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Epimedin B exerts neuroprotective effect against MPTP-induced mouse model of Parkinson's disease: GPER as a potential target

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ABSTRACT

Mitochondrial dysfunction and oxidative stress play important roles in the neuropathogenesis of Parkinson's disease (PD). Epimedin B, the second highest active ingredient in the flavonoids of *Herba Epimedii*, has been proven effective in treating osteoporosis and oxaliplatin-induced peripheral neuropathy. The present study aims to investigate the neuroprotective effects of Epimedin B in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridin (MPTP)-induced mouse model of PD, and the involvement of G protein-coupled estrogen receptor (GPER)-mediated anti-apoptosis as well as anti-endoplasmic reticulum stress. Molecular docking revealed that Epimedin B could directly bind to GPER at the same site as GPER agonist G1 and the binding energy was − 7.3 kcal/mol. Epimedin B treatment ameliorated MPTP-induced motor dysfunction and alleviated the decreased contents of DA with its metabolites in the striatum and the loss of tyrosine hydroxylase-immunoreactive (TH-IR) neurons in the substantial nigra pars compacta (SNpc). Epimedin B treatment markedly prevented MPTP-induced changes in apoptosis-related protein Bcl-2 and Bax as well as endoplasmic reticulum stress-related protein glucose-regulated protein 78 (GRP78) and C/EBP homologous protein (CHOP). Pharmacological blockade with GPER antagonist G15 could antagonize these neuroprotective effects of Epimedin B on the nigrostriatal system. Moreover, the anti-apoptosis and anti-endoplasmic reticulum stress effects of Epimedin B against MPTP toxicity were significantly reduced in GPER knockout ($GPER^{-/-}$) mice. The present study provides the first evidence that Epimedin B can protect against MPTP-induced PD mice model. GPER may be a potential target for the neuroprotective effect of Epimedin B against PD.

1. Introduction

Parkinson's disease (PD), one of the most common neurodegenerative disorders, is characterized by progressive loss of dopaminergic neurons in the midbrain substantia nigra (SN) [1,2]. Epidemiological studies have shown that the prevalence of PD in men is about twice as much as that in women [3]. The gender differences seem to be related to the neuroprotective effect of estrogen. There is increasing evidence confirming the involvement of estrogen and estrogen signaling pathways in neuroprotection [4,5]. However, the adverse effects of estrogen replacement therapy limit its clinical application.

Estrogen exerts its physiological and pathophysiological effects through genomic and rapid nongenomic signaling pathways mediated by classical nuclear estrogen receptors (ERs) and G protein-coupled estrogen receptor (GPER, also known as GPR30), respectively [6]. The nuclear ERα and ERβ, which act as transcriptional factors, combine with estrogen or estrogen-like natural compound to regulate gene transcription in estrogen target tissues. GPER acts through heterotrimeric G-proteins and transactivate adenylate cyclase, phospholipase C and growth factor receptor/PI3K/Akt or MAPK/ERK signaling [7]. Several lines of evidence have demonstrated that GPER may be a therapeutic target for the treatment of obesity, diabetes, vascular pathology, and

Abbreviations: CHOP, C/EBP homologous protein; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; ERα, estrogen receptor α; ERβ, estrogen receptor β; ERs, estrogen receptors; GPER, G protein-coupled estrogen receptor; GRP78, Glucose-regulated protein 78; HPLC, High-performance liquid chromatography; MPP^+, 1-methyl-4-phenylpyridinium; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PD, Parkinson's diseases; SN, substantial nigra; SNpc, substantial nigra pars compacta; TH, tyrosine hydroxylase; TH-IR, tyrosine hydroxylase-immunoreactive.

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advanced cancer [8–10]. Therefore, screening natural products for GPER will provide an opportunity to develop new therapeutic strategies to strengthen the benefits of estrogen while limiting its potential adverse side effects.

Herba Epimedii, a traditional Chinese medicine, has many pharmacological functions such as tonifying the kidney, strengthening bones, protecting cardio-cerebral vessels, anti-cancer, anti-oxidization, immunomodulation, and neuroprotection [11–16]. Xu et al. have demonstrated that extracts of Epimedium can exert anti-osteoporosis effects by directly regulating the 11 estrogen-related targets and a set of target proteins in five estrogen-related pathways [17]. The active ingredients of *Herba Epimedii* include Icariin, Epimedin B, Baohuoside I and Epimedin A and so on [18,19]. Epimedin B is the second highest active ingredient in flavonoids of *Herba Epimedii* and has the advantages of hypotoxicity [20,21]. Epimedin B, as a major component of neuroprotective ingredients, can significantly reduce mechanical allodynia and hyperalgesia induced by oxaliplatin [22]. The potential mechanism of the neuroprotective effect of Epimedin B is unknown. Recently, Epimedin B has been proved to be effective in preventing osteoporosis via regulating PI3K-Akt, MAPK, and peroxisome proliferator-activated receptor (PPAR) signaling pathways in a mouse osteoporosis model [21]. Xiao et al. have reported that Epimedin B can exert ER-dependent bone protective effects in UMR-106 cell. However, competitive radioligand binding assay has shown that Epimedin B cannot directly bind to ERα or ERβ [23]. Based on the characteristics of flavonoids, we hypothesized that the biological effects of Epimedin B might be related to the estrogen membrane receptor GPER. Our previous study has shown that total flavonoid of *Herba Epimedii* can protect against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridin (MPTP)/1-methyl-4-phenylpyridinium (MPP^+)-induced dopaminergic neuronal apoptosis both in vivo and in vitro [19]. However, there is no reported study of the possible effects of Epimedin B against neurotoxins-induced PD and its relationship with GPER.

Therefore, in the present study, we investigated the potential neuroprotective effects of Epimedin B in rescuing DA neuron injury and the underlying mechanisms in an MPTP-induced mouse model of PD by using computer molecular docking, pharmacological blockade, and GPER knockout mice.

2. Materials and methods

2.1. Reagents

Epimedin B (source of *Epimedium brevicornum Maxim*, purity ≥ 98%, powder) was obtained from Tauto Biotech Co., Ltd (E-0369, Shanghai, China). MPTP(M0896) was purchased from Sigma-Aldrich (St Louis, MO, USA). G15 was obtained from Tocris Bioscience (3678, Bristol, UK). Antibody against tyrosine hydroxylase (TH) was purchased from Millipore (#AB152, Bedford, MA, USA, 1:2000). Antibodies against Bax (#2772, 1:2000), Bcl-2 (#3498, 1:1000), C/EBP homologous protein (CHOP) (#5554, 1:1000), and β-actin (#3700, 1:8000) were supplied by Cell Signaling Technology Inc. (Hertfordshire, England). Antibody against glucose-regulated protein 78 (GRP78) was obtained from Affinity Biosciences (#AF5366, Sacramento, CA, USA, 1:2000). Anti-rabbit and anti-mouse HRP conjugated secondary antibodies were purchased from Absin Bioscience Inc. (Shanghai, China, 1:5000). All other chemicals were obtained from commercial sources.

2.2. Molecular docking between Epimedin B and GPER

Molecular docking was performed to investigate the interaction between Epimedin B and GPER. We constructed a three-dimensional (3D) model structure of GPER in mice based on the structure of G protein-coupled receptors (GPCRs) and GPER protein sequence by the use of GPCR-I-Tasser (https://zhanggroup.org//GPCR-I-TASSER/). GPER protein sequence (Q8BMP4) was retrieved from the Universal Protein Resource (UniProt knowledgebase). The molecular structure for Epimedin B (PubChem CID 5748393) was downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/) in sdf format. GPER and the ligands were processed by Autodock Tools (ver. 1.5.6), and the results were saved as pdbqt files. Autodock Vina (ver. 1.1.2) was used for semi-flexible docking, and the best conformation was selected as the final docking conformation. The binding energy was evaluated by using the AutoDock Vina energy evaluation function in score-only mode. Binding affinity was expressed as binding free energy (kcal/mol).

2.3. Animals and treatment

9–12-week-old male C57BL/6 mice were obtained from Henan Skbex Biotechnology Co., Ltd. (Henan, China). GPER-targeted knockout mice were generated by the CRISP-Cas9 gene editing technology (BRL Medicine Inc, Shanghai, China). As shown in Fig. 1A, $GPER^{+/-}$ mice were bred to C57BL/6 to establish germline by backcrossing. Genotypes of the young mice were identified by single nucleotide polymorphisms (SNPs) (Fig. 1B). In this study, 9–12-week-old male wild-type ($GPER^{+/+}$) and GPER knockout ($GPER^{-/-}$) mice were selected for experiments. Animals were group-housed under a 12-h light-dark cycle with free access to water and food. All procedures were conducted according to the Guide for the Care and Use of Laboratory Animals (NIH publications No. 80–23, revised 1978). The animal experimentation protocol in the present study was approved by the Laboratory Animal Platform of Biomedical Centre, Qingdao University, and all efforts were made to minimize animal suffering and the number of animals used.

Exp 1. Neuroprotective effect of different doses of Epimedin B on MPTP-induced PD mice.

Thirty mice were randomly divided into five groups: (1) Control group, (2) MPTP group, (3) Epimedin B (1 mg/kg) +MPTP group, (4) Epimedin B (10 mg/kg) +MPTP group, (5) Epimedin B (20 mg/kg) +MPTP group, n = 6 mice per group. Mice were treated with different doses of Epimedin B (1, 10, 20 mg/kg) or saline with 1% DMSO by oral gavage on days 1–8. On day 4, after Epimedin B treatment, mice were given MPTP (15 mg/kg) intraperitoneally four times with intervals of 2 h [24]. On day 9, the mice were sacrificed after the behavioral test and the brains were removed for HPLC analysis and western blot analysis.

Exp 2. The blocking effect of G15 on the neuroprotection of Epimedin B on nigrostriatal system.

Fifty mice were randomly divided into five groups: (1) Control group, (2) MPTP group, (3) Epimedin B+MPTP group, (4) G15 +Epimedin B+MPTP group, (5) Epimedin B group, n = 10 mice per group. The mice were anesthetized with sodium pentobarbital (40 mg/kg) [25] and stereotaxic surgery was performed. The procedures of drug administration were the same as experiment 1 except for G15 treatment. In G15 +Epimedin B+MPTP group, 1 μl G15 (0.5 μg/μl) was stereotaxically injected into the lateral ventricle 1 h before Epimedin B treatment for 8 days. And in the other groups, mice were given microinjection with 1 μl of normal saline containing 15% DMSO into the lateral cerebral ventricle for 8 days [24]. On day 9, fifty mice were used for behavior tests. After that, thirty mice were decapitated and the SN was used for western blot assay. Twenty mice were transcardially perfused with normal saline and 4% paraformaldehyde (PFA) sequentially after anesthesia with sodium pentobarbital. Then the brains were used for immunohistochemistry of tyrosine hydroxylase-immunoreactive (TH-IR) in SNpc.

Exp 3. Effect of GPER knockout on the neuroprotection of Epimedin B in nigrostriatal system.

$GPER^{+/+}$ and $GPER^{-/-}$ mice were randomly divided into three groups, respectively. (1) Control group, (2) MPTP group, (3) Epimedin B+MPTP group, n = 6 mice per group. The procedures were the same as in experiment 1. Mice were treated with Epimedin B (10 mg/kg) or saline with 1% DMSO by oral gavage on days 1–8. On day 4, mice were given

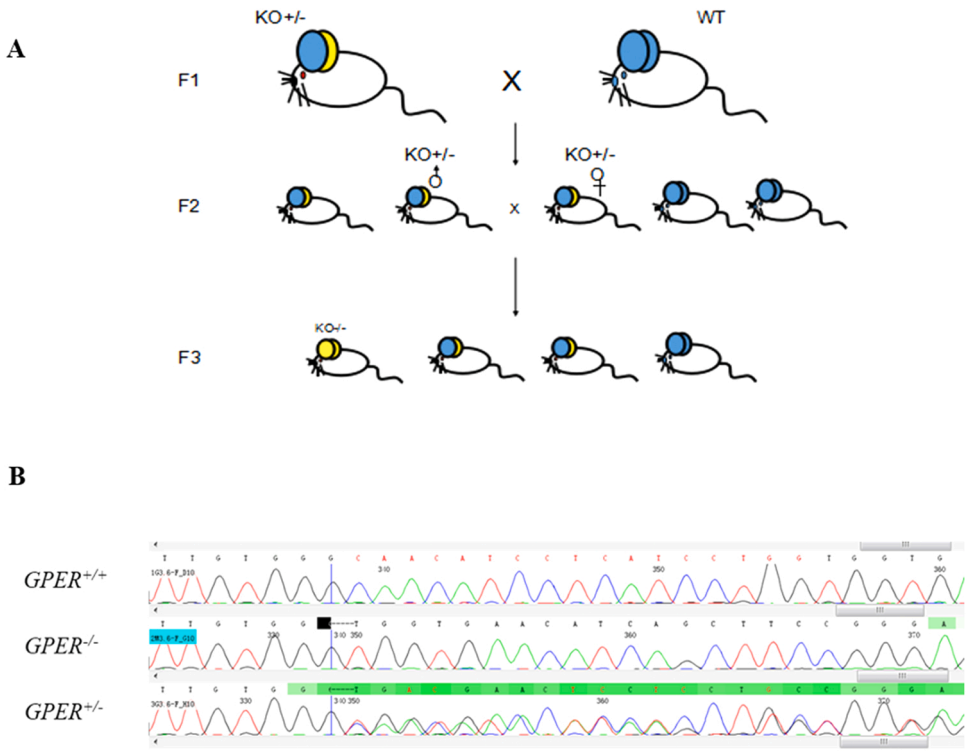

Fig. 1. Breeding and phenotyping of GPER knockout mice. *GPER*$^{+/-}$ mice were bred to C57BL/6 wild-type mice to establish germline by backcrossing (A). Genotypes of young mice were identified by single nucleotide polymorphisms (SNPs) (B).

MPTP intraperitoneally four times with intervals of 2 h. On day 9, the mice were sacrificed after the behavioral test and the brains were removed for western blot analysis.

2.4. Behavior test

After 8 days of experimental dosing, motor functional performance was assessed using the rotarod test and pole test.

2.4.1. Rotarod test

Motor coordination was evaluated on the rotarod apparatus. Before Epimedin B treatment, mice were pre-trained for 3 days. On the test day, the parameters were set as follows: initial speed, 4 rpm; maximum speed, 40 rpm throughout 300 s. The duration that the mice remained on the rotarod was automatically recorded.

2.4.2. Pole test

The pole test evaluated motor deficits in PD models. Briefly, mice were placed with their head facing upside on top of a rough-surfaced pole (1 cm in diameter and 50 cm in height). The time required for the mice to turn completely downward (T-turn) and climb down to the floor (T-total) was recorded.

2.5. High-performance liquid chromatography (HPLC)

Striatum tissue samples were homogenized in 0.1 ml liquid A (0.4 M perchloric acid) and centrifuged (12,000 rpm, 4 °C) for 20 min. The supernatant was collected and mixed with a half-volume of solution B (20 mM citramalic acid-potassium, 300 mM dipotassium phosphate, 2 mM EDTA-2Na). And then the samples were placed on ice for 1 h and centrifuged (12,000 rpm, 4 °C) for 20 min. The supernatant was collected to detect the contents of DA, DOPAC and HVA using HPLC (Waters Corp., Milford, MA, USA).

2.6. TH immunohistochemistry staining

Immunohistochemistry was performed as described previously [26]. Briefly, the midbrains containing the substantial nigra pars compacta (SNpc) were fixed in 4% paraformaldehyde for 4–6 h. After that, brains were transferred into 30% sucrose and stored at 4 °C for 48 h. Frozen coronal sections were serially cut through the SN with a freezing cryostat (Leica, German). The 12 adjacent frozen brain slices (20 μm thick; section was from bregma −2.46 mm to bregma −4.04 mm) of the same coronal section were selected in strict accordance with the mouse brain atlas. DAB staining was used to detect the expression of TH in the SNpc. The counting on 12 sections of each mouse was performed blindly on an Olympus microscope.

2.7. Western blotting

Western blotting was carried out as previously described [27]. The SN tissues were lysed on ice for 40 min in RIPA lysis buffer containing protease inhibitors. Lysates were centrifuged at 12,000 rpm for 20 min at 4 ℃, and a BCA colorimetric protein assay kit analyzed the supernatants with a full-wavelength spectrophotometer (Thermo Scientific). Protein samples were boiled for 5 min and then electrophoresed on 12.5% SDS-PAGE and transferred to PVDF membranes (Immobilin P; Millipore Corp.). The blots were blocked with a blocking solution (5% skim milk in TBST containing 20 mM Tris-HCl, 150 mM NaCl, and 0.1% Tween-20) at RT for 1 h. The blots were incubated with primary antibodies against TH (1:2000), Bax (1:2000), Bcl-2 (1:1000), GRP78 (1:2000), CHOP (1:1000) and β-actin (1:8000) at 4 ℃ overnight. After washing, the blots were immersed in horseradish peroxidase (HRP)-coupled secondary antibody (1:5000) for 1 h at room temperature. The antibody complexes were detected with enhanced chemiluminescence (ECL) reagent and visualized by Imager (UVP Biospectrum 810).

2.8. Statistical analysis

Data were presented as the mean ± SD. Statistical analyses were carried out using One-way ANOVA followed by Tukey's post hoc test. Two-way ANOVA analysis followed by post hoc Bonferroni/Dunn test was used to analyze the statistical significance of experiments on gene knockout experiments (GraphPad Prism 8.0 Software, USA). $P < 0.05$ was considered statistically significant.

3. Results

3.1. Epimedin B binds to GPER

In order to validate the binding energy between Epimedin B and GPER, we firstly carried out a molecular docking study. Fig. 2A, which depicted the selected GPER 3-D model and the corresponding Ramachandran Plot, showed that 99.3% of the residues were within the allowed region (Fig. 2B). This model was used to pursue docking studies on GPER agonist G1 (Fig. 2C) and Epimedin B (Fig. 2D). As shown in Fig. 2E, as expected, G1 could bind to the GPER N-terminal hydrophobic structure through hydrogen bond and hydrophobic accumulation, and

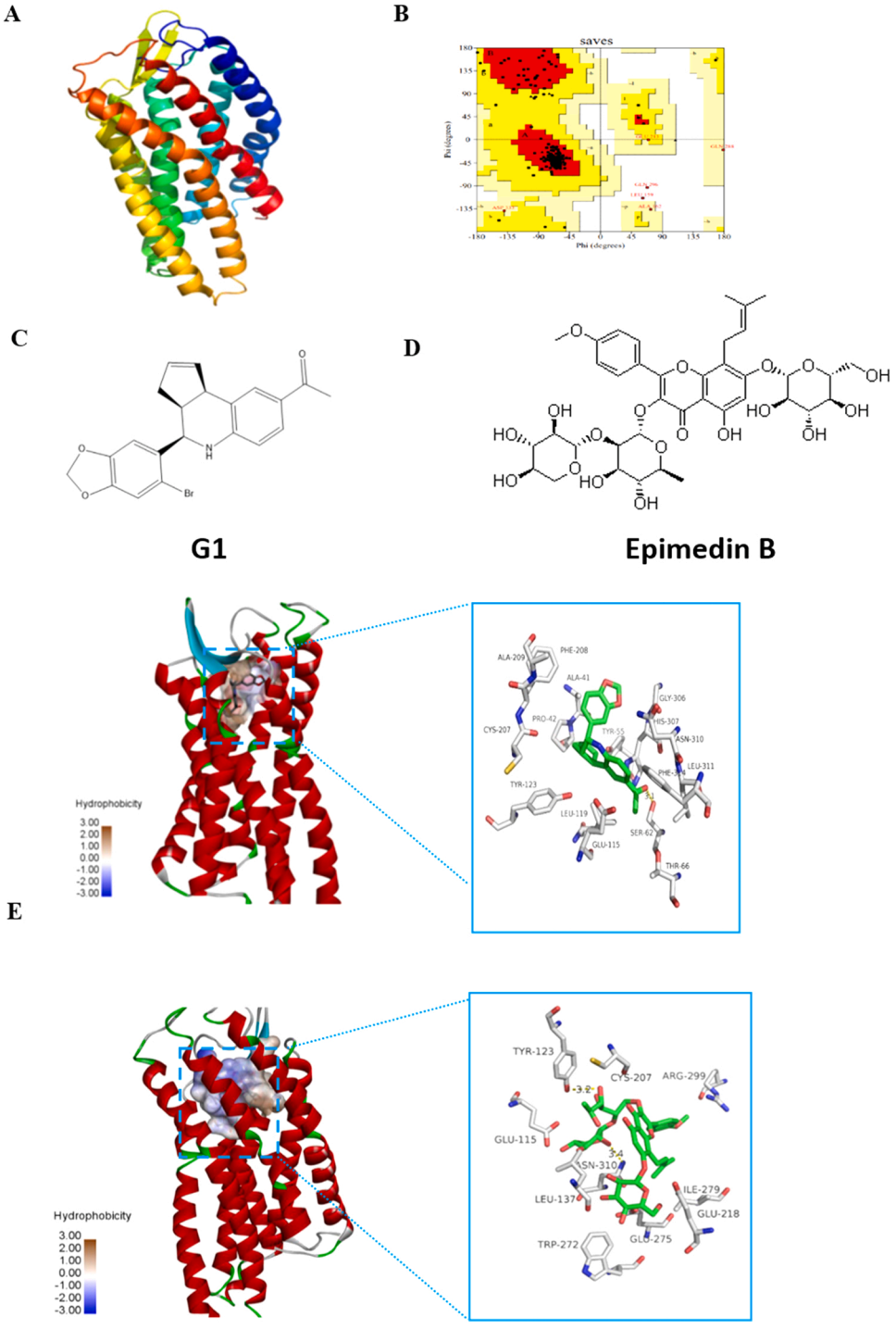

Fig. 2. Molecular docking of GPER with G1 and Epimedin B. (A) Structure of GPER obtained through homology modeling by using the web server GPCR-I-TASSER. (B) Ramachandran plot was used for evaluating the accuracy of GPER protein structure. (C) Formular structure of G1. (D) Formular structure of Epimedin B. (E) Molecular Docking of the N-terminal domain of GPER with G1. (F) Molecular Docking of the N-terminal domain of GPER with Epimedin B. The binding energy between GPER and G1 or Epimedin B was − 9.8 kcal/mol and − 7.3 kcal/mol, respectively.

the binding energy of G1 to GPER was − 9.8 kcal/mol. Epimedin B could also bind to the N-terminal hydrophobic core of GPER, and the binding energy was − 7.3 kcal/mol (Fig. 2F).

3.2. Epimedin B ameliorates MPTP-induced motor deficits in mice

The effects of different doses of Epimedin B (1, 10, 20 mg/kg) against MPTP-induced motor deficits were evaluated according to the experimental design shown in Fig. 3A. The motor skill performance of mice was evaluated by the rotarod test (Fig. 3B) and pole test (Fig. 3C&D). In the rotarod test, the shortened latency to fall in the MPTP model group was significantly increased by Epimedin B (10 mg/kg & 20 mg/kg) treatment. In the pole test, the total time to descend the pole (T-total) and the time to orient down (T-turn) were significantly prolonged after MPTP treatment, indicating MPTP-induced bradykinesia and incoordination, while Epimedin B (10 mg/kg & 20 mg/kg) treatment for 8 days significantly attenuated the MPTP-induced prolonged duration of T-total and T-turn.

3.3. Epimedin B inhibits MPTP-induced dopaminergic neuronal damage

To confirm the neuroprotective effects of Epimedin B on DA neurons, we detected the protein expressions of TH in SN and the contents of DA and its metabolites DOPAC and HVA in the striatum using western blot and HPLC, respectively. Our results clearly showed that MPTP treatment resulted in a significant decrease in the TH protein expression relative to the control group in SN. In contrast, treatment of Epimedin B at both doses of 10 mg/kg and 20 mg/kg could protect against the neurotoxicity of MPTP (Fig. 4A) in PD mice model. In detail, MPTP treatment significantly decreased the contents of DA (Fig. 4B), DOPAC (Fig. 4C) and HVA (Fig. 4D) approximately by 65.3%, 79.4% and 64.0%, respectively. Treatment with 10 mg/kg Epimedin B significantly increased the content of DA, DOPAC by 34.4%, 19.2%, respectively. Epimedin B treatment increased the content of HVA by 17.8% , but the increase did not reach statistical significance. Treatment with 20 mg/kg Epimedin B could slightly but not significantly increase the content of DA, DOPAC and HVA compared with the MPTP group. Based on these results, 10 mg/kg Epimedin B was chosen for subsequent experiments.

3.4. GPER is involved in the neuroprotective effects of Epimedin B against MPTP-induced motor deficits

Molecular docking revealed that Epimedin B might directly bind to GPER. In order to prove that GPER was a potential neuroprotective target for Epimedin B, GPER antagonist G15 was microinjected into the lateral cerebral ventricle along with gastric infusion of Epimedin B (Fig. 5A). The results of the behavioral test showed that co-treatment with G15 could significantly attenuate the neuroprotective effects of Epimedin B against MPTP-induced motor deficits. Treatment with Epimedin B alone did not affect motor function compared with the control group (Fig. 5 B-D).

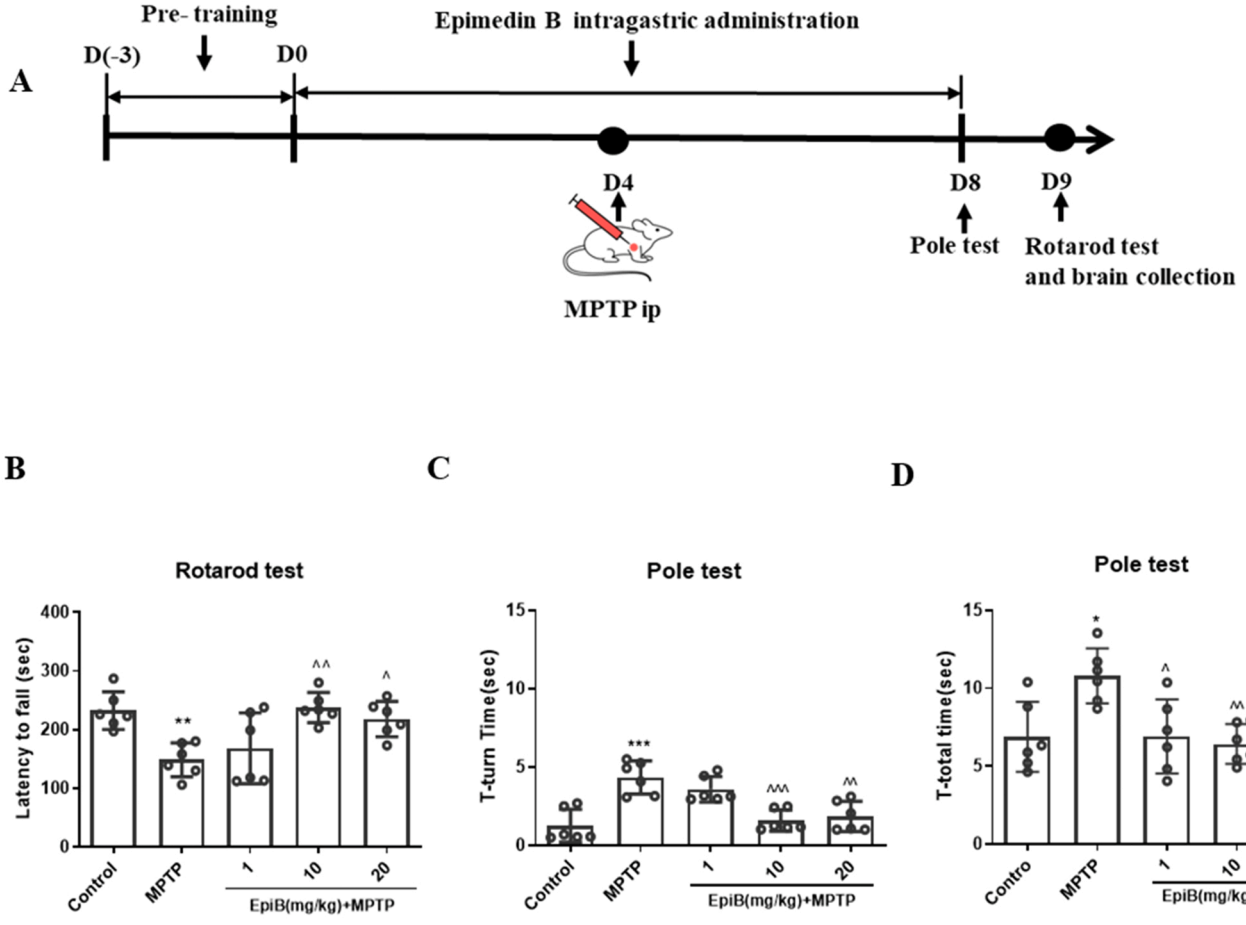

Fig. 3. Epimedin B ameliorates MPTP-induced motor deficits in mice. (A) The scheme of the experimental procedure for the neuroprotective effect of different doses of Epimedin B on MPTP-induced PD mice. (B) The latency to fall on the rotarod apparatus was recorded by the rotarod test. (C) The time to descend the pole (T-total) was recorded by Pole test. (D) The time to turn heads down in the pole (T-turn) was recorded by Pole test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs control group. $^{\wedge\wedge}P < 0.01$, $^{\wedge\wedge\wedge}P < 0.001$ vs MPTP group. Data are expressed as means ± SD (n = 6).

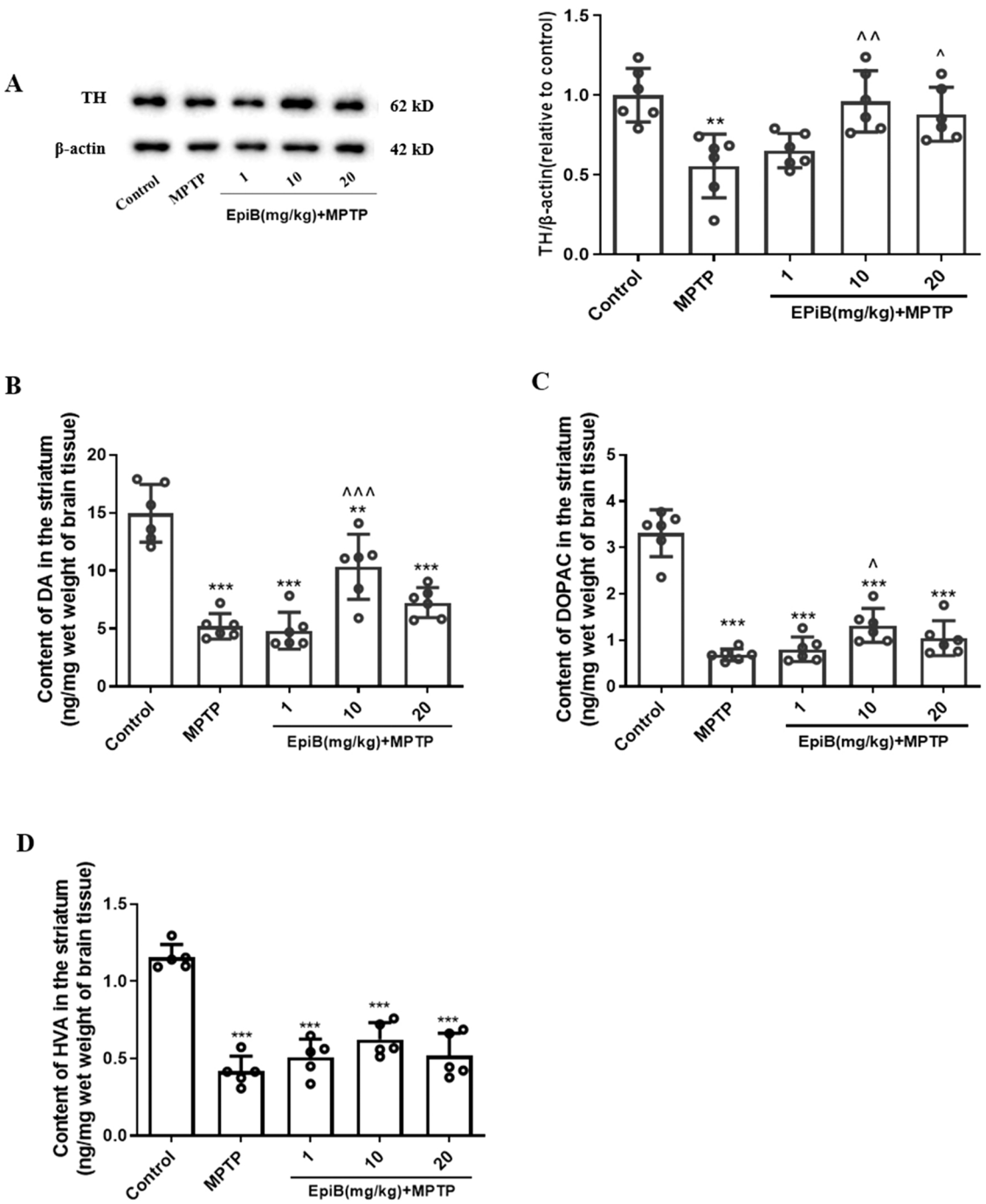

Fig. 4. Epimedin B inhibits MPTP-induced DA neuron damage. The mice were treated with vehicle or three different doses of Epimedin B (1, 10, 20 mg/kg) by oral gavage for 8 days. Three days after the Epimedin B pretreatment, MPTP injection (four times with intervals of 2 h, i.p) was performed at 2 h after the intragastric administration of Epimedin B or vehicle. Eight days after Epimedin B treatment, the mice were sacrificed and the tissues of substantia nigra and striatum were collected for western blot and HLPC assay, respectively. (A) The protein expression of TH. (B-D) Dose-dependent effects of Epimedin B on the content of DA, DOPAC and HVA in the striatum. $^{**}P < 0.01$, $^{***}P < 0.01$ vs control group. $\hat{}P < 0.05$, $\hat{}\hat{}P < 0.01$, $\hat{}\hat{}\hat{}P < 0.001$ vs MPTP group. Data are expressed as means ± SD (n = 6).

3.5. GPER mediates the neuroprotective effects of Epimedin B on MPTP-induced DA neuronal damage

To further clarify the involvement of GPER in the protective effects of Epimedin B on DA neurons, we used immunohistochemistry and western blot technique to detect the survival of TH neurons and TH protein expression in SN. MPTP treatment resulted in 36.1% decrease of the dopaminergic neurons. Epimedin B treatment significantly increased the survival of TH-IR neurons and the survival ratio was 91.6%, while the neuroprotective effect of Epimedin B was antagonized by co-treatment with G15, and the survival rate was only 68.3% of the control group (Fig. 6A&B). Immunoblotting results also showed that Epimedin B treatment significantly antagonized the MPTP-induced decrease of TH protein level and this effect could also be reversed by G15 treatment (Fig. 6C). Epimedin B treatment alone did not exert any effect on the DA neuron survival compared with the control group.

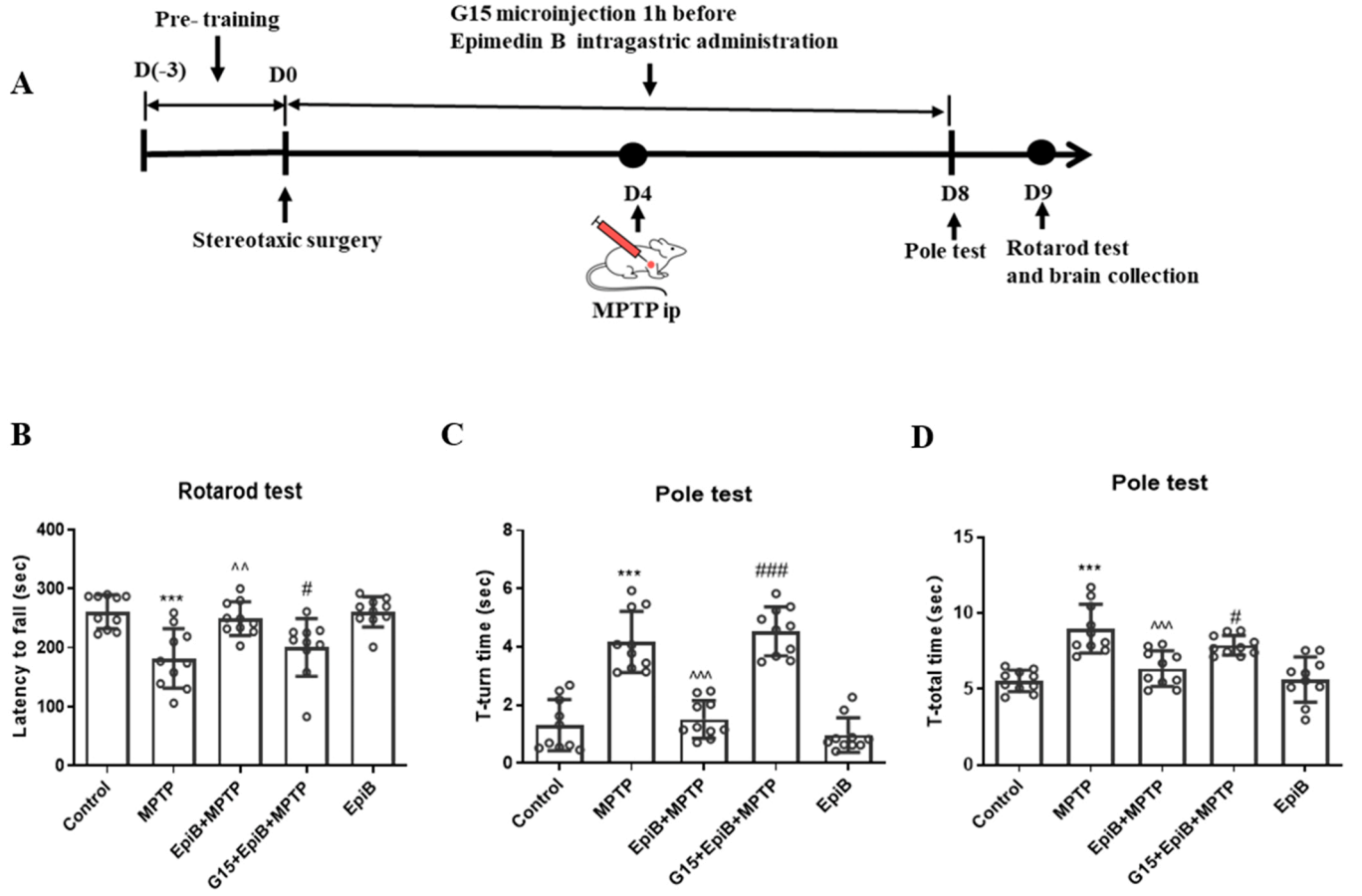

Fig. 5. GPER is involved in the improvement of Epimedin B on MPTP-induced motor deficits. (A) The scheme of the experimental procedure for the antagonist effect of G15. (B) The latency to fall on Rotarod was detected by the rotarod test. (C, D) The time of turning head and climbing down was detected by pole test. $^{***}P < 0.001$ vs control group, $^{\wedge\wedge}P < 0.01$, $^{\wedge\wedge\wedge}P < 0.001$ vs MPTP group, $^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$ vs Epi B+MPTP group. Data are expressed as means ± SD (n = 10).

3.6. Epimedin B exerts anti-apoptotic and anti-endoplasmic reticulum stress effects on MPTP-induced PD mice through GPER

There are accumulated evidences that support the involvement of apoptosis and endoplasmic reticulum stress in the pathological progression of PD. To investigate the anti-apoptotic and anti-endoplasmic reticulum stress effects of Epimedin B in MPTP-induced PD mice, the protein levels of a Bcl-2, Bax, GRP78 and CHOP were detected by western blot. As shown in Fig. 7, MPTP significantly increased the protein expressions of Bax, GRP78, CHOP and decreased the protein level of Bcl-2, indicating the involvement of apoptosis and endoplasmic reticulum stress in MPTP neurotoxicity. Epimedin B could reverse the above changes induced by MPTP. Moreover, G15 could block the neuroprotective effects of Epimedin B. Similarly, Epimedin B treatment alone did not affect the protein expressions of Bax, Bcl-2, GRP78 or CHOP compared with the control group.

3.7. GPER deficiency attenuates the effects of Epimedin B against MPTP-induced motor deficits

To further demonstrate the involvement of GPER in the neuroprotective effects of Epimedin B against PD, *GPER*$^{-/-}$ mice were employed in the following studies. As shown in Fig. 8, GPER deficiency had no significant effect on the latency to fall in the rotarod test or the T-total time or T-turn time in the pole test when comparing between the control group of *GPER*$^{+/+}$and *GPER*$^{-/-}$ mice. MPTP treatment could induce motor impairments in both *GPER*$^{+/+}$ and *GPER*$^{-/-}$ mice. Epimedin B treatment could alleviate MPTP-induced motor deficits in *GPER*$^{+/+}$ mice, but its protective effects disappeared in *GPER*$^{-/-}$ mice.

3.8. GPER deficiency abolishes the protective effect of Epimedin B on DA neurons

To explore the effect of GPER deficiency on neuronal survival, the protein expression of TH in SN was detected. Western blot results revealed that the mouse genotype (presence or absence of GPER) did not influence the TH protein expression in SN. Epimedin B treatment could rescue the MPTP-induced down-regulation of TH protein expression in *GPER*$^{+/+}$ mice. However, the neuroprotective effect of Epimedin B against MPTP-induced neurotoxicity was abolished in *GPER*$^{-/-}$ mice (Fig. 9).

3.9. GPER deficiency attenuates the anti-apoptotic and anti-endoplasmic reticulum stress effects of Epimedin B

We next investigated the role of GPER gene knockout on the anti-apoptotic and anti-endoplasmic reticulum stress effects of Epimedin B. As shown in Fig. 10, GPER deficiency had no effect on protein expressions of Bax, Bcl-2, GRP78 or CHOP in the SN of mice when comparing between the control group of *GPER*$^{+/+}$and *GPER*$^{-/-}$ mice. Epimedin B treatment significantly antagonized the MPTP-induced changes of Bax, Bcl-2, GRP78 and CHOP protein levels in *GPER*$^{+/+}$ MPTP mice, while the anti-apoptosis and anti-endoplasmic reticulum stress effects of Epimedin B were significantly inhibited in *GPER*$^{-/-}$ mice. Thus, the results further suggest that GPER plays an important role in the neuroprotective effect of Epimedin B.

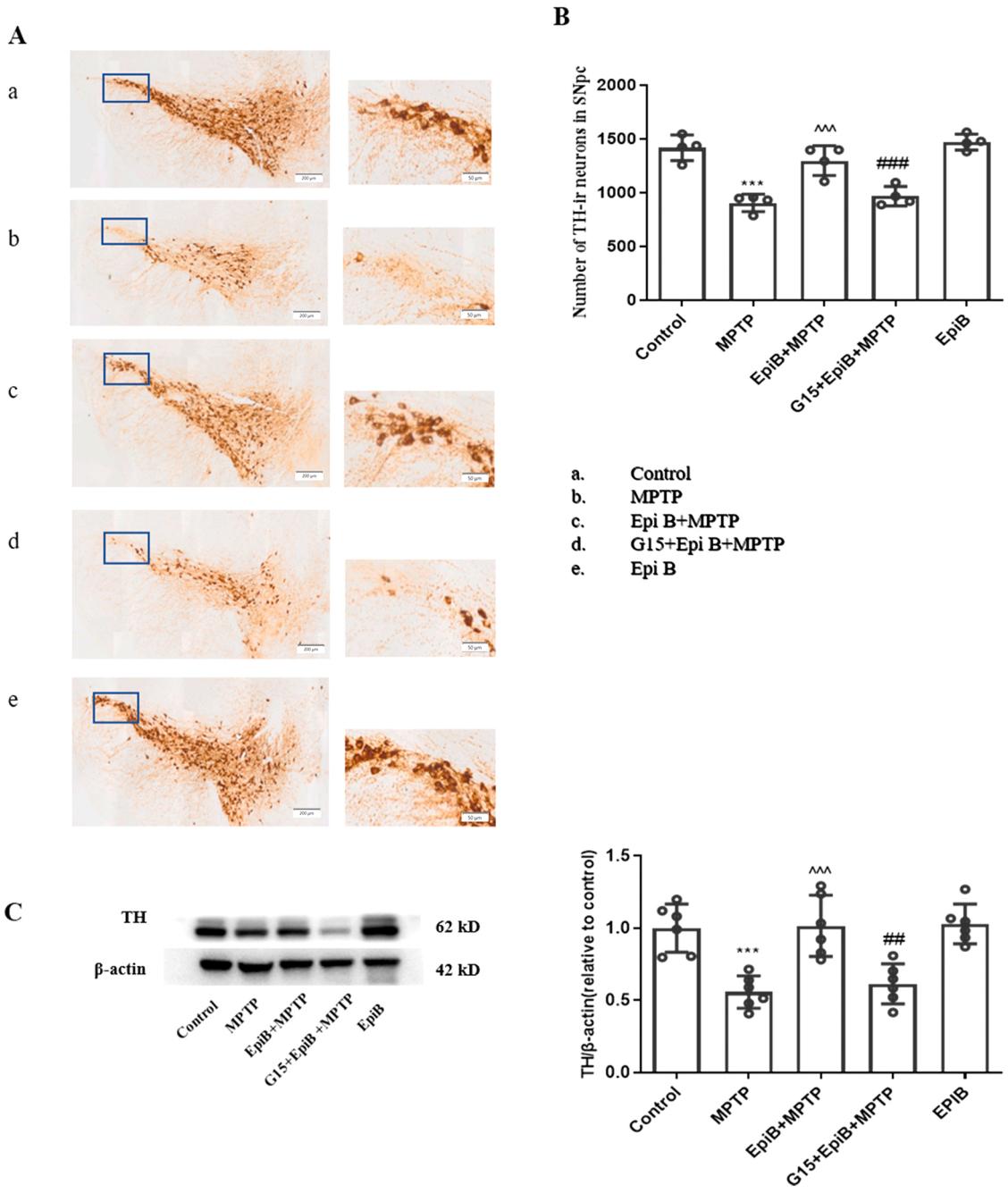

Fig. 6. GPER mediates the neuroprotective effects of Epimedin B on MPTP-induced DA neuron damage. (A) The microphotographs of TH-immunoreactive neurons in the SNpc. (a) Control group, (b) MPTP group, (c) Epi B+MPTP group, (d) G15 +Epi B+LPS group, (e) Epi B group. Scale bar, 200 μm or 50 μm. (B) Quantitative analysis of TH-immunoreactive neurons in the SN. Data are expressed as means ± SD, (n = 4). (C) The protein expression of TH was examined using western blot in the SN. Data are expressed as means ± SD, (n = 6). $^{***}P < 0.001$ vs control group, $^{\wedge\wedge\wedge}P < 0.001$ vs MPTP group, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs Epi B+MPTP group.

4. Discussion

Total flavonoids are the major active ingredients of *Herba Epimedii*, and they have been demonstrated to process pharmacological effects such as anti-aging, anti-inflammation, anti-osteoporosis, anti-oxidative stress, and neuroprotection [28–33]. Epimedin B is the second highest active ingredient in flavonoids after icariin [33]. The present work aimed at evaluating the neuroprotective effects of Epimedin B on dopaminergic neurons and the potential underlying mechanism in the MPTP-induced mouse model of PD. Our finding demonstrated the following: (1) Molecular docking study revealed that Epimedin B could directly bind to GPER at the same site as GPER agonist G1. (2) Epimedin B suppressed MPTP-induced nigral DA neuron injury via anti-apoptotic and anti-endoplasmic reticulum stress. (3) Pharmacological blockade of GPER with GPER antagonist G15 significantly inhibited the neuroprotective effects of Epimedin B. (4) Using *GPER*$^{-/-}$ mice, our results clearly demonstrated that GPER deficiency significantly abolished the neuroprotective effects of Epimedin B against MPTP-induced dopaminergic neurotoxicity. These findings provide the first evidence that Epimedin B exerts neuroprotective effect against MPTP-induced mouse model of PD and that GPER acts as a potential target.

MPTP-induced mouse model of PD is the most commonly used animal model of PD. MPTP can be metabolized to MPP^+, which induces the death of DA neurons by causing a series of insults, such as oxidative

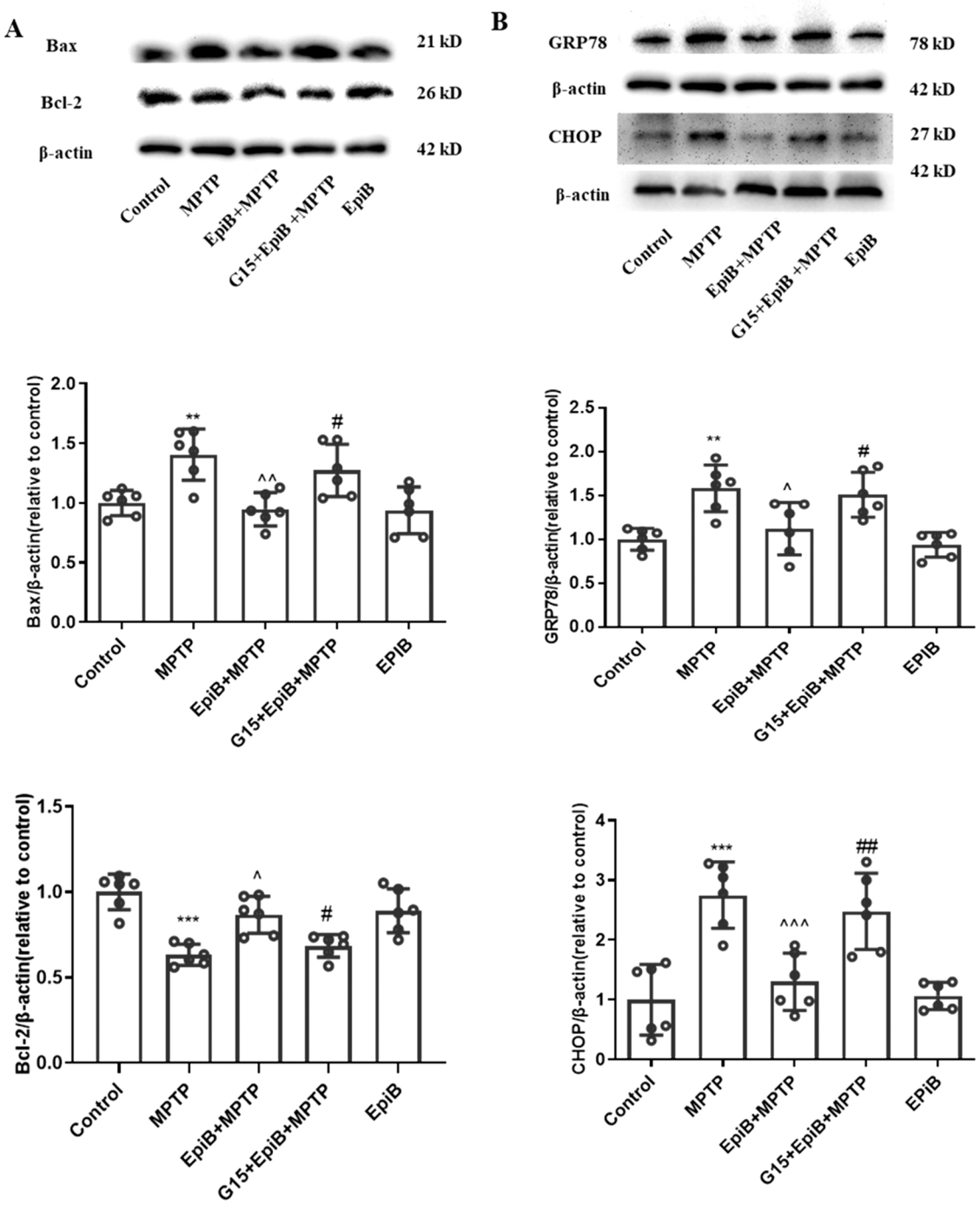

Fig. 7. Epimedin B exerts anti-apoptotic and anti-endoplasmic reticulum stress effects on MPTP-induced PD mice through GPER. Western blot assay was used to assess the effect of Epimedin B on the protein expressions of apoptosis-related protein Bax and Bcl-2 (A), and endoplasmic reticulum stress-related protein GRP78 and CHOP (B). Data are expressed as means ± SD, (n = 6). $^{**}P < 0.01$, $^{***}P < 0.001$ vs control group, $^{\wedge}P < 0.05$, $^{\wedge\wedge}P < 0.01$, $^{\wedge\wedge\wedge}P < 0.001$ vs MPTP group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs Epi B+MPTP group.

stress, mitochondrial dysfunction, inflammation, and excitotoxicity [34,35]. In the present study, MPTP was intraperitoneally injected four times with an interval of 2 h to generate the PD mouse model. MPTP treatment significantly reduced the number of DA neurons and the protein expression of TH in SN. The results of HPLC showed that MPTP could decrease the contents of DA and DOPAC in the striatum. Rotarod and pole tests showed that the dysregulated nigrostriatal system caused dyskinesia in mice. Epimedin B treatment could rescue MPTP-induced loss of DA neurons and motor impairments. Evidence from cellular and animal models of PD or postmortem brains of PD patients has shown that endoplasmic reticulum stress is involved in the pathophysiological changes in PD [36,37]. Under normal conditions, endoplasmic reticulum stress plays an important role in maintaining protein function, which could promote cell survival by reducing misfolded proteins. However, severe endoplasmic reticulum stress can disrupt endoplasmic reticulum function and induce apoptosis [36,38]. Tsujii et al. reported MPTP/MPP^{+} treatment increased the expressions of endoplasmic reticulum stress-related factors, such as GRP78 and CHOP [39]. In this study, we also investigated the changes in GRP78 and CHOP, as well as apoptosis-related proteins Bax and Bcl-2 in MPTP-induced PD mice

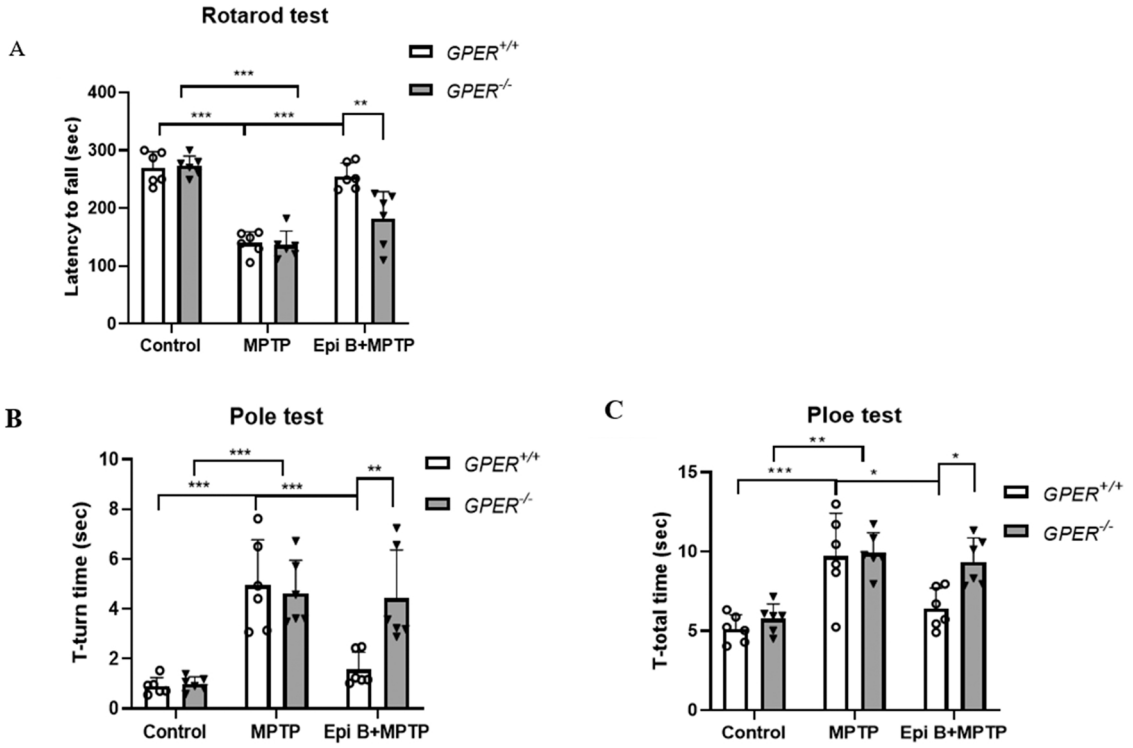

Fig. 8. GPER deficiency attenuates the improvement of Epimedin B against MPTP-induced motor deficits. Rotarod test and pole test were used to evaluate motor coordination in *GPER*$^{+/+}$ and *GPER*$^{-/-}$ mice. (A) The latency to fall on Rotarod. (B&C) The time of turning head and climbing down the pole. Data are expressed as means ± SD, (n = 6). $^{*}P < 0.05$, $^{**}P < 0.01$ $^{***}P < 0.001$ represent the comparisons between the different treatment groups.

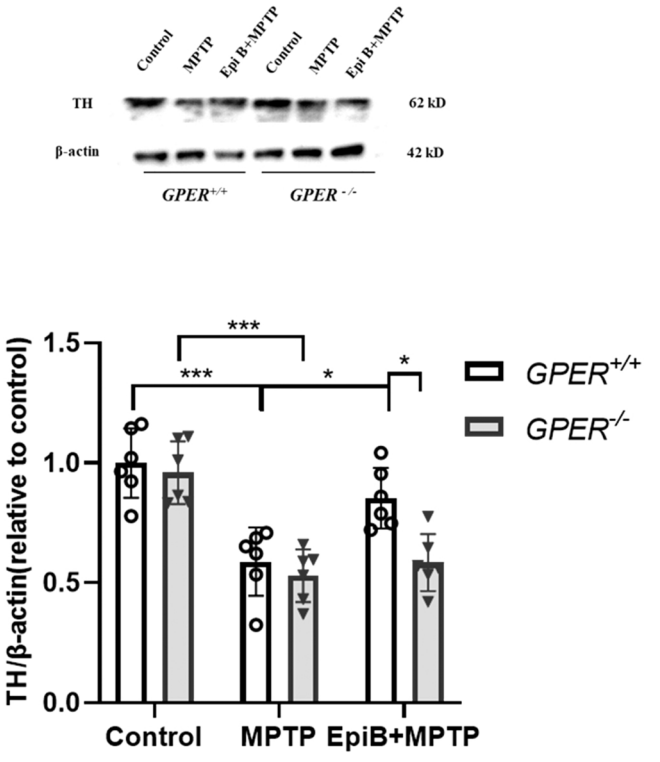

Fig. 9. GPER deficiency abolishes the protective effect of Epimedin B on DA neurons. Brain tissues of SN were harvested after the behavioral test. The protein level of TH in SN was examined by Western blot analysis. Data are expressed as means ± SD (n = 6). $^{*}P < 0.05$, $^{***}P < 0.001$ represent the comparisons between the different treatment groups.

model. The results showed that GRP78, CHOP and Bax protein expressions were up-regulated, and Bcl-2 protein expression was down-regulated after MPTP injection, and these effects could be reversed by Epimedin B treatment. These data indicated that Epimedin B could protect against MPTP-induced neurotoxicity by inhibiting apoptosis and endoplasmic reticulum stress.

Phytoestrogens such as flavonoids, lignans, and steroids, as a kind of natural product, have estrogenic properties and can scavenge free radicals and anti-oxidant stress (Sirotkin and Harrath, 2014). Epimedin B, with a basic structure similar to the 8-isoprenylated flavonoid, is a flavonoid phytoestrogen. It was reported that Epimedin B could stimulate cell proliferation and cell differentiation in pre-osteoblastic UMR-106 cells, which were blocked by ER inhibitor ICI182,780. However, Epimedin B could not directly bind to nuclear ERs, nor could it activate phosphorylation of ERα in estrogen response element-dependent manner [23]. GPER is a major mediator of estrogen's rapid cellular effects throughout the body [40]. We speculated that Epimedin B might exert its estrogen-like effects through GPER-mediated signaling pathway. The results of molecular docking firstly confirmed that Epimedin B could directly bind to the N-terminal hydrophobic structure of GPER at the same site as GPER agonist G1 and the binding energy was − 7.3 kcal/mol, indicating GPER might be a potential target of Epimedin B.

GPER is widely expressed in numerous tissues and can regulate cell proliferation, endoplasmic reticulum stress, and apoptosis in reproductive system and nervous system. A recent study confirmed that G1, a selective agonist of GPER, could increase endoplasmic reticulum stress and induce cell death in the estrogen receptor-positive breast cancer cell line MCF-7 [41]. Liu et al. reported G1 could activate GPER and induce endoplasmic reticulum stress as well as the mitochondrial-related apoptosis in colon cancer cells [42]. In the central nervous system, a large body of evidence showed that estrogen could protect nigrostriatal DA neurons by activating GPER. G1 could protect DA neurons against MPTP/MPP^{+}, a protection similar to that exerted by E2. However, these

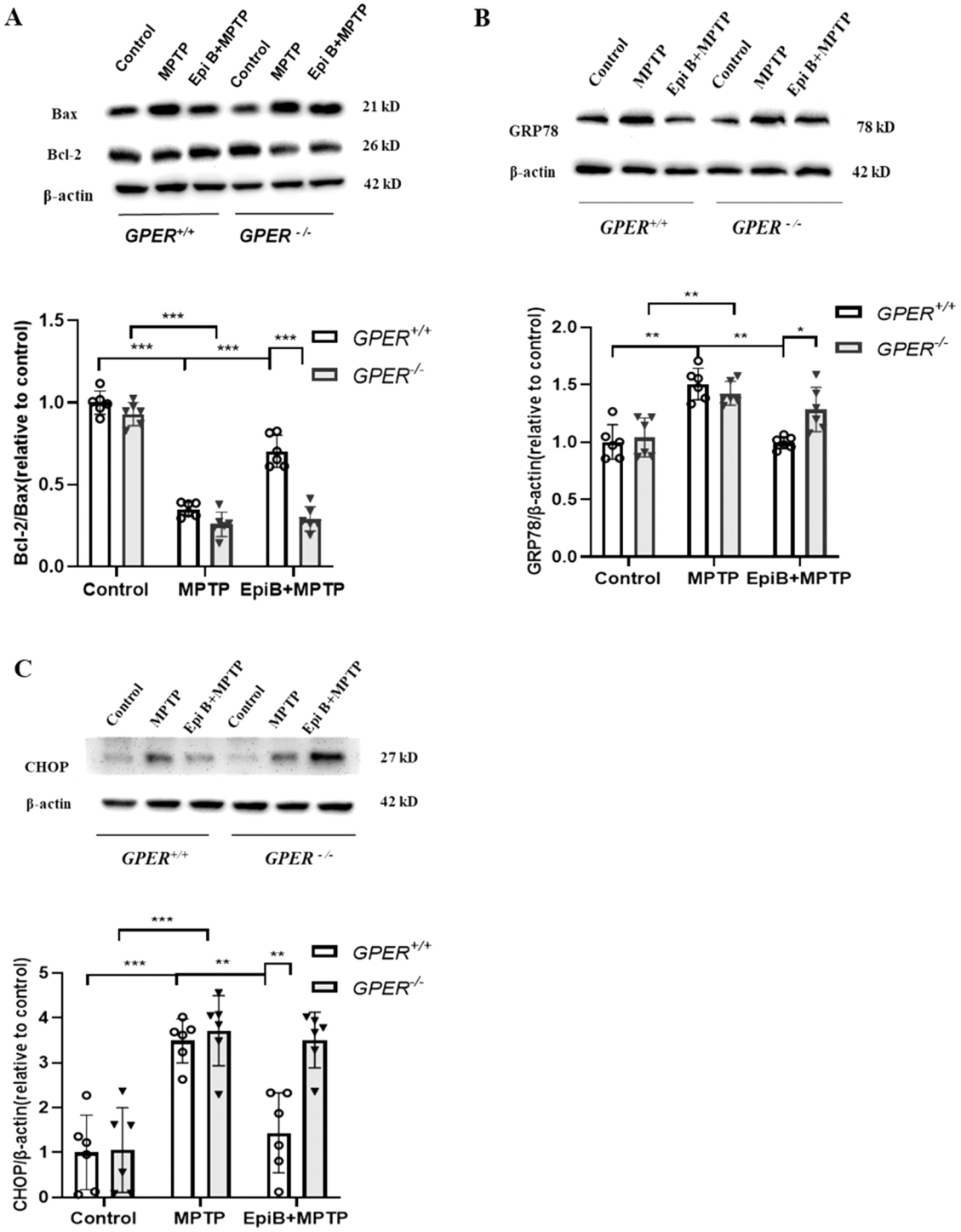

Fig. 10. GPER deficiency attenuates the anti-apoptotic and anti-endoplasmic reticulum stress effects of Epimedin B. Brain tissues of SN were harvested after the behavioral test. Western blot assay was performed to determine the levels of apoptosis-related protein Bax and Bcl-2 (A) and endoplasmic reticulum stress-related protein GRP78 (B) and CHOP (C). Data are expressed as means ± SD, (n = 6). $^{*}P < 0.05$, $^{**}P < 0.001$, $^{***}P < 0.001$ represent the comparisons between the different treatment group.

neuroprotective effects of E2 and G1 disappeared in the presence of G15 [40,43,44]. In addition, GPER activation also increased the expression of GDNF, BDNF and Bcl-2 in the striatum and protected DA neurons in the SN of MPTP mice [45–47]. Han et al. reported that GPER agonist G1 could suppress neuron apoptosis and inhibit the expression of endoplasmic reticulum stress-related proteins in the hippocampus after cerebral ischemia/reperfusion (I/R) injury [48]. In the primary cultured murine retinal ganglion cells, GPER activation could decrease apoptosis and the expressions of endoplasmic reticulum stress-related proteins [49]. These results suggested that GPER activation could protect neurons against apoptosis and endoplasmic reticulum stress as well as promote cell survival. In the present study, we used G15, an antagonist of GPER, to examine the involvement of GPER in the anti-apoptosis and anti-endoplasmic reticulum stress of Epimedin B in MPTP-induced mouse model of PD. According to our results, G15 could significantly antagonize the neuroprotective effects of Epimedin B against

MPTP-induced behavioral disorders, dopaminergic neuronal damage, and apoptotic and endoplasmic reticulum stress. In order to further confirm the exact role of GPER in the neuroprotective effect of Epimedin B, we developed the GPER KO mouse (*GPER*$^{-/-}$). Consistent with the research of others, we observed the obese phenotype in the male *GPER*$^{-/-}$ mice [50]. Compared with the wild-type (*GPER*$^{+/+}$) mice, GPER deletion didn't affect the motor ability of mice, or the protein expressions of TH, Bax, Bcl-2, GRP78 and CHOP in SN, suggesting that GPER deletion did not affect the sensitivity of mice to neurotoxin MPTP. Moreover, the neuroprotective effects of Epimedin B against MPTP were significantly inhibited in *GPER*$^{-/-}$ mice. These findings further supported GPER as a potential target for the neuroprotective effect of Epimedin B.

In conclusion, our data provide the first evidence that GPER may be a potential target for the neuroprotective effect of Epimedin B against MPTP-induced DA neuron injury in mice. This study may provide a new strategy for PD treatment.

CRediT authorship contribution statement

Mei Zhang: Conceptualization, Methodology, Data curation, Formal analysis, Writing – original draft. **Zi-Fan Hu:** Conceptualization, Methodology, Software, Validation. **Xiao-Li Dong:** Reviewing and Editing. **Wen-Fang Chen:** Supervision, Project administration, Writing – review & editing, Funding acquisition.

Conflict of interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] L.V. Kalia, A.E. Lang, Parkinson's disease, Lancet 386 (9996) (2015) 896–912.
[2] S.G. Reich, J.M. Savitt, Parkinson's disease, Med. Clin. N. Am. 103 (2) (2019) 337–350.
[3] S. Cerri, L. Mus, F. Blandini, Parkinson's disease in women and men: what's the difference? J. Park. Dis. 9 (3) (2019) 501–515.
[4] J.W. Simpkins, M. Singh, C. Brock, A.M. Etgen, Neuroprotection and estrogen receptors, Neuroendocrinology 96 (2) (2012) 119–130.
[5] S. Maioli, K. Leander, P. Nilsson, I. Nalvarte, Estrogen receptors and the aging brain, Essays Biochem. 65 (6) (2021) 913–925.
[6] M. Rouhimoghadam, A.S. Lu, A.K. Salem, E.J. Filardo, Therapeutic perspectives on the modulation of g-protein coupled estrogen receptor, GPER, function, Front. Endocrinol. 11 (2020), 591217.
[7] L.A. Bean, L. Ianov, T.C. Foster, Estrogen receptors, the hippocampus, and memory, Neuroscientist 20 (5) (2014) 534–545.
[8] G. Sharma, C. Hu, D.I. Staquicini, J.L. Brigman, M. Liu, F. Mauvais-Jarvis, R. Pasqualini, W. Arap, J.B. Arterburn, H.J. Hathaway, E.R. Prossnitz, Preclinical efficacy of the GPER-selective agonist G-1 in mouse models of obesity and diabetes, Sci. Transl. Med. 12 (528) (2020).
[9] E.R. Prossnitz, M. Barton, The G-protein-coupled estrogen receptor GPER in health and disease, Nat. Rev. Endocrinol. 7 (12) (2011) 715–726.
[10] Z.J. Chen, W. Wei, G.M. Jiang, H. Liu, W.D. Wei, X. Yang, Y.M. Wu, H. Liu, C. K. Wong, J. Du, H.S. Wang, Activation of GPER suppresses epithelial mesenchymal transition of triple negative breast cancer cells via NF-kappaB signals, Mol. Oncol. 10 (6) (2016) 775–788.
[11] J.H. Cho, J.Y. Jung, B.J. Lee, K. Lee, J.W. Park, Y. Bu, Epimedii herba: a promising herbal medicine for neuroplasticity, Phytother. Res. 31 (6) (2017) 838–848.
[12] J. Cai, Y. Tian, R. Lin, X. Chen, Z. Liu, J. Xie, Protective effects of kidney-tonifying Chinese herbal preparation on substantia nigra neurons in a mouse model of Parkinson's disease, Neural Regen. Res. 7 (6) (2012) 413–420.
[13] M. Chen, J. Wu, Q. Luo, S. Mo, Y. Lyu, Y. Wei, J. Dong, The anticancer properties of herba epimedii and its main bioactive componentsicariin and icariside II, Nutrients 8 (9) (2016).
[14] Y. Guo, X. Wang, J. Gao, Simultaneous preparation and comparison of the osteogenic effects of epimedins A - C and Icariin from Epimedium brevicornu, Chem. Biodivers. 15 (4) (2018), e1700578.
[15] S.C. Sze, Y. Tong, T.B. Ng, C.L. Cheng, H.P. Cheung, Herba Epimedii: anti-oxidative properties and its medical implications, Molecules 15 (11) (2010) 7861–7870.
[16] J. He, S. Zang, N. Liu, M. Ji, D. Ma, C. Ji, Epimedium polysaccharides attenuates hematotoxicity by reducing oxidative stress and enhancing immune function in mice model of benzene-induced bone marrow failure, Biomed. Pharmacother. 125 (2020), 109908.
[17] F. Xu, Y. Ding, Y. Guo, B. Liu, Z. Kou, W. Xiao, J. Zhu, Anti-osteoporosis effect of Epimedium via an estrogen-like mechanism based on a system-level approach, J. Ethnopharmacol. 177 (2016) 148–160.
[18] N. Xu, G. Zhou, X. Li, H. Lu, F. Meng, H. Zhai, Geographical classification of Epimedium based on HPLC fingerprint analysis combined with multi-ingredients quantitative analysis, Biomed. Chromatogr. 31 (5) (2017).
[19] L. Wu, Z.R. Du, A.L. Xu, Z. Yan, H.H. Xiao, M.S. Wong, X.S. Yao, W.F. Chen, Neuroprotective effects of total flavonoid fraction of the Epimedium koreanum Nakai extract on dopaminergic neurons: in vivo and in vitro, Biomed. Pharmacother. 91 (2017) 656–663.
[20] R. Zhong, Y. Chen, J. Ling, Z. Xia, Y. Zhan, E. Sun, Z. Shi, L. Feng, X. Jia, J. Song, Y. Wei, The toxicity and metabolism properties of herba epimedii flavonoids on laval and adult zebrafish, Evid. Based Complement. Altern. Med. 2019 (2019), 3745051.
[21] X. Diao, L. Wang, Y. Zhou, Y. Bi, K. Zhou, L. Song, The mechanism of Epimedin B in treating osteoporosis as revealed by RNA sequencing-based analysis, Basic Clin. Pharmacol. Toxicol. 129 (6) (2021) 450–461.
[22] H.M. Lin, L.F. Lin, M.Y. Sun, J. Liu, Q. Wu, Topical delivery of four neuroprotective ingredients by ethosome-gel: synergistic combination for treatment of oxaliplatin-induced peripheral neuropathy, Int. J. Nanomed. 15 (2020) 3251–3266.
[23] H.H. Xiao, C.Y. Fung, S.K. Mok, K.C. Wong, M.X. Ho, X.L. Wang, X.S. Yao, M. S. Wong, Flavonoids from Herba epimedii selectively activate estrogen receptor alpha (ERalpha) and stimulate ER-dependent osteoblastic functions in UMR-106 cells, J. Steroid Biochem. Mol. Biol. 143 (2014) 141–151.
[24] L.J. Yuan, X.W. Wang, H.T. Wang, M. Zhang, J.W. Sun, W.F. Chen, G protein-coupled estrogen receptor is involved in the neuroprotective effect of IGF-1 against MPTP/MPP(+)-induced dopaminergic neuronal injury, J. Steroid Biochem. Mol. Biol. 192 (2019), 105384.
[25] C.A. Laferriere, D.S. Pang, Review of intraperitoneal injection of sodium pentobarbital as a method of euthanasia in laboratory rodents, J. Am. Assoc. Lab Anim. Sci. 59 (3) (2020) 254–263.
[26] L.-X. Liu, W.-F. Chen, J.-X. Xie, M.-S. Wong, Neuroprotective effects of genistein on dopaminergic neurons in the mice model of Parkinson's disease, Neurosci. Res. 60 (2) (2008) 156–161.
[27] X.-W. Wang, L.-J. Yuan, Y. Yang, M. Zhang, W.-F. Chen, IGF-1 inhibits MPTP/MPP-induced autophagy on dopaminergic neurons through the IGF-1R/PI3K-Akt-mTOR pathway and GPER, Am. J. Physiol. Endocrinol. Metab. 319 (4) (2020) E734–E743.
[28] Z. Meng-Ru, S. Ruo-Xuan, Y. Ming-Yang, T. Tong, Z. Lei, Y. Ying-Bo, X. Bao-Guo, Antagonizing astrocytic platelet activating factor receptor-neuroinflammation for total flavone of epimedium in response to cuprizone demyelination, Int. Immunopharmacol. 101 (Pt A) (2021), 108181.
[29] B. Wu, X. Xiao, S. Li, G. Zuo, Transcriptomics and metabonomics of the anti-aging properties of total flavones of Epimedium in relation to lipid metabolism, J. Ethnopharmacol. 229 (2019) 73–80.
[30] W.P. Zhang, Y.Y. Deng, J.X. Ren, J.J. Li, T. Chen, S.S. Gao, [Mechanisms of total flavones of Epimedium on oxidative stress induced by myocardial ischemia/reperfusion injury in rats], Zhongguo Zhong Yao Za Zhi 41 (18) (2016) 3400–3405.
[31] Z.K. Xu, H.M. Yin, F. Li, J.P. Qin, S.L. Gu, Y. Wu, Z.Z. Wang, W. Xiao, [Determination of flavonoids constituents in epimedium total flavone capsule and research on its anti-osteoporosis activity], Zhongguo Zhong Yao Za Zhi 43 (15) (2018) 3140–3144.
[32] D. Zhang, J. Zhang, C. Fong, X. Yao, M. Yang, Herba epimedii flavonoids suppress osteoclastic differentiation and bone resorption by inducing G2/M arrest and apoptosis, Biochimie 94 (12) (2012) 2514–2522.
[33] W.F. Chen, S.K. Mok, X.L. Wang, K.H. Lai, W.P. Lai, H.K. Luk, P.C. Leung, X.S. Yao, M.S. Wong, Total flavonoid fraction of the Herba epimedii extract suppresses urinary calcium excretion and improves bone properties in ovariectomised mice, Br. J. Nutr. 105 (2) (2011) 180–189.
[34] M. Mustapha, C.N. Mat Taib, MPTP-induced mouse model of Parkinson's disease: a promising direction of therapeutic strategies, Bosn. J. Basic Med. Sci. 21 (4) (2021) 422–433.
[35] X.S. Zeng, W.S. Geng, J.J. Jia, Neurotoxin-induced animal models of parkinson disease: pathogenic mechanism and assessment, ASN Neuro 10 (2018), 1759091418777438.
[36] R. Ghemrawi, M. Khair, Endoplasmic reticulum stress and unfolded protein response in neurodegenerative diseases, Int. J. Mol. Sci. 21 (17) (2020).
[37] D.M. Arduino, A.R. Esteves, S.M. Cardoso, C.R. Oliveira, Endoplasmic reticulum and mitochondria interplay mediates apoptotic cell death: relevance to Parkinson's disease, Neurochem. Int. 55 (5) (2009) 341–348.
[38] C.A.D. Costa, W.E. Manaa, E. Duplan, F. Checler, The endoplasmic reticulum stress/unfolded protein response and their contributions to Parkinson's disease physiopathology, Cells 9 (11) (2020).
[39] S. Tsujii, M. Ishisaka, M. Shimazawa, T. Hashizume, H. Hara, Zonisamide suppresses endoplasmic reticulum stress-induced neuronal cell damage in vitro and in vivo, Eur. J. Pharmacol. 746 (2015) 301–307.
[40] E.R. Prossnitz, M. Barton, Estrogen biology: new insights into GPER function and clinical opportunities, Mol. Cell Endocrinol. 389 (1–2) (2014) 71–83.
[41] D.H. Vo, R. Hartig, S. Weinert, J. Haybaeck, N. Nass, G-protein-coupled estrogen receptor (GPER)-specific agonist G1 induces ER stress leading to cell death in MCF-7 cells, Biomolecules 9 (9) (2019).

[42] Q. Liu, Z. Chen, G. Jiang, Y. Zhou, X. Yang, H. Huang, H. Liu, J. Du, H. Wang, Epigenetic down regulation of G protein-coupled estrogen receptor (GPER) functions as a tumor suppressor in colorectal cancer, Mol. Cancer 16 (1) (2017) 87.
[43] A. Bessa, F.L. Campos, R.A. Videira, J. Mendes-Oliveira, D. Bessa-Neto, G. Baltazar, GPER: a new tool to protect dopaminergic neurons? Biochim. Biophys. Acta 1852 (10 Pt A) (2015) 2035–2041.
[44] M. Bourque, M. Morissette, M. Côté, D. Soulet, T. Di Paolo, Implication of GPER1 in neuroprotection in a mouse model of Parkinson's disease, Neurobiol. Aging 34 (3) (2013) 887–901.
[45] M. Bourque, M. Morissette, T. Di Paolo, Raloxifene activates G protein-coupled estrogen receptor 1/Akt signaling to protect dopamine neurons in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mice, Neurobiol. Aging 35 (10) (2014) 2347–2356.
[46] M. Bourque, M. Morissette, T. Di Paolo, Neuroprotection in Parkinsonian-treated mice via estrogen receptor alpha activation requires G protein-coupled estrogen receptor 1, Neuropharmacology 95 (2015) 343–352.
[47] C. Roque, J. Mendes-Oliveira, C. Duarte-Chendo, G. Baltazar, The role of G protein-coupled estrogen receptor 1 on neurological disorders, Front. Neuroendocr. 55 (2019), 100786.
[48] Z.W. Han, Y.C. Chang, Y. Zhou, H. Zhang, L. Chen, Y. Zhang, J.Q. Si, L. Li, GPER agonist G1 suppresses neuronal apoptosis mediated by endoplasmic reticulum stress after cerebral ischemia/reperfusion injury, Neural Regen. Res. 14 (7) (2019) 1221–1229.
[49] R. Li, Y. Wang, P. Chen, J. Meng, H. Zhang, G-protein-coupled estrogen receptor protects retinal ganglion cells via inhibiting endoplasmic reticulum stress under hyperoxia, J. Cell Physiol. 236 (5) (2021) 3780–3788.
[50] G. Sharma, C. Hu, J.L. Brigman, G. Zhu, H.J. Hathaway, E.R. Prossnitz, GPER deficiency in male mice results in insulin resistance, dyslipidemia, and a proinflammatory state, Endocrinology 154 (11) (2013) 4136–4145.