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Original Article

Curcumin protects from oxidative stress and inhibits α-synuclein aggregation in MPTP induced parkinsonian mice

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Abstract: While curcumin has been reported to provide several health medicinal benefits, its effects on brain, particularly during diseased condition, is known very less. To evaluate the contribution of curcumin against neuropathological implications of Parkinson's disease, mice were experimented in the present study. Both MPTP (M) and MPTP plus Curcumin (MC) group received 30 mg/Kg body weight MPTP intraperitoneally (I.p.), consecutively for 7 days at a regular interval of 24 hours and MC group additionally received curcumin (50 mg/kg body weight, I.p.) 1 hour prior to MPTP while, control mice were given saline (0.9% NaCl) during treatment period at similar time intervals. Result of MPTP treatment caused severe motor impairments, reduced tyrosine hydroxylase (TH), and dopamine level was found in M group. While, treatment with curcumin showed improved behavioral manifestations and increased striatal DA level, paralleling with reduced cellular stress parameters. In summary, present study suggest the neuroprotective efficacy of curcumin in MPTP induced neuropathology in MPTP Parkinsonism.

Keywords: Parkinsonism, MPTP, α-synuclein, curcumin, behavioral abnormalities, motor deficits

Introduction

Parkinson's disease (PD) is the second most prevalent neurodegenerative disorder that affects almost 0.01% of global population over the age of 60 [1]. The most prominent phenotypic characteristic of the disease is motor impairments, which includes rigidity, dyskinesia, gait imbalance, and tremor at rest [2]. Such motor deficits are also evident in parkinsonian toxin induced experimental animal models [3]. Clinical feature of PD includes dopaminergic neuronal loss in substantia nigra pars compacta [4] and as a result depleted level of dopamine in striatum has been reported [5]. Pathological hallmark of PD is the presence of Lewy bodies, which consists of insoluble and fibrous aggregates of α-synuclein with some other filaments [6]. Moreover, it has been shown that, soluble oligomers of α-synuclein are also possessing toxic potency in PD pathology [6, 7]. PD is determined by the progressiveness of the disease pathology and the etiology of the disease is consisting handful of influencer like: oxidative stress, mitochondrial dysfunction, inflammation, and apoptosis [8]. It has been shown that, cellular stress level during the disease progress has uniquely been altered by the involvement of dopamine (DA)-a crucial neurotransmitter responsible motor coordination [9, 10]. DA undergo the auto-oxidation process, which produce reactive oxygen species (ROS), which exacerbates the stress level in neuron [11, 12]. Moreover, production of quinones from such auto-oxidation also has neurotoxic effects, which adds woods on fire in PD [11, 13]. Besides, depletion of DA levels in striatum, tyrosine hydroxylase (TH)-a marker for live neurons, also has found to reduce immunosensitivity during PD progress [14, 15]. As striatum region of brain is known for its high energy metabolism due to enormous turnover of DA, always maintains a high levels of free radical concentration than any other tissue in the body [16]. Additional recruitment of free radicals initiates the breakdown of cellular homeostasis in striatal region and pathological implications has been shown to emerge out [17]. The ratio-

nale of understanding from this phenomena, mitochondrial targeted toxins have been introduced to prepare desired model formation in PD. It has been reported that, MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)-a neurotoxin, causes mitochondrial impairments which results into free radical generation and increased oxidative stress in neuron [18]. The molecular mechanisms behind such cytotoxic insult by MPTP holds the involvement of monoamine oxidase B (MAO-B), which converts MPTP into its toxic form MPP^+, which is able to enter into DA-ergic neurons with the help of DA transporter and vesicular monoamine transporter 2 (VMAT2) and blocks the mitochondrial complex-I activity [4]. Such blockade results into increased generation of reactive oxygen species (ROS) and less energy turnover [19]. To salvage from such unavoidable situation, potential herbal product with antioxidant activity, was the major search to researchers. Encouraging research has shown that curcumin-a polyphenol is having the antioxidant, anti-inflammatory, anticarcinigenic potency [20-22] and also have been shown to be effective against neurological implications [23, 24]. Moreover, curcumin effectively can cross the blood brain barrier (BBB), which is the most crucial condition to be a therapeutic approach for PD [25]. However, role of curcumin in MPTP-induced parkinsonian pathology in mice, have been less documented. Besides, several health benefits curcumin also reported without any side effects, which apprise its global acceptance in daily diet [26-28]. As the major therapeutic concern of PD is the formation and conversion of α-synuclein into its toxic state [29-31], inhibition of these processes is an immediate need for conclusive PD therapy. Interestingly, curcumin has been reported to have the potency to inhibit the aggregates of toxic protein [32-35]. Hence, it has been selected for the present study. Taken together, our present study was destined to explore whether curcumin provides neuroprotection by the virtue of its neuroprotective potency in MPTP induced parkinsonian mice.

Materials and methods

Experimental animals and ethical statement

Studies were conducted with male C57BL/6 mice (about 8 weeks old) weighing between 23 and 25 g, taken from institutional animal house. Six animals were housed per cage and were maintained in a temperature-controlled (25°C) room under 60% humidity, and 12 h diurnal cycle. Mice had *ad libitum* access to food and water. All experiments were performed in accordance with Guidelines on the Proper Care and Use of Animals in Laboratory Research and approved by Linyi people's Hospital animal ethics committee.

MPTP and curcumin treatment

The mice were injected intraperitoneally (i.p.) 7 times (for seven consecutive days, 24 hours apart) with 30 mg/kg/day MPTP (M group) and control group received a corresponding volume of saline (0.9% NaCl in ddH_2O). One hour prior to each MPTP injection, the MPTP plus curcumin treated groups (MC group) were treated with an intraperitoneal injection of curcumin at 50 mg/kg body weight for seven consecutive days. Biochemical tests were performed on day seven two hours after the last injection and for immunohistochemical tests separate sets of mice were dissected on day 14 to provide sufficient time for MPTP toxicity on brain histology. All the animals were sacrificed with minimum pain and their brains were processed for further analysis.

Materials

MPTP, Curcumin, Sodium chloride (NaCl). Dopamine, Hydrochloric acid (HCl), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), ethylenediaminetetraacetic acid disodium salt (EDTA), and heptane sulfonic acid (Sigma, St. Louis, MO, USA), double distilled Water (Milli-Q system, Bedford, MA), Acetonitrile (SISCO Research Laboratories), Dimethyl sulfoxide (DMSO), Silver nitrate (Merck), Potassium dichromate, Formaldehyde (Qualigens), Orthophosphoric acid (Fisher scientific), Chloral hydrate (Fluka).

Behavioral and neuromuscular assessment

Akinesia: Akinesia is the latency to initiate all four limb movement was measured in seconds [36]. The test was dismissed if the latency exceeded 180 s. Initially, animals were allowed to acclimatize for 3 min on the test platform (30×40 cm^2). Electronic stopwatch was used to minimize the manual error and experiment was performed thrice for each group.

Catalepsy: Catalepsy- the inability of the animal to correct an externally imposed posture, was measured by placing the animals on a wooden platform while its hind limbs were placed on wood and fore limbs on ground [37]. Latency time was measured when mouse moved its hind limbs from wooden platform (3 cm). Acclimatization time was for 3 min and the test was terminated if the latency exceeds more than 180 sec.

Hang test: The effect of curcumin on neuromuscular strength was analysed by grid hang test [38]. Animals were placed on a gridded horizontal surface and after successful gripping the platform was inverted, which make the animal to hang from the surface. Hang score was measured by the time the mouse is able to hang just before it lands on the ground. Intensive care was taken to minimise the injury during landing.

Biochemical analysis

Estimation of superoxide dismutase (SOD) activity: The activity of SOD was assessed following the method as previously described [39]. Xanthine and xanthine oxidase served as superoxide generator and nitro blue tetrazolium is used as superoxide indicator. Briefly, the reaction mixture consists of 960 µL of 50 mM sodium carbonate buffer (pH 10.2) containing 0.1 mM of EDTA, 0.1 mM of xanthine, 20 µL of xanthine oxidase, 0.025 mM of NBT, and 20 µL of brain supernatant. The absorbance is measured spectrometrically at 560 nm and the activity was expressed as units/min/mg protein.

Determination of catalase (CAT) activity: Catalase activity was measured by determining the rate of decomposition of hydrogen peroxide (H_2O_2) at 240 nm. In brief, assay mixture consisted of 50 µL of 1 M Tris-Hcl buffer (pH 8.0) containing 5 mM of EDTA, 900 µL of 10 mM H_2O_2, 30 µL of water, and 20 µL of the supernatant. Rate of decomposition of hydrogen was measured at 240 nm spectrometrically. Enzyme activity is expressed as nmol of H_2O_2 decomposed/min/mg protein [40].

Estimation of neurotransmitter and metabolites: As diurnal variations cause alteration in biogenic amines, the mice were sacrificed in the morning. Dissected striatum was collected and processed for the analysis of neurotransmitters with an HPLC based electrochemical detector. The tissue was sonicated (at 50 Hz for 10 s) in ice-cold 0.1 M $HClO_4$ containing 0.01% EDTA (1 mg of tissue/10 lL of $HCLO_4$). The supernatant collected after centrifugation at 17500×g for 10 min was injected (10 lL) into the HPLC system (Bioanalytical Systems Inc., West Lafayette, IN, USA). The flow rate was 0.7 mL/min, and the electrochemical detection was performed at +0.74 V. The composition of the mobile phase was 8.65 mM heptane sulfonic acid, 0.27 mM EDTA, 13% acetonitrile, 0.43% triethylamine, and 0.32% orthophosphoric acid [41].

Estimation of lipid peroxidation: Lipid peroxidation was evaluated by measuring the thiobarbituric acid reactive substances (TBARS) according to the thiobarbituric acid (TBA) test described earlier [42] with the a few modifications. After incubations in the perfusion system, brain samples were added to ice-cold deionized water containing 5 ml of butylated hydroxytoluene (BHT) dissolved in methanol for HPLC grade (10%, w/v). Water was previously gassed with 100% N_2 for 60 min to remove oxygen. Tissue homogenates were prepared on ice using a teflon pestle (10-12 strokes) and centrifuged at 3000×g for 15 min. The supernatant was retrieved. 20 µl of supernatant was taken to determine the amount of proteins using the procedure described previously [43]. Supernatant of 350 µl was taken to precipitate soluble and membrane proteins and obtain deproteinizated membranes. TCA was then added (250l) and centrifuged at 3000×g for 15 min. During this test temperature was maintained at 4°C and 5 µl of TBA reagent (0.67% TBA and 0.05N NaOH in 50 ml of deionized water previously gassed for 60 min with 100% N_2) was added to 5 ml of the resulting supernatant. This solution was placed under a stream of nitrogen at 4°C for 1 min and was afterwards heated at 100°C for 15 min. This stage was carried out on a N_2 gas phase obtained by displacing the air gas phase with 100% N_2 before closing the tubes. After heating, the tubes were cooled in a water bath at room temperature. Clear solutions were obtained with this procedure suitable for direct spectrophotometric measurement at 532 nm. A Perkin-Elmer Spectrophotometer Lambda 3B was used. The formation of TBARS was

expressed as malondialdehyde equivalents (MDA) per mg protein. The absorbance of the upper phase at 532 nm was read. In these fractions, proteins were also measured in supernatants of cerebellar slice homogenates.

Estimation of GSH and GSSG: Reduced glutathione in brain tissue homogenate was estimated according to a previously described procedure [44]. Homogenate was centrifuged at 16,000×g for 15 min at 40°C. Supernatant (0.5 mL) was added to 4 mL of ice-cold 0.1 mM 5,5-dithiobis [2-nitrobenzoic acid] solution in 1 M phosphate buffer (pH-8) and the absorbance was observed at 412 nm. For GSSG estimation, sonicated brain homogenate was taken in 10 volumes of deionized water. 20 µl of tissue homogenate 5 ml of a solution containing 1.43 M sodium borohydride, 1.5 mM EDTA, 66 mM NaOH and 1 of n-amyl alcohol was added, and incubated at 40°C in a water bath for 30 min. Proteins were precipitated by addition of 25 µl of ice-cold 0.4 M perchloric acid, and separated after centrifugation at 18,000×g for 15 min at 4°C. The supernatant was filtered and the sample was then allowed to a reaction with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) to form the mixed disulfide GS-TNB and the chromophore 5-thio-2-nitrobenzoic acid (TNB); this is followed by back-reduction of GS-TNB to GSH by GR and NADPH (prevailing reaction) or by direct reaction of GS-TNB with any GSH still present in the assay mix. At this point, the conditions are present for recurrence of this reaction in a self-sustained closed cycle and for analysis of the steady-state production of TNB by spectrophotometry at a 412 nm wavelength. The concentration of GSH was determined by subtracting GSSG from total GSH. GSH/GSSG ratio was estimated to interpret the evidence of redox unbalance in striatum.

Immunohistochemical and histological studies

Perfusion and tissue processing: Animals were sacrificed and perfused via intra-cardial infusion with ice cold phosphate buffered saline (1X) followed by 4% paraformaldehyde (pH 7.4). Brain was dissected out from the skull and fixed in 4% paraformaldehyde for 24 h at 4°C. Before taking section, the brains were transferred to 30% sucrose solution in PBS for overnight. The brain tissue was then embedded in cryoglue and sliced into 20 µm coronal sections containing entire SN for immunohistochemical study.

TH Immunohistochemistry and haematoxylin-eosin (H-E) histological analysis: Animals were anaesthetized with chloral hydrate (400 mg/kg; i.p.) and perfused intracardially with phosphate buffer saline (PBS, pH 7.4) followed by 4% paraformaldehyde. Brains were removed and kept in 4% paraformaldehyde, transferred to 30% sucrose and coronal sections (35 µm thickness) passing through striatum, hippocampus and cortex were taken using Cryotome (0620E Cryostat, Thermo Shandon, United Kingdom). The sections were rinsed three times with 0.1 M PBS (pH 7.4), incubated in 1% H_2O_2 in PBS, permeabilized with 0.4% Triton X-100, blocked for with 8% BSA containing 0.1% Triton X-100, incubated with the primary antibody (1:100) in PBS, containing 4% BSA for overnight at 4°C and then incubated with secondary anti-rabbit IgG-conjugated horseradish peroxidase antibody (1:500) in PBS containing 2% BSA for 1h at room temperature. Visualization was performed by incubation in 3,3-diaminobenzidine for 5 min and the sections were photographed by using a Fluorescent microscope (Nikon, Japan). While Haematoxylin-Eosin staining was performed according to the procedure described earlier [45, 46].

Statistical measures

The behavioural test data were statistically evaluated for significance employing one-way ANOVA followed by Newman Keul's post-hoc analysis. Neurochemical data were analysed for significance using Student's t-test. Results are given as mean ± S.E.M. Value of $P \leq 0.05$ was considered significant.

Results

Effect of MPTP and curcumin on bodyweight

MPTP treated mice showed parkinsonian behavioural and neuromuscular abnormalities, which were prominent from the second MPTP injection and such deficits existed till their sacrifice. Significant loss of body weight was observed from the second day of MPTP administration (**Figure 1A**). However, control and MC mice did not showed any marked differences.

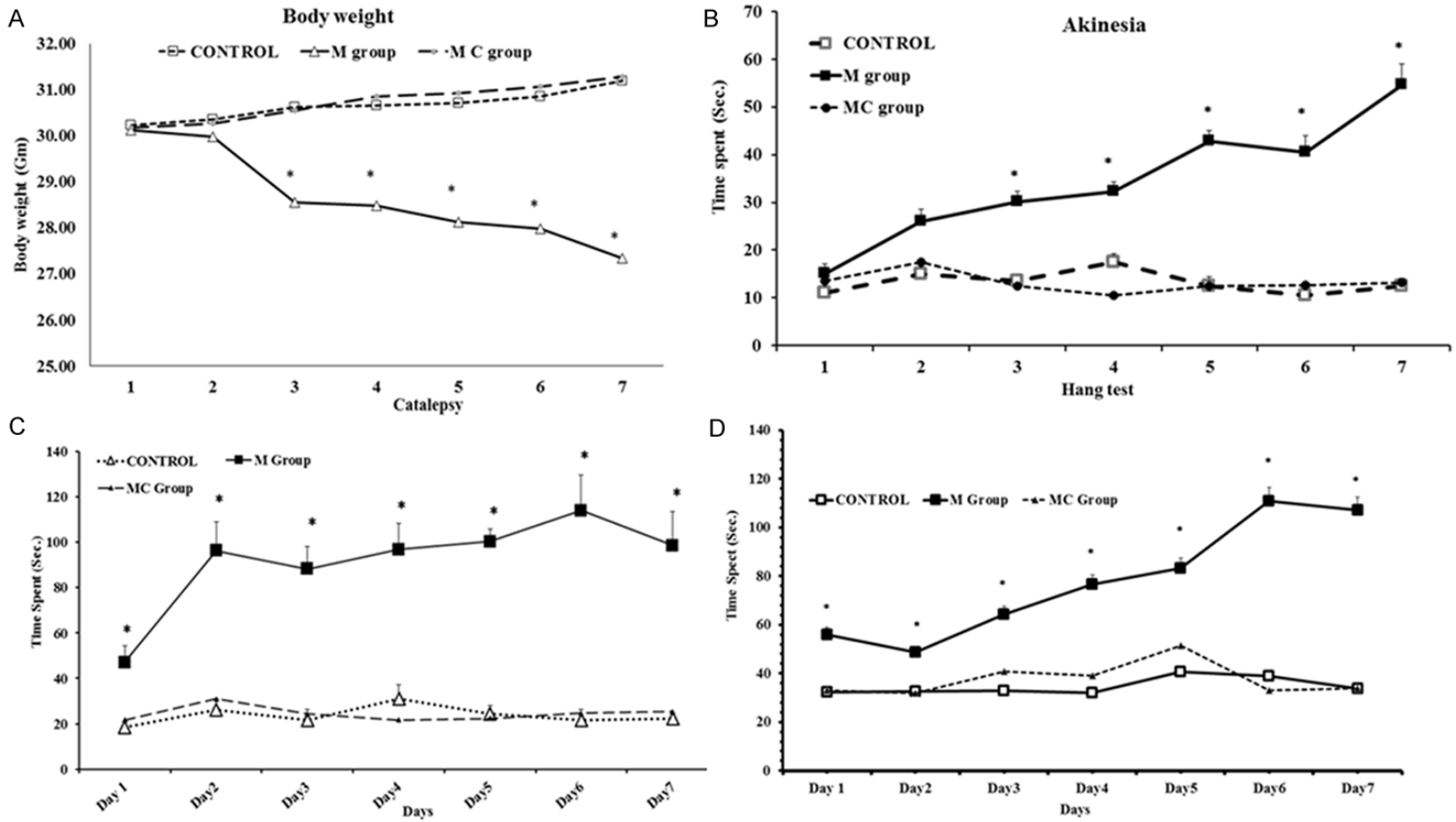

Figure 1. Effect of MPTP and curcumin on behaviour. A. Effect of MPTP and Curcumin on body weight: Body weight was measured every day before food. B. Effect of MPTP and Curcumin on akinetic behaviour. Akinesia was measured each day one hour after administration of MPTP with or without curcumin in mice. Mice received only MPTP showed akinesia i.e., latency to move all the four limbs. The highest akinetic score was on day 7 after MPTP injection. C. Effect of MPTP and Curcumin on cataleptic behaviour. Test for cataleptic behaviour was undertaken 2 hours after the administration of MPTP with or without curcumin in mice. D. Effect of MPTP and Curcumin on hang test performance. Effect of MPTP on the neuromuscular strength mice was tested on a horizontal grid apparatus, on each day 4 hours after the treatment of MPTP. Total time taken to climb down was recorded. All the results given are mean ± S.E.M., n = 6 (*P ≤ 0.05, with control).

Effect of MPTP and curcumin on akinetic behaviour

MPTP administration caused akinesia in the MPTP treated mice (**Figure 1B**). After second administration of MPTP the akinetic behaviour in these animals were visible. While, MC group of mice showed no significant difference in latency time with control. Increasing tendency of akinetic behaviour was observed among M group with the maximum latency score at day 7 with 64% more latency with control mice.

Effect of MPTP and curcumin on cataleptic behaviour

Catalepsy was observed among the M group of mice after first dose of MPTP injection. Control group of mice did not show any cataleptic behaviour. Latency in catalepsy test among M group showed increasing tendency with time and observed data was significantly different from control group (**Figure 1C**). Though, MC mice showed little latency but, it was not at all statistically significant form control mice. Highest latency in M group was observed on day 7 with 58% more latency with control.

Effect of MPTP and curcumin on neuromuscular strength

MPTP treatment caused neuromuscular impairments after second injection. M group of mice spent more time in contact with the supporting wall. However, behavioural performance of control and MC group was not effected significantly. In MPTP treated mice highest time taken to touch the ground from the horizontal supporting was recorded on day 6, which was 115 ± 1.98, and was 71.25% more than control animals of the same day (**Figure 1D**).

Effect of MPTP and curcumin on superoxide dismutase and catalase activity

MPTP treatment caused a significant increase in SOD activity (1.18 ± 1.21) in the striatum in M group. Co-treatment of MPTP with curcumin showed control like activity of SOD in MC group. Moreover, it was not statistically significant (0.653 ± 1.43) with the control mice (**Figure 2A**). MPTP injection caused a significant increase in catalase activity in the striatum. While, MC group showed non-significant increase of catalase activity with control group (**Figure 2B**).

Effect of MPTP and curcumin on neurotransmitter and metabolites

While solely MPTP decreased 57% striatal DA level, co-administration with curcumin didn't cause any DA depletion. Moreover, DA level in MC group showed almost similar level with control (**Figure 2C**). M group of mice showed significantly higher turnover of DA Comparatively, co-administration with curcumin with MPTP showed control like turnover trend (**Figure 2D**), indicates potential of curcumin to reduce the toxicity induced by MPTP.

Effect of MPTP and curcumin on lipid peroxidation

The TBARS level in Control group animals was found to be 2.63 ± 0.060 nmoles of MDA/mg protein. Curcumin co-administration with MPTP in MC animals found significantly ($P<0.05$) decreased TBARS content of brain homogenate from striatum. While, only MPTP treated animals (M Group) resulted in a significant ($P<0.05$) increase in TBARS level in striatum of animals as compared to Control group (**Figure 2E**).

Effect of MPTP and curcumin on GSH and GSSG content

MPTP injection (i.p.) in M group of mice caused a decrease in the GSH levels in the striatum. Co-administration of curcumin with MPTP in MC group significantly uplifts these levels. We also measured the levels of GSSG to get the total glutathione content as the total glutathione level is actually the sum of GSH and GSSG and also indicates the oxidative stress levels in brain (**Figure 2F**). In MC group GSSG/GSH ratio was similar to that of control group, indicates the compensatory effect of curcumin on total glutathione level (**Figure 2F**).

Effect of MPTP and curcumin on Immunohistopathology

Tyrosine hydroxylase (TH)-a rate-limiting enzyme for the synthesis of DA, is the marker for alive and functional dopaminergic neurons. TH is found abundantly in striatum, where DA-ergic nerve fibers and perikarya has been found to be saturated with its presence. In the present study, MPTP administration in mice caused severe loss of TH immunoreactivity in the striatum as compared to the control. Co-administration of curcumin with MPTP caused an increase in TH immunoreactivity in

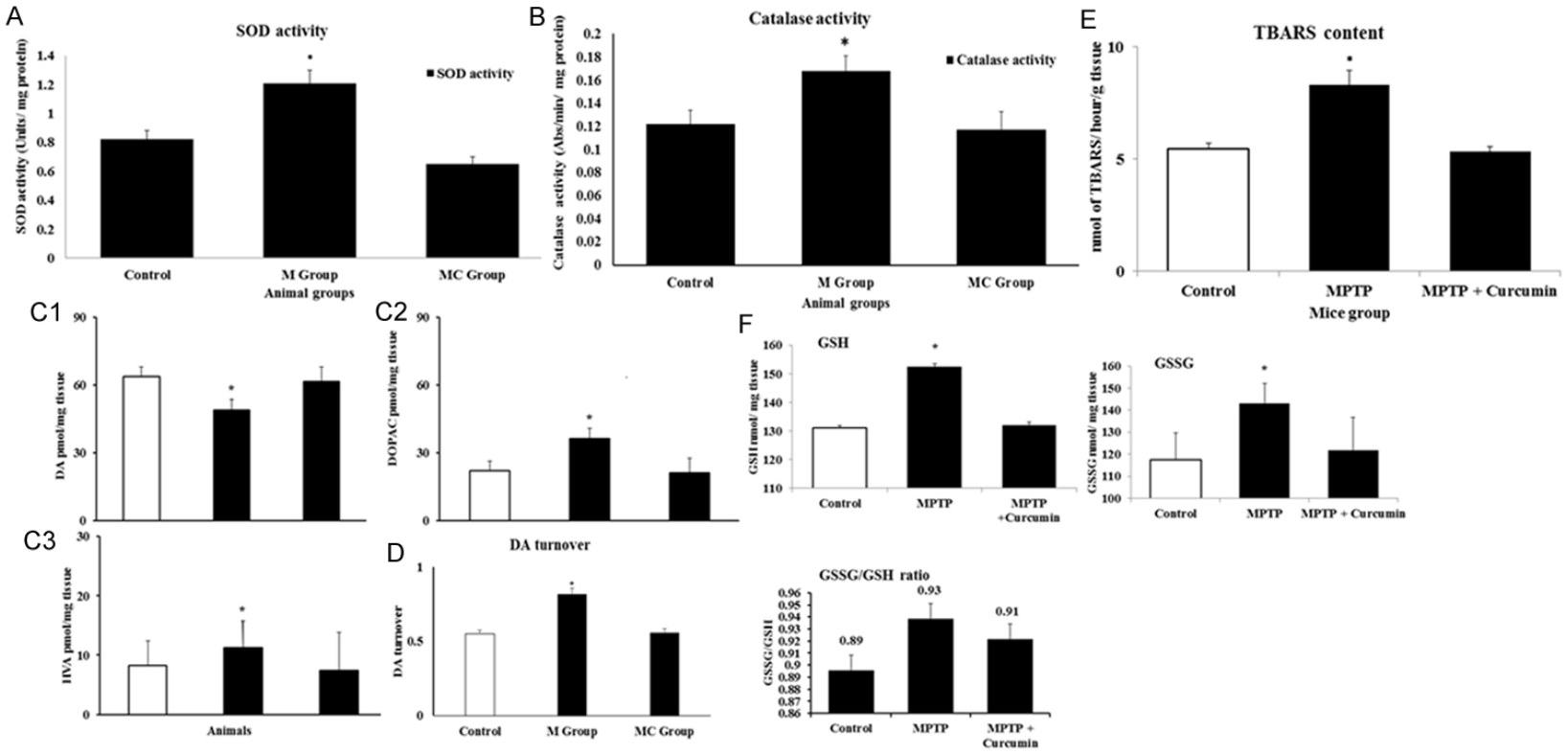

Figure 2. Effect of MPTP and curcumin on biochemical status. Effect of MPTP and Curcumin on SOD (A) and catalase (B) levels. Brain homogenate was processed to measure SOD and catalase enzyme activity analysis, which was determined employing a spectrophotometric procedure. The absorbance is measured spectrometrically at 560 nm for SOD and 240 nm for catalase and the enzyme activity was expressed as units/min/mg protein for SOD and nmol of H_2O_2 decomposed/min/mg protein for catalase. (C) Effect of MPTP and Curcumin on Dopamine level. Dopamine and its metabolites 3,4-dihydroxyplehanyl acetic acid (DOPAC) and homovanillic acid (HVA) were assayed employing a sensitive HPLC-electrochemical procedure in the striata of both drug treated and drug naive mice on day 7 following the last dose of drug treatment. (D) Effects of MPTP and Curcumin on dopamine turnover. Administration of MPTP and curcumin on dopamine turnover were computed as the ratio of the metabolites levels to the neurotransmitter [DA turnover = (HVA + DOPAC): DA]. (E) Effect of MPTP and Curcumin on lipid peroxidation. Brain homogenate was processed and TBARS content was determined employing a spectrophotometric procedure. Lipid peroxidation is expressed as nmoles of MDA/mg protein. (F) Effect of MPTP and Curcumin on GSH and GSSG content. Brain homogenate was processed to measure GSH and GSSG content, which was estimated by the production of TNB with spectrophotometry at a 412 nm wavelength. The concentration of GSH was determined by subtracting GSSG from total GSH. GSH/GSSG ratio was estimated to interpret the evidence of redox unbalance in striatum. All the results given are mean ± S.E.M., n = 6 (*P ≤ 0.05, with control).

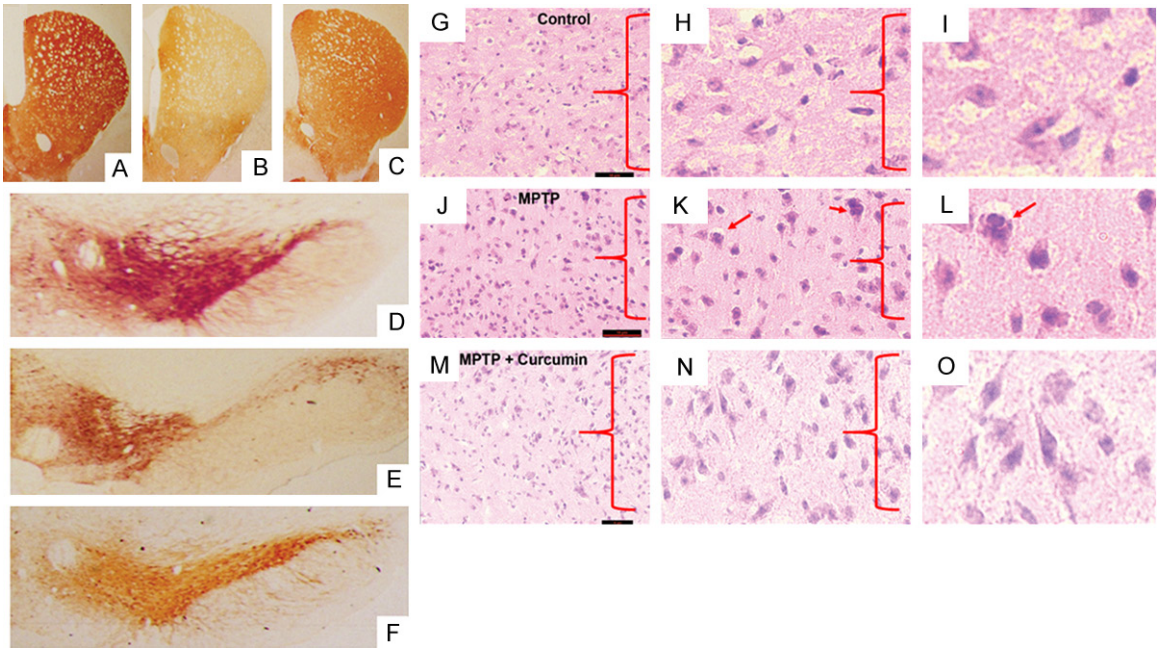

Figure 3. TH immunohistochemistry and H-E histochemistry. Overnight cryopreserved (30% sucrose solution) brain sections (coronal, 20 mm thick) passing through the striatum and substantia nigra were cut on a cryostat and were incubated with primary antibody (anti-rabbit TH polyclonal 1:1000), followed by HRP-conjugated secondary antibody (goat anti-rabbit IgG 1:300). (B) is the striatum of (M) mice, lacking TH immunereactivity and TH positive striatum were in control (A) and MC (C) mice. Substantia nigra of M mice also negative to immune staining (E). While, (D) and (F) are the sections from substantia nigra of the control and MC mice showed TH immunepositivity respectively. H-E stained Histological sections from striatum region showed the presence of Lewy bodies in (M) mice (J-L), which are absent in control (G-I) and MC mice (M-O).

the nerve fibers of the striatum as compared to the control striatum (**Figure 3A-F**). MPTP-induced loss in immunoreactivity was attenuated by curcumin co-administration. During visualization the brain section under light microscopy, M group of mice brain stained with H-E staining revealed presence of Lewy body in MPTP treated group (**Figure 3J-L**) while, MC and control group did not showed any trace of Lewy body.

Discussion

The result of the present study indicates that curcumin-a dietary food ingredient used mainly by Asian ethnic population, provides neuroprotection from MPTP induced toxic pathology. The study demonstrated for the first time that curcumin, when co-administered with MPTP, compensates the neurotoxicity and also provides neuroprotection with its anti-inflammatory, anti-oxidant and anti-aggregation potency, which was evident from neuromuscular performances as well as biochemical analysis.

Our result shows that curcumin possesses neuroprotective properties in animal model of PD induced by MPTP, which is an established model of Parkinsonism. Our study also encourages the use of curcumin for the treatment of PD. Previously it was showed that MPTP animal model of PD suffers from severe motor abnormalities, which were determined by classical parkinsonian neuro-behavioural phenomena like: akinesia, catalepsy [47], are also evident from the present study. Neuromuscular disabilities were reported in experimental animal models of PD as well as human PD patient [48, 49]. Such neuromuscular deficits was evident in our MPTP treated mice. Thus, validates the success of PD model preparation in the present study.

MPTP treatment showed severe behavioural abnormalities as we found in our present (**Figure 1B-D**), is consistent with other drug induced behavioural alteration in PD [50, 51]. Curcumin co-administration with MPTP did not alter any behavioural manifestation and showed almost similar result with the control mice (**Figure 1A-D**). Such finding indicates curcumin is successful to cope up with the pathological implications of MPTP at behavioural

level. The cause behind the weight loss among the MPTP injected mice could be due to role of MPTP on impairment of energy metabolism and suppression of cell's survival pathways (**Figure 1A**). Similar weight loss was evident in previous report of drug induced neurotoxicity [18] and our result is consistent with their findings.

MPTP treatment showed significant decrease in DA level in striatum region of brain (**Figure 2C**). This is might be due to toxic effects of MPTP in physiological environment, which in turn produce more toxic MPP^+ and after reaching the cytosol it binds to mitochondrial complexes and blocks the respiratory chain function [19]. While, co-administration of curcumin didn't showed any effect on DAergic neurons (**Figure 2C**). This may be due to the fact that curcumin is reported to upregulate the DA synthesis [52, 53] and its transporter expression [52, 54-56], which not only helps the mechanism of MPP^+ in to neurons but, also takes it out of the dopaminergic neurons. Hence, reduces the toxicity of MPTP, as reflected in no effect in behavioural manifestation of the animals co-administrated with curcumin. Such co-administration of curcumin with MPTP did not affect the levels of striatal DA, provides the basis of behavioral as well as neuromuscular no alteration among the MC mice group.

MPTP treatment in M group increased the cellular stress levels as evident from our result on increasing the levels of SOD and catalase in striatum (**Figure 2A**, **2B**). As cellular defense mechanism gets activated in presence of toxic substance, SOD and catalase activity increased to cope up the situations [57, 58]. It is known that MPTP effects on mitochondrial complexes as a result increased ROS production takes places and initiates other pathological cycles in neuron [59, 60]. While, striatal region MC mice did not altered the oxidant balance due to co-administration of curcumin (**Figure 2A**, **2B**). The reason behind such phenomena might be due to the anti-oxidant potency of curcumin and its physiological metabolites [61-64], which might have compensated the antioxidant need while MPTP and its metabolites were active in the neurons.

During toxic insult it was seen that oxidative stress increased and paralleling with the oxidative radical. Cellular antioxidant enzyme production also increases in such situation to cope up the adverse situation. During the process of cellular antioxidant response glutathione plays an important role. Glutathione also plays crucial role in DA turnover and pathogenesis of PD. Increased levels of oxidized glutathione (GSSG) represents the altered cellular stress level. The GSH/GSSG ratio indicates the cellular redox state, which is determined by levels of GSSG and relative de novo synthesis of GSH. In our study, M group of mice showed reduced levels of redox state and co-treatment with curcumin maintains the redox level like control mice (**Figure 2F**). Hence, it is suggestive from the present study that curcumin could be a potential therapeutic strategy for cellular stress mediated pathology as well as PD. MPTP insult have increased the levels of lipid peroxidation in M group (**Figure 2E**). While, treatment of curcumin with MPTP showed non-significant levels of lipid peroxidation. Interestingly, our result showed the opposite result those were reported earlier [58, 65]. Hence, our result also documents lipid peroxidation as a neurotoxic step in the pathology of MPTP induced Parkinsonism.

Immunohistological studies revealed that MPTP treatment successively reduced the TH expression in striatum but there were no change in MC group of mice (**Figure 3A-F**), indicates the potency of curcumin in maintaining normalization of DA synthesis and release. Control mice also did not showed any change in histopathology. Though, from Haematoxylin-Eosin (H-E) staining presence of 'Lewy body' is visible among the brain of MPTP treated mice group at fourteenth day (**Figure 3J-L**) but, MC mice (**Figure 3M-O**) brain did not showed any trace of 'Lewy body', suggests that MPTP induced aggregation of α-synuclein which ultimately forms Lewy body was inhibited by the co-administration of curcumin. Previous reports have shown that curcumin is having the ability to inhibit the pathological aggregation of α-synuclein. This fact might be the cause behind lacking Lewy body formation in MC group of mice. Taken together, our results indicate that curcumin is capable of reducing the toxic exposure induced by MPTP. Thus, future therapeutics might take curcumin as therapeutic agent for Parkinsonian pathology. Depending upon the administration strategy it is also suggestive that, curcumin in daily diet could reduce the chance of PD.

Acknowledgements

Hai-Ying Zhao and Qin-Liang Xu has given the concept of the present work. Xiang-Jun Xia and Yong-Gang Lian performed the experiment. Hai-Ying Zhao and Xiang-Jun Xia prepared the manuscript.

Disclosure of conflict of interest

None.

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