel mode of dopaminergic neuroprotection by *G. morella* in PD by targeting the mitochondrial dysfunctions and neuroinflammation, which are considered to be intricately associated with the loss of dopaminergic neurons.

Keywords *Garcinia morella* · Motor abnormalities · Dopamine · Dopaminergic neuroprotection · Mitochondrial dysfunctions · Inflammation

Ankumoni Dutta and Banashree Chetia Phukan contributed equally to this work.

✉ Sanjeev Kumar
skpuniya@gmail.com

✉ Anupom Borah
anupomborahh@gmail.com

[1] Department of Life Science and Bioinformatics, Assam University, Silchar 788011, Assam, India

[2] Department of Zoology, Pandit Deendayal Upadhyaya Adarsha Mahavidyalaya (PDUAM), Behali, Biswanath, Assam, India

[3] Department of Zoology, Dhemaji College, Assam, India

[4] Department of Zoology, Pandit Deendayal Upadhyaya Adarsha Mahavidyalaya (PDUAM), Eraligool, Karimganj, Assam, India

[5] Department of Zoology, Patharkandi College, Assam, India

[6] Department of Microbiology, Assam University, Silchar, Assam, India

[7] Department of Pharmacology and Toxicology, National Institute of Pharmaceutical Education and Research (NIPER), Ahmedabad, Gandhinagar, Gujarat, India

[8] Department of Zoology, Cachar College, Silchar, Assam, India

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Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized mainly by motor behavioural abnormalities (Hayes 2019; Jankovic 2008). The primary pathology associated with PD is the degeneration of dopamine-containing neurons in the substantia nigra pars compacta (SNpc) region of the midbrain, leading to decreased release of the neurotransmitter dopamine from its axon terminals in the striatum (Dauer and Przedborski 2003; Dawson and Dawson 2003; Lees et al. 2009). The impaired dopaminergic neurotransmission results in the cardinal motor behavioural abnormalities such as bradykinesia, rigidity, postural instability, and gait impairment (Gibb and Lees 1988; Jankovic 2008). Till date, these PD symptoms are being managed by the dopamine precursor molecule levodopa to maintain the level of striatal dopamine (Nagatsua and Sawadab 2009). In the absence of an alternate therapy to prevent dopaminergic degeneration in PD, these drugs are being used despite their detrimental side-effects after chronic use (Thomas 2009). Therefore, a novel therapeutic strategy for preventing dopaminergic neuronal loss and motor deficits in PD is of prime importance.

Garcinia morella, belonging to the family Clusiaceae, is a common constituent of Ayurvedic medicine, beneficial in mitigating a wide range of inflammatory disorders (Choudhury et al. 2018). Analytical studies have revealed its rich storage of bioactive compounds with potent anti-oxidant and anti-inflammatory values, and has recently been found effective in attenuating liver damage, cardiovascular disorders, atherosclerosis, and abnormal cell proliferations (Aravind et al. 2016; Choudhury et al. 2018; Gogoi et al. 2017; Patar 2015). In lipopolysaccharide stimulated macrophages, *G. morella* fruit extract reduced the release of inflammatory mediators, *viz.* nitric oxide and cytokines (Choudhury et al. 2018). Enhanced release of free radicals was also shown to be inhibited by the fruit extract in different anti-oxidant screening systems (Choudhury et al. 2018; Gogoi et al. 2017; Sarma et al. 2016). Furthermore, recent molecular docking studies and *in vivo* studies have also indicated the applicability of *Garcinia* fractions in the treatment of motor symptoms linked with PD, by one of its phytoconstituent, garcinol (Mazumder et al. 2018; Chetia Phukan et al. 2022). However, the role of *G. morella* fruit extract remained un-investigated in neurodegenerative diseases like PD. Evidences suggests mitochondrial dysfunctions and inflammation with characteristic glial cell and nNOS hyperactivation as the primary mechanisms of dopaminergic neurodegeneration in PD (Birla et al. 2019; Paul and Borah 2017; Vivekanantham et al. 2015; Yates 2015). Free radical generation and mitochondrial dysfunctions are intricately associated with one another and are regarded as the significant pathogenic events in PD (Dexter and Jenner 2013; Panov et al. 2005). Dysfunction of mitochondrial complexes lead to enhanced production of free radicals, further deteriorating the pathological conditions in PD (Keane et al. 2011). Moreover, inflammation is also considered as another major pathogenesis of the disease (De Virgilio et al. 2016; Paul et al. 2017a). Following brain injury, glial cells of the brain become hyperactive and release a series of inflammatory mediators causing damage to the surrounding neurons (Ducourneau et al. 2014; Zhang et al. 2019). With the progression of the disease, the damaged neurons gradually activate the glial cells and this cycle continues in PD (Pekny and Pekna 2014; Yates 2015; Zhang et al. 2019). Again neuronal nitric oxide synthase (nNOS), responsible for the production of neuronal messenger nitric oxide (NO), is another significant marker of the neuronal inflammatory stress (Giulivi 2003). Elevated nNOS activity is associated with glial cell activation and inhibition of mitochondrial complexes activity, and is thus a key factor in exaggerating the pathological conditions in PD (Hope et al. 1991; Madathil et al. 2013; Paul and Borah 2017). Thus, a therapy targeting all these molecular pathways responsible for neurodegeneration in PD is of utmost importance. In the present study, the therapeutic potential of *G. morella* extract to protect against dopaminergic neurodegeneration and ameliorate motor abnormalities was investigated in an experimental mouse model of PD. Additionally, the effect of *G. morella* extract on mitochondrial dysfunctions and inflammatory processes were investigated.

Materials and methods

Garcinia morella fruit extract preparation

Fresh *Garcinia morella* (Gaertn.) Desr. fruits were collected from Assam (India) [Coordinates: 26.6981° N, 93.9670° E] in April 2017 and taxonomical classification was done and the specimen was submitted to Assam University, Silchar (Herbarium No. AUSL 3112). The fruit rinds were cut into small pieces and air-dried in shade. The dried rind pieces were then ground using a mixer grinder and used for further experiment. Water extract was prepared by soaking 100 g of rind powder in 1000 ml of water and then stirred vigorously and allowed to stand for 24 h at 4 °C. Subsequently, it was filtered by Whatman filter paper and stored at 4 °C, following Sarma et al. (2016). The filtrate was then administered to the mice as per their body weight. Here, the fruit-water-extract (fruit-water-extract: GME) implies the filtrate recovered after soaking the material in water overnight. After that, the dose was quantified by evaporating the extract, which

was found to be 400 ± 14 mg/kg. The quantification process was performed four times. The yield of the dried extract was 38–40% of the weight of the dried rind powder of the fruit.

Animals

Male Swiss albino mice of weight between 21 and 27 g were housed in a controlled environment of 24 ± 2 °C temperature and 60 ± 5% humidity and were given standard feed and water ad libitum. The experimental protocols met the National Guidelines and were approved by the Institutional Animal Ethics Committee of Assam University, Silchar (IEC/AUS/2013–052; AUS/IAEC/2017/PC/01).

Chemicals

1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP; M0896), dopamine hydrochloride (H8502), hydrogen peroxide (H_2O_2;V800211), perchloric acid ($HClO_4$; 380,083), 3, 3-diaminobenzidine (DAB) liquid substrate system (code: D3939) and poly-L-lysine were purchased from Sigma-Aldrich Co (St. Louis, MO, USA). Acetonitrile, ethylene diamine tetra acetic acid disodium salt (EDTA), Nicotinamide adenine dinucleotide (Reduced) disodium salt (NADH; 77,268), nitrobluetetrazolium (NBT; 48,898), orthophosphoric acid, paraformaldehyde, triethylamine and Triton X-100were purchased from SISCO Research Laboratories (Mumbai, India). Rabbit anti-tyrosine hydroxylase (TH) antibody (ab112), anti-Glial fibrillary acidic protein (GFAP) antibody (ab7260), and Donkey serum were purchased from Abcam(Cambridge, UK). Anti-rabbit goat secondary antibody tagged with horseradish peroxidase (HRP) (code: ap307p) was purchased from Millipore Co. (USA). All other chemicals used in the study were of extra pure grade and purchased from SISCO Research Laboratories (Mumbai, India).

Experimental design

Mice were randomly divided into four groups: Control (CON), PD (MPTP, 30 mg/kg i.p.),GME (400 mg/kg b.w), and GME-administered PD group (400 mg/kg b.w). MPTP was given at 30 mg/kg b.w. on day 1, followed by the next dose on day 2 at 16 h interval to develop the mouse model of PD (Paul et al. 2017b). CON animals were injected with only 0.9% saline. GME (400 mg/kg b.w) was administered orally after 2 h following 2nd dose of MPTP, which was followed by the next dose of GME (400 mg/kg b.w) 6 h apart. The same routine dose was followed until the 5th day and a total of seven doses of GME were administered. On the 4th day of the experiment following the first dose of MPTP, animals were subjected to motor behavioural tests (Akinesia, Catalepsy, and Rearing behaviour), following which mice were sacrificed on the 5th day after 2 h of GME or vehicle administration. The behavioural tests were performed by assessors who were blind to the experimental groups.

Invitro anti-oxidant activity screening

To assess the formation of hydroxyl radical, in vitro screening was performed using Fe^{2+}–EDTA/ascorbate (ferrous ascorbate) system. This anti-oxidant screening system was developed and performed using High Performance Liquid Chromatography coupled with Electrochemical detector system (HPLC-ECD, Waters) following the method described by Maharaj et al. (2005) with slight modifications. Salicylic acid was used to convert hydroxyl radical into its hydroxylation products 2,3- and 2,5-dihydroxybenzoic acid (DHBA) and the quantity of 2,3- and 2,5- DHBA assessed signifies the amount of hydroxyl radical formed in the system. The concentration of the chemicals in the ferrous ascorbate system are: phosphate buffer (50 mM, pH 7.2), EDTA (240 µM), ascorbate (1 mM), and ferrous sulfate (200 µM). Different concentrations of GME (100, 250, and 400 µg/ml) were added to the system to analyse its effect on the levels of hydroxyl radical, which was compared with melatonin (40 µg/ml). Reaction mixtures were incubated at 37 °C in dark for 1 h, following which 0.75 mM salicylic acid was added to it. The reaction was stopped after 5 min by diluting (1:9) the mixtures in 0.1 M ice-cold perchloric acid. Ten microlitre of each sample was injected into the HPLC-ECD and hydroxyl radical adducts of salicylic acid, 2,3-DHBA and 2,5-DHBA were estimated. The composition of the mobile phase used for estimation was 8.65 mM heptane sulfonic acid, 0.27 mM EDTA, 13% acetonitrile, 0.43% triethylamine, and 0.32% phosphoric acid. The flow rate was kept at 0.7 ml/min.

Analysis of motor behaviour

Akinesia

Akinesia is measured as the latency (in sec) of animals to move all four limbs and the test was stopped if the latency of animals exceeded 180 s. The animals were acclimatized to a wooden platform (40 cm × 40 cm × 30 cm) before the beginning of the test. The latency of the animals to move all four limbs was noted using a stopwatch, following standard protocols (Haobam et al. 2005; Paul et al. 2017b).

Catalepsy

Catalepsy is the inability of the animals to correct an externally imposed posture. The animals were kept on a flat surface with their hind limbs placed on a wooden block of 3 cm height. Using a stopwatch, the time taken by animals

to move their hind limbs to the flat surface was recorded, following standard protocols (Haobam et al. 2005; Paul et al. 2017b).

Rearing behaviour

Animals were placed in a glass cylinder (height = 30 cm, diameter = 20 cm) for 5 min. While in the cylinder, animals typically rear and engage in exploratory behaviour by placing their forelimbs along the wall of the cylinder, which was counted. To be counted as a rear, the animal had to raise the forelimbs above the shoulder level and make contact with the cylinder wall with either one or both forelimbs. Withdrawal of the forelimb from the cylinder wall and contact with the bottom surface of the cylinder was required before another rear score, following Tapias et al. (2014).

Analysis of dopamine and its metabolites

To analyze the effect of GME on the levels of dopamine and its metabolites, the mice were sacrificed by decapitation on the 5th day of the experiment. Whole brains from mice were removed and the striata were dissected out. The tissues were weighed and sonicated in 0.4 M chilled perchloric acid (1:10 weight/volume), followed by centrifugation at 12,000 × g for 10 min at 4°C. The supernatant (10 µl) was injected into HPLC-ECD system, keeping the flow rate at 0.7 ml/min. Calibration curves were prepared by injecting known concentrations of the standard, prepared in the same 0.1 M perchloric acid. The composition of the mobile phase was the same as that used in the in vitro determination of hydroxyl radical (Muralikrishnan and Mohanakumar 1998).

Immunohistochemistry

Animals were deeply anesthetized and perfused with 50 ml of ice-cold PBS (0.1 M, pH 7.4) and followed by 50 ml of 4% (w/v) paraformaldehyde prepared in PBS. Brains were dissected out, kept overnight in the same fixative, and cryoprotected in 30% (w/v) sucrose solution. Thirty micron thick coronal sections passing through the striatum (NCP) and substantia nigra (SN) were made using cryotome (Thermo Shandon, UK). Coronal sections were then rinsed with 0.1 M Tris-buffered saline (pH 7.4), incubated in 3% H_2O_2 in TBS, permeabilized with 0.3% Triton X-100, and blocked with 10% donkey serum. Sections passing through both NCP and SN sections were assessed for Tyrosine hydroxylase (TH) and Glial fibrillary acidic protein (GFAP) immunohistochemistry. Then, the sections were incubated overnight with polyclonal rabbit anti-TH or rabbit anti-GFAP primary antibody (1:500 for TH and 1:1000 for GFAP) in TBS containing 2% donkey serum and 0.3% Triton X-100 for overnight at 4 °C. The sections were then washed with TBS and incubated with HRP-conjugated anti-rabbit secondary antibody (1: 1000) in TBS containing 2% donkey serum and 0.3% Triton X-100 for 1 h at room temperature over a shaker following Paul et al. (2017b). Colour development was performed by incubating the sections in the diaminobenzidine-liquid substrate system and then sections were washed, dehydrated, mounted in DPX, and photographed with Trinocular Microscope (Nikon, Japan). The intensity of the colour was measured and number of cells was counted using the Fiji Version of ImageJ software by calculating the optical density, following Schindelin et al. (2012).

Mitochondrial complex II histoenzymology

For histoenzymology, mice were anesthetized and perfused intracardially with 50 ml ice-cold PBS (0.1 M, pH-7.4) followed by 10% (50 ml) ice-cold glycerol. Brains were removed and kept overnight in 30% (w/v) sucrose solution. Twenty micron thick coronal sections passing through the striatum were cut using Cryotome and transferred to poly-L-lysine coated slides. Mitochondrial complex II histoenzymology was initiated by activating the sections in PBS (0.1 M, pH 7.4) for 10 min at 30ºC. Following this, the sections were incubated in the reaction mixture containing 0.05 mol/L phosphate buffer (pH 7.4), 0.05 mol/L sodium succinate and 0.3 mol/L NBT. Then sections were washed in the PBS, mounted in glycerol, and photographed immediately, following standard protocols (Mazumder et al. 2019; Riddle et al. 1992). Photography was performed under bright filled illumination using a digital SLR camera attached with a microscope (Eclipse Ci, Nikon, Japan). The intensity of colour of the photographs was measured using Fiji Version of ImageJ software by calculating the optical density, following Schindelin et al. (2012).

nNOS histoenzymology

Neuronal NOS histoenzymology in brain tissues was performed following Madathil et al. (2013) with slight modifications. Striatal and SN sections in the coated slides were washed and permeabilised with 0.3% Triton X-100 (in 0.5 M Tris–HCl buffer, pH 7.4) for 5 min. Then sections were incubated with reaction mixture (0.5 M Tris–HCl buffer, 1.5 mM β-NADPH, 0.25 mM NBT, 0.25% Triton X-100, and 15 mM $CaCl_2$) in the dark at 37 °C for 60 min. Sections were then washed in PBS, dried, mounted with glycerol, and photographed with Trinocular Microscope (Nikon, Japan). nNOS positive neurons were counted using ImageJ software, as per standard procedure (Madathil et al. 2013; Schindelin et al. 2012).

Statistical analysis

Statistical analysis was performed employing Student's t-test (two-tailed) and two-way repeated measures ANOVA using GraphPad Prism version 7.0. The distribution of data points

has been shown using whisker's box plots, developed using Graph Pad prism software. The results are expressed as mean ± SEM and *p ≤ 0.05 was considered statistically significant.

Results

GME exhibit dose dependent hydroxyl radical scavenging activity

Different concentrations of *G. morella* fruit extract (GME) were assessed for hydroxyl radical scavenging activity compared to the well-known antioxidant, melatonin. When co-incubated with the reaction mixture, GME showed significant hydroxyl radical scavenging activity than the naïve reaction mixture (Control). When incubated at concentrations 40, 100, 250, 400, and 600 µg/ml, GME causes a significant decrease in the generation of 2,5-DHBA and 2,3-DHBA, compared to their respective controls. The antioxidant activity of GME at 400 and 600 µg/ml were comparable to that of melatonin at 40 µg/ml. However, at 400 µg/ml, scavenging activity was found to be more stable compared to other doses. Melatonin showed 76% and 78% decrease in 2,5-DHBA and 2,3-DHBA production respectively, while GME at 400 and 600 µg/ml showed 82.97% and 76.50% decrease of 2,5-DHBA, and 80.33% and 79.10% decrease of 2,3-DHBA respectively as compared to their controls. The results thus highlighted the dose-dependent hydroxyl radical scavenging efficacy of GME (Fig. 1).

GME significantly ameliorates motor behavioural abnormalities in PD mice

Motor behavioural tests were performed on the 4th day of the experimental setup which revealed motor behavioural deficits with significantly increased akinesia and catalepsy scores, and a decreased number of rears in the PD group of mice. GME administration in PD mice significantly reduced the akinesia and catalepsy scores by 39.32% and 47.09% respectively, and increased the number of rears by 1.66–folds. However, the behavioural scores remained unaffected after GME administration in the naïve mice (Fig. 2).

GME ameliorated the levels of dopamine and its metabolites in PD mice

The levels of the neurotransmitter dopamine and its metabolites were analyzed from the striatal region of the brain in all the groups of animals using HPLC-ECD. MPTP caused significant depletion of striatal dopamine and its metabolites– DOPAC and HVA levels in the PD mice as compared to the control group, thus validating our model as PD. Administration of GME in PD mice significantly increased the levels of dopamine, DOPAC, and HVA by 2.0–, 1.4– and 1.33 – folds respectively compared to the PD group. Further, the dopamine turnover rate, calculated as the ratio of the metabolites to the neurotransmitter,

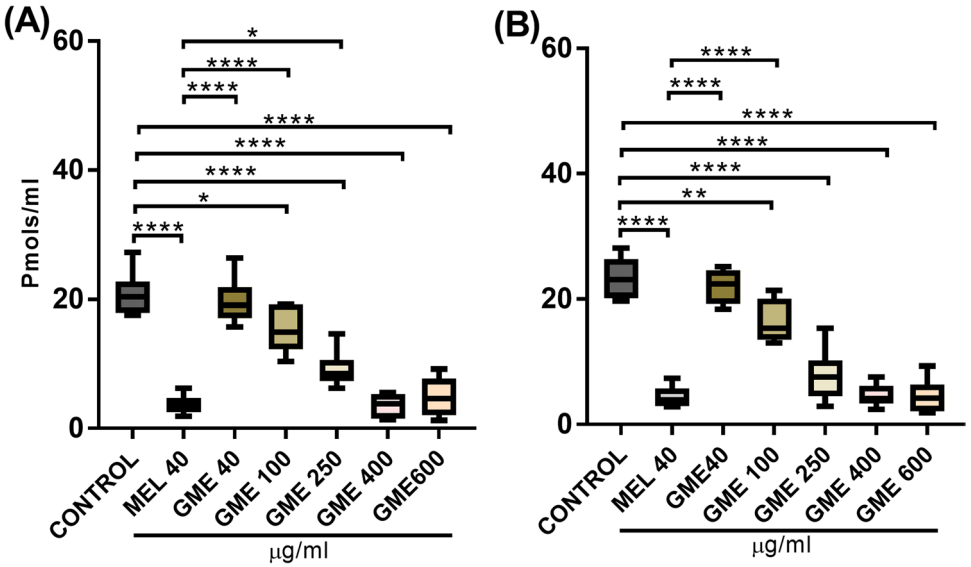

Fig. 1 Efficacy of GME in an *invitro* hydroxyl radical generating system: In the ferrous ascorbate system, hydroxyl radical scavenging activity of GME at different concentrations were tested in comparison to the known anti-oxidant melatonin and expressed in terms of (**A**) 2,5 DHBA and (**B**) 2,3 DHBA production at pmols/ml. Distributions of values are shown in box plot. The box extends from 25 to 75th percentile, the horizontal line inside the box represent median, and the whiskers indicates minimum to maximum values. ****p ≤ 0.00001,***p ≤ 0.0001, **p ≤ 0.009, and *p ≤ 0.05 are considered statistically significant in comparison between two groups (n = 6)

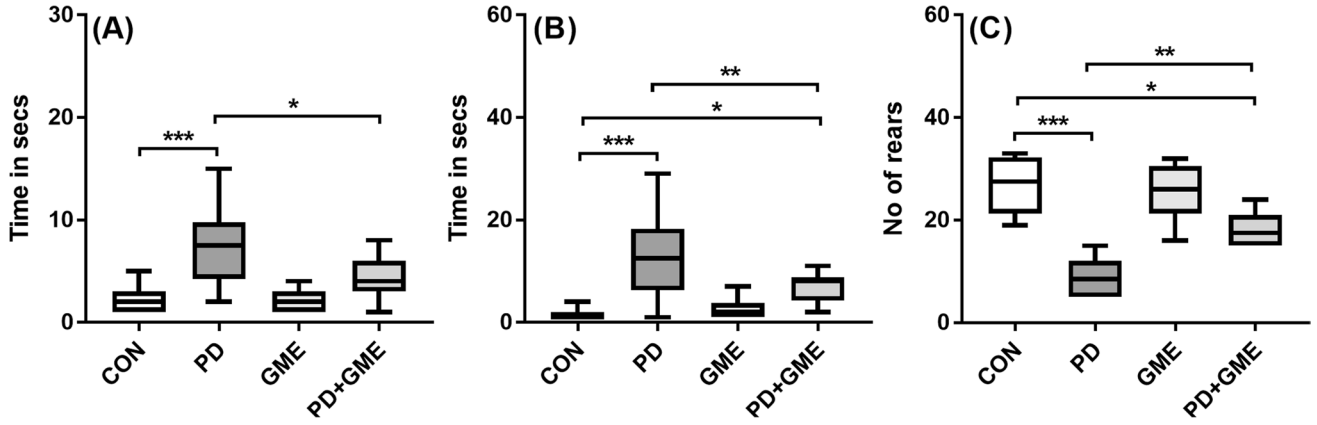

Fig. 2 Effect of GME on motor behaviour of PD mice: Motor behavioural tests (**A**) Akinesia, (**B**) Catalepsy, and (**C**) Rearing behaviour were performed on the 4th day of the experiment. GME was found to ameliorate the observed motor behavioural abnormalities in the PD group of mice. Distributions of values are shown in box plot. The box extends from 25 to 75th percentile, the horizontal line inside the box represent median, and the whiskers indicates minimum to maximum values. ***$p \leq 0.0001$,**$p \leq 0.009$, and *$p \leq 0.05$ are considered statistically significant in comparison between two groups ($n = 6$)

was found to be significantly high in the parkinsonian mice compared to control. Interestingly, GME administered PD mice exhibited no difference in dopamine turnover rate as compared to the PD only mice. Therefore, GME treatment in the PD mice though upregulated the striatal dopamine level, did not change the dopamine turnover rate. Moreover, no difference was noted on the striatal dopamine and its metabolites between the GME and the control group of mice (Fig. 3).

GME prevented the loss of nigral dopaminergic neurons and striatal TH immunoreactivity in PD mice

The status of the nigrostriatal dopaminergic neurons was assessed by immunohistochemistry of TH, the rate-limiting enzyme of dopamine synthesis, which is regarded as the marker of dopaminergic neurons (Daubner et al. 2011). Administration of MPTP in mice caused significant loss of nigral TH-positive dopaminergic neurons and depletion in striatal TH-immunoreactivity as compared to corresponding brain regions of the control group of mice. GME administration in PD mice protected against the MPTP-induced degeneration of dopaminergic neurons and increased the neuronal count by 1.2- fold in the substantia nigra region of the brain. Striatal TH-immunoreactivity was also found to be increased by 1.8-fold in the GME treated mice as compared to the PD only group. However, in the naïve animals administered with the fruit extract, no significant change was observed in nigral dopaminergic neurons count as well as in the TH-immunoreactivity as compared to the control mice. Therefore, GME treatment was found to exhibit neuroprotective efficacy against nigrostriatal dopaminergic degeneration in PD (Fig. 4).

GME upregulated the nigrostriatal mitochondrial complex II activity in PD mice

Histoenzymology was performed to determine the activity of the mitochondrial complexes II from the brain of the mice. In the nigrostriatal areas of PD mice, complex-II activity was found to be significantly decreased as determined by measuring the optical density of the NCP region as compared to the control group. The complex II activity was also significantly less in the SN region of the PD group of mice. GME administered PD mice exhibited increased striatal as well as nigral complex II activity by 1.56-fold and 1.43-fold respectively as compared to PD only group of mice. Thus, it may be argued that the administration of GME can be effective against the loss of mitochondrial complexe II activity in PD mice (Fig. 5).

GME decreased the GFAP immunoreactivity in nigrostriatum of PD mice

A significant increase in the number of GFAP-positive cells, as analysed from GFAP-immunoreactivity, was noted in the NCP and SN regions of the brain in the PD group of mice, which was 4.11-fold and 2.72-fold respectively, as compared to the corresponding brain regions of control mice. Importantly, GME treatment to the parkinsonian mice significantly reduced the MPTP-induced activation of GFAP in astrocytes in NCP and SN by 35% and 29.16% respectively, compared to the PD group. However, no significant change in the number of GFAP positive astrocytes was observed in the fruit extract alone treated mice in comparison with the control group. Thus, our result indicated that GME could prevent the hyper-activation astrocytes in the brain of PD mice (Fig. 6).

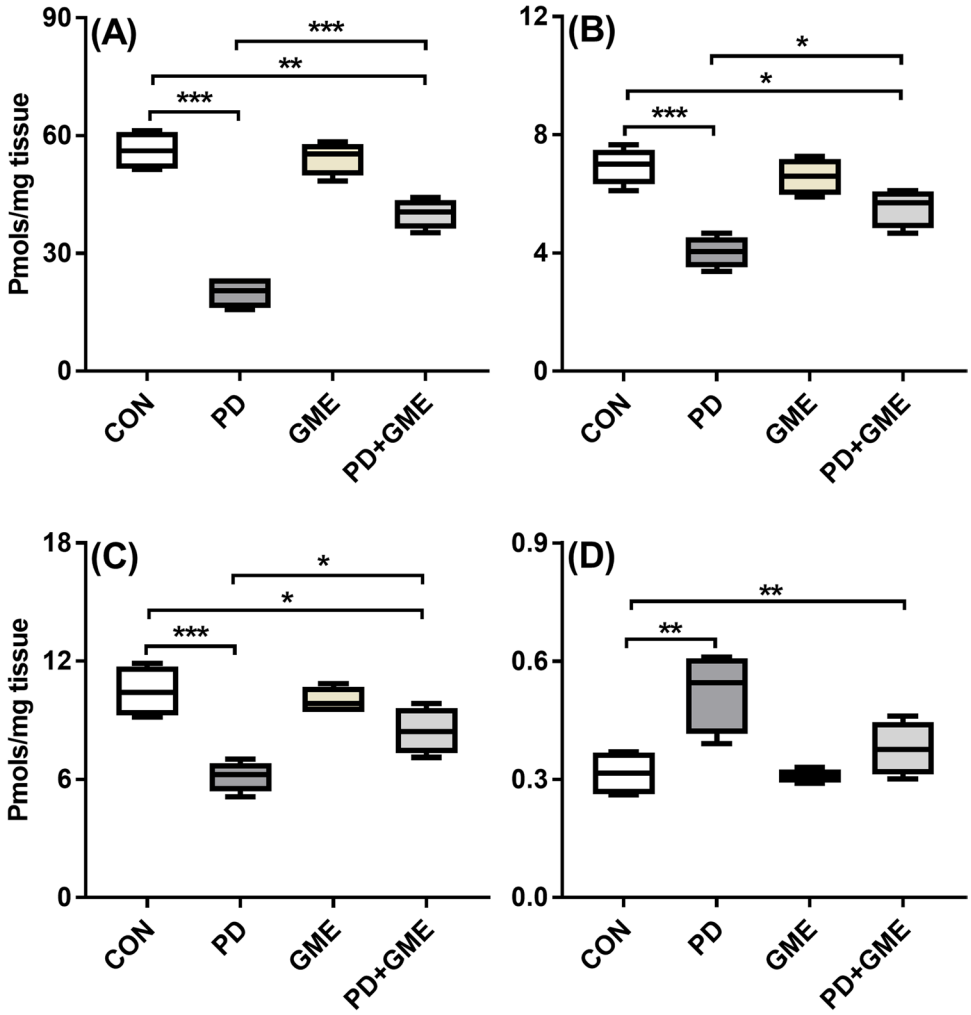

Fig. 3 Effect of GME on dopamine and its metabolites in PD mice: (**A**) Dopamine, (**B**) DOPAC, (**C**) HVA and (**D**) Dopamine turn over were analysed on the 5th day of the experiment by HPLC ECD technique. GME recovered the dopamine and its metabolites (DOPAC and HVA) levels in PD mice. Distributions of values are shown in box plot. The box extends from 25 to 75th percentile, the horizontal line inside the box represent median, and the whiskers indicates minimum to maximum values. ***$p \leq 0.0001$,**$p \leq 0.009$, and *$p \leq 0.05$ are considered statistically significant in the comparison between two groups ($n = 6$)

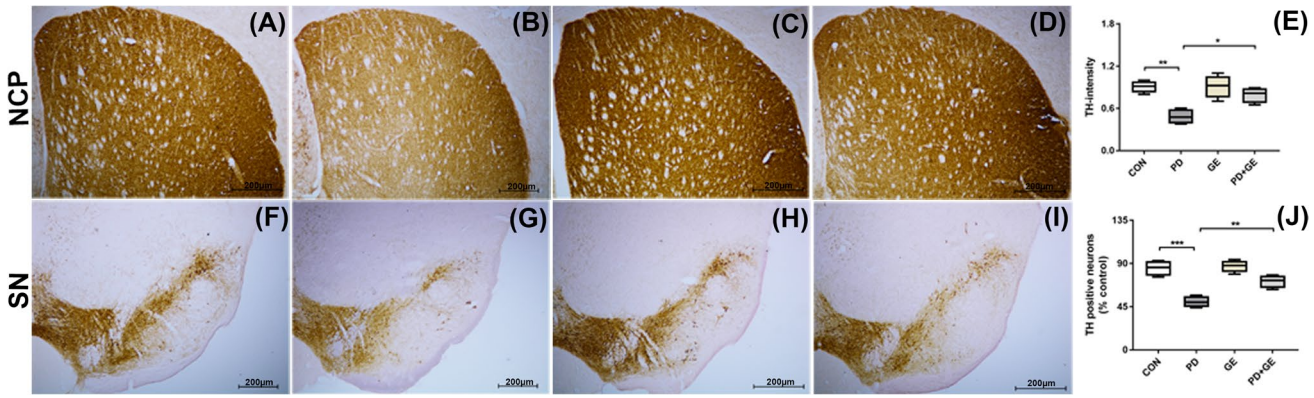

Fig. 4 Effect of GME on tyrosine hydroxylase (TH)-immunoreactivity in the striatum (NCP) and TH-positive substantia nigral (SN) neurons of PD mice: GME significantly prevented the nigrostriatal dopaminergic neurons from degeneration. Representative photographs from (**A-D**) striatum [NCP], and (**F-I**) substantia nigra [SN] from CON, PD, GME, PD + GME (left to right) groups showing TH immunohistochemistry. Quantification of relative optical density of TH immunohistochemistry in (**E**) NCP and (**J**) TH positive neurons in SN, was done using ImageJ software. Distributions of values are shown in box plot. The box extends from 25 to 75th percentile, the horizontal line inside the box represent median, and the whiskers indicates minimum to maximum values. ***$p \leq 0.0001$,**$p \leq 0.009$, and *$p \leq 0.05$ are considered statistically significant in the comparison between two groups ($n = 6$)

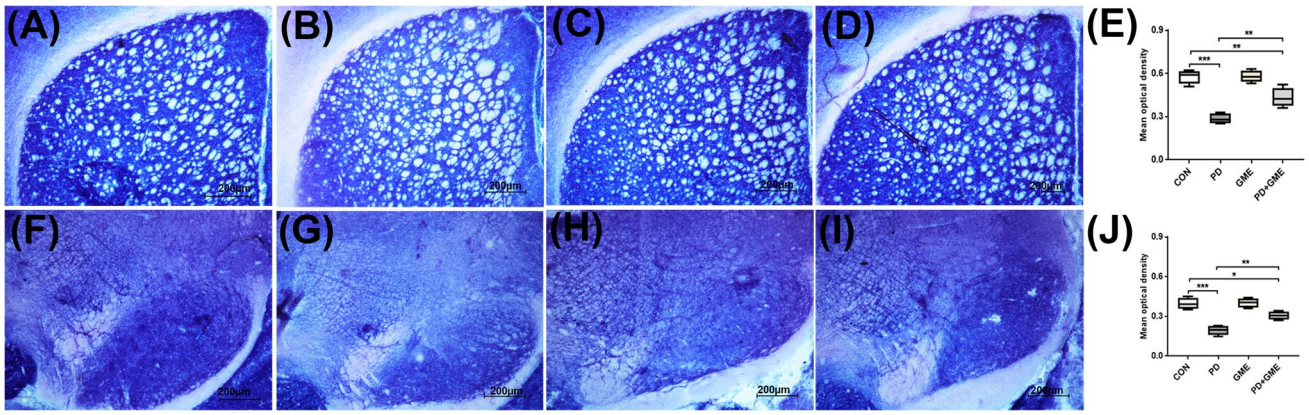

Fig. 5 Effect of GME on mitochondrial complex II activity of PD mice: GME significantly recovered the depletion of mitochondrial complex II activity of PD mice. Representative photographs from (**A**-**D**) striatum [NCP], and (**F**-**I**) substantia nigra [SN] from CON, PD, GME, PD + GME (left to right) groups showing mitochondrial complex II histoenzymology. Quantification of relative optical density in (**E**) NCP and (**J**) SN was done using ImageJ software. Distributions of values are shown in box plot. The box extends from 25 to 75th percentile, the horizontal line inside the box represent median, and the whiskers indicates minimum to maximum values. $^{***}p \leq 0.0001$,$^{**}p \leq 0.009$, and $^{*}p \leq 0.05$ are considered statistically significant in the comparison between two groups ($n = 6$)

GME decreased the number of nNOS positive neurons in nigrostriatum of PD mice

nNOS is regarded as a marker of inflammatory stress in neurons (Paul and Borah 2017) and is analysed by nNOS histoenzymology. The numbers of nNOS-positive neurons were found to be noticeably increased in the NCP and SN regions of the brain in the PD mice compared to the control mice. Administration of GME in PD mice however, significantly reduced the number of nNOS-active neurons in both NCP and SN regions of the brain in comparison to the PD mice. Therefore, GME was observed to protect against the increased activation of nNOS enzyme in the nigrostriatal neurons in PD mice, thus, highlighting its anti-inflammatory potential (Fig. 7).

Discussion

Natural products have always remained the roots of new drug discovery for various pathological conditions (Harvey et al. 2015). While plant extracts when tested in experimental models of PD have always been found promising in targeting the multiple pathological pathways associated with the disease which is the primary requirement for the development of novel therapeutic strategy

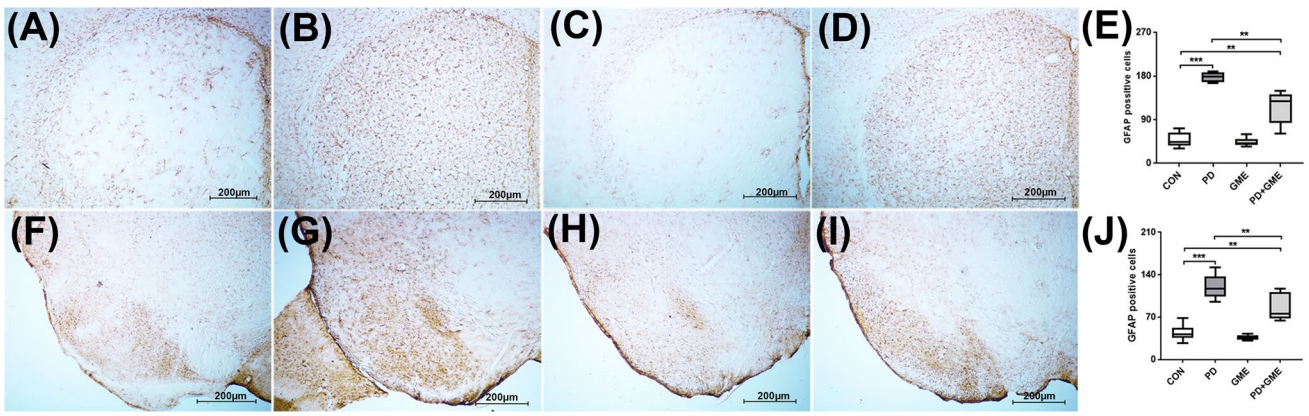

Fig. 6 Effect of GME on an increased number of GFAP positive astrocytes in nigrostriatal areas of PD mice: GME significantly normalised the enhancement of GFAP immunoreactivity in PD mice. Representative photographs from (**A-D**) striatum [NCP] and (**F-I**) substantia nigra [SN] from CON, PD, GME, PD + GME (left to right) groups showing GFAP immunoreactivity. Quantification of GFAP positive cells in (**E**) NCP and (**J**) SN was done using ImageJ software. Distributions of values are shown in box plot. The box extends from 25 to 75th percentile, the horizontal line inside the box represent median, and the whiskers indicates minimum to maximum values. $^{***}p \leq 0.0001$,$^{**}p \leq 0.009$, and $^{*}p \leq 0.05$ are considered statistically significant in the comparison between two groups ($n = 6$)

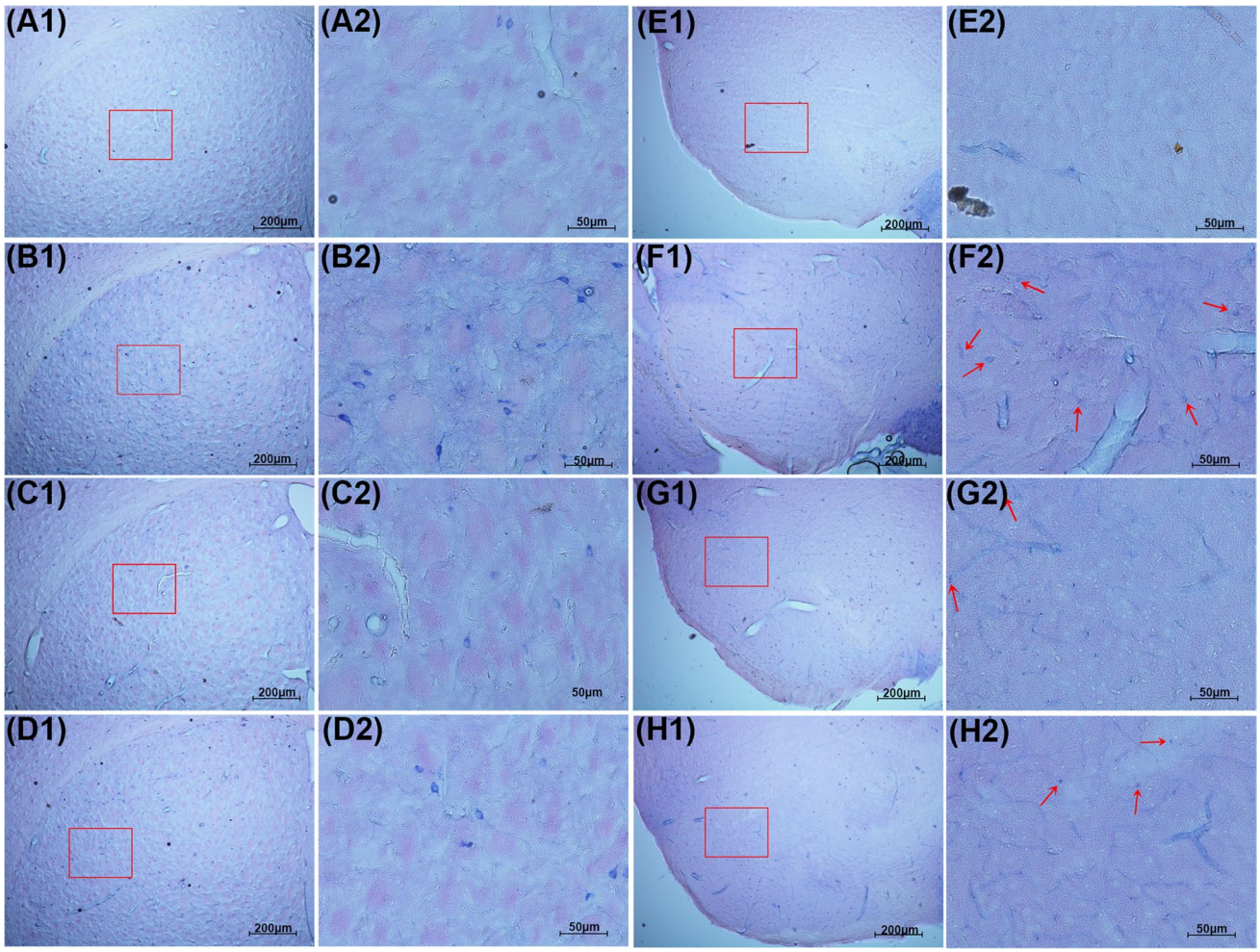

Fig. 7 Effect of GME on increased number of nNOS positive neurons in PD mice: Representative photographs from striatum (NCP) of (A1, A2) CON, (B1, B2) PD, (C1, C2) GME, and (D1, D2) PD + GME; and SN of (E1, E2) CON, (F1, F2) PD, (G1, G2) GME, and (H1, H2) PD + GME groups showing nNOS histoenzymology. Annotated rectangles denote the area of comparison between the groups. The photographs of the second and fourth columns were taken in 20 × magnification and of first and second were taken in 4 × magnification

(Litvinenko et al. 2019; Pathak- Gandhi and Vaidya 2017; Skovronsky et al. 2006). Keeping in mind the rich storage of bioactive compounds in *G.morella* and its potential antioxidant and anti-inflammatory potentials, the present study was designed to assess the neuroprotective efficacy of *G. morella* fruit extract (GME) on dopaminergic neurodegeneration and the underlying mechanisms in the MPTP model of PD. The most important findings of our study are the neuroprotective effects of GME over the dopaminergic neuronal death via recovering the mitochondrial complex dysfunction and normalizing the enhanced inflammatory stress in the MPTP mouse model of PD. Consequently, recovery of depleted dopamine level and parkinsonian behavioural abnormalities was also prominent in the same model following GME treatment.

Studies have suggested fruit extracts from different plant species including a *Garcinia sp.* could ameliorate the motor deficits observed in the rodent model of PD (Antala et al. 2012; Gokul and Muralidhara 2014; Kasture et al. 2009). Antala et al. 2012) highlighted the efficiency of *G. indica* in ameliorating 6-OHDA-induced motor abnormalities in a rodent model of PD. Loss of involuntary movements or akinesia, and catalepsy/rigidity with postural instability are the standard motor behavioural parameters used to assess the parkinsonian motor abnormalities in experimental models of PD (Haobam et al. 2005). Additionally, rearing behaviour test is performed as a functional measure of alteration in dopamine neurotransmission (Cannon et al. 2009; Tapias et al. 2014). In the present study, we found that GME administration in PD mice attenuated the motor behavioural deficits as evident from reduced akinetic and cataleptic behavior along with the increase in number of rears (Fig. 2). Thus, our findings for the first time highlighted the therapeutic potential of fruit extract of *G. morella*

in ameliorating parkinsonian behavioural deficits, which are in compliance with earlier studies, which suggested that fruit extracts from different plant species with medicinal properties may be a prospective therapeutic strategy for PD (Amro et al. 2018; Prakash et al. 2014). The motor behavioural activities are regulated by the neurotransmitter dopamine released by the axon terminals of dopaminergic neurons located in the striatal region of the brain, and depletion in dopamine and its metabolite levels is considered as a basal biochemical marker of PD (Naskar et al. 2015; Sengupta et al. 2011; Wilson et al. 1996). Our experimental results have indicated an elevation in striatal dopamine and its metabolites level in GME administered PD mice (Fig. 3A, B and C), which is in consistence with the previous studies which have also demonstrated similar findings using raw plant extract or their derivatives (Bi et al. 2015; Zhu et al. 2010). Antala et al. (2012) also highlighted the therapeutic efficacy of fruit extract of *G. indica* in up-regulating the depleted dopamine levels in an experimental model of PD (Antala et al. 2012). However, GME treatment could not attenuate the increased dopamine turnover rate as observed in the PD mice (Fig. 3D). Previously, a study has surmised that garcinol, a constituent of *Garcinia sp.,* might be capable of replenishing the dopamine level in PD by inhibiting its catabolizing enzyme Monoamine Oxidase-B (MAO-B) (Mazumder et al. 2018). For several decades, MAO-B inhibitors have been prescribed in PD cases to prevent the dopamine breakdown in terminals and made the monoamine available for a longer time (Birkmayer et al. 1977; Dezsi and Vecsei 2017). Noticeably, from our findings, it can be suggested that dopamine recovery in the PD mice in our study is not resulting from slow breakdown, hence unlikely due to the inhibition of the MAO-B enzyme.

In the brain of PD patients as well as in experimental models, severe degeneration of dopaminergic cell bodies in the substantia nigra pars compacta and the terminals in striatum are found to be the main pathogenesis (Grünblatt et al. 2000; Yan et al. 2017). From several decades, studies have been aimed to target the neurodegenerative process of the disease; however very few of them are convincing and many are limited to the recovery of dopamine and subsequent motor abnormalities (Lange et al. 1994; Olanow et al. 2008). In this study, we investigated the effect of GME on the status of nigrostriatal dopaminergic neurons in PD by TH-immunohistochemistry (Fig. 4). TH is the rate-limiting enzyme for the synthesis of the neurotransmitter dopamine, which directly indicates the status of the available and degenerated dopaminergic neurons in brain (Daubner et al. 2011). Significant loss of dopaminergic neurons and their terminals ingrain the nigrostriatal region was evident in PD mice in our study, which is consistent with the earlier studies (Liu et al. 2015; Paul et al. 2017b). Importantly, GME treatment in PD mice was found to protect against dopaminergic neurodegeneration throughout the nigrostriatal pathway of the brain. Our study, therefore, clearly points out the neuroprotective role of GME in preventing the death of dopaminergic neurons in PD. Moreover, our results also indicated that dopamine recovery as prevalent in the GME treated PD mice is the result of dopaminergic neuroprotection rather than the MAO-B inhibitory potential.

Dysfunction of mitochondrial complexes plays a major role in degeneration of dopaminergic neurons in PD (Dexter and Jenner 2013; Panov et al. 2005). Shreds of evidences indicated the significant role of dysfunction of mitochondrial complexes in PD pathogenesis (Beyer 1992; Jastroch et al. 2010; Takeshige and Minakami 1979). Studies from the past decades have highlighted the involvement of complex-II in the disease progression. Impairment of complex II or complex II + III activity was reported in the platelets of PD patients as well (Haas et al. 1995; Yoshino et al. 1992). Dysfunction of mitochondrial complexes lead to generation of reactive oxygen species, and depletion of ATP (Dexter and Jenner 2013; Farshbaf 2017). Inhibition of the mitochondrial complex II damages the neuronal system by increasing the entry of calcium ions preceded by enhance activity of N-methyl-D-aspartate receptors (Liot et al. 2009; Tieu et al. 2003). Our study demonstrated a significant recovery of complex II inhibition by GME in PD mice (Fig. 5). Thus, the present study highlighted mitochondrial complex II as one of the therapeutic targets of PD and also to the best of our knowledge for the first time investigated and demonstrated the ameliorative effect of GME on the same.

Inflammation is considered as another major pathogenesis in PD (De Virgilio et al. 2016; Paul et al. 2017a). Oxidative stress, that affects the vulnerability of neurons, is known to further cause inflammation in neurodegenerative diseases, including PD (Lugrin et al. 2014). In order to evaluate the effect of GME on the underlying inflammatory conditions in the PD mice, we assessed the brain inflammatory markers nNOS and GFAP positive cells in the nigrostriatal areas of the brain. Enhanced activation of glial cells especially astrocytes, exemplified by the over-expression of GFAP, in response to a brain injury is a major neuroinflammatory event (Ducourneau et al. 2014). Following increased activation, a series of inflammatory mediators are released from the astrocytes causing damage to the neighboring neuronal cells (Ducourneau et al. 2014; Zhang et al. 2019). Thereafter, damaged neurons further activate the glial cells and generate a cycle which eventually leads to the progressive loss of neuronal cells in PD (Pekny and Pekna 2014; Zhang et al. 2019). Noticeably, increase in nNOS activity is also known to worsen the pathological conditions in PD by glial cell activation and inhibition of mitochondrial complexes (Hope et al. 1991; Madathil et al. 2013; Paul and Borah 2016). In addition, enhanced expression of nNOS is linked with the increase in nuclear translocation of NF-κB, which is

known to mediate the inflammatory pathways (Rathnasamy et al. 2014). Interestingly, our findings highlighted that GME treatment in the PD mice could reduce both GFAP active astrocytes and nNOS positive neurons in the nigrostriatal areas of the brain, thus indicating its ability to ameliorate hyperactivation of glia and nNOS positive cells (Figs. 6 and 7). In agreement with our findings, *G. morella* fruit extract has been reported to exhibit anti-inflammatory properties by inhibiting the release of inflammatory cytokines and nitric oxide in lipopolysacchride stimulated macrophages (Choudhury et al. 2018). Therefore, our studies indicate that GME could target the inflammatory pathways of neurodegeneration in PD.

Several bioactive compounds with promising therapeutic capabilities have been isolated from *G. morella* (Table 1, supplementary file). Among all the reported compounds, hydroxycitric acid, garcinol, kaempferol, epicatechin, amentoflavone, apigenin, isovitexin, orientin, caffeic acid, and luteolin are expected to play the key role in conferring neuroprotection which is exhibited by *G. morella* extract in our study (Choudhury et al. 2018; Pandey et al. 2015; Bheemaiah and Kushalappa 2019; Murthy et al. 2020). Fascinatingly, these compounds could cross the blood–brain barrier and are known to ameliorate mitochondrial dysfunctions, inflammation, and oxidative stress via modulating different cell signalling pathways (Cao et al. 2017; Chetia Phukan et al. 2022; Siddique and Jyoti 2017; Bitu Pinto et al. 2015; Pan et al. 2020; Wang et al. 2017; Siddique 2021; Liu et al. 2019; Ohia et al. 2001). Prevention of dopaminergic neuronal loss was shown in both cell culture and animal models with these compounds by upregulation of Nrf2 and PI3K/Akt signaling pathways, thereby reducing the oxidant and inflammatory responses and promoting cell survival and growth (Chung et al. 2019; Chetia Phukan et al. 2022; Hou et al. 2022; Cao et al. 2017; Li et al. 2020; Cheng et al. 2016; Liu et al. 2019; Pan et al. 2020; Xiao et al. 2018; Tsai et al. 2011; Ma et al. 2020; Antala et al. 2012). The referred compounds from *G. morella* were also reported to provide neuroprotection by suppressing the NF-κB and MAPK signaling pathways and subsequent inflammatory processes (Hou et al. 2022; Chung et al. 2019; Lv et al. 2016; Xiao et al. 2017; Ajiboye et al. 2019; Ma et al. 2020; Li et al. 2020; Kim et al. 2004; Wang et al. 2020). Therefore, it can be suggested that by virtue of these compounds, GME might have controlled the mitochondrial dysfunctions and inflammatory processes via modulating the Nrf2/PI3K/Akt/NF-κB/MAPK signaling pathways. However, the observed dopaminergic neuroprotection may be due to sole or synergistic effects of the compounds in GME, which needs to be delineated in further studies. Nevertheless, our experimental outcome demonstrated the neuroprotective role of *G. morella* in an animal model of PD.

Conclusion

Taken together, our study presented a novel mode of neuroprotection by *Garcinia morella* in a rodent model of PD. *G. morella* fruit extract targeted the mitochondrial dysfunctions and inflammatory stress that are considered as major pathophysiologies leading to the degeneration of nigrostriatal dopaminergic neurons in PD. In addition, the present study also suggests that recovery of dopamine and subsequent behavioural abnormalities observed in GME treated PD mice is resulting from the prevention of dopaminergic neuronal loss.

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Author contribution The idea and concept of the proposed study were designed by MKM, RP and AB. In vivo experiments were carried out by BCP and AD. In vitro experiment was performed by AD and BCP. Data interpretation, statistical analysis and script writing were performed by AD and BCP. The manuscript was edited by BCP, MKM, SK, RR, JN and AB. Studies performed were critically evaluated by MKM, RP, AC, PB, SK, DK and AB.

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Data availability The datasets generated during and/or analysed during the current study are available from the first author (duttaankur56@gmail.com) and corresponding author on reasonable request.

Code availability Not applicable.

Declarations

Ethics approval The experimental protocols met the National Guidelines and were approved by the Institutional Animal Ethics Committee of Assam University, Silchar (IEC/AUS/2013–052; AUS/IAEC/2017/PC/01).

Consent to participate Not applicable.

Consent for publication Not applicable.

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