

# Neuroprotective effects of piperine on the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced Parkinson's disease mouse model

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**Abstract.** Parkinson's disease (PD) is second only to Alzheimer's disease as the most common and debilitating age-associated neurodegenerative disorder. Currently, no therapy has been shown to unequivocally retard or arrest the progression of the disease. The aim of the present study was to investigate the protective effect of piperine on the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced Parkinson's mouse model. For MPTP treatment, the animals received repeated intraperitoneal injections (i.p.) of MPTP (30 mg/kg) solution for 7 days. Piperine (10 mg/kg) was administered orally for 15 days including 8 days of pretreatment. Motor behavior analysis was conducted with the rotarod test. The Morris water maze (MWM) was used to assess the cognitive learning ability of the mice. A histological examination was subsequently conducted. The results demonstrate that piperine treatment attenuated MPTP-induced deficits in motor coordination and cognitive functioning. Piperine also prevented MPTP-induced decreases in the number of tyrosine hydroxylase-positive cells in the substantia nigra. Additionally, piperine reduced the number of activated microglia, expression of cytokine IL-1 $\beta$ , and oxidative stress following MPTP treatment. An anti-apoptotic property of piperine was identified by maintaining the balance of Bcl-2/Bax. In conclusion, the results show that piperine exerts a protective effect on dopaminergic neurons via antioxidant, anti-apoptotic, and anti-inflammatory mechanisms in an MPTP-induced mouse model of PD. Thus, piperine is a potential therapeutic treatment for PD.

## Introduction

Parkinson's disease (PD) is a neurodegenerative disorder characterized by progressive and selective degeneration of dopamine (DA) neurons in the substantia nigra pars compacta (SNpc), a region that controls movement (1,2). The initial symptoms of PD include basal tremor, muscular rigidity, bradykinesia, cognitive impairment, postural abnormalities and instability (3). The cause of PD remains undefined. However, a number of environmental, immune (4), and genetic (5) cues have been associated with the onset of this disease. Accumulative evidence has revealed many biochemical processes and molecular mechanisms that are involved in mediating neuronal cell death in PD. These processes and mechanisms include oxidative stress, apoptosis, inflammation, mitochondrial dysfunction (6,7) and ubiquitin-proteasome system dysfunction (8).

1-Methyl-4-phenylpyridinium (MPP<sup>+</sup>), the active metabolite of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), is a neurotoxin that selectively destroys nigrostriatal DA neurons *in vivo* as shown in studies using rodents and non-human primates (9). MPP<sup>+</sup> induces apoptotic cell death by releasing cytochrome *c*, leading to the opening of the mitochondrial permeability transition pore (MTP) and subsequently activating caspases (10-13). Oxidative stress is also involved in dopaminergic neuronal cytotoxicity by the observation that infusion of MPP<sup>+</sup> into the brain increases hydroxyl radicals and the formations of lipid peroxides in the striatum (14).

Although PD symptoms can be effectively treated by DA replacement therapy, the current treatments are not successful in altering the progression of the disease (15). Additionally, long-term treatment with a DA agonist or levodopa leads to severe motor deficits, such as motor fluctuation and dyskinesia, and non-motor adverse reactions, such as cardiac arrhythmia, DA dysregulation syndrome, abdominal discomfort, PD dementia, and sleep disorders (1). Therefore, the optimal strategy is to identify a drug with neuroprotective traits that exhibits few or no adverse side effects.

Piperine (1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]-(E,E)-piperidine 1-piperonylpiperidine) is a pungent nitrogenous alkaloid present in black pepper (*Piper nigrum*), long pepper (*Piper longum*) and other Piper species fruits (family Piperaceae) (16). It has anti-inflammatory (17),

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antioxidant (18), antipyretic, gastroprotective and antidiarrheal properties in rodents (19,20). Pharmacological studies have reported that piperine possesses anticancer and antioxidative properties (21,22). In some countries of Asia, *Piper longum* L. has also been applied in folk medicine to ameliorate asthma, intestinal disorder, and poor peripheral blood circulation (23). Piperine possesses powerful antidepressant (24) properties and protects against cognitive impairment in animal models of Alzheimer's disease (25). Piperine has also been reported to inhibit MPP<sup>+</sup>-induced mitochondrial dysfunction and cell death in PC12 cells (26). However, whether piperine exerts neuroprotective effects against the MPTP-induced mouse model of PD remains to be reported.

In the present study, we hypothesized that piperine, consistent with its antioxidant property, exerted anti-parkinsonian effects by attenuating neuronal oxidative stress, apoptosis, and inflammation. For this purpose, we assessed the ability of piperine to protect against MPTP-induced motor and cognitive impairments in the rotarod and Morris water maze (MWM) tests as well as MPTP-induced reductions of dopaminergic neurons in SNpc. To determine the mechanism of the observed effects, we assayed lipid peroxidation by measuring activity of the oxidative stress marker malondialdehyde (MDA) and the antioxidant enzyme superoxide dismutase (SOD). Microglial activation, the pro-inflammatory cytokine interleukin-1 $\beta$  (IL-1 $\beta$ ), and expression of the pro-apoptotic protein Bax and the anti-apoptotic peptide Bcl-2 were also assessed.

## Materials and methods

**Experimental animals and treatment.** Male C57BL/6 mice weighing 18–20 g were obtained from JXJ Experimental Animal Co., Ltd., Shanghai, China (2010002601739) and kept in a room maintained on a 12 h light/dark cycle and temperature of 20–22°C with food and water available *ad libitum*. To minimize discomfort and pain for the animals, experimental procedures were carried out in accordance with the European Community's Council Directive of 24 November 1986 (86/609/EEC). The mice were randomly divided into 3 groups (n=9): normal saline-treated controls (NS), piperine-treated 10 mg/kg body weight MPTP-induced group (P+M), and the group treated with MPTP alone (MPTP). Piperine was dissolved in 5% carboxymethylcellulose sodium solution. The mice were treated with piperine (10 mg/kg; Selleck Chemicals, Houston, TX, USA) by daily intragastric administration for 15 days. For the MPTP treatment, the mice received intraperitoneal injection of MPTP hydrochloride (30 mg/kg; Sigma-Aldrich, St. Louis, MO, USA) dissolved in normal saline once daily for 7 successive days, starting the 8th day of piperine treatment. After the behavioral testing was conducted, the animals were sacrificed and their brains were dissected and prepared for immunohistochemical staining (4 mice from each group were sacrificed by cervical dislocation) or western blot analysis (the remaining mice were sacrificed by perfused fixation) (for experimental schedules, see Fig. 1).

**Rotarod test.** To measure motor coordination, the mice were assessed on the rotarod apparatus (27). Prior to the test session, each mouse received 30 min daily training for two successive days (speed 0.17 x g). The rotational speed was increased to

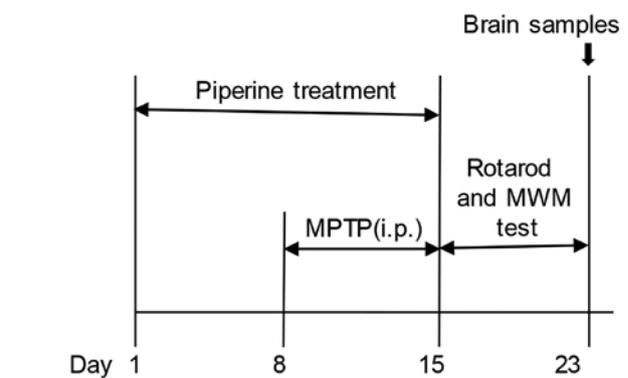


Figure 1. Schematic timeline of the experimental paradigm.

0.21 x g on the third day. Each mouse was placed in a separate lane of the rotarod (3 cm in diameter) and the time they remained on the rotating bar was recorded. The maximum time was 6 min/trial. Each mouse was given three trials on the rotating bar, and the average retention time for each mouse was used for comparison. The examiner conducting the rotarod test was blind to the treatment.

**MWM test.** For the MWM test, a stainless steel cylindrical tank (120 cm in diameter) surrounded by a wall 40 cm high and filled with homothermal water (22°C) was used. A plastic platform, 8 cm in diameter, was submerged 1.5 cm below the water surface with its base fixed to the floor of the tank. Four large unique navigation markers were placed above the edge of each quadrant of the tank as geographical cues prior to releasing the animals into the water.

In the hidden platform acquisition test, on each of four consecutive days mice underwent four swimming sessions. For each session, the mice were placed facing the wall of the pool and released from a starting point pseudo-randomly chosen from the four predetermined positions into the water. The time mice spent to reach the platform was recorded as the escape latency. If any mouse failed to reach the platform within 60 sec, they were guided and placed on the platform for 20 sec by the experimenter and the escape latency was recorded as 60 sec. The platform location remained constant throughout the test. For a particular day for an individual mouse, the average time spent over the four sessions was utilized as the latency score. The 4 day averages were then measured for each group to evaluate spatial learning ability.

On the fifth day, an additional 1 min session occurred with the platform removed (probe session). The mice were placed in the diagonal quadrant of the hidden platform originally located. Site crossings (the number of times animals crossed the original platform location) were recorded and used to indicate the degree of memory maintenance.

The results were analyzed with the analysis system of Morris water Maze (Huaibei Zhenghua Biological Equipment, Anhui, China). Two series of MWM tests were conducted by two professional technicians who were blind to the treatments.

**Immunohistochemical staining procedures.** Brain tissue was prepared for immunohistochemical staining. Briefly, the mice were perfused under chloral hydrate anesthesia through the ventriculus sinister with normal saline, followed by 4%

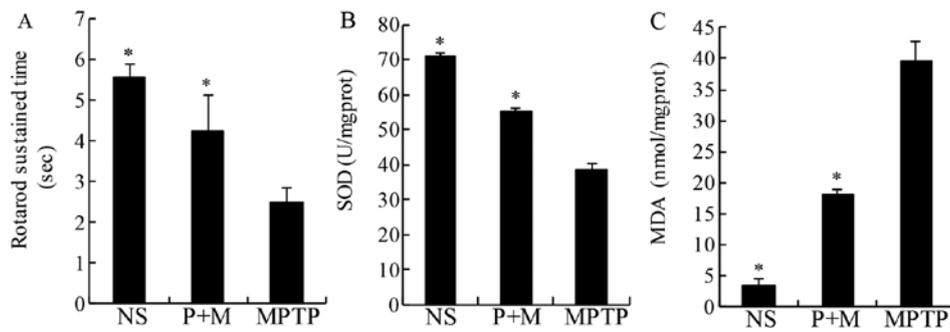


Figure 2. (A) Mice were placed on the rotating rod and the length of time they remained on the rotating rod was recorded. Piperine-treated mouse showed more sustained than MPTP group. (B) Levels of malondialdehyde (MDA) in the midbrain of mice was measured by a thiobarbituric acid reactive substances assay kit, and expressed as nmol/mg protein obtained from 5 mice/group. Piperine inhibited lipid peroxidation (MDA generation) induced by MPTP. (C) Superoxide dismutase (SOD) activity in the midbrain of mice was measured spectrophotometrically and expressed as units per milligram of protein obtained from 5 mice/group. Piperine restored the levels of SOD. Values are expressed as mean  $\pm$  standard deviation, \* $P < 0.05$ , compared to MPTP.

ice-cold paraformaldehyde for ~20 min. After antigen retrieval and phosphate-buffered saline (PBS) rinse, the brain sections were incubated at 37°C overnight with rabbit monoclonal anti-tyrosine hydroxylase (TH) (1:600), rabbit anti-IL-1 $\beta$  (1:200) or rabbit anti-Iba-1 (1:200) (all from Abcam, Qatar, Kingdom of Saudi Arabia). The following day, the brain sections were again rinsed with PBS and incubated with the appropriate biotinylated secondary antibody, goat anti-mouse/rabbit HRP-labeled (K5007; Dako, Glostrup, Denmark) for 50 min. The sections were then washed and stained with a DAB staining kit (Dako). After Harris hematoxylin staining and dehydration, the stained sections were mounted and analyzed under an optical microscope (Nikon, Tokyo, Japan).

**Assay for MDA activity.** Activity of the lipid peroxidation product MDA was measured by using a thiobarbituric acid reactive substances assay kit (Jiancheng Bioengineering, Nanjing, China) according to manufacturer's instructions. The assay was performed using a homogenate of midbrain tissues in physiological saline according to the given protocol. The supernatant was prepared by centrifugation at 9,184 x g for 10 min at 4°C (Smart R17; Hanil Science Inc., Incheon, Korea). Absorbance at 532 nm was measured using a microplate reader (Synergy HT; BioTek, Winooski, VT, USA). The MDA content was calculated according to the equation in the protocol and expressed in nanomoles per milligram protein.

**Assay for SOD activity.** The aforementioned midbrain tissue homogenates were also used to study SOD activity. Total SOD activity was determined spectrophotometrically using a SOD assay kit (Jiancheng Bioengineering) and calculated according to the manufacturer's instructions. In this study, a SOD unit was defined as the amount that reduced the absorbance at 550 nm by 50%. The result was expressed as SOD units per milligram protein.

**Western blot analysis of IL-1 $\beta$ , Bax and Bcl-2.** Brain tissues were prepared as previously described (28-30). Briefly, the midbrain tissues were homogenized overnight in an ice-cold lysis buffer containing a protease inhibitor cocktail (Sigma-Aldrich). Homogenates were centrifuged for 30 min at 13,225 x g at 4°C (Smart R17; Hanil Science Inc.) and the resulting supernatant contained the cytosolic fraction. Protein concentration was

measured with a BCA Protein assay kit (Biosharp, Shanghai, China). After boiling the samples at 95°C for 5 min, 35  $\mu$ g of protein was loaded in each lane with a gel loading buffer containing 10% glycerol, 62.5 mM Tris-HCl, pH 6.8, 50 mM dithiothreitol, 2% sodium dodecyl sulfate (SDS) and 0.1% (w/v) bromophenol blue. Western blot analysis was conducted as described by Burnette (31), with little modification. Proteins were resolved using SDS-polyacrylamide gel electrophoresis (SDS-PAGE) with 12% acrylamide resolving gel for IL-1 $\beta$ , Bcl-2 and Bax, and then transferred to polyvinylidene difluoride membranes (Millipore, Billerica, MA, USA). The membranes were blocked in TNE containing 5% skim dry milk in Tris-buffered saline and 0.05% Tween-20 detergent for 4 h. The primary antibodies were diluted in blocking buffer. Anti-IL-1 $\beta$  (1:500; rabbit monoclonal), anti-Bax (1:500; mouse monoclonal), and anti-Bcl-2 (1:500; mouse polyclonal) (all from Abcam) were used. The nitrocellulose membranes were then incubated overnight with primary antibodies at 4°C. After washing three times, the membranes were incubated with secondary antibodies, goat-anti-mouse-HRP-labeled (1:1,000; Boster Biotechnology, Wuhan, China) for 2 h at room temperature. The color was revealed by recovering DAB (50 mg/100 ml + 30% H<sub>2</sub>O<sub>2</sub>) reagent on the membranes. Conserved protein ( $\beta$ -actin) was measured to assay equal loading of protein. The pixels were measured using the Chemidoc XRS imaging system (Universal Hood II; Bio-Rad Laboratories, Hercules, CA, USA).

**Statistical analysis.** Results are presented as mean  $\pm$  standard deviation. The comparisons among groups were evaluated by one-way ANOVA, followed by LSD tests using SPSS 17.0 (SPSS, Inc., Chicago, IL, USA).  $P < 0.05$  was considered statistically significant.

## Results

**Effect of piperine on rotarod in MPTP-induced parkinsonian mice.** The aim of the antioxidant therapy in PD is to decrease functional impairments (32). Thus, in the present study, the rotarod was used to directly assess motor coordination. As expected, MPTP treatment significantly decreased the latency to fall off the rotating rod relative to NS mice, an effect that was significantly prevented by pretreatment with piperine (10 mg/kg) (Fig. 2A).

Table I. The performance of the mouse model in the probe trial of the MWM test.

Group (n=9)	Escape latency (second)				Site crossings
	1st day	2nd day	3rd day	4th day	5th day
NS	41.43±6.24 <sup>a</sup>	31.07±4.20 <sup>a</sup>	30.75±1.76 <sup>a</sup>	23.23±1.31 <sup>a</sup>	2.75±1.00 <sup>a</sup>
MPTP	58.06±3.82	48.1±6.00	47.36±2.10	46.65±5.73	0.5±0.58
Piperine	50.77±8.84 <sup>a</sup>	40.21±4.25 <sup>a</sup>	38.37±2.13 <sup>a</sup>	37.42±1.44 <sup>a</sup>	1.83±0.31 <sup>a</sup>

<sup>a</sup>P<0.05 vs. MPTP. MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NS, normal saline-treated controls.

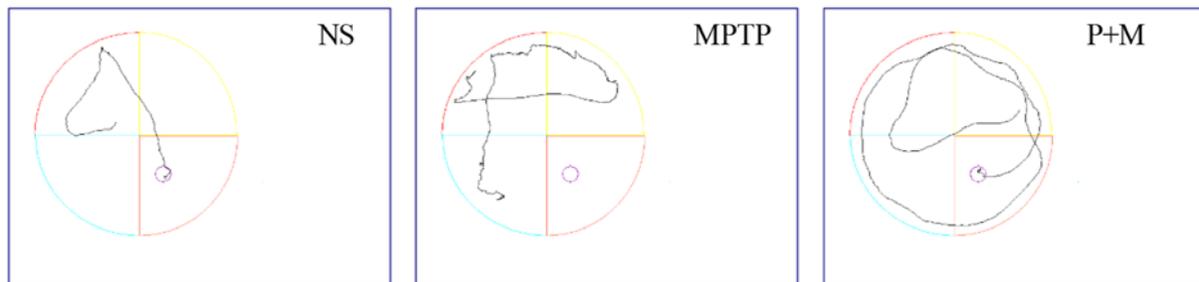


Figure 3. The track of the mouse model in the Morris water maze test was recorded and analyzed with the analysis system of Morris water Maze. MPTP-induced mice failed to find or experienced difficulty in finding the platform within the set time and piperine exerted a protective effect against this phenomenon.

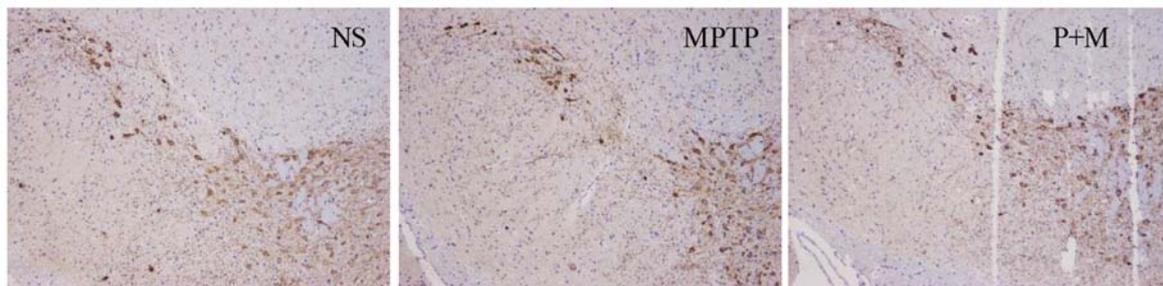


Figure 4. Piperine protects dopaminergic neurons in the MPTP-induced mouse model of Parkinson's disease. Brain sections were processed for immunostaining with antibodies against tyrosine hydroxylase (TH). An obvious decrease in TH-positive cells was observed in the substantia nigra in MPTP-treated mice and this phenomenon was significantly prevented by piperine treatment.

*Effect of piperine on the MWM test in MPTP-induced parkinsonian mice.* Various cognitive impairments including deficits in learning and memory are common clinical symptoms of PD, so the MWM was used to assess learning and recall. The mean escape latency decreased gradually over repeated days in all the groups (Table I and Fig. 3), and the mean escape latencies of the NS and piperine groups were significantly shorter compared to the MPTP group. These results indicated that MPTP impairs learning ability in the MWM test and piperine pretreatment was able to protect against this impairment. In the probe trial, the MPTP group showed decreased site crossings ( $P<0.05$ ), suggesting that piperine is capable of improving the spatial memory ability of PD mice in the MWM test (Table I).

*Piperine protects DA neurons against MPTP-induced neurotoxicity.* Brain sections were immunostained for TH, a marker for DA neurons. The result was expressed as the number of TH-positive neurons in the SNpc. TH-positive cells were

significantly decreased in the SNpc after MPTP administration relative to the NS group ( $P<0.05$ ), demonstrating that MPTP induced dopaminergic neuronal toxicity. However, piperine treatment (10 mg/kg) clearly protected against MPTP-induced dopaminergic neuronal death in the SNpc (Fig. 4).

*Piperine attenuates inflammation in MPTP-treated brains.* Previous findings have shown that MPTP treatment induces degeneration of DA neurons due to the induction of pro-inflammatory cytokine secretion by activated microglia (33,34). Therefore, we examined the expression of IL-1 $\beta$ , a representative pro-inflammatory cytokine, and Iba-1 as a marker of activated microglia. Immunohistochemical data (Fig. 5B) and western blot analysis (Fig. 5C and D) demonstrated that piperine significantly suppressed the expression of IL-1 $\beta$  in the SNpc of MPTP-treated brains. The Iba-1-positive cells showed that activated microglia were apparently present in the SNpc of the MPTP group. The number and morphological phenotype

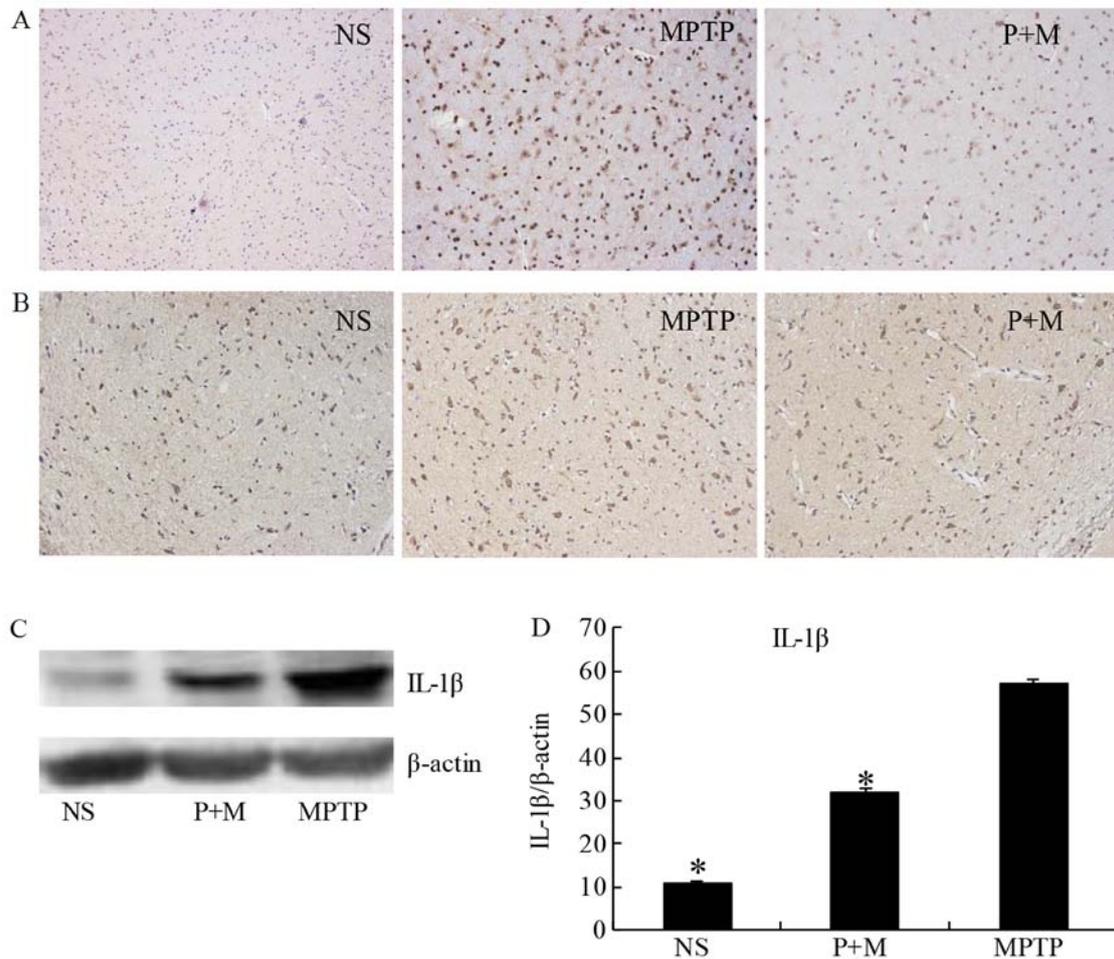


Figure 5. Piperine attenuates inflammation in the substantia nigra (SN) of the MPTP-treated brains. (A) Iba-1-immunostaining was performed on brain sections for microglia. Piperine alleviated microglia activating caused by MPTP. (B) Brain tissue was stained with the anti-interleukin-1 $\beta$  (IL-1 $\beta$ ) antibody in the SN. MPTP notably increased the expression of IL-1 $\beta$  in the SN of the mice and the increase was partly blocked by piperine. (C) Western blot analysis of IL-1 $\beta$  expression in the SN. (D) Densitometric analysis of western blot analyses of IL-1 $\beta$ . Densitometry was measured by Quantity One 1-D analyze software (Universal Hood II; Bio-Rad Laboratories, Hercules, CA, USA). Values are presented as mean  $\pm$  standard deviation, \*P<0.05, compared to MPTP.

of activated microglia were obviously alleviated following piperine pretreatment (Fig. 5A).

**Piperine attenuates oxidative stress in MPTP-treated brains.** MDA is a marker of lipid peroxidation and oxidative stress. The level of lipid peroxidation indicated by MDA was significantly increased in the midbrain of MPTP-treated mice compared to the NS group (Fig. 3). The increase was significantly prevented by piperine pretreatment (Fig. 2B). Similarly, treatment with piperine markedly increased SOD activity in the mouse midbrain samples while MPTP only induced modest increases compared to NS-treated mice (Fig. 2C). These results suggested that piperine attenuated the oxidative stress induced by MPTP.

**Piperine attenuates apoptosis in the brains of MPTP-treated mice.** To confirm the anti-apoptotic potential of piperine, we evaluated the expression of the pro-apoptotic protein Bax and the anti-apoptotic protein Bcl-2 in the midbrain tissue. The balance of pro-apoptotic and anti-apoptotic signals from the Bcl-2 family can be influenced by members of the caspase family, which can determine whether neurons undergo apoptosis. Following MPTP treatment, Bcl-2 expression was

reduced in the midbrain compared to the NS group, while piperine pretreatment prevented this decrease (Fig. 6A and B). Similarly, MPTP treatment alone increased Bax expression relative to the NS group, an effect significantly attenuated by pretreatment with piperine (Fig. 6A and C). The ratio of the anti-apoptotic/pro-apoptotic proteins demonstrated that piperine significantly attenuated MPTP-induced apoptosis by a reduced expression of pro-apoptotic proteins (Fig. 6D). These results suggested that piperine had an anti-apoptotic effect on MPTP-induced neuronal cell death.

## Discussion

It has been reported that piperine has antioxidant and anti-inflammatory activity (35,36). Lee *et al* studied the inhibitory effect of piperine against the cytotoxicity of MPP<sup>+</sup> in PC12 cells (26). Previous findings have shown that piperine plays a novel role in the neuroprotection for PD. In the present study, the results demonstrated that piperine pretreatment improved motor ability and cognitive performance, and the mechanism of its neuroprotection may be by inhibiting MPTP-induced neurotoxicity due to a reduction of oxidative stress, apoptosis, and inflammation. Similar to

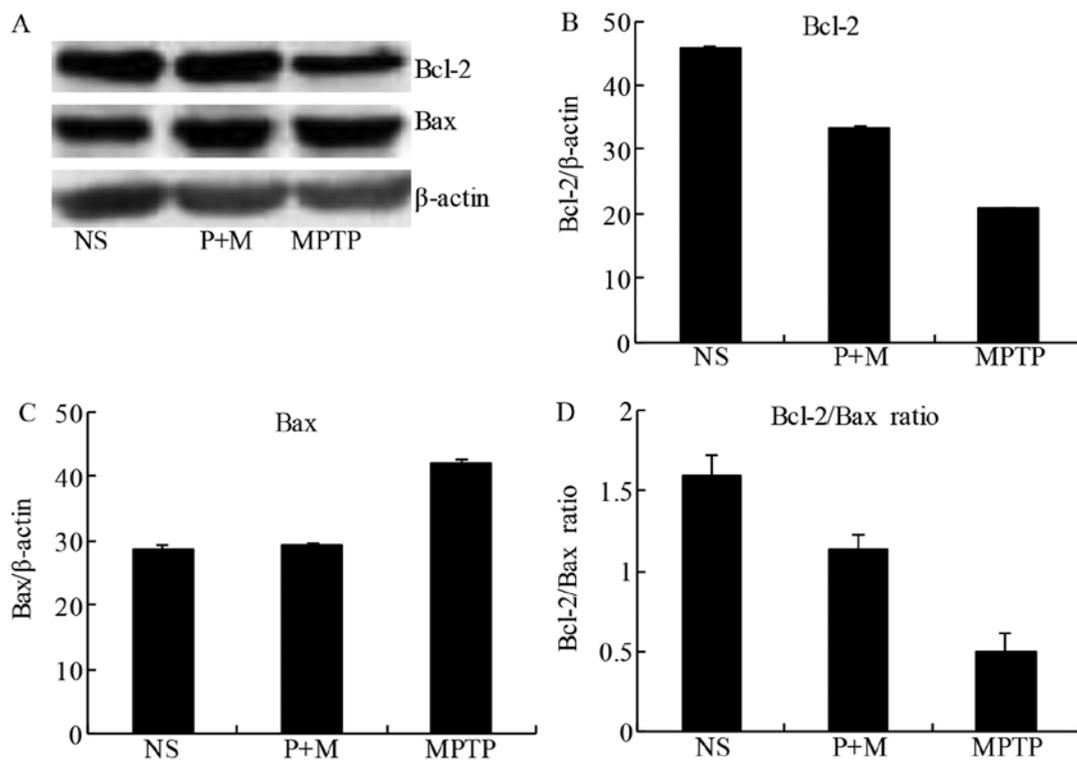


Figure 6. Effect of piperine on apoptotic signaling proteins. (A) Protein levels of Bcl-2 and Bax were analyzed by western blot analysis. Piperine blocked MPTP-induced decreases in the anti-apoptotic protein Bcl-2 and MPTP-induced activation of the pro-apoptotic protein Bax. (B and C) Densitometric analysis of western blot analyses of Bcl-2 anti-apoptotic protein and Bax pro-apoptotic protein. (D) Ratio of pixel counts of blots of Bcl-2 and Bax. The balance between anti-apoptotic and pro-apoptotic proteins controls the fate of neuronal survival or apoptosis. Densitometry was measured using Quantity One 1-D analysis software. Values are expressed as mean  $\pm$  standard deviation, \* $P$ <0.05, compared to MPTP.

our results, Shrivastava *et al* (17) have reported that piperine exerts a protective effect through antioxidant, anti-apoptotic, and anti-inflammatory mechanisms in the 6-OHDA-induced Parkinson's rat model. These results suggested that piperine is a promising therapy for PD patients. However, in some studies on C57BL/6J mice, MPTP has been reported to produce an almost complete, permanent and selective nigrostriatal DA depletion similar to that observed in humans with PD and primates (37,38), particularly in chronic administration paradigms (39,40). Thus, in this study, we used a MPTP-induced Parkinson's disease mouse model to explore the neuroprotective effects of piperine.

Motor function impairments have been identified following MPTP treatment in mice (41,42). In the present results, we have shown that in the rotarod test, a widely used method to assess motor coordination in laboratory rodents, piperine pretreatment attenuated MPTP-induced reduction in the fall latency. However, similar to our results, Shrivastava *et al* have shown that piperine showed motor deficit improvement in 6-OHDA-treated rats (17). This finding is of crucial importance in providing information concerning the qualitative aspects of walking movements (43). Cognitive impairment and dementia have been particular challenges in addition to the functional impairment caused by motor symptoms for patients with PD, placing patients under increasing strain (44). The impairment may be mild (mild cognitive impairment) or severe enough to be defined as dementia (PD dementia) (45,46). The results of the MWM test showed that piperine may improve learning and recall in the MPTP-intoxicated mice. To the best of our

knowledge, this is the first study to show that piperine is capable of improving cognitive impairment in a PD mouse model and, at least in part, can improve the learning and memory of animals.

Accumulating evidence suggests that neuroinflammation in the brain plays an important role in the pathogenesis of PD (47-49). Autopsy studies have shown that greater numbers of reactive microglia were identified in the substantia nigra of PD patients, particularly in areas of maximal neurodegeneration (50). A large number of activated microglia have also been detected in MPTP-induced PD animal models (51). Overactivation of microglia is an important element of neuroinflammation. Activated microglia can release deleterious compounds such as pro-inflammatory cytokines (IL-1 $\beta$ ), which may exert a direct deleterious effect on DA neurons (52), and are believed to contribute to neurodegenerative processes (53,54). It has been shown that piperine has anti-inflammatory activity and is capable of suppressing lipopolysaccharide-induced inflammation (55). Thus, we examined the activation of glial cells and the level of cytokines IL-1 $\beta$  in the midbrain of PD mice. We observed that piperine pretreatment significantly prevented MPTP-induced activation of microglia and ameliorated the levels of IL-1 $\beta$ . These results demonstrate that piperine played a neuroprotective role mediated by anti-inflammatory effects in the MPTP-induced mouse model of PD.

Oxidative stress is widely accepted to play a role in the development and progression of PD and MPTP-mediated parkinsonism (35,36). Previous findings have demonstrated

that neuronal lipids (56), nucleic acids (57) and proteins (58), which are extensive in the brains of PD patients, are particularly damaged by free radical oxidation. Our results have shown that pretreatment with piperine clearly elevated the SOD levels and decreased lipid peroxidation. These results suggest that this antioxidant property may be involved in the neuroprotective effects against MPTP neurotoxicity.

Apoptosis is involved in the pathogenesis of cell death in PD (59). Mitochondrial defects following cytotoxic stimuli can be closely associated with apoptosis (60). The pro-apoptogenic Bax, which can form a channel by itself for translocation to mitochondria, can trigger the release of the apoptogenic factor cytochrome *c* from mitochondria into the cytoplasm (61,62). Contrary to Bax, the anti-apoptotic Bcl-2 is able to prevent the release of cytochrome *c* from mitochondria by functioning as a docking protein or by direct blockade of the MTP opening (61,63). The balance between pro- and anti-apoptotic proteins is crucial in apoptosis and cell survival. We observed that the expression of Bax and Bcl-2 in the midbrain from the piperine and MPTP groups showed opposite trends. Furthermore, the ratio between anti-apoptotic Bcl-2 and pro-apoptotic Bax suggested that piperine played a positive role in cell survival by inhibiting apoptosis. Thus, the results suggest that piperine has anti-apoptotic activity against MPTP neurotoxicity in this mouse model of PD.

In conclusion, results of the present study have shown that piperine inhibits MPTP-induced neurotoxicity in mice. Piperine reduced MPTP-induced oxidative stress by decreasing the expression of lipid peroxidation (shown by a reduction of MDA expression) while increasing SOD. It also prevented MPTP-induced alterations in the balance of Bcl-2 and Bax. In addition, piperine controlled the overactivation of microglia and inhibited inflammation by reducing the levels of cytokine IL-1 $\beta$ . Taken together, the results suggest that piperine has therapeutic potential as a treatment for PD and other neurodegenerative disorders.

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