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Neuroprotective effects of *Polygonum multiflorum* on nigrostriatal dopaminergic degeneration induced by paraquat and maneb in mice

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Abstract

The neuroprotective effects of *Polygonum multiflorum* extract (PME) and its two fractions, ethanol-soluble PME (PME-I) and -insoluble PME (PME-II), on the degeneration of nigrostriatal dopaminergic neurons induced by a combination of paraquat and maneb (PQMB) were investigated in male C57BL/6 mice. The mice were treated twice a week for 6 weeks with intraperitoneal injections of PQMB. This combination caused a reduction of spontaneous locomotor activity, motor incoordination, and declines of dopamine level in the striatum and tyrosine hydroxylase-positive neurons in the substantia nigra. Administration of PME and PME-I once daily for 47 days during 6 weeks of PQMB treatment and last 8 days after PQMB significantly attenuated the impairment of behavioral performance and the decrease in striatal dopamine level and substantia nigral tyrosine hydroxylase-positive neurons in the PQMB-treated animals, whereas the administration of PME-II had no effect on these behavioral, neurochemical and histological indices. The present findings suggest that PME has a beneficial influence on parkinsonism induced by PQMB and that the effects of PME are attributable to some substance(s) included in the ethanol-soluble fraction of PME (PME-I).
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Keywords: *Polygonum multiflorum*; Parkinson's disease; Paraquat; Maneb; Tyrosine hydroxylase; Dopamine neuron

1. Introduction

Parkinson's disease (PD) is a progressive, age-related, neurodegenerative disorder characterized by tremors, rigidity, akinesia, and cognitive impairment (Basic et al., 2004). The most distinctive pathophysiology of this disease is the selective degeneration of the nigrostriatal dopaminergic system in the brain. In addition to genetic factors, several environmental factors have been implicated in the pathogenesis of PD including exposure to environmental herbicides (Rajput et al., 1987; Semchuk et al., 1992; Liou et al., 1997; Gorell et al., 1998; Tanner et al., 1999).

The pathogenesis of PD appears to partly involve mitochondrial dysfunction (Clejan and Cederbaum, 1989), since mitochondria play an important role in neuronal cell survival and death and ATP with a critical role in the mitochondrial respiratory chain provides protection to dopaminergic neurons (Kang et al., 1997; Laurens et al., 2003). Moreover, oxidative stress, for example, due to free radicals generated by the metabolism of dopamine by monoamine oxidase-B (MAO-B), also plays a role in the pathogenesis of PD, since MAO-B inhibitors have a neuroprotective effect in cellular and animal models of PD (Magyar and Haberle, 1999).

Polygonum multiflorum Thunb. (PM) is a medicinal herb which has long been used as a constituent of traditional Chinese prescriptions aiming at treatment of age-related

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diseases such as cognitive impairment and PD. Numerous reports have demonstrated that PM extract (PME) exhibits a variety of pharmacological effects such as antioxidative action (Chiu et al., 2002), free radical scavenging effects (Chen et al., 1999), the inhibition of monoamine oxidase (MAO) activity (Yang, 1996), improving memory (Chan et al., 2002, 2003), etc. These pharmacological properties of the plant prompted us to hypothesize that PM may have a beneficial effect on PD. In the present study, to confirm this hypothesis, we have investigated the effects of PM on behavioral, neurochemical, and neurohistological alterations in mice caused by combined treatment (PQMB) of paraquat (PQ) and manganese ethylene-bisdithiocarbamate (maneb, MB), a useful animal model of PD which exhibits age-related, progressive, and irreversible neuronal cell damage in the substantia nigra and includes an environmental factor (Thiruchelvam et al., 2000, 2002, 2003).

2. Materials and methods

2.1. Drugs

Drugs used were as follows: paraquat (Tokyo Kasei Kogyo CO. Ltd, Tokyo, Japan), maneb and benserazide (Wako, Osaka, Japan), L-DOPA (Sigma, St. Louis, MO), l-isoproterenol hydrogen D-tartrate (Nacalai tesque, Kyoto, Japan), rabbit anti-tyrosine hydroxylase (anti-TH) polyclonal antibody (Chemicon International, Inc., Temecula, CA), goat anti-rabbit Ig conjugated to peroxidase labeled-dextran polymer (Dako EnVision+®, Cytomation, Kyoto, Japan), emodin, and 2,3,5,4′-tetrahydroxystilbene 2-*O*-β-D-glucopyranoside (the National Institute for Control of Pharmaceutical and Biological Products, China).

2.2. Plant material

Dried roots of PM were purchased from Shenyang Company of Traditional Crude Drugs (Shenyang, China) and was authenticated by Dr. Qi-shi Sun, Pharmacognosy Department, Shenyang Pharmaceutical University. Dried roots of *P. multiflorum* (2.5 kg) were extracted 3 times with 6 L of 75% ethanol. The extract was pooled and evaporated under reduced pressure and then lyophilized, yielding PM extract (PME) as a powder (385 g, yield = 15.9%). The PME was further divided into ethanol-soluble and -insoluble fractions using 100% ethanol under sonication. The fractions were evaporated and then lyophilized under vacuum, yielding PME-I (ethanol-soluble, yield = 49.0%) and PME-II (ethanol-insoluble, yield = 43.7%). For the assessment of neuroprotective effects, powdered PME and its fractions were dissolved in distilled water immediately before administration.

2.3. HPLC analysis of PME-I

The chemical constituents of PME-I were analyzed using HPLC (Fig. 1). The PME-I was dissolved in methanol and its 10 μl was subjected to HPLC. Emodin and 2,3,5,4′-tetrahydroxystilbene 2-*O*-β-D-glucopyranoside were identified based on UV absorption (351 nm) and retention times of authentic compounds. The mobile phase was NaH_2PO_4 buffer (pH 5.8) : acetonitrile (80 : 20).

2.4. Animals and drug treatment

Male C57BL/6 mice (6 weeks old) were purchased from Japan SLC (Shizuoka, Japan) and housed in an animal room

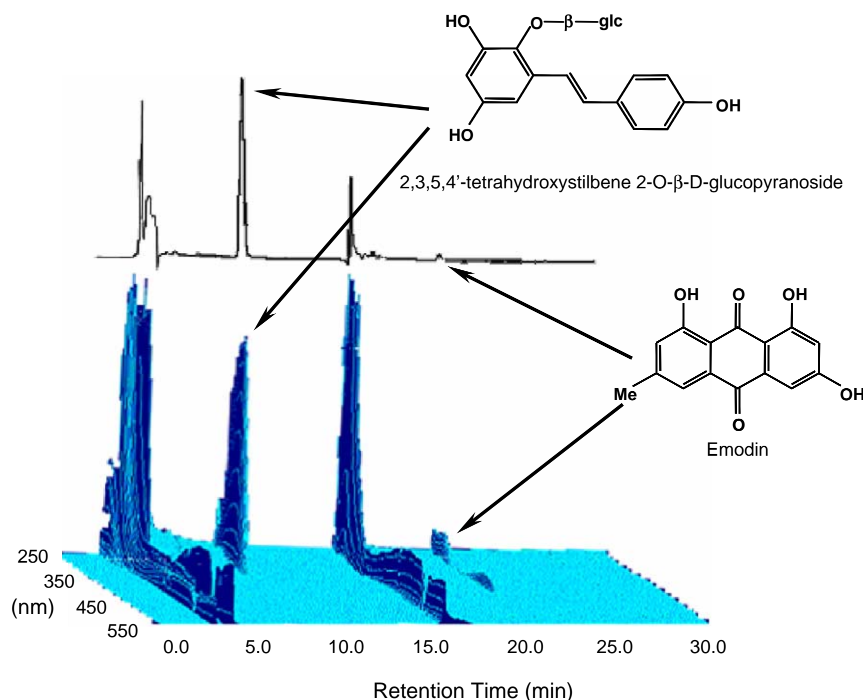

Fig. 1. Two- and three-dimensional HPLC patterns of PME-I. PME-I dissolved in methanol was subjected to HPLC and separated in a mobile phase of NaH_2PO_4 buffer (pH 5.8) : acetonitrile (80 : 20). Emodin and 2,3,5,4′-tetrahydroxy-stilbene-2-*O*-β-D-glucopyranoside were identified based on retention times and UV spectra at 351 nm.

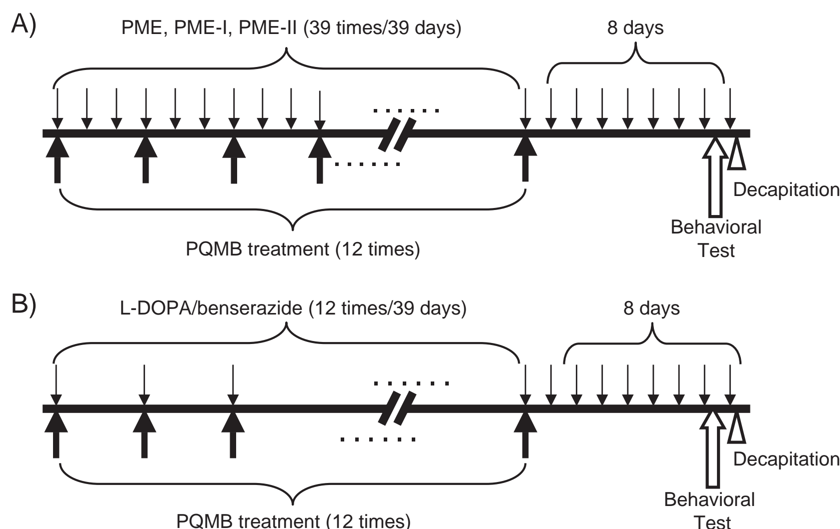

Fig. 2. Time-line of drugs treatment and experimental schedule. The PQMB treated group received i.p. injections of paraquat (PQ: 8 mg/kg)+maneb (MB: 24 mg/kg) twice a week (every Monday and Thursday) a total of 12 times (39 days) and oral administrations of distilled water or test drugs; PME, PME-I, PME-II, and L-DOPA/benserazide. (A) PME (400 and 800 mg/kg) and its fractions PME-I and -II (250 and 500 mg/kg each) were orally administered once daily for 47 days (during the 39-day period of PQMB treatment and on day 8 after PQMB treatment). (B) L-DOPA/benserazide (50 and 12.5 mg/kg, respectively) was orally administered 30 min prior to PQMB a total of 12 times and once daily for 8 days after PQMB treatment. Behavioral tests were performed at 7th day after the last injection. Animals were decapitated at 8 days after the last injection for neurochemical and histological examination.

maintained under constant temperature (23–25 °C) with a 12 : 12 light : dark cycle. Food and water were available ad libitum. Mice were acclimatized to the laboratory room and handling for 2 weeks before the start of the experiments, and then randomly divided into 2 groups: a vehicle treated group and a PQMB treated group. The drug administration schedule is shown in Fig. 2. These groups received i.p. injections of saline or a combination of PQ (8 mg/kg) and MB (24 mg/kg) (PQMB) at around 9:30 AM two times a week (on Monday and Thursday) for a total of 6 weeks. Moreover, distilled water or the test drugs (PME, PME-I, and PME-II), except L-DOPA/benserazide, were administered perorally (p.o.) to the PQMB treated group daily at around 9:00 AM (30 min before PQMB) for 47 days. L-DOPA/benserazide (50 and 12.5 mg/kg, respectively) was given p.o. to the PQMB treated group at around 9:00 AM (30 min before PQMB) twice a week (on Monday and Thursday) for 6 weeks (12 times), and then once daily for 8 days before the experiments. Behavioral experiments were performed at 7 days after the last injection of PQMB. The animals were decapitated for neurochemical and histological examination at 8 days after the last injection of PQMB.

All experiments were conducted in accordance with the Guiding Principles for Care and Use of Animals in the Field of Physiological Science of the Physiological Society of Japan and had approval of the Institutional Animal Use and Care Committee of Toyama Medical and Pharmaceutical University.

2.5. Behavioral experiments

2.5.1. The pole test

The pole test was used to analyze behavioral abnormalities of the animals according to a method described previously (Abe et al., 2001). Briefly, the animals were positioned head upward near the top of a rough-surfaced iron pole (12 mm in diameter and 55 cm in height). The time taken until they turned completely downward (defined as T_{turn}) and the time until they had climbed down to the floor (T_{LA}) were recorded as indices of motor coordination deficit. Performances in the pole test were measured at 7 days after the last injection of PQMB. This test was conducted 1 h after the administration of test drugs.

2.5.2. Spontaneous motor activity

Immediately after the pole test, spontaneous motor activity was measured using a computerized locomotion detection system equipped with photosensers (Scanet SV-10®, MATYS, Toyama, Japan) as previously described (Asakura et al., 1992, 1993). Seven days after the last injection of PQMB, mice were individually placed in a transparent cage ($25 \times 48 \times 18$ cm) and numbers of horizontal and vertical movements were recorded every 2 min over a 20-min observation period.

2.6. Neurochemical and histological experiments

The mice were killed by decapitation on day 8 after the last PQMB treatment. Brains were quickly removed and divided coronally in two parts; the one including the striatum was used for the analysis of DA and dihydroxyphenylacetic acid (DOPAC) and the other including the substantia nigra was embedded in dry-ice and kept at −80 °C until the immuno-histochemical examination.

2.6.1. Measurement of dopamine and DOPAC levels in the striatum

Two striata were rapidly dissected out from the brain on an ice-cold glass plate and frozen in liquid nitrogen. Samples were

weighed and homogenized in Polytron homogenizer (PT-10, Kinematica, Switzerland) in 1 ml ice-cooled 0.1 N perchloric acid containing 0.2 μg/ml of l-isoproterenol hydrogen D-tartrate as an internal standard and 0.1 mM disodium EDTA. The homogenate was centrifuged at 10,000 ×g at 4 °C for 20 min. The supernatant was filtered through a 0.45 μm filter (Type HV, Nihon Millipore, Japan) and stored at −80 °C until assayed. Dopamine and DOPAC levels were measured by high-performance liquid chromatography (HPLC) with an electrochemical detector (ECD) (Eicom, Kyoto, Japan). The mobile phase consisted of 0.1 M acetate–citrate buffer (pH 3.9) containing 0.716 mM 1-octane sulfonic acid sodium salt, 0.013 mM disodium EDTA, and 15% (v/v) methanol. The mobile phase was delivered at a constant rate of 0.5 ml/min by a EICOM Model EP-10 pump through a SC-5ODS column (3.0×150 mm; Eicom, Kyoto, Japan) placed in a heater box (30 °C). The operating potential was 500 mV versus Ag/AgCl. The signal from the detector was recorded and the data was analyzed using a Power Chrom® (ver. 2.2).

2.6.2. Immunohistochemistry of tyrosine hydroxylase-positive neurons in the substantia nigra

The coronal section (12 μm thick) passing through substantia nigra was cut for immunohistochemistry using a cryostat (LEICA CM300, Germany). Briefly, the frozen sections were placed in 4% paraformaldehyde (PFA) containing 10 mM phosphate-buffered saline (PBS, pH 7.4) and then treated with 0.3% hydrogen peroxide in 10 mM PBS. The slices were washed twice in 10 mM PBS for 5 min, then pre-incubated for 60 min with goat IgG blocking reagent. The sections were incubated with rabbit anti-tyrosine hydroxylase (anti-TH) polyclonal antibody (1:250) at 4 °C for 48 h. After a 5-min rinse in 10 mM PBS 3 times, the sections were incubated with goat anti-rabbit Ig conjugated to peroxidase labeled-dextran polymer (the second antibody) at room temperature for 2 h. The immunoreaction was visualized using 50 mM Tris–HCl buffer (pH 7.6) containing 0.05% diaminobenzidine and 0.01% hydrogen peroxidase. Negative control sections were treated in the same way without anti-TH. Three sections containing the same positional substantia nigra pars compacta were chosen. The numbers of TH-positive neurons were counted by an experimental blind to the treatment. Cells to be measured were verified as neurons based on criteria that included a visible nucleus and at least one distinct projection (e.g., a dendrite or initial axon segment). There were no size-related criteria (Muramatsu et al., 2003).

2.7. Statistics

All values obtained are expressed as the mean±S.E.M. All data were analyzed statistically using the one-way analysis of variance (ANOVA) followed by the Dunnett test for multiple

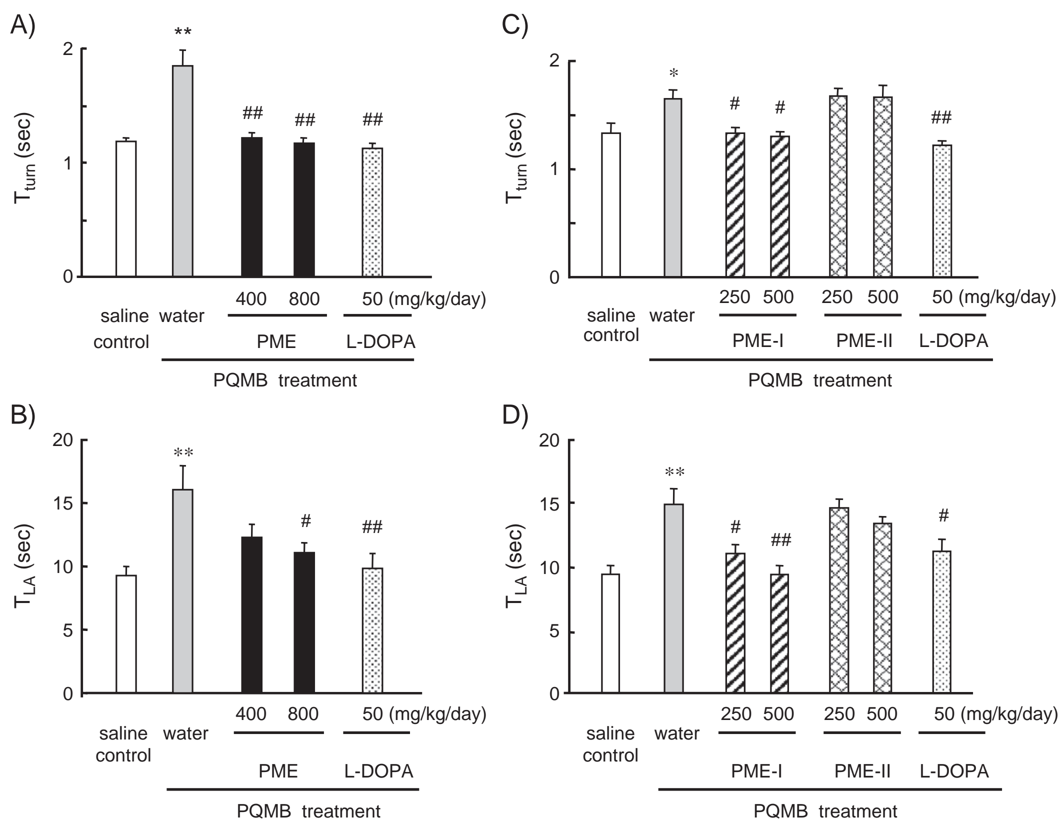

Fig. 3. The pole test performance of mice pretreated with PQMB with or without test drugs. The pole test was conducted as described in the text and the time until mice turned completely downward (T_{turn}; A and C) and the time until they climbed down to the floor (T_{LA}; B and D) were determined. Each column represents the mean±S.E.M. (A: n=6–7; B: n=7–9). *P<0.05, **P<0.01 vs. the animals pretreated with saline alone (saline control). #P<0.05, ##P<0.01 vs. the animals pretreated with PQMB alone (PQMB-treated water group).

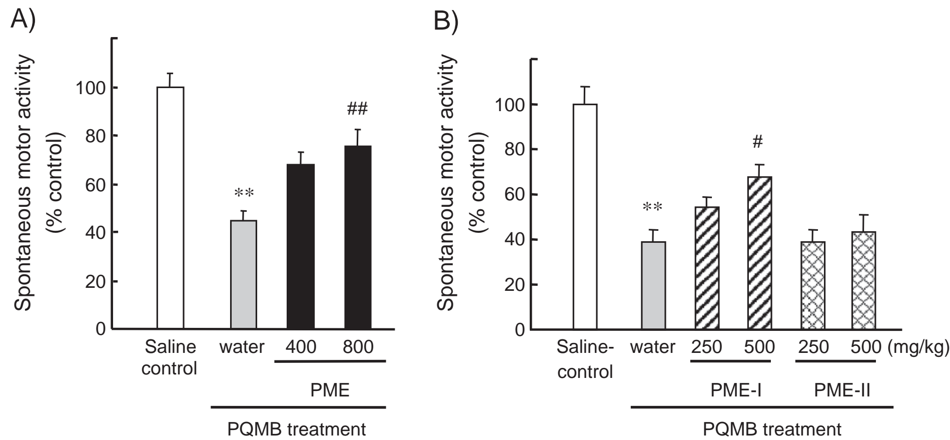

Fig. 4. Effects of PME (A), PME-I, and PME-II (B) on PQMB-induced impairment of spontaneous motor activity in mice. Measurement of spontaneous motor activity was conducted as described in the text at 7th day after the last treatment with PQMB. Spontaneous motor activity was measured for 20 min. Each column represents the mean ± S.E.M. (A: $n=6-7$; B: 7–9). *$P<0.05$, **$P<0.01$ vs. the animals pretreated with saline alone (saline control group). #$P<0.05$, ##$P<0.01$ vs. the animals pretreated wih PQMB alone (PQMB-treated water group).

comparisons among different groups (SPSS, ver. 11.5). Differences with $P<0.05$ were considered significant.

3. Results

3.1. The pole test

In the pole test, the animals which had received repeated treatment with PQMB showed significantly prolonged T_{turn} (Fig. 3A and C) and T_{LA} (Fig. 3B and D) as compared to the control animals which had received saline alone during the same period, indicating an induction of bradykinesia (Fig. 3). Repeated administration of L-DOPA/benserazide (50 mg/kg, p.o.) prevented PQMB treatment-induced impairment of performance in the pole test and significantly reduced T_{turn} [$F_{L\text{-}DOPA}(2,20)=9.858$; $P<0.01$] (Fig. 3A) and T_{LA} [$F_{L\text{-}DOPA}(2,20)=16.666$, $P<0.01$] (Fig. 3B). PME (400 and 800 mg/kg, p.o.) and PME-I (250 and 500 mg/kg, p.o.), as well as L-DOPA, significantly reversed the PQMB treatment-induced prolongation of T_{turn} [$F_{PME}(4,27)=22.939$, $P<0.01$;

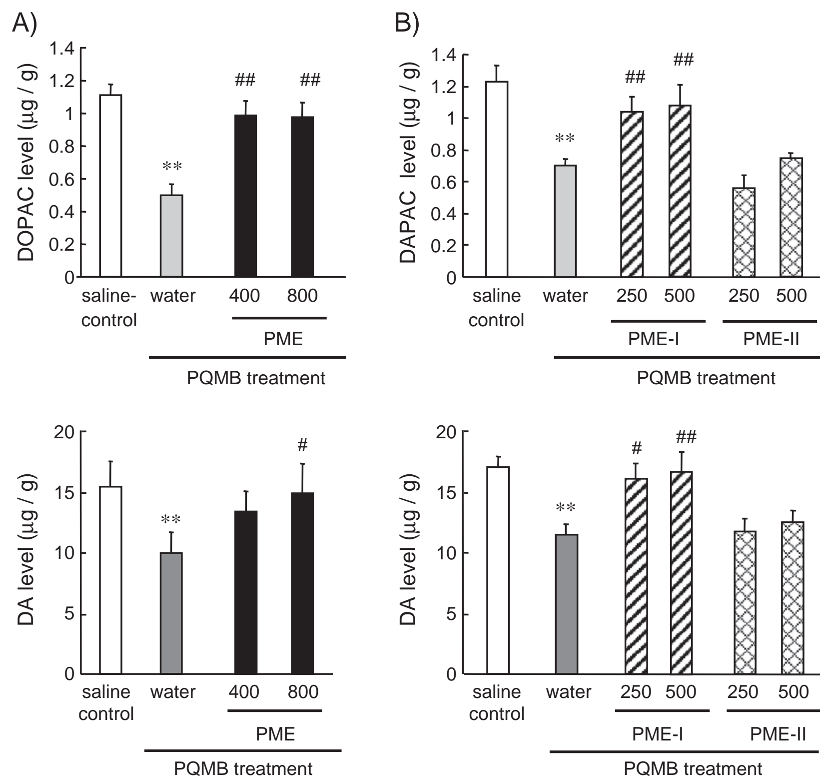

Fig. 5. Effects of PME, PME-I, and PME-II on PQMB-induced alterations in DA and DOPAC levels in the striatum. The animals were decapitated on day 8 after the last injection of PQMB or saline and the striatal tissues were dissected out. Each column represents the mean ± S.E.M. (A: $n=6-7$; B: $n=7-9$). *$P<0.05$, **$P<0.01$ vs. saline-control group. #$P<0.05$, ##$P<0.01$ vs. the animals pretreated with PQMB alone (PQMB-treated water group).

$F_{\text{PME-I}}(6,47)=7.363$, $P<0.01$] (Fig. 3A and C) and T_{LA} [$F_{\text{PME}}(4,27)=6.271$, $P<0.01$; $F_{\text{PME-I}}(6,47)=10.143$, $P<0.01$] (Fig. 3B and D). In contrast, repeated administration of PME-II (250 and 500 mg/kg, p.o.) had no effect on the performance of PQMB-treated animals in the pole test (Fig. 3C and D).

3.2. Locomotor activity

Fig. 4 shows the effects of PME and its ethanol-fractionated samples, PME-I and -II, on the PQMB-induced alteration of spontaneous motor activity in mice. The spontaneous motor activities during a 20-min observation period were compared at 7 days after the last treatment with PQMB or saline. The spontaneous motor activity of mice pretreated with PQMB alone was significantly reduced as compared with the activity of the mice pretreated with saline alone (saline control). Systemic administration of PME (400 and 800 mg/kg, p.o.) and PME-I (250 and 500 mg/kg, p.o.) dose-dependently reversed the effect of PQMB [PME: $F_{(3,22)}=15.587$, $P<0.01$; PME-I: $F_{(5,41)}=14.629$, $P<0.01$], whereas PME-II (250 and 500 mg/kg, p.o.) had no effect on the PQMB-induced suppression of spontaneous motor activity.

3.3. Changes in striatal DA and DOPAC levels following PQMB treatment

Changes in the amounts of DA and DOPAC were analyzed on day 8 after the last injection of PQMB. As shown in Fig. 5, the repeated administration of PQMB significantly reduced levels of DA and DOPAC in the striatum as compared to those measured in the mice treated with vehicle alone. The striatal DA level was significantly decreased to about 30–40% and the DOPAC level to about 50–60% of the control value. PME (400 and 800 mg/kg, p.o.) dose-dependently attenuated the reduction in striatal DA [$F_{\text{PME}}(3,22)=4.300$, $P<0.05$] and DOPAC [$F_{\text{PME}}(3,22)=9.134$, $P<0.01$] levels caused by PQMB treatment. Among the fractionated samples from PME (400 and 800 mg/kg, p.o.), PME-I (250 and 500 mg/kg, p.o.) but not PME-II (250 and 500 mg/kg, p.o.) markedly prevented the decrease in DA [$F_{\text{PME-I}}(5,41)=6.195$, $P<0.01$] and DOPAC [$F_{\text{PME-I}}(5,41)=9.443$, $P<0.01$] levels induced by PQMB in the striatum.

3.4. Immunohistochemistry

Fig. 6 shows immunostaining of TH-positive neurons in the substantia nigra pars compacta of mice. The PQMB treatment produced a marked reduction in the number of TH-positive neurons in this area. However, repeated treatment with PME (400 and 800 mg/kg, p.o.) [$F_{(4,24)}=5.330$, $P<0.01$] and PME-I (250 and 500 mg/kg p.o.) [$F_{(6,47)}=26.273$, $P<0.01$] significantly reversed the PQMB-induced reduction in the number of TH-positive neurons in the substantia nigra. In contrast, the administration of PME-II (250 and 500 mg/kg p.o.) did not affect the number of TH-positive neurons as compared to the treatment with PQMB alone.

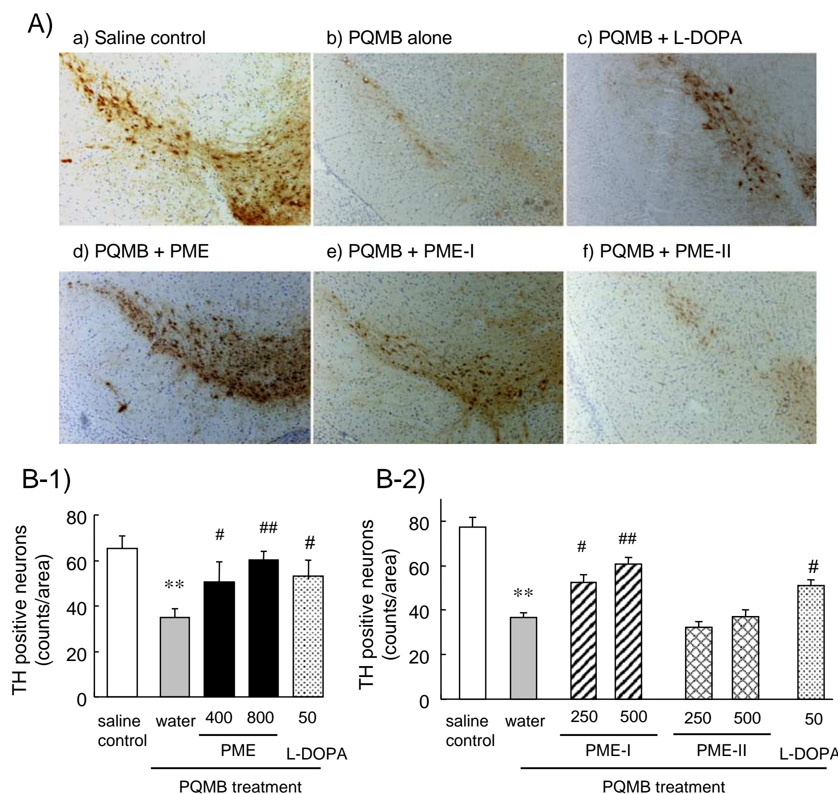

Fig. 6. Effects of PME, PME-I, and PME-II on the PQMB-induced decrease in tyrosine hydroxylase (TH)-positive neurons in the substantia nigra compacta. The numbers of TH positive neurons were counted on day 8 after the last injection of PQMB or saline. (A) Represent immunohistochemical data (original magnification: ×100). (B) Summary of the effect of test drugs on PQMB-induced damage of TH-positive neurons. Each column represents the mean ± S.E.M. (B-1: $n=5–6$; B-2: $n=7–9$). *$P<0.05$, **$P<0.01$ vs. saline-control group. #$P<0.05$, ##$P<0.01$ vs. the animals pretreated with PQMB alone (PQMB-treated water group).

4. Discussion

This study demonstrated that PME, an extract of *P. multiflorum* Thunb. which has long been used as a constituent of traditional Chinese prescriptions for the treatment of age-related diseases, attenuated behavioral abnormalities induced in mice by PQMB as an animal model of PD. Moreover, it was revealed that this preventive effect of PME was closely related to the protection of nigrostriatal dopaminergic neurons from PQMB-induced neurotoxicity in the brain and was mainly attributable to the ethanol-soluble constituent(s) of PME.

A number of animal models have been developed to clarify the pathogenesis of PD. One model uses 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a dopaminergic neurotoxin. However, the effects of MPTP are often acute and reversible (Saitoh et al., 1987; Date et al., 1990), and such properties limit the ability of this toxin to reproduce a progressive disorder like PD (Thiruchelvam et al., 2003). Recently it was demonstrated that PQ (Manning-Bog et al., 2002; McCormack et al., 2002; Di Monte, 2003), a herbicide with structural similarity to MPTP, and MB (Zhang et al., 2003), a fungicide, both selectively cause damage in the nigrostriatal dopaminergic system, leading to neuronal cell death in the substantia nigra (Manning-Bog et al., 2002; McCormack et al., 2002; Di Monte, 2003; Chanyachukul et al., 2004). Moreover, the combination of PQ and MB (PQMB) produced a more potent neurotoxic effect on the nigrostriatal dopaminergic system than either compound given alone (Thiruchelvam et al., 2000), in an age-related, progressive, and irreversible manner in mice (Thiruchelvam et al., 2000, 2002, 2003), providing a valuable environmental factor model with behavioral, pathological, and neurochemical features remarkably similar to those of PD. Based on these neurotoxic properties of PQMB, we elucidated the effect of PME on the behavioral, neurochemical, and histochemical alterations caused by PQMB treatment in mice.

In this study, repeated treatment of PQMB in C57BL/6 mice produced behavioral abnormalities, i.e., motor incoordination in the pole test and a reduction of spontaneous motor behavior, and degeneration of nigrostriatal dopaminergic systems. These behavioral and neurochemical alterations are consistent with previous reports (Thiruchelvam et al., 2000, 2003) and indicate the induction of bradykinesia, a hallmark of PD phenotype caused by the neurotoxic insult of central dopaminergic systems (Liou et al., 1996, 2001). Interestingly, the bradykinesia and degeneration of nigrostriatal dopaminergic neurons following PQMB treatment were significantly suppressed by chronic administration of PME, as well as by repeated treatment with L-DOPA, a DA precursor. These findings indicate that PME exhibits protective activity against PD-like symptoms probably by inhibiting PQMB-induced damage of the nigrostriatal dopaminergic system. Moreover, the protective effect of PME in an animal model of PD supports the availability of this plant for clinical treatment in PD.

The exact mechanism by which PME prevents PQMB-induced behavioral and neurological deficits remains unclear. However, at least a couple of factors seem to be involved in the effects of PME. First, PQMB reportedly has a neurotoxic effect by preferentially acting on the nigrostriatal DA system (Thiruchelvam et al., 2000). This toxicity appears to in part involve the generation of oxygen free radials that exert neurotoxic effects by disrupting mitochondrial complex 1 activity (Fukushima et al., 1993; Tawara et al., 1996). Considering reports that PME exhibited antioxidative activity in vivo (Yang, 1996; Chiu et al., 2002), it is likely that the reduction of oxygen free radicals by PME is involved in the prevention of PQMB-induced neurotoxicity in the nigrostriatal dopaminergic system. Second, constituent(s) included in PME and PME-I may be able to facilitate the operation of the dopaminergic system, for instance, by increasing the synaptic DA level in the striatum, as well as the level of L-DOPA, and/or by attenuating the function of cholinergic neurons in the striatum which counteracts the activity of the striatal dopaminergic system, and thereby improves behavioral deficits caused by PQMB treatment. In fact, there is a report demonstrating a suppressive effect of PME on monoamine oxidase (MAO) activity (Yang, 1996). To clarify the exact mechanisms underlying the action of PME in PQMB-treated animals, further investigations are required.

It should be noted that PME and PME-I (an ethanol-soluble fraction of PME) exhibited protective effects on PQMB-induced behavioral and neurological deficits, while PME-II, an ethanol-insoluble fraction, did not. Taken together with the fact that the effective dose range was reduced by ethanol fractionation, it is very likely that ethanol-soluble constituents of PME play an important role in the effect of PME found in this study. Interestingly, our HPLC analysis revealed that 2,3,5,4′-tetrahydroxystilbene 2-*O*-β-D-glucopyranoside and emodin are included in PME-I as characteristic constituents of PME (Grech et al., 1994; Lin et al., 2003). Stilbene glucoside reportedly has strong free radical scavenging activity and an ATPase inhibitor effect (Grech et al., 1994) in vitro and in vivo (Chen et al., 1999; Ryu et al., 2002). Moreover, in a previous study (Chiu et al., 2002), emodin exhibited antioxidative activity and prevented carbon tetrachloride-induced hepatotoxicity in mice. Oxidative stress and mitochondrial dysfunction have been implicated in the PQMB-induced apoptosis of DA neurons (Brooks et al., 1999; McCormack et al., 2002; Barlow et al., 2005). Together, the present findings suggest that these constituents of PME contribute to prevent the PQMB-induced apoptosis of nigrostriatal dopaminergic neurons via their antioxidative activity and regulation of the mitochondrial respiratory chain. Nevertheless, the detail mechanism underlying the action of PME needs to be investigated further.

In conclusion, the present results demonstrated that the ethanol soluble fraction of PME (PME-I) exhibited a protective effect on PQMB-induced damage of the substantia nigral dopaminergic system in mice, an animal model of the PD phenotype, and suggest that this medicinal plant may be beneficial in preventing PD.

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