

Exploring Extracts from *Wendlandia Uvariifolia* Subsp. *Chinensis* (Merr.) Cowan as Natural PDE5 Inhibitors with Potent Anti-Erectile Dysfunction Activity

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Abstract

Wendlandia uvariifolia subsp. *chinensis* (Merr.) Cowan (*W. u. chinensis*) is a traditional medicinal plant used by the Li ethnic group in Hainan Province, China, for the treatment of herpes zoster. However, its pharmacological properties remain unclear. This study aimed to evaluate its phosphodiesterase type 5 (PDE5) inhibitory activity, identify the active components, and explore its therapeutic potential for erectile dysfunction (ED). Through high-throughput screening of 1200 traditional Chinese herbal medicines, this plant was identified as a potential PDE5 inhibitor. After activity evaluation and gas chromatography-mass spectrometry analysis, its petroleum ether fraction (PE) exhibited the strongest PDE5 inhibitory activity, and its main component, phytol, was identified as a novel PDE5 inhibitor with an unreported structural framework. PE could induce endothelial cells to produce nitric oxide *in vitro* (NO). PE significantly improved the sexual behavior indicators, hormone levels, and penile tissue of paroxetine-induced ED rats *in vivo*, while increasing the intracavernous pressure/mean arterial pressure (ICP/MAP) ratio and reducing fibrosis in diabetes-related ED (DMED) rats. This study confirms that the PE fraction of *W. u. chinensis* has potent PDE5 inhibitory activity, providing a basis for the development of natural therapeutic drugs for ED and herbal medicine.

Introduction

Erectile dysfunction (ED) is defined as the persistent inability to achieve or maintain an adequate penile erection for satisfactory sexual performance, which can profoundly undermine patients' self-confidence, intimate relationships, and overall quality of life [1]. It affects more than 150 million men worldwide, representing a growing public health challenge [2], and its prevalence is increasing not only among middle-aged and elderly men but also in younger populations [3]. The etiology of ED is multifactorial, encompassing psychological factors such as chronic stress, anxiety, and depression; physiological abnormalities including vascular disorders, hormonal imbalance, and neurological dysfunction; as well as adverse drug reactions and unhealthy lifestyles. These factors, alone or in combination, contribute to the rising incidence of ED in younger cohorts [4, 5]. Long-term administration of antidepressants can induce sexual dysfunction and thereby exacerbate patients' physical and psychological burden [6]. Paroxetine (PAR), a selective serotonin reuptake inhibitor (SSRI), is a well-recognized culprit, frequently causing erectile impairment and delayed ejaculation [7], making SSRI-induced ED a clinically relevant model for evaluating candidate therapeutics. In parallel, diabetes mellitus represents another major risk factor for ED, with hyperglycemia-induced endothelial injury, oxidative stress, and fibrosis leading to diabetic mellitus-associated ED (DMED) [8], a subtype notoriously refractory to conventional therapy. Given these challenges, the development of safer, more effective, and long-term-applicable therapeutic agents for ED is of considerable clinical importance.

Phosphodiesterase type 5 (PDE5), the main PDE subfamily in the smooth muscle of the penile corpus cavernosum, specifically hydrolyzes Cyclic guanosine monophosphate (cGMP) to control penile erection. By increasing cGMP concentration, PDE5 inhibitors (PDE5Is) encourage vasodilation, support smooth muscle relaxation, and as a result, mitigate ED symptoms [9]. Currently, PDE5Is are still the preferred

option for ED patients, providing considerable enhancement of erectile function in the early stages [10]. While existing PDE5Is, such as sildenafil (Sil), tadalafil, vardenafil, and avanafil, are associated with side effects such as heartburn, headache, facial flushing, and allergic reactions. Moreover, these agents can potentially lead to hepatic and renal impairment. Additionally, limitations such as inadequate selectivity, poor aqueous solubility, relatively short half-life, and therapeutic resistance in certain patient subsets further constrain their clinical utility [11, 12]. There is still a challenge in developing a PDE5 inhibitor that is highly effective, has few side effects, and is suitable for long-term administration.

With potential advantages in safety and affordability, Chinese herbal medicine is an attractive option for the development of PDE5 inhibitors that are both effective and well-tolerated [13–16]. Thus, we established an in-house extract library of 1,200 traditional Chinese herbal medicines, contained with southern herbs, northern herbs and Li ethnic herb, and a high-through put PDE5 inhibition activity screening were carried out. *Wendlandia uvariifolia* subsp. *chinensis* (Merr.) Cowan (*W. u. chinensis*), a perennial woody plant of the *Wendlandia* genus in the *Rubiaceae* family, is primarily distributed across the subtropical regions of Guangdong, Guangxi, and Hainan provinces in China, and demonstrated notable inhibitory activity against PDE5. It typically grows in mountainous or hilly areas, thriving in warm and humid environments, and is characterized by glossy lanceolate leaves and dense clusters of small white flowers. Despite its wide distribution, modern pharmacological research on this species remains scarce. In Li ethnic medicine, however, *W. u. chinensis* holds notable therapeutic significance, having been traditionally prescribed by Li physicians in Qiongzong Li and Miao Autonomous County, Hainan Province, particularly for the treatment of herpes zoster. This ethnomedicinal use aligns with the broader pharmacological profile observed in certain members of the *Rubiaceae* family, which have been reported to possess heat-clearing, blood-cooling, and hemostatic properties [17, 18]. Nevertheless, *W. u. chinensis* has not been systematically investigated, especially in relation to ED, for which no prior studies have been documented. Given the increasing interest in exploring traditional medicinal plants as novel therapeutic resources, this study sought to comprehensively assess the PDE5 inhibitory potential of *W. u. chinensis*, characterize its bioactive constituents, and elucidate its efficacy and underlying mechanisms in validated ED animal models.

Material and methods

Plant material, Chemicals and reagents

The leaves of *W. u. chinensis* were collected from Qiongzong Li and Miao Autonomous County, Hainan Province, China (19.04° N, 109.78° E). The species was authenticated by Kunming Institute of Botany, Chinese Academy of Sciences. The total DNA was extracted from the leaf samples of branch segments at the bud stage, followed by PCR amplification of the ITS sequence fragment and bidirectional sequencing (Supporting Information S1). Thus, this plant was identified as *W. u. chinensis*.

PAR was obtained from Macklin Biochemical Technology Co., Ltd. (Shanghai, China, Cat. No. B873253). Sil (Cat. No. R286565), ethanol (Cat. No. A040901), petroleum ether (Cat. No. D070281), and ethyl

acetate (Cat. No. A022002) were purchased from Adamas Reagent Co., Ltd. (Shanghai, China). Dimethyl sulfoxide (DMSO, Cat. No. G75927B) and Tween 20 (Cat. No. G89190B) were purchased from Titan Scientific Co., Ltd. (Shanghai, China). PEG 300 (Cat. No. CP8187) was purchased from Yuanye Bio-Technology Co., Ltd. (Shanghai, China). All other chemicals and reagents were of analytical grade.

Preparation of *W. u. chinensis* fractions

Fresh leaves were washed, air-dried, and pulverized into fine powder. 5 kg of the powdered leaves were extracted with 75% ethanol (3×10 L) at room temperature for 24 h, followed by filtration. The filtrate was concentrated under vacuum to obtain the *W. u. chinensis*-EtOH (EtOH, crude extract), which was then suspended in distilled water (3 L) and successively partitioned with petroleum ether (3×3 L), and ethyl acetate (3×3 L) to obtain fractions: *W. u. chinensis*-petroleum ether (PE), *W. u. chinensis*-ethyl acetate (EA) and *W. u. chinensis*-water (H_2O). Each fraction was evaporated under reduced pressure, lyophilized, and stored at -20 °C in the dark, providing a material basis for subsequent efficacy evaluations.

GC-MS analysis

Gas chromatography-mass spectrometry (GC-MS) analyses were conducted on an Agilent 8890-5977C system equipped with three core components: an automated thermal desorption unit, a programmable purge-and-trap concentrator, and a J&W DB-5ht capillary column ($30\text{ m} \times 0.25\text{ mm i.d.}$, $0.25\text{ }\mu\text{m}$ film thickness; Agilent Technologies). The analytical protocol employed hydro inert ion source as carrier gas with a constant flow rate of 1.0 mL/min . The temperature program consisted of three sequential phases: (i) initial isothermal hold at 40 °C for 2 min, (ii) dynamic ramp from 40 °C to 280 °C at 8 °C/min, (iii) final conditioning at 280 °C for 8 min. Mass spectrometric detection was performed in full-scan mode with electron impact ionization (70 eV), covering a mass range of m/z 29-600. The ion source and quadrupole analyzer were maintained at 230 °C and 150 °C respectively to ensure optimal ionization efficiency and mass resolution.

PDE5A inhibitory assay

The PDE5A inhibitory activity was determined using a liquid scintillation counting method, with $^3\text{H-cGMP}$ as the substrate and Sil as the positive control. The assay buffer contained 1.0 mM MgCl_2 (Bide Pharmatech Ltd., Shanghai, China; Cat. No. BD136984), 50 mM Tris (Beyotime Biotechnology Co., Ltd., Shanghai, China; Cat. No. ST761) 7.5 , 1.0 mM DTT (Yeasen Biotechnology Co., Ltd., Shanghai, China; Cat. No. BD20311ES10). PDE5A protein was diluted with the assay buffer and kept on ice before use. The reaction mixture ($60\text{ }\mu\text{L}$) contained assay buffer, diluted $^3\text{H-cGMP}$, and test samples at different concentrations. After the PDE5A protein were added in the mixture and incubation at room temperature for 15 min, the reaction was terminated by adding ZnSO_4 (0.2 M , $200\text{ }\mu\text{L}$, Bide Pharmatech Ltd., Shanghai, China; Cat. No. BD157254) and Ba(OH)_2 (0.2 M , $200\text{ }\mu\text{L}$, Energy Chemical, Shanghai,

China; Cat. No. A17300), followed by centrifugation at 14,000 rpm for 15 min. The supernatant (430 μ L) was mixed with scintillation liquid (2.5 mL), and radioactivity was measured using a liquid scintillation counter. The inhibition rate was calculated as follows: Inhibition Rate = $[1-(C_0-C_I)/(C_0-C_E)] \times 100\%$, where C_0 is the substrate control, C_E is the PDE5 enzyme control, and C_I is the inhibitor-treated group.

Molecular docking

Molecular docking was performed using AutoDock Tools 1.5.7. The 3D structures of the target proteins were retrieved from the PDB database, while the ligand structures were obtained from PubChem and optimized using Chem3D software with energy minimization. The proteins were prepared by removing water molecules, adding polar hydrogens, and assigning Kollman charges. The docking grid box was centered on the active site residues, with dimensions adjusted to encompass the entire binding pocket. Docking simulations were run with an exhaustiveness of 8, and the top-scoring poses (lowest binding energy, kcal/mol) were visualized and analyzed using PyMOL to evaluate hydrogen bonds, hydrophobic interactions, and binding affinities.

Cell viability

Human Umbilical Vein Endothelial Cells (HUVEC, Shanghai Meiyuan Biological Technology Co., Ltd., Cat. No. CC-Y1285) was cultured in DMEM (HyClone, Logan, Utah, USA, Cat. No. 10566-016) medium supplemented with 10% fetal bovine serum (Gibco, Thermo Fisher Scientific, Waltham, MA, USA; Cat. No. A5256701) and 1% penicillin/streptomycin (Wuhan Servicebio Technology Co., Ltd., Wuhan, China; Cat. No. G4014-100ML) at 37 °C and 5% CO₂. HUVEC were seeded in 96-well plate and treated with different *W. u. chinensis* extracts, including EtOH, PE, EA and H₂O for 24 hours. And then, 10 μ L of CCK8 (TargetMol, Shanghai, China; Cat. No. C0005) was added to the 96-well plate and incubated at 37 °C for 1 hour. The absorbance at 450 nm was measured using a spectrophotometer.

Cell treatment

The HUVEC cells were randomly divided into seven groups: the control group, the model group, the Sil group, the EtOH group, the PE group, the EA group, and the H₂O group. The control group was cultured under standard growth conditions. The model group was incubated in a medium containing 700 μ M H₂O₂ for 24 hours. The Sil group (50 μ M), EtOH group (100 μ g/mL), PE group (100 μ g/mL), EA group (100 μ g/mL), and H₂O (100 μ g/mL) group were pre-treated for 24 hours, followed by the addition of 700 μ M H₂O₂ to the medium and a further 24 hours of incubation. Nitric oxide (NO) levels in the culture medium supernatant were measured using the Griess assay.

Toxicity test

Ten male Sprague-Dawley (SD) rats (20 weeks old, 580-620g) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd.. Rats were randomly assigned to two groups: the Control group (n = 5) and the PE group (1 g/kg, n = 5). PE was administered intragastrically once daily for seven consecutive days. The extract was dissolved in saline containing 5% DMSO, 40% PEG300, and 5% Tween 20. Body weight was recorded before the first administration (day 0) and monitored daily throughout the treatment period (days 1–7).

Body weight change rate (%) = ((daily weight - baseline weight) / baseline weight) × 100%.

PAR-induced ED assay

All experimental protocols and procedures were conducted following “Guide for the Care and Use of Laboratory Animals” (National Institutes of Health Publication, revised 1996, No. 86-23, Bethesda, MD). Sixty specific pathogen-free (SPF) Wistar rats (220-240g, 30 males and 30 females) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. After seven days of acclimatization, the male rats were randomly divided into five groups (n=6 per group): Control, PAR (10 mg/kg, Shanghai Macklin Biochemical Technology Co., Ltd., Shanghai, China; Cat. No. B873253), PAR + Sil (5 mg/kg, Shanghai VastSail Biotechnology Co., Ltd., Shanghai, China; Cat. No. R286565), PAR + PE (100 mg/kg), and PAR + PE (200 mg/kg). All groups were administered the respective treatments intragastrically once daily for 21 days. PAR was dissolved in 5% DMSO, with the remaining volume consisting of saline. Sil and the test extract were dissolved in a saline solution containing 5% DMSO, 40% PEG300, and 5% Tween 20. After the sexual function assays, the rats were euthanized by cervical dislocation under anesthetized with Zoletil 50 (50 mg/kg, i.m., Virbac, France, Cat. No. H001), and their penis, epididymis, and blood were collected. Blood samples were taken from the abdominal aorta, centrifuged at 3,000 rpm for 15 minutes at 4 °C to separate the serum. For histological analysis, one-third of the penis was preserved in 4% paraformaldehyde, while the remaining tissue was stored at -80 °C for subsequent experiments.

Sexual behavior assay

After the last gavage was completed, the sexual behavior experiment was conducted at 7 p.m. The experimental method is summarized as follows: female rats were intramuscularly injected with 2 µg/kg estradiol benzoate injection (Shanghai Quanyu Biotechnology Co., Ltd., Shanghai, China; Cat. No. 24.337) 48 h before formal sexual behavior and 1 mg/kg progesterone injection (Jiangxi Bolai Pharmaceutical Co., Ltd., Jiangxi, China; Cat. No. 20240302) 4 h before to make the female rats in estrus. The sexual behavior experiment was carried out in a quiet room with dark red light. First, male rats were placed in transparent plastic boxes (30 × 15 × 15 cm). After waiting for 5 minutes, female rats were introduced. Researchers began observing and recording parameters related to the sexual behavior of male rats during the first 30 minutes, with the entire procedure being video-recorded using high-definition cameras for subsequent analysis. These include: mount latency (ML): the time from the start of being caged with the female rat to the first mounting; mount frequency (MF): the number of mountings on the female rat

from the first mounting to before ejaculation; ejaculation latency (EL): the time from the first insertion to ejaculation; ejaculation frequency (EF): the number of insertions into the vagina before ejaculation.

Hematoxylin and Eosin (H&E) staining and Masson staining

The penile corpus cavernosum tissues were fixed in 4% paraformaldehyde (Wuhan Servicebio Technology Co., Ltd., Wuhan, China; Cat. No. BL539A) for 24 hours, followed by paraffin embedding, and sectioning at 4 µm thickness using a microtome, dehydration through a graded ethanol series. The slices were then washed three times in distilled water (5 min each). Finally, according to the manufacturer's instructions, sections were stained with H&E solution (Wuhan Servicebio Technology Co., Ltd., Wuhan, China). Masson staining was conducted according to the manufacturer's instructions (Wuhan Servicebio Technology Co., Ltd., Wuhan, China). The ratio of smooth muscle to collagen in the rat cavernous tissue sections was analyzed using Image J.

Sperm analysis

The left epididymis of rats was placed in a centrifuge tube containing 1 mL of HEPES (Wuhan Servicebio Technology Co., Ltd., Wuhan, China; Cat. No. G4210) culture medium (placed at room temperature in advance). After cutting up the epididymis, incubate it at 37 °C, 5% CO₂ and saturated humidity for 20 min to allow the sperm to swim out fully. Sperm motility and count were assessed under a 40 × microscope, with motility categories as follows: I, Rapid progressive motility; II, Slow/sluggish progressive motility; III, Non-progressive motility; IV, Immotile. Sperm motility and Sperm motility rate were calculated as:

$$\text{sperm motility} = (I + II) / (I + II + III + IV) \times 100\%$$

$$\text{sperm survival} = (I + II + III) / (I + II + III + IV) \times 100\%$$

Detection of Hormones and Enzymes

According to the instructions of the enzyme-linked immunosorbent assay (ELISA) kit (Byabscience Biotechnology Co.,Ltd., Nanjing, China), We determined mouse serum sex hormone levels, including testosterone (Cat. No. BY-WJZF1224), luteinizing hormone (LH, Cat. No. BY-WJZF1591), follicle-stimulating hormone (FSH, Cat. No. BY-ER334438). The penile tissue homogenates were prepared according to the instructions of the kit for Adenosine deaminase (ADA, Cat. No. BY-ER337173) detection. The ADA content of corpus cavernosum was measured according to manufacturer's recommendation (Byabscience Biotechnology Co.,Ltd., Nanjing, China).

Detection of NO, cGMP and PDE5

First, blood samples were collected from the abdominal aorta and centrifuged at 3000 rpm for 15 minutes to separate serum. According to the manufacturer's instructions, use the Griess reagent to determine the content of NO in the serum (Beyotime Biotechnology Co., Ltd., Shanghai, China; Cat. No. S0021S). The content of cGMP in the penis were determined by ELISA kit (Byabscience Biotechnology Co., Ltd., Nanjing, China; Cat. No. BY-WJZF0408). Cut the penile tissue into pieces smaller than 0.5 mm³. Homogenize the tissue using a magnetic bead homogenizer and centrifuge for 30 minutes (4°C, 12,000 g). Collect the supernatant. The content of PDE5 in the penile homogenate were determined by ELISA kit (Byabscience Biotechnology Co., Ltd., Nanjing, China; Cat. No. BY-ER337483).

Immunohistochemical (IHC) analysis

Following a standard protocol, IHC staining was carried out as previously reported [19]. Penile tissues were obtained to prepare 4 µm-thick paraffin sections. After deparaffinization, antigen recovery and endogenous peroxidase activity blocked, the sections were incubated with primary antibody against phosphorylated cyclic AMP response element-binding protein (p-CREB) (1:800, Absin Biotechnology Co., Ltd., Shanghai, China; Cat. No. abs154911) at 4 °C overnight. A secondary antibody (Rabbit IgG, Yeasen Biotechnology Co., Ltd., Shanghai, China; Cat. No. 33101ES60) was added dropwise to cover the tissue, followed by incubation at room temperature for 50 minutes. Subsequently, DAB staining was conducted, nuclear staining was performed, dehydration, and mounting. Finally, the tissue sections were photographed using a biological microscope.

DMED model

Fifty male Sprague-Dawley (SD) rats (4 weeks old, 160-180 g) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd.. After five days of acclimatization, rats were randomly assigned to two groups: the normal con group (n = 8) and the model group (n = 42). The model group received a high-fat, high-sugar diet for 4 weeks, followed by an intraperitoneal injection of streptozotocin (STZ, 35 mg/kg, Sigma-Aldrich, Saint Louis, Missouri, USA, Cat. No. 572201) to induce diabetes. After an additional 4 weeks on a high-fat diet, rats with fasting blood glucose (FBG) levels >11.1 mmol/L were considered diabetic. Blood glucose was detected at four time periods: 0 min before glucose loading (50% glucose solution, 2 g/kg), and 30 min, 60 min, and 120 min after loading. A line graph of time-blood glucose value was drawn to calculate the area under the curve. In week 11, an apomorphine (APO) test was performed. After weighing the animals, they were placed in transparent boxes respectively. The room light was dimmed, and the room was kept as quiet as possible. The animals were allowed to adapt to the environment for 10 min. After subcutaneous injection of APO (100 µg/kg, Aladdin Biochemical Technology Co., Ltd., Shanghai, China; Cat. No. R2866565), the rats were observed for 30 min. Licking the lower abdomen, glans congestion, exposure, retraction of the foreskin and penile swelling were counted as erection. Rats without erection and with a blood glucose level exceeding 7.2 mmol/L were considered DMED rats. Additionally, diabetic rats displayed characteristic symptoms of polydipsia,

polyphagia, and polyuria, further confirming the successful establishment of the DMED model. Among the 42 rats injected with STZ, 11 died post-injection, while the remaining 31 were subjected to an APO test. Of these, 20 rats failed to exhibit erectile responses, verifying successful DMED induction. 20 DMED rats were randomly divided into groups: model group, Sil group (10 mg/kg), the PE group (150 mg/kg), and the EtOH group (150 mg/kg). Treatments were administered once daily for 28 days. After the rats were anesthetized with isoflurane (4-5% is used for induction, 1-2% is used for maintenance, RWD Life Science Co., Ltd., Shenzhen, China; Cat. No. R510-22-10), erectile function was assessed by measuring the ratio of intracavernous pressure (ICP) and mean arterial pressure (MAP) with biological signal acquisition instrument (BL-420N, Techman, Chengdu, China). During the operation, midline incisions on the abdomen and lateral incisions on the neck were made. After the experiment, euthanasia was performed by cervical dislocation under anesthesia. Blood samples were collected from the abdominal aorta, centrifuged at 3,000 rpm for 15 minutes at 4 °C to separate the serum. For histological analysis, the penis was preserved in 4% paraformaldehyde. Histological analysis included H&E staining and Masson's trichrome staining. The DMED model animal experiment was conducted from November 3, 2023, to February 27, 2024.

Statistical analysis

All data were processed and analyzed using GraphPad Prism10.2. H&E, Masson staining and IHC results were analyzed by Image J software, with IHC data expressed as mean optical density. Statistical comparisons between groups were performed using Student's t-test or one-way analysis of variance (ANOVA), followed by post hoc multiple comparisons where applicable. Data are presented as mean $\pm$ standard error of the mean (SEM). A p-value of less than 0.05 was considered to indicate a statistically significant difference.

Results

PDE5 inhibitory activity of the extract of the *W. u. chinensis* and chemical characterization of the PE extract of the *W. u. chinensis*

Through screening our in-house library of 1,200 traditional Chinese medicinal plant extracts, *W. u. chinensis* was identified as a promising candidate with PDE5 inhibitory activity (Figure 1A). EtOH exhibited 79.4% inhibition at 2 μ g/mL, but showed limited activity at the lower concentration of 0.2 μ g/mL (2.5%, Figure 1A and Table 1). In contrast, PE maintained strong inhibitory activity, achieving a notable 40.6% inhibition at 0.2 μ g/mL, indicating the enrichment of active constituents in the PE fraction.

To identify the chemical constituents, PE was subjected to GC-MS analysis. As shown in the chromatogram (Figure 1B), twelve major peaks were identified and classified into three structural classes: terpenoids (compounds 1, 2, and 7), aromatic esters (compounds 4 and 5), and fatty

compounds (compounds 3, 6, and 8-12) (Figure 1C, Table 1 and Supporting Information Table S1). Among them, seven compounds (4-8, 10, and 11) were confirmed using authentic standards (Figure S1). Notably, one long-chain aliphatic terpenoid phytol (7) and one long-chain aliphatic acid linoleic acid (8) exhibited remarkable PDE5 inhibition with the ratio 87.2% and 68.1% at the concentration 2 $\mu\text{g/mL}$, respectively. Furthermore, the half maximal inhibitory concentration (IC_{50}) values of phytol and linoleic acid were characterized as of $2.5 \pm 0.1 \mu\text{mol/L}$ and $15.5 \pm 0.3 \mu\text{mol/L}$, respectively (Table 1).

[Insert Figure 1 near here]

[Insert Table 1 near here]

Molecular docking of phytol (7) and linoleic acid (8) with PDE5

Given the *in vitro* PDE5 inhibitory activities, phytol and linoleic acid were subjected to molecular docking analysis to elucidate their potential binding modes. Both compounds exhibited favorable binding free energies (phytol: -6.9 kcal/mol; linoleic acid: -6.0 kcal/mol), indicative of stable interactions within the catalytic pocket of PDE5 (PDB: 2H24) [20]. Phytol demonstrated superior binding stability relative to linoleic acid, consistent with its higher inhibitory potency, which can be attributed to its extensive hydrophobic contacts and additional Pi-alkyl interactions with key aromatic residues PHE786 and PHE820. In both cases, conventional hydrogen bonds were formed with the critical residue GLN817, further reinforcing ligand-protein stability. The docking poses revealed that phytol engages multiple stabilizing interactions, hydrophobic, Pi-alkyl , and hydrogen bonding, suggesting that it may serve as a novel PDE5 inhibitory scaffold (Figure 2A-B). Despite the notable activity of these individual constituents, the PE fraction exhibited substantially greater PDE5 inhibition, implying possible synergistic effects among its components. Consequently, subsequent *in vitro* and *in vivo* pharmacological evaluations focused on PE as the primary candidate for anti-ED efficacy assessment.

[Insert Figure 2 near here]

W. u. chinensis increase the contents of NO in HUVEC cells

NO is a crucial signaling molecule in the vascular system, acting as a vasodilator and protective factor against oxidative stress [21]. Importantly, NO plays an essential role in penile erection by inducing relaxation of cavernous smooth muscle and facilitating blood flow into the corpus cavernosum [22]. Furthermore, studies have shown that ED often occurs with a decrease in endothelial nitric oxide synthase (eNOS) activity, leading to a reduction in NO production [23]. CCK8 assay results revealed that cell viability remained high with *W. u. chinensis* extracts, indicating no apparent toxicity (Figure 3A-D). Based on these findings, a concentration of 100 $\mu\text{g/mL}$ was selected for subsequent experiments. To evaluate the protective effect of *W. u. chinensis*, an oxidative injury model in HUVEC cells was

established using H₂O₂ (700 µM). Notably, subsequent treatments with various fractions of *W. u. chinensis* effectively restored NO levels, among which the PE demonstrated the most pronounced recovery, significantly elevating NO content (Figure 3E), consistent with the obtained PDE5 inhibitory activity.

[Insert Figure 3 near here]

Effects of *W. u. chinensis*-PE on sexual function in PAR-induced ED rats

To validate the *in vivo* therapeutic potential of PE, we employed a well-established model of ED induced by chronic PAR, known to cause sexual side effects (Figure 4A). Based on preliminary dose-escalation studies and prior tolerability evaluations, two doses of PE, 100 and 200 mg/kg, were selected to assess dose-response relationships while ensuring safety and bioactivity. Throughout the experimental period, all groups exhibited a steady increase in body weight, with no statistically significant differences observed among groups, suggesting that PE is well tolerated and does not induce systemic toxicity (Figure 4B). The results of the acute toxicity test were also the same, with no significant differences. (Figure S2). The sexual behavior index is one of the most intuitive and important indicators for evaluating the sexual ability of experimental animals. To evaluate sexual behavior, rats were placed with sexually receptive females and observed for key behavioral patterns, including immobility, mounting, intromission, and ejaculation (Figure 4C). Behavioral scoring showed that PAR-treated rats exhibited significant sexual dysfunction, as evidenced by prolonged ML and EL, as well as reduced MF and ejaculation EF. Notably, administration of either Sil (5 mg/kg) or PE at both 100 and 200 mg/kg significantly ameliorated these impairments. (Figure 4D). To further evaluate erectile tissue remodeling, Masson staining was performed to quantify smooth muscle and collagen content in corpus cavernosum tissues. PAR treatment led to a pronounced reduction in the smooth muscle-to-collagen ratio, a hallmark of fibrotic degeneration associated with ED. Both Sil and PE treatments significantly restored this ratio, with histological architecture resembling that of the control group (Figure 4E-F).

[Insert Figure 4 near here]

Effects of *W. u. chinensis*-PE on hormones regulation and sperm parameters in PAR-induced ED rats

Sex hormone homeostasis is integral to male reproductive health. PAR administration significantly suppressed serum levels of LH and FSH, along with a moderate decline in testosterone (Figure 5A-C), reflecting disruption of the hypothalamic-pituitary-gonadal axis. Treatments with Sil (5 mg/kg) and PE (100 and 200 mg/kg) restored these hormone levels, demonstrating normalization of endocrine function. In the PAR group, an increase in ADA levels was observed. This enzyme is not only a marker of

inflammation and oxidative stress, but also plays a regulatory role in vascular function [24]. Dysregulation of ADA activity has been implicated in the pathogenesis of erectile dysfunction [25]. Both doses of PE significantly reduced ADA levels, suggesting superior anti-inflammatory, antioxidant and ability to maintain penile erection properties (Figure 5D). Sperm quality, encompassing parameters such as count, motility, and viability, is a fundamental determinant of male fertility and reproductive success. In the PAR-treated group, all three indices were markedly reduced, reflecting severe impairment of spermatogenic function (Figure 5E-G). Specifically, sperm count exhibited a pronounced decline, accompanied by significant reductions in both motility and survival rate, indicating that chronic paroxetine exposure disrupts not only sperm production but also functional competence. Treatments with Sil (5 mg/kg) and PE (100 and 200 mg/kg) significantly ameliorated these deficits. Microscopic examination further confirmed these findings, with representative images illustrating a higher proportion of morphologically intact and motile sperm in the PE-treated groups compared with the PAR group (Figure 5H), where immotile or morphologically abnormal sperm predominated.

[Insert Figure 5 near here]

Effects of *W. u. chinensis*-PE on the NO/cGMP signaling pathway in PAR-induced ED rats

Penile erection is mediated by the NO/cGMP signaling cascade, which induces smooth muscle relaxation and vasodilation. PAR-treated rats exhibited a significant reduction in both NO and cGMP levels in penile tissue (Figure 6A-B), indicating impaired signaling. Treatments with PE (100 and 200 mg/kg) significantly increased NO and cGMP concentrations, partially restoring erectile signaling. In parallel, elevated expression of PDE5 in the PAR group was suppressed by PE, consistent with restored NO/cGMP signaling (Figure 6C). IHC analysis further demonstrated that p-CREB, a downstream transcription factor regulated by cGMP, was downregulated in the model group but significantly upregulated by Sil and PE treatments (Figure 6D-E).

Collectively, the *in vivo* findings indicate that PE exhibited significant therapeutic effects against ED *in vivo*.

[Insert Figure 6 near here]

Pharmacological effects of *W. u. chinensis* in DMED rats

DMED presents a greater clinical challenge, occurring earlier and with increased severity compared to other forms of ED. To further assess therapeutic efficacy, a DMED rat model was established and erectile function was evaluated by measuring the ratio of intracavernosal pressure (ICP) to mean arterial pressure (MAP) during cavernous nerve stimulation (Figure 7A). PE administration increased the ICP/MAP ratio compared with the model group, consistent with the protective effect observed in the PAR

model (Figure 7B and 7E). Compared with the control group, the testosterone level and sperm count of rats in the model group were significantly decreased. After the intervention with sildenafil ethanol and PE, the serum testosterone level and sperm count of rats showed a certain upward trend (Figure 7C-D). H&E staining demonstrated that PE markedly increased penile cross-sectional area and circumference in DMED rats (Figure 7F). Moreover, Histological analyses further confirmed that PE improved corpus cavernosum structure, increased smooth muscle content, and reduced fibrosis (Figure 7G). Collectively, these findings confirm that PE exerts robust protective and restorative effects on erectile function in both PAR-induced and DMED models.

[Insert Figure 7 near here]

Discussion

This study provides the first systematic pharmacological evaluation of *W. u. chinensis* as a PDE5 inhibitor with significant therapeutic effects against ED. Historically, this plant has been valued in Li ethnic medicine of Hainan Province for the treatment of herpes zoster, but its pharmacological profile has remained largely unexplored. Here, we not only confirmed the PDE5 inhibitory activity of PE but also identified phytol as one of its bioactive constituents. Phytol, known for its anti-inflammatory and antioxidant effects [17, 18], exhibited potent PDE5 inhibitory activity ($IC_{50} = 2.5 \pm 0.1 \mu\text{mol/L}$) in our assays. This finding expands the chemical diversity of PDE5 inhibitors beyond traditional heterocyclic scaffolds and provides a novel structural lead for future medicinal chemistry optimization.

The NO/cGMP signaling axis is a central mediator of penile erection. PDE5 inhibitors such as Sil, tadalafil, vardenafil, and avanafil exert their effects by preventing cGMP degradation, thereby sustaining smooth muscle relaxation and promoting penile blood flow [11, 26]. However, clinical use of current PDE5 inhibitors is often limited by systemic side effects, low target selectivity, and reduced responsiveness in comorbid conditions such as diabetes [12, 27]. In our work, PE administration significantly elevated NO and cGMP levels, restored CREB expression, and reduced ADA activity in penile tissues, collectively facilitating smooth muscle relaxation and erectile function. These biochemical effects parallel those observed with Sil, suggesting that PE could serve as a promising natural alternative with a potentially improved safety profile. Additionally, PE demonstrated good biosafety in acute toxicity tests in rats, although further studies are required to evaluate its potential side effects similar to those seen with current PDE5 inhibitors.

Importantly, PE showed consistent efficacy in two mechanistically distinct ED models. In the PAR-induced ED model, which simulates SSRI-associated sexual dysfunction, PE treatment markedly improved sexual behavior parameters, normalized serum testosterone, LH, and FSH levels, and attenuated oxidative stress and inflammatory responses in erectile tissues. Histological analysis confirmed restoration of smooth muscle architecture and suppression of fibrosis, indicating preservation of erectile tissue integrity. Given the high prevalence of SSRI-induced ED and the paucity of effective treatments, PE's profile supports its potential as an adjunctive therapy in this setting [28, 29]. Similarly, in

the diabetic ED model, PE significantly improved erectile hemodynamics, as shown by increased ICP/MAP ratios upon cavernous nerve stimulation. Histopathological evaluation revealed enhanced smooth muscle content and reduced collagen deposition, further supporting its protective role in maintaining erectile tissue structure under metabolic stress.

Despite these promising findings, several limitations remain. First, while phytol exhibits potent activity as a single compound, further structural optimization is necessary to enhance its potency and pharmacokinetic properties. Future research should also focus on optimizing the extraction and purification processes for PE, refining formulation strategies, and conducting comprehensive safety and pharmacokinetic evaluations. These efforts will be essential in advancing PE as a viable therapeutic option and contribute to the modernization and industrial utilization of traditional medicinal resources from the Li ethnic group.

Conclusions

This study is the first to report *W. u. chinensis* as a natural source of PDE5 inhibitors and to identify phytol as a previously unrecognized structural scaffold with potent PDE5 inhibitory activity. PE demonstrated low cytotoxicity *in vitro*, enhanced NO production, and produced robust therapeutic effects in both PAR-induced and diabetic ED rat models. These benefits were reflected in improved erectile function, normalization of reproductive hormone profiles, and mitigation of oxidative and inflammatory stress. Collectively, these findings position PE as a promising botanical candidate for ED therapy, offering a novel chemical scaffold, validated preclinical efficacy, and strong potential for advancement toward standardized drug development.

Abbreviations

ADA, adenosine deaminase; ALB, albumin; C-T-P, compound-target-pathway; CREB, cyclic AMP response element-binding protein; cGMP, cyclic guanosine monophosphate; DMSO, dimethyl sulfoxide; DMED, diabetes mellitus-associated erectile dysfunction; ED, erectile dysfunction; EtOH, *Wendlandia uvariifolia* subsp. *chinensis* (Merr.) Cowan ethanol extract; EA, *Wendlandia uvariifolia* subsp. *chinensis* (Merr.) Cowan ethyl acetate extract; EF, ejaculation frequency; ESR1, estrogen receptor; eNOS, endothelial nitric oxide synthase; ERK, extracellular regulated protein kinases; FSH, follicle-stimulating hormone; GC-MS, gas chromatography-mass spectrometry; H₂O, *Wendlandia uvariifolia* subsp. *chinensis* (Merr.) Cowan water extract; HUVEC, human umbilical vein endothelial cells; H&E, hematoxylin and eosin staining; IHC, immunohistochemical analysis; ICP, intracavernosal pressure; IL6, interleukin-6; JNK, c-Jun N-terminal kinase; LH, luteinizing hormone; ML, mount latency; MF, mount frequency; MAP, mean arterial pressure; MAPK1, mitogen-activated protein kinase 1; MAPK3, mitogen-activated protein kinase 3; MAPK14, mitogen-activated protein kinase 14; NOS, inducible nitric oxide synthase; PAR, paroxetine; PDE5, phosphodiesterase type 5; PPI, protein-protein interaction; PI3K-Akt, phosphoinositide 3'-kinase-protein kinase B; PE, *Wendlandia uvariifolia* subsp. *chinensis* (Merr.) Cowan petroleum ether extract; RELA, transcription factor p65; SSRI, selective serotonin reuptake inhibitor; Sil, sildenafil; SPF, specific

pathogen-free; TNF, tumor necrosis factor; TCM, traditional Chinese medicine; TCMSP, traditional Chinese medicine systems pharmacology; *W. u. chinensis*, *Wendlandia uvariifolia* subsp. *chinensis* (Merr.) Cowan.

Declarations

Author Contribution Statement

The authors declare that there is no conflict of interest. Baoli Li: Conceptualization, Methodology, Writing-review & editing, Supervision, Project administration. Qian Zhou: Writing-review & editing, Validation, Supervision, Project administration, Formal analysis, Conceptualization. Hai-Bin Luo: Conceptualization, Supervision, Resources, Project administration. Wenhui Gu: Investigation, Writing-original draft, Methodology, Validation, Software, Data curation. Lingyu Wu: Investigation, Methodology, Project administration. Zhiguo Yang: Visualization, Validation, Writing - Original Draft. Zhongbin Cheng: Methodology, Investigation. Wenwen Liu: Software, Formal analysis. Yi-You Huang: Resources, Formal analysis. Dan Liu: Formal analysis. Faliang An: Formal analysis, Investigation. Jian Li: Conceptualization, Supervision, Project administration.

Ethical Approval

All animal experimental protocols were reviewed and approved by the Animal Committee of Hainan University (IACUC approval numbers: HPIACUC2024069 for the PAR-induced ED model, HPIACUC2023060 for the DMED model, and HPIACUC2024023 for the acute toxicity test). All procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health, Bethesda, MD, USA; revised 1996, Publication No. 86 – 23). Every effort was made to minimize animal suffering and to reduce the number of animals used.

Competing Interests

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.

Acknowledgments

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Data Availability Statement

All data generated or analyzed during this study are included in this published article.

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Table

Table 1 Inhibition activity of the *W. u. chinensis* extracts and chemical constituents of PE against PDE5A

Fractions/Compd.	t_R /min	Formula	PDE5A inhibition (%)		IC ₅₀ (μmol/L)
			2 μg/mL	0.2 μg/mL	
EtOH	_c	_c	79.4	2.54	_c
PE	_c	_c	86.7	40.6	_c
EA	_c	_c	77.2	0.2	_c
H ₂ O	_c	_c	85.9	12.0	_c
1	23.258	C ₂₀ H ₃₈	7.2	_c	_c
2	23.333	C ₂₀ H ₄₀	<i>n.s.</i> ^b	_c	_c
3	23.574	C ₂₀ H ₃₈ O ₂	<i>n.s.</i> ^b	_c	_c
4	24.608	C ₁₈ H ₂₈ O ₃	12.5	_c	_c
5	24.827	C ₁₆ H ₂₂ O ₄	10.2	_c	_c
6	25.160	C ₁₈ H ₃₆ O ₂	20.4	_c	_c
Phytol (7)	26.603	C ₂₀ H ₄₀ O	87.2	20.2	2.5±0.1
Linoleic acid (8)	26.800	C ₁₈ H ₃₂ O ₂	68.1	10.1	15.5±0.3
9	26.878	C ₁₈ H ₃₀ O ₂	48.5	_c	_c
10	27.110	C ₁₈ H ₃₆ O ₂	30.8	_c	_c
11	27.211	C ₂₀ H ₃₄ O ₂	25.9	_c	_c
12	27.471	C ₂₀ H ₄₀ O ₂	11.7	_c	_c
Sil ^a	_c	_c	54.7 ^a	_c	_c

- a Positive control, at a concentration of 2 nmol/L;
- b No significant inhibition was observed under the tested conditions;
- c Value was not tested.

Figures

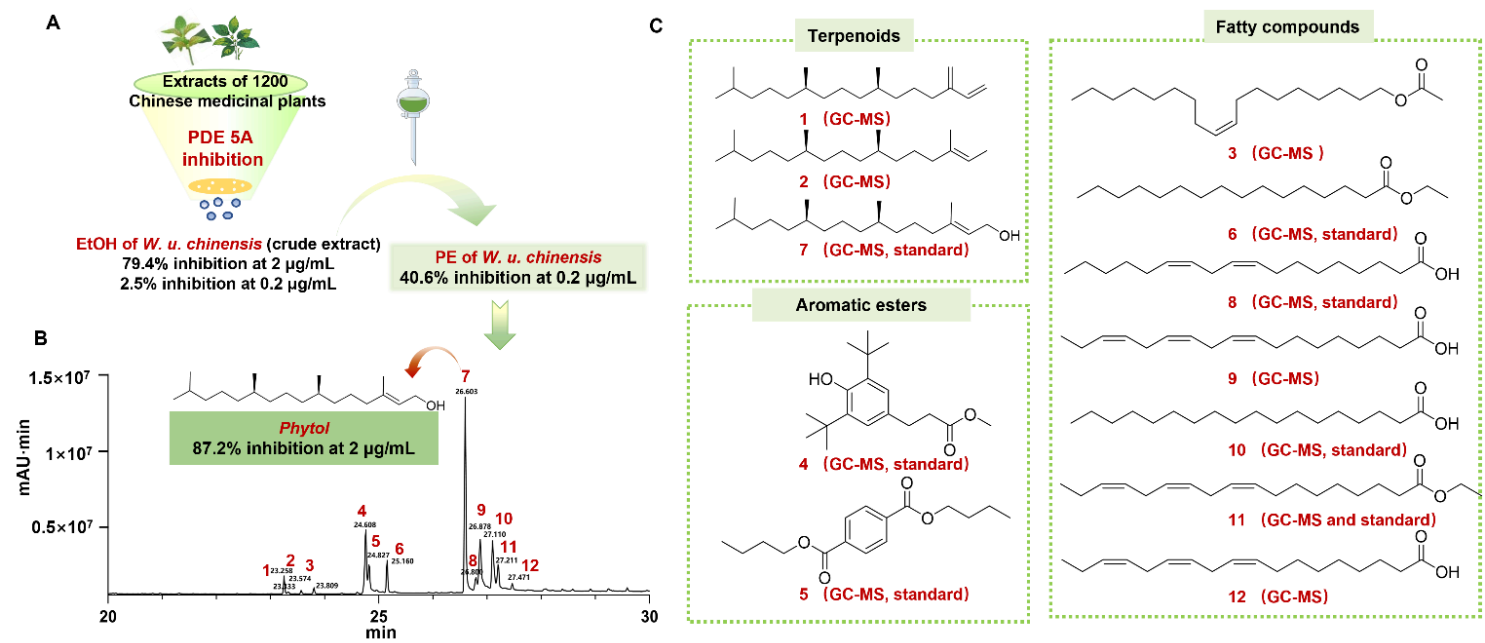


Figure 1

Phytochemical profiling and PDE5 inhibitory activity of PE. (A) Schematic workflow of bioactivity-guided fractionation and PDE5 inhibitory screening of *W. u. chinensis*. (B) GC-MS chromatogram of the PE fraction. Peak numbers correspond to compounds listed in Table 1. (C) Representative chemical structures of major compounds identified in the PE fraction, categorized as terpenoids, aromatic esters, and fatty compounds based on GC-MS and co-injection with standards.

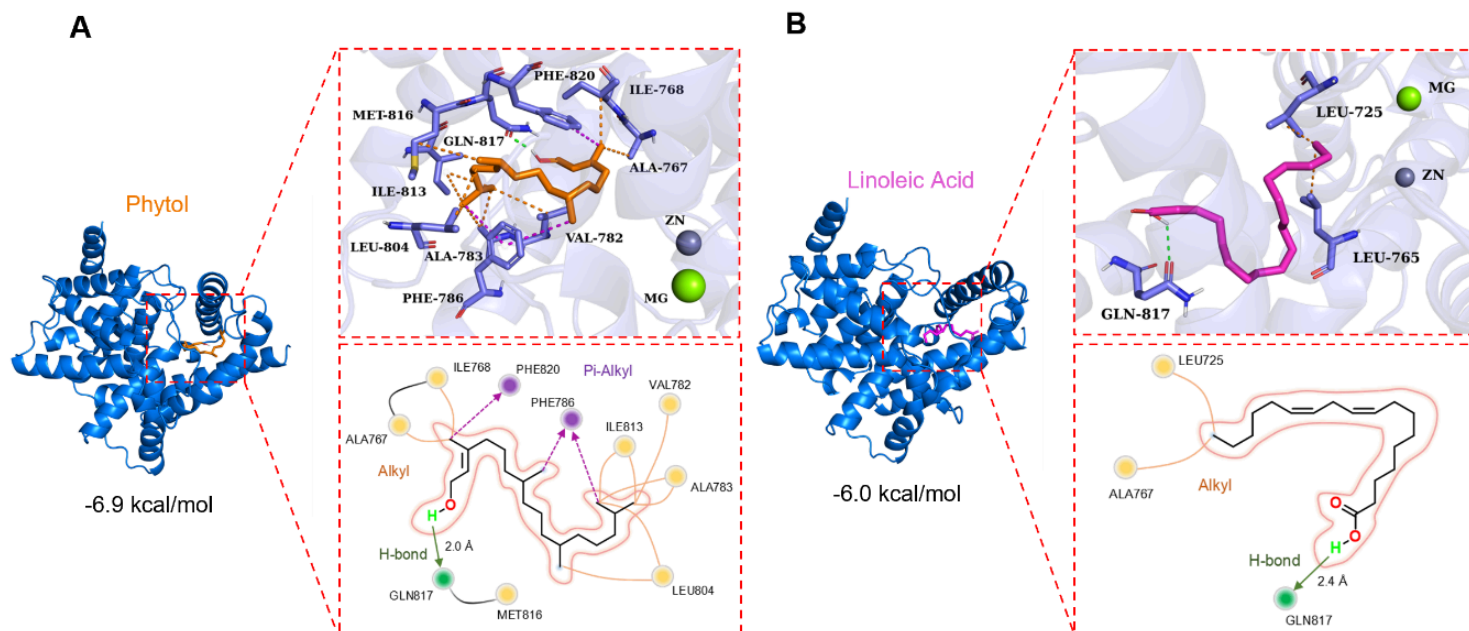


Figure 2

Molecular docking analysis of PDE5 with key bioactive compounds. (A) Binding conformation of phytol in the catalytic pocket of PDE5. (B) Binding conformation of linoleic acid with PDE5. (PDB: 2H24).

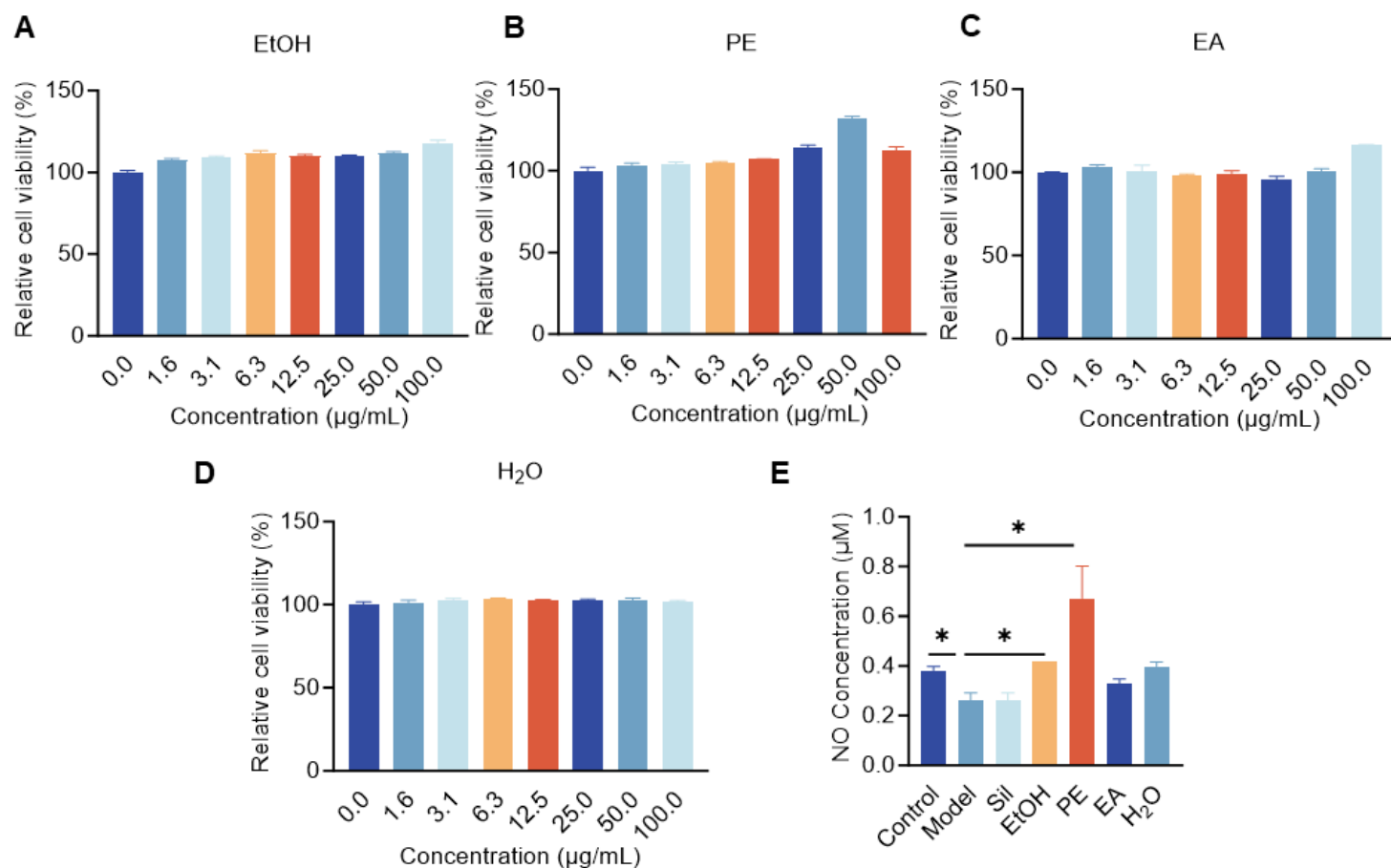


Figure 3

Protective effects of *W. u. chinensis* against H₂O₂-induced endothelial NO depletion in HUVECs. (A-D) Cell viability following treatment with increasing concentrations of the extracts of the *W. u. chinensis* (0-100 µg/mL) for 24 h. (E) Effects of *W. u. chinensis* (100 µg/mL) extracts on NO levels in HUVECs exposed to 700 µM H₂O₂. Data are presented as means ± SEM (n = 3). *p < 0.05, compared with model group.

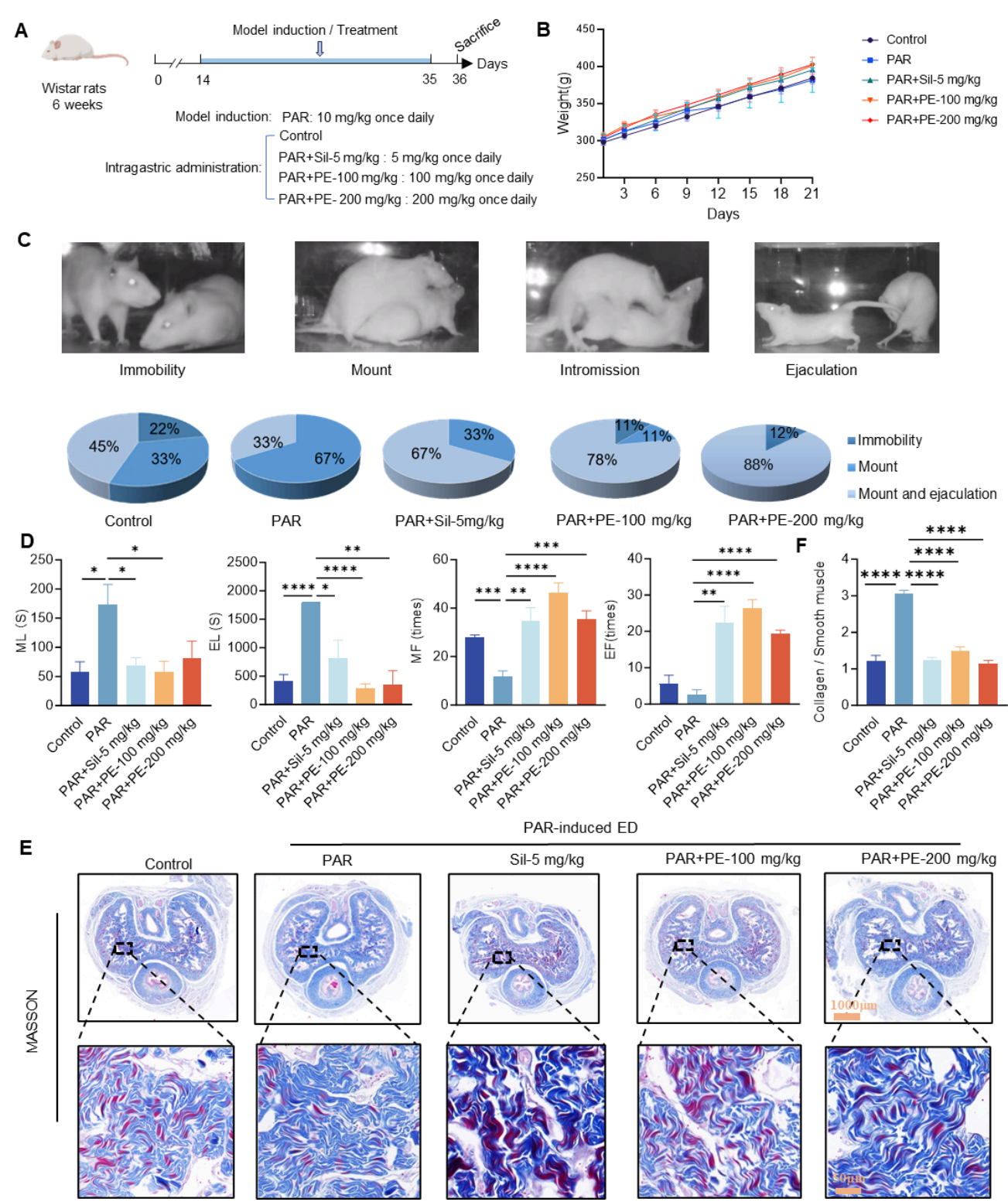


Figure 4

Evaluation of PE for PAR-induced ED rats. (A) Schematic representation of the PAR-induced ED rat model. (B) Changes in body weight over the experimental period. (C) Representative behavioral states during the sexual behavior test, including immobility, mounting, intromission, and ejaculation. (D) Quantification of key sexual behavior parameters: mount latency (ML), ejaculation latency (EL), mount frequency (MF), and ejaculation frequency (EF). (E) The ratio of collagen to smooth muscle contents in penile section of rats. (F) Representative images of Masson staining. n = 6 for B-D, n=4 for E-F independent experiments and data are presented as means ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.001, compared with PAR group.

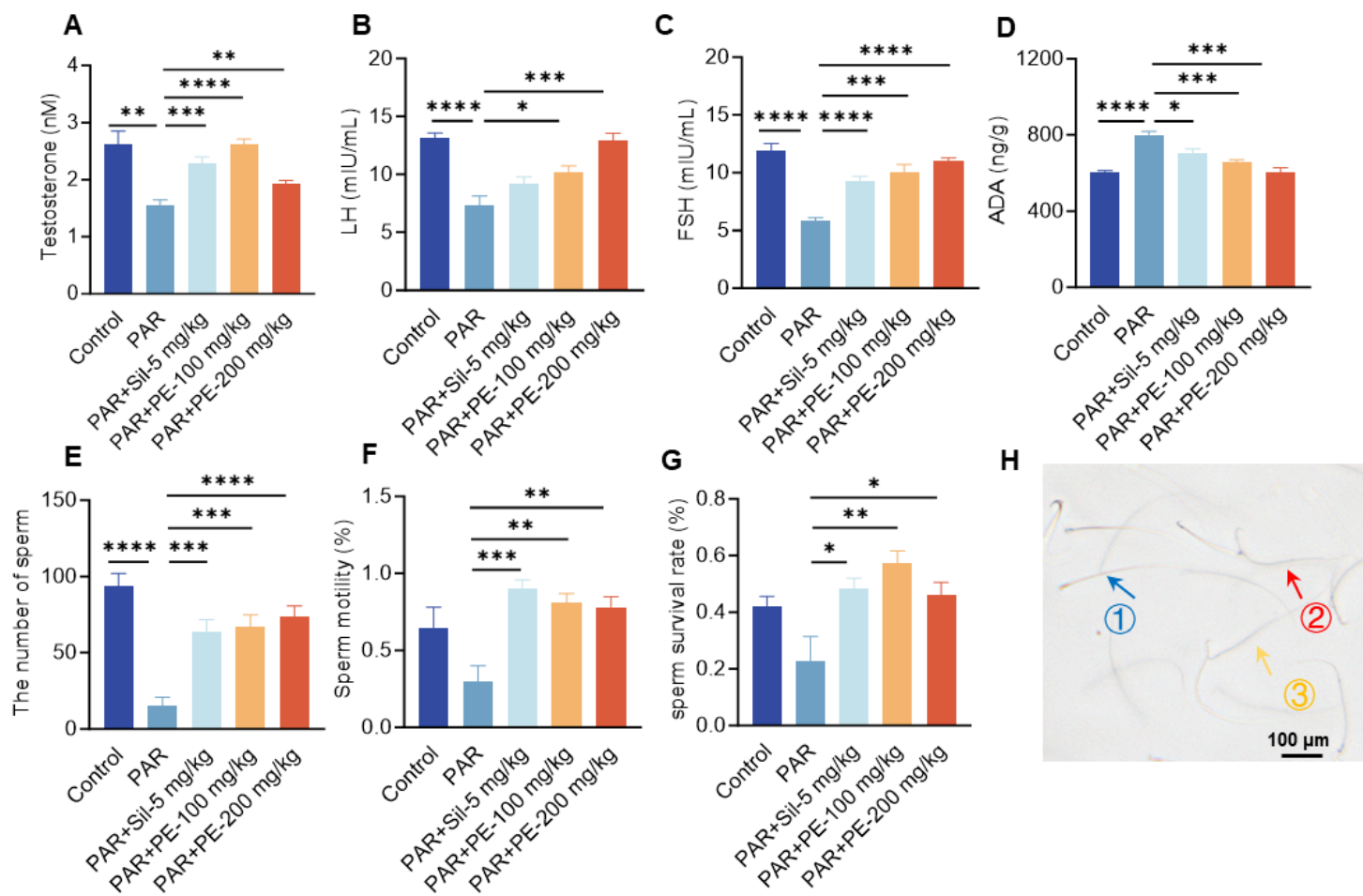


Figure 5

Effects on hormones, ADA, and sperm quality in PAR-induced ED rats. (A-D) Testosterone, LH, FSH, and ADA levels in penile tissue. (E-G) Sperm count, motility, and survival rate. (H) Representative microscopic image of sperm in the control group. □ Rapid progressive motility sperm; □ Slow/sluggish progressive motility sperm; □ Immotile sperm. n = 6 independent experiments and data are presented as means ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.001, compared with PAR group.

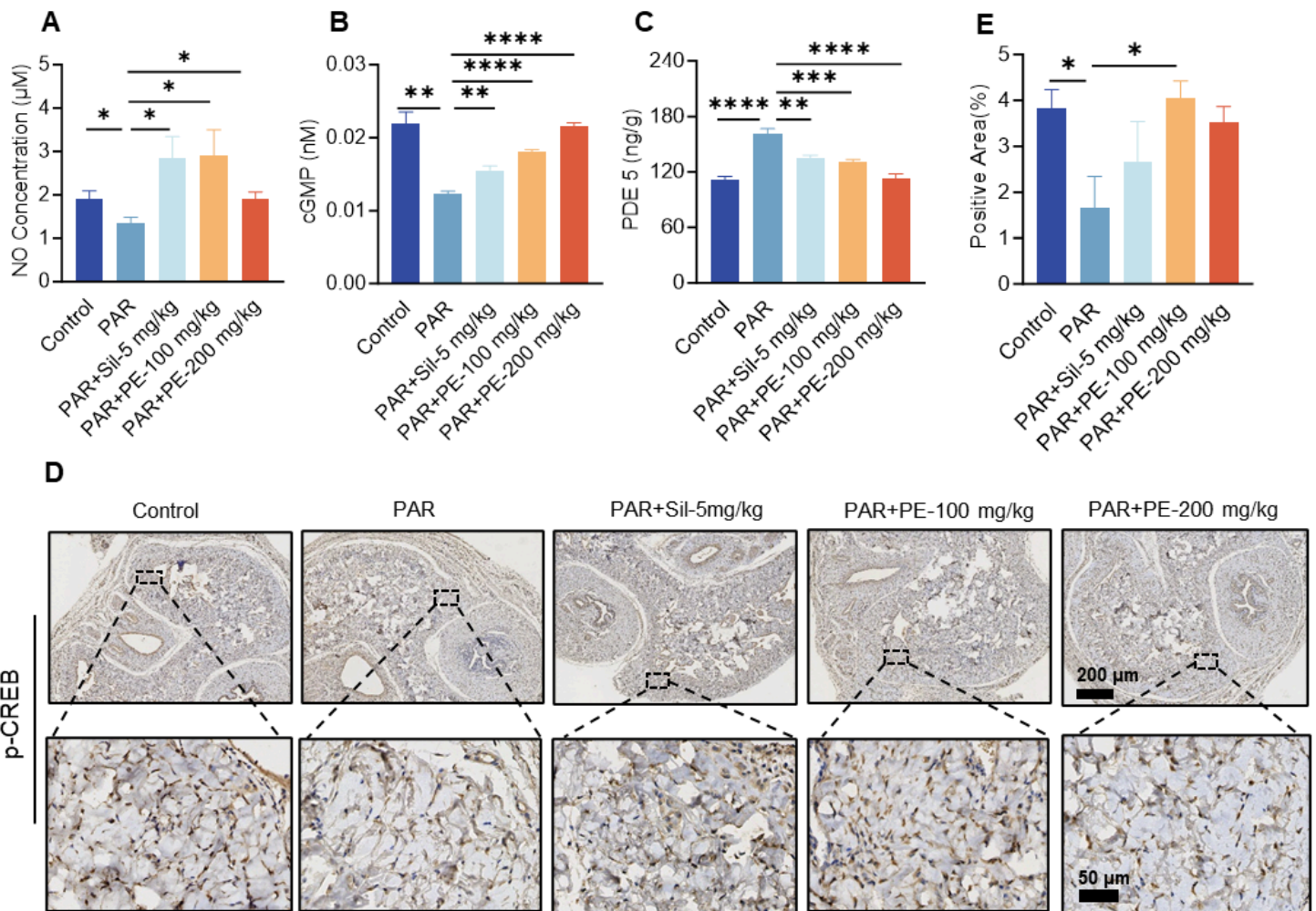


Figure 6

Effects of PE on the NO/cGMP pathway in PAR-induced ED rats. (A-B) NO and cGMP levels in penile tissue. (C) PDE5 and ADA expression. (D) IHC staining of p-CREB in penile tissue (scale bars: 400 μm for upper images, 50 μm for magnified insets). (E) Quantitative analysis of p-CREB expression. $n = 6$ independent experiments and data are presented as means $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$, compared with PAR group.

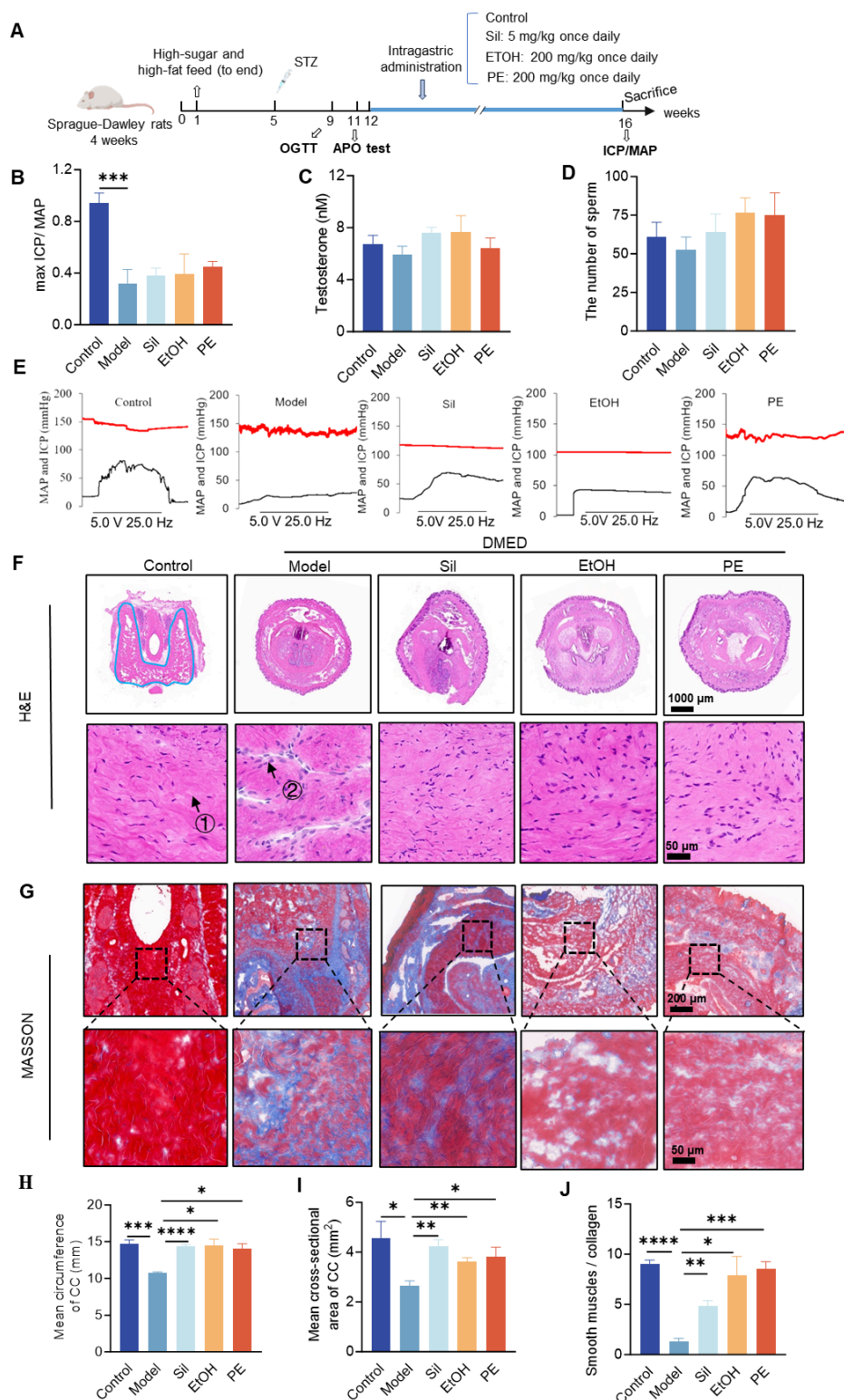


Figure 7

Metabolic status and erectile function evaluation in DMED rats. (A) Schemes of the DMED rat model. (B) Ratios of max ICP/MAP for each group. (C) The level of testosterone in rat serum. (D) The number of sperm. (E). Representative ICP traces following CN stimulation (stimulation parameters: 25 Hz; 5.0 V; 1 min). (F) Representative images of H&E staining, where blue indicates clustered corpora cavernosa, $\boxtimes$ The interstitial spaces are narrow; $\boxtimes$ Disorganized smooth muscle fibers with increased interstitial space.

(G) Representative Masson's staining images, showing smooth muscle (red) and collagen fibers (blue). (H-I) Quantification of circumference and cross-sectional area of the paired corpus cavernosum. (J) Quantitative analysis of fibrosis based on Masson's staining. n = 5 for B-E, n=4 for F-J, independent experiments and data are presented as means $\pm$ SEM. *0.01 < p < 0.05, **0.001 < p < 0.01, ***p < 0.001, and ****p < 0.001.

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