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Original Research Article

Cuminum cyminum Linn (Apiaceae) extract attenuates MPTP-induced oxidative stress and behavioral impairments in mouse model of Parkinson's disease

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Abstract

Purpose: *To evaluate the protective effects of Cuminum cyminum Linn (Apiaceae, CCY) against 1-methyl-4 phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP)-induced oxidative stress and behavioral impairments in mouse model of Parkinson's disease (PD).*
Methods: *MPTP-intoxicated mice model of PD was used for evaluating the effect of CCY extract on behavioral deficits through rota rod, passive avoidance and open field tasks. The effect of CCY extract on oxidative stress levels were assessed by estimating enzyme status, including superoxide dismutase (SOD), catalase (CAT) and lipid peroxidation(LPO) in brain tissues of MPTP-induced mice.*
Results: *MPTP (25 mg/kg, i.p.)-treated mice resulted in a significant ($p < 0.001$) behavioral deficit in locomotor behavior (from 56.24 ± 1.21 to 27.64 ± 0.94) and cognitive functions (from 298 ± 3.68 s to 207.28 ± 4.12 s) compared with their respective control groups. Administration of CCY extract (100, 200 and 300 mg/kg, p.o.) for three weeks significantly and dose-dependently improved ($p < 0.001$ at 300 mg/kg) locomotor and cognitive deficits in MPTP-treated mice. CCY treatment also significantly ($p < 0.001$ at 300 mg/kg) inhibited MPTP-induced decrease in antioxidant enzyme levels (superoxide dismutase and catalase) and lipid peroxides in mice brain tissues.*
Conclusion: *CCY extract exhibits strong protection against MPTP-induced behavioral deficit through enhancement of antioxidant defense mechanisms. Therefore, CCY may be developed as a therapeutic strategy in the treatment of neurodegeneration seen in PD.*

Keywords: *Cuminum cyminum, Neurodegeneration, Catalase, Superoxide dismutase, Oxidative stress, Parkinson's disease*

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INTRODUCTION

Parkinson's disease (PD) is a progressive neurodegenerative disease characterized by a loss of dopaminergic neurons in the substantia nigra (SN) region of the brain [1]. Although the etiology of PD remains unknown, generation of reactive oxygen species (ROS) caused by oxidative stress together with reduced antioxidant defense system in nigrostriatal dopaminergic pathway is widely considered as one of the major cause of neuronal death [2]. Currently available drugs do not halt or slow down the progressive neurodegeneration seen in PD. These drugs also produce deleterious side-effects on long term usage. Therefore, alternative therapy using natural herbs having neuroprotective effects might be necessary in PD treatment [3].

Cuminum cyminum Linn (*C. cyminum*) Apiaceaeis is an annual herb widely used as a traditional medicine for the treatment of several disorders, such as toothaches, dyspepsia, diarrhea, epilepsy and jaundice [4]. Pharmacologically, *C. cyminum* has been reported to possess anti-diabetic, anti-bacterial, hepatoprotective, neuroprotective, anti-epileptic, hypolipidemic, anti-stress anti-oxidant and memory - enhancing properties [5-7]. Recently, *C. cyminum* was also reported to exhibit potent immunomodulatory, nephroprotective, anti-stress and memory enhancing properties [8-10]. But so far their protective effect in neurodegenerative disorders such as PD has not been studied.

The neurotoxin, 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), is well studied for its ability to induce oxidative damage and mitochondrial dysfunction in the nigrostriatal dopaminergic system in animal models, resembling the idiopathic PD in humans [11]. MPTP-induction has been well reported for the development of cognitive and behavioral deficits in both non-human primates and mice models [12,13]. Therefore, MPTP-induced neurotoxicity in mice is considered as the most useful functional model of Parkinsonism [13].

In the present study, the protective effect of *C. cyminum* against MPTP-induced behavioral deficits in mouse model of PD was investigated. In addition the effect of CCY on the antioxidant enzyme status in MPTP-induced mouse brain tissues was also evaluated to correlate its neuroprotective effect.

EXPERIMENTAL

Chemicals and reagents

MPTP hydrochloride and thiobarbituric acid were purchased from Sigma Chemical Co. USA. All other chemicals used were of analytical grade. Stock solutions of all chemicals were prepared in distilled water and the dilutions were made fresh on the day of the experiment.

Preparation of extract

Dried fruit of *C. cyminum* procured in the month of August 2008 were obtained from herbal market, Vijayawada, India. The fruit was authenticated by Dr. K.N. Vidyadar, a Botanist at VJ's College of Pharmacy and Research Institute, Andhra University, Rajahmundry, India and a voucher specimen (CCY-RJY/08) was deposited in Pharmacognosy department of the same institute. The fruit (1 kg) was powdered in a conventional mixer and grinder and extracted with boiling water (5 L) for 30 min in a Soxhlet apparatus (Borosil Glass Works Ltd, Mumbai, India). The filtrate was evaporated under vacuum below 70 °C in a rotary vacuum evaporator (Buchi R-210, Mumbai, India) to give a final yield of 8.33 %. The obtained *C. cyminum* extract named as CCY here after was dissolved in sterile distilled water, filtered on 0.22 µm filters and stored at -20 °C until use.

Animals

Adult male Swiss albino mice weighing between 20-25 g were obtained from the Animal House of National Institute of Nutrition, Hyderabad, India. The animals were housed in an air-conditioned animal room at 23 ± 2 ^{o}C with 12/12 h light/dark photoperiod, and given free access to food (Nutravet, Hyderabad) and water *ad libitum*.

The animals were kept for seven days in laboratory for habituation. All animal experiments were performed under the guidelines of Principles of Laboratory Animal Care (NIH publication No. 85-23, revised 1985) [14] and the Institutional Animal Care and Use Committee, Vishnu Institute of Pharmaceutical Education and Research, India (Reg no. 1358/ERe/S/10/ CPCSEA).

Experimental design

The mice were divided into five groups (n = 15), i.e., vehicle, MPTP (25 mg/kg), MPTP + CCY 100 mg/kg, MPTP + CCY 200 mg/kg and MPTP + ITE 300 mg/kg. MPTP 25 mg/kg (i.p.) was administered along with probenecid 250 mg/kg (i.p.) for five consecutive days to induce chronic Parkinsonian symptoms in mice as described previously [15]. Different doses of CCY (100, 200 and 300 mg/kg) were prepared freshly by dissolving in distilled water and administered on day 1 (1 h prior to MPTP administration) and continued up to 21 days through oral gavage (p.o.).

Locomotor behavioral paradigm was evaluated during the course of MPTP administration (Day 5) and also at the last phase of the study (Day 20). Cognitive paradigm using passive avoidance test was evaluated on day 20 (acquisition) and day 21 (retention). Open field test were evaluated on day 21. At the end of the study mice were sacrificed and their brains were isolated by cardiac perfusion. The protocol for perfusion and tissue processing was performed as described previously [16]. On day 22, all the brain tissues were rinsed in ice-cold isotonic saline, homogenized with 1 mL of ice-cold 0.1 M

phosphate buffer saline (pH 7.4) and centrifuged at -4 °C for 15 min. aliquots of homogenates were used for estimation of superoxide dismutase (SOD), catalase (CAT) and lipid peroxide (LPO) levels.

Rotarod test

In rotarod test (ROTA-ROD for mice 7650 by UGO Basile, Varese, Italy), the beam revolves around its longitudinal axis and the animal walks or runs forward in synchrony. After adaptation for 5 min, the mice were placed on a horizontal plastic rod rotating at a speed of 10 rpm for a maximum of 10 min. The period (s) that each mouse was able to maintain its balance walking on the top of the rod was measured.

Passive avoidance test

A step through type passive avoidance test apparatus (GEMINI, Model PACS-30, San Diego instruments Int., USA) was used to evaluate the effects of CCY extract on learning and memory as described previously [17]. The shuttle box is divided into two chambers of equal size (23.5 cm × 15.5 cm × 15.5 cm) separated by a guillotine door. Mice were placed initially in the light chamber with the door open. They displayed exploratory behavior, and then entered the dark compartment. Upon entering the dark compartment, the door closed automatically. Training was repeated until the mouse entered the dark compartment within 20 s (training trial). Twenty four hours after the training trial, the mouse was placed in the illuminated chamber. When the mice entered the dark chamber, electric foot shock (1 mA) was delivered for 3 s through the grid floor and the door was closed automatically (acquisition trial). The mouse was again placed in the dark chamber, 24 h after the acquisition trial and the latency time to enter the dark chamber was measured for 300 s (retention trial). If the mice did not enter the dark chamber within the cutoff time (300 s), it was assigned a latency value of 300 s.

Open-field test

Open-field consisted of a square arena of 40 cm × 40 cm and a wall 35 cm high. The square arena was divided into 16 sub-squares. The test was initiated by placing the mouse at the center of the arena. The behavior of the mouse was then observed for 5 min. After each test, the apparatus was thoroughly cleaned with cotton pad wetted with 70 % ethanol. The number of line crossings (crossing the square boundaries with both forepaws), rearing (standing on its hind legs), grooming (rubbing the body with paws or mouth and rubbing the head with paws) and duration of immobility were measured as described previously [18].

SOD assay

The ability to scavenge the superoxide radicals in the brain homogenate generated by auto-oxidation of pyrogallol in the alkaline medium was measured. Each 3mL reaction mixture contained 2.8 mL of potassium phosphate buffer (0.1 M, pH 7.4), 0.1mL tissue homogenate and 0.1 mL pyrogallol solution (2.6 mM in 10 mMHCl). Increase in the absorbance at 325 nm was recorded spectrophotometrically for a period of 5 min at 30 s interval (UV- 1601, Shimadzu). One unit of SOD is defined as the amount of enzyme required to cause 50 % inhibition of pyrogallol autoxidation under the assay conditions.

CAT assay

CAT activity was assessed by the method described previously [16]. Briefly, catalase activity is measured by the decomposition of hydrogen peroxide (H_2O_2) or by liberation of oxygen (O_2). The decrease in the absorbance by H_2O_2 as a function of time is used to follow the catalase-peroxide reaction. Reaction mixture consisted of 2.9 mL of 10 mM H_2O_2 in 50 µM Potassium phosphate buffer (pH 7) and 0.1 mL of tissue homogenate. Decrease in the absorbance at 240 nm was recorded spectrophotometrically for 3 min (UV- 1601, Shimadzu). The results were expressed as units of CAT activity/mg of protein.

LPO assay

The LPO content in the brain homogenate was determined by the spectrophotometric method as described previously [19]. Tissue homogenate (0.2 mL) was added to a mixture of 0.2 mL of 8.1 % SDS, 1.5 mL of 20 % acetic acid solution adjusted to pH 3.5 with NaOH, and 1.5 mL of 0.8 % aqueous solution of thiobarbituric acid (TBA). The final mixture volume was adjusted to 4.0 mL with distilled water, and then heated at 95 °C for 60 min in a water bath. After cooling, 1 mL of distilled water and 5.0 mL of the mixture of n-butanol and pyridine (15:1, v/v) were added to the final reaction mixture and shaken vigorously. After centrifugation at 4000 rpm for 10 min, the absorbance of organic layer was measured spectrophotometrically (UV- 1601, Shimadzu) at 532 nm. LPO was expressed in terms of nanomoles of malondialdehyde (MDA) per mg of protein.

Statistical analysis

All data are presented as mean ± SEM. Statistical analysis was performed with SAS statistical software (SAS Institute, Cray, NC, USA) using one-way analysis of variance, followed by Dunnett's tests. $P < 0.05$ was considered statistically significant.

RESULTS

Effect of CCY extract on MPTP-induced locomotor impairment in mice

As shown in Fig 1, MPTP toxicity significantly ($p < 0.001$) impaired locomotor activity son day 5 (9.85 ± 1.35) and day 20 (27.64 ± 0.94). Administration of CCY extract at various doses (100, 200 and 300 mg/kg) did not influence the MPTP-induced locomotor deficits on day 5. However, on day 20 CCY extract (100, 200 and 300 mg/kg) significantly attenuated the decreased locomotor activity in a dose-dependent fashion (37.21 ± 0.92; $p < 0.05$, 40.26 ± 1.13; $p < 0.05$ and 47.54 ± 1.19; $p < 0.001$ at 100, 200 and 300 mg/kg, respectively).

Effect of CCY extract on MPTP-induced passive avoidance in mice

As shown in Fig 2, no significant differences were observed in latency time in any group in the absence the aversive foot-shock stimulus (acquisition trial). The latency time was significantly increased in retention trial (298 ± 3.68 s) compared to acquisition trial (18.26 ±1.97 s) in control trained group. In addition, the latency to enter the dark compartment was significantly decreased 24 h after foot shock in MPTP-treated mice compared with control mice (control mice: 298.5 ± 1.5 s; MPTP-treated mice: 207.28±4.12 s, $p < 0.001$ vs. control mice). However, CCY extract treatment at indicated doses (100, 200 and 300 mg/kg) significantly attenuated the decreased latency time (238.63 ± 7.78 s; $p < 0.05$, 248.94 ± 8.89 s; $p < 0.05$ and 269.26 ± 8.21 s; $p < 0.01$) at 100, 200 and 300 mg/kg, respectively.

Effect of CCY extract on open field test in MPTP-exposed mice

MPTP-treated mice showed a significant ($p < 0.001$) impairment in all the four parameters tested when compared with normal control group (Figure 3). However, CCY significantly attenuated the MPTP-induced reduction in line crossing number, rearing number and grooming behavior in mice. The effects observed at indicated doses were dose-dependent and the highest effect was observed at 300 mg/kg dose ($p < 0.001$). Furthermore, the increased immobility time in MPTP-induced mice group was significantly lowered ($p < 0.001$ at 300 mg/kg) in CCY treated groups dose-dependently.

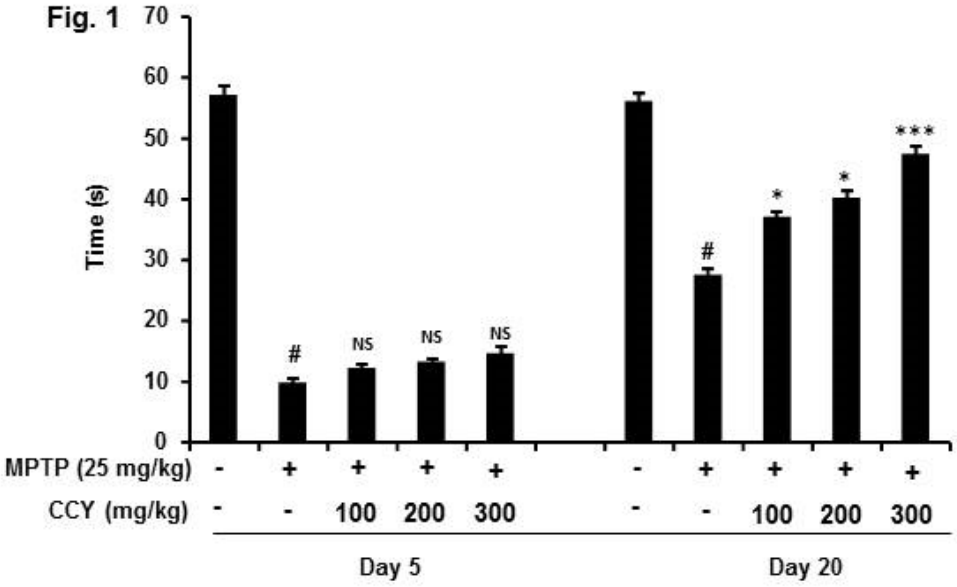

Figure 1: Effect of CCY extract on the MPTP-induced locomotor impairment in mice**.** Rotarod performance in different experimental groups on day 5 and day 20 was shown; $^{\#}p < 0.001$ compared with their respective untreated groups. NS: Not significant; $^{*}p<0.05$ and $^{***}p<0.001$ compared with MPTP-induced group. Data expressed as mean ± SEM (n = 5) using one-way ANOVA followed by Dunnett's test; MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; CCY: *Cuminum cyminum* extract

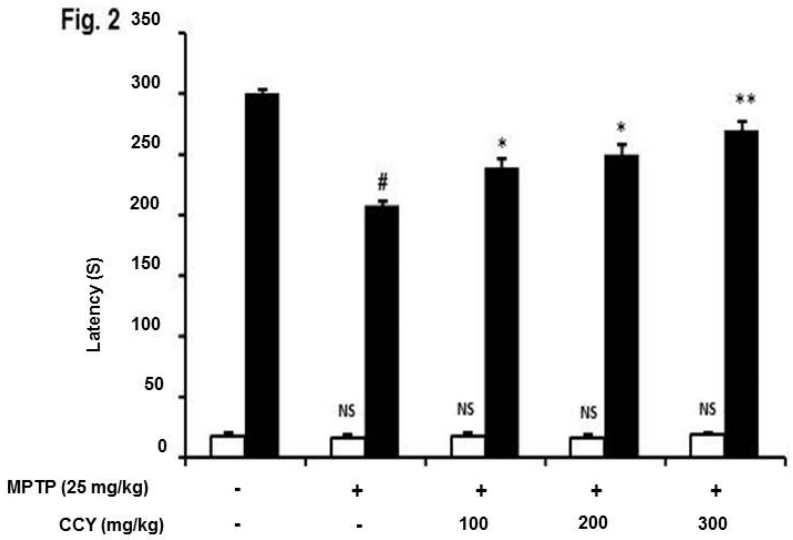

Figure 2: Effect of CCY extract on the MPTP-induced cognitive impairment in mice. Latency times (s) in acquisition (trial 1) was carried on day 20 and retention (Trial 2) was carried 24 h after trial 1 (Day 21). □: Acquisition, ■: Retention. $^{\#}p < 0.001$ compared with untreated group. NS: Not significant. $^{*}p<0.05$ and $^{**}p<0.01$ compared with MPTP-induced group. Data expressed as mean ± S.E.M. (n=5) using one-way ANOVA followed by Dunnett's tests using Graph Pad Prism v5.01 software. MPTP: 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine; CCY: *Cuminum cyminum* extract

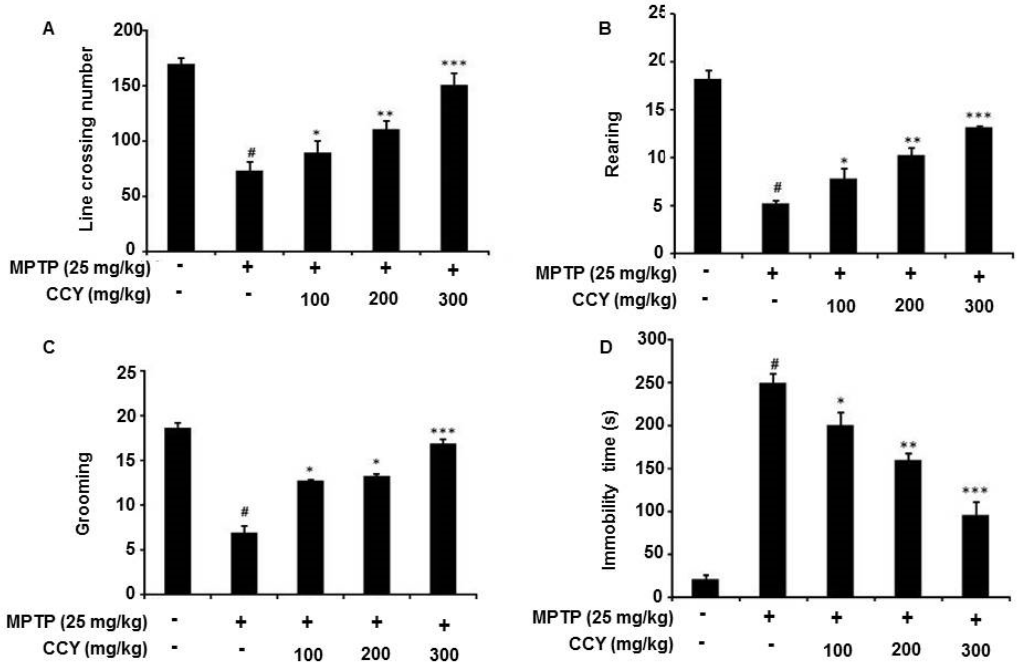

Figure 3: Effect of CCY extract on the MPTP-induced open-field performance in mice**.** A: Line crossing, B: Rearing, C: Grooming and D: immobility time was measured in different experimental groups. $^{\#}p < 0.001$ compared with untreated group; $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$compared with MPTP-induced group. Data are expressed as mean ± SEM (n = 5) using one-way ANOVA followed by Dunnett's test. MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; CCY: *Cuminum cyminum* extract

Effect of CCY extract on antioxidant enzymes in MPTP-treated mouse brain tissue

As shown in Fig. 4, significant decrease in the activities of SOD and CAT was observed in MPTP-treated group ($p < 0.001$) when compared with their respective control groups (Fig 4A and B). Furthermore, LPO levels were significantly ($p < 0.001$) increased in MPTP-induced group (Fig 4C). However, CCY treated groups (100, 200 and 300 mg/kg) dose dependently attenuated these changes. Although 100 mg/kg dose of

CCY did not show significant effect in altering the SOD and LPO activity, the effects were dose-dependent. The highest effect was observed at 300 mg/kg dose ($p < 0.001$).

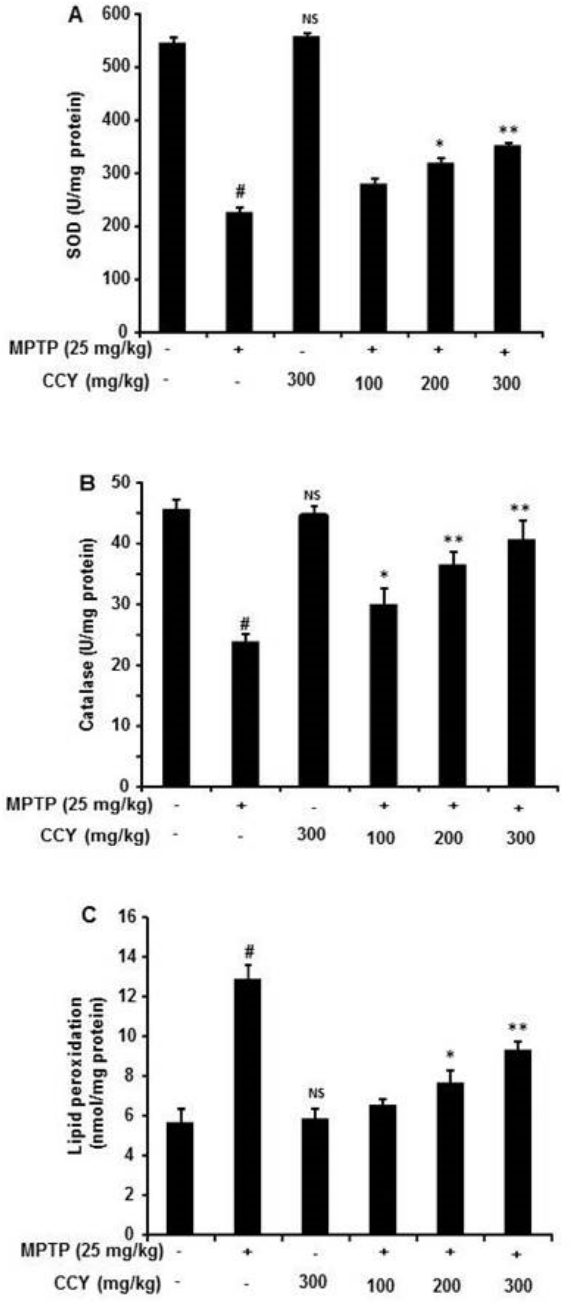

Figure 4: Effect of CCY extract on the anti-oxidant enzyme levels in MPTP-induced mice. The antioxidant enzymes levels in different experimental groups were shown. A: Superoxide dismutase (SOD), B: catalase (CAT) and C: Lipid peroxides (LPO); $^{\#}p < 0.001$ compared with untreated group. $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$compared with MPTP-induced group. Data expressed as mean ± S.E.M. (n=5) using one-way ANOVA followed by Dunnett's tests. MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; CCY: *Cuminum cyminum* extract

DISCUSSION

In the present study, CCY extract attenuated the cognitive and behavioral impairments in MPTP-treated mouse model of PD. The enhanced cognitive and behavioral function exhibited by CCY extract treatment was strongly supported by the reduced brain oxidative stress in MPTP-induced mice. It is well documented that MPTP-injected mice led to a decreased latency to fall on an accelerating rota rod apparatus, reflecting diminished balance and loss of muscular coordination [20]. Locomotor dysfunction including tremors, rigidity and bradykinesia are various kinds of clinical symptoms of PD [21]. Since the behavioral effects are intertwined with the degree of neuronal dysfunction, its assessment is a more powerful endpoint in evaluating neuroprotection.

In the present study, results from behavioral assessment through rota rod test suggest that long term treatment of CCY extract improved muscular activity and locomotion. It is well known that MPTP-induction to non-human primates and mice develop cognitive deficits [12,13]. Therefore, testing the behavioral function provides a sensitive evaluation of the CCY's ability to provide neuroprotection. In agreement, MPTP-induced mice significantly altered the cognitive performance with poor performance in passive avoidance and open field tasks. Mice administered 300 mg/kg CCY extract showed a significant improvement compared with lower doses (100 and 200 mg/kg).

MPTP damage mitochondria, proteins and lipids thereby altering the anti-oxidant enzyme status in the brain [22]. Therefore, estimating the antioxidant enzyme status in brain might be beneficial in understanding the degree of MPTP-intoxication in animal models. Furthermore, reports also revealed that antioxidant supplementation attenuated the reduced dopamine (DA) level and behavioral deficits in Parkinsonian mice [20,23,24]. In the present study, the oxidative stress in MPTP-induced Parkinsonian mice was measured by determining the activity of SOD, CAT and LPO levels in the mouse brain tissues. CCY extract treatment ameliorated the MPTP-induced reduction in the activities of antioxidant enzymes and resulted in reduced levels of oxidative stress. CCY extract effectively improved antioxidant enzyme activities in MPTP treated animals especially at the dose of 300 mg/kg. The anti-oxidant effects observed in this study were in agreement with our earlier reported works, that *C. cyminum* extract has ability to scavenge free radicals [6,10].

The major active constituents of CCY extract are cuminol, cymine, cuminaldehyde, limonene, eugenol, α & β-pinenes, terpenes and glucosides [25]. Some of these compounds are known to possess antioxidant activities [26-28]. These compounds might act individually or in a synergistic manner in delivering neuroprotective effect.

CONCLUSION

The findings of this study indicate that CCY extract attenuates MPTP-induced cognitive and behavioural impairments in mouse model of PD. Further, MPTP-induced alteration in anti-oxidative enzyme levels (SOD, CAT and LPO) was reversed in PD mouse brain tissues treated with CCY. Regulation of antioxidant defense mechanisms by CCY may partly be responsible for its neuroprotective effect in MPTP-induced PD mice.

ACKNOWLEDGEMENT

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REFERENCES

1. *Meissner WG, Frasier M, Gasser T, Goetz CG, Lozano A, Piccini P. Priorities in Parkinson's disease research. Nat Rev 2011; 10: 377-393.*
2. *Heh-In Im EN, Lee ES, Hwang YJ, Kim YS. Baicalein protects 6-OHDA-induced neuronal damage by suppressing oxidative stress. Kor J PhysiolPharmacol 2006; 10: 309–315.*
3. *Chaturvedi RK, Shukla S, Seth K, Chauhan S. Neuroprotective and neurorescue effect of black tea extract in 6-hydroxydopamine-lesioned rat model of Parkinson's disease. Neurobiol Disease 2006; 22: 421–434.*
4. *Nostro A, Cellini L, Di Bartolomeo S, Di Campli E, Grande R, Cannatelli MA. Antibacterial effect of plant extracts against Helicobacter pylori. Phytother Res 2005; 19: 198-202.*
5. *Satyanarayana S, Sushruta K, Sarma GS, Srinivas N, Subba Raju GV. Antioxidant activity of the aqueous extracts of spicy food additives--evaluation and comparison with ascorbic acid in in-vitro systems. J Herb Pharmacother 2004; 4(2): 1-10.*
6. *Sushruta K, Satyanarayana S, Srinivas N, Rajasekhar J. Evaluation of the blood-glucose reducing effects of aqueous extracts of the selected umbelliferous fruits used in culinary practices. Trop J Pharm Res 2006; 5: 613-617.*
7. *Johri RK. Cuminum cyminum and Carum carvi: An update. Phcog Rev 2011; 5: 63-72.*
8. *Mahesh CM, Gowda KPS, Gupta AK. Protective action of Cuminum cyminum against gentamicin- induced nephrotoxicity. J Pharmacy Res 2010; 3: 753-757.*
9. *Chauhan PS, Satti NK, Suri KA, Aminab M, Bania S. Stimulatory effects of Cuminum cyminum and flavonoid glycoside on Cyclosporine-A and restraint stress induced immune-suppression in Swiss albino mice. Chem-Biol Interact 2010; 185: 66-72.*
10. *Koppula S, Choi DK. Cuminum cyminum extract attenuates scopolamine-induced memory loss and stress-induced urinary biochemical changes in rats: a noninvasive biochemical approach. Pharm Biol 2011; 49(7): 702-708.*
11. *Javitch JA, Uhl GR, Snyder SH. Parkinsonism-inducing neurotoxin, N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine:Characterization and localization of receptor binding sites in rat and human brain. Proc Natl Acad Sci USA 1984; 81:4591–4595.*
12. *Yabuki Y, Ohizumi Y, Yokosuka A, Mimaki Y, Fukunaga K. Nobiletin treatment improves motor and cognitive deficits seen in MPTP-induced Parkinson model mice. Neurosci 2014; 259: 126–141.*
13. *Schneider JS. Modeling Cognitive Deficits Associated with Parkinsonism in the Chronic-Low-Dose MPTP-Treated Monkey. In: Levin ED, Buccafusco JJ, editors. Animal Models of Cognitive Impairment. Boca Raton (FL): CRC Press; 2006. Chapter 9.*
14. *Derrell C. "Guide for the care and use of laboratory animals. Institute of laboratory animal resources. National Academy Press, Washington DC, USA 1996.*
15. *Jackson-Lewis V, Przedborski S. Protocol for the MPTP mouse model of Parkinson's disease. Nat Protoc 2007; 2(1): 141-151.*
16. *Patil SP, Jain PD, Ghumatkar PJ, Tambe R, Sathaye S. Neuroprotective effect of metformin in MPTP-induced Parkinson's disease in mice. Neurosci 2014; 26; 277: 747-754.*
17. *Shen Z, Wang G, Lin SZ. Two-way shuttlebox avoidance conditioning and brain NADH in rats. PhysiolBehav 1990; 48: 515–517.*
18. *Gould TD, Dao DT, Kovacsics CE. The open field test. Neuromethods 2009; 42: 1–20.*
19. *Ohkawa H, Nobuko O, Yagi K Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979; 95: 351–358.*
20. *Moon M, Kim HG, Hwang L, Seo JH, Kim S, Hwang S, Kim S, Lee D, Chung H, Oh MS, et al. Neuroprotective effect of ghrelin in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson's disease by blocking microglial activation. Neurotox Res 2009; 15: 332–347.*
21. *Jankovic J. Parkinson's disease: Clinical features and diagnosis. J Neurol Neurosurg Psychiat 2008; 79: 368–376.*
22. *Perier C, Vila M. Mitochondrial biology and Parkinson's disease. Cold Spring Harb Perspect Med 2012; 4: 1–19.*
23. *Khan MM, Hoda MN, Ishrat T, Ahmad A, Khan MB, Khuwaja G, Raza SS, Safhi MM, Islam F. Amelioration*

of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced behavioural dysfunction and oxidative stress by Pycnogenol in mouse model of Parkinson's disease. Behav Pharmacol 2010; 21: 563–571.

24. *Chung YC, Kim SR, Park JY, Chung ES, Park KW, Won SY, et al. Fluoxetine prevents MPTP-induced loss of dopaminergic neurons by inhibiting microglial activation. Neuropharmacol 2011; 60: 963–974.*
25. *Gohari AR, Saeidnia S. A Review on Phytochemistry of Cuminum cyminum seeds and its Standards from Field to Market. Pharmacogn J 2011; 3(25): 1.*
26. *Bettaieb I, Bourgou S, Wannes WA, Hamrouni I, Limam F, Marzouk B. Essential Oils, Phenolics, and Antioxidant Activities of Different Parts of Cumin (Cuminum cyminum L.). J Agric Food Chem 2010; 58 (19): 10410–10418.*
27. *Rebey IB1, Zakhama N, Karoui IJ, Marzouk B. Polyphenol composition and antioxidant activity of cumin (Cuminum cyminum L.) seed extract under drought. J Food Sci 2012; 77(6): C734-C739.*
28. *Al-Juhaimi FY, Ghafoor K. Extraction optimization and in vitro antioxidant properties of phenolic compounds from Cumin (Cuminum cyminum L.) seed. Int Food Res J 2013; 20(4): 1669-1675.*