Astragaloside IV Protects Detrusor from Partial Bladder Outlet Obstruction-Induced Oxidative Stress by Activating Mitophagy through AMPK-ULK1 Pathway

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1. Introduction

Voiding dysfunction is a common health problem in the general population. As a vital component of the excretory system, the smooth muscle layer of the bladder wall (the detrusor smooth muscle) endows it with contractile function. With the extension of average life, the prevalence of underactive detrusor function, which stems from numerous etiologies such as benign prostatic hyperplasia (BPH) [1], is reported to be as high as 48% in adults [2]. The increased prostate volume compresses the urethra and leads to bladder outlet obstruction (BOO). Clinical assay suggests that the severity of BOO is highly associated with the oxidative stress level in the bladder wall [3]. Oxidative stress reportedly blocks the muscarinic receptor and reduces the detrusor contractility [4], as well as induces protein damage and activates apoptotic pathways [5], hence results in deterioration in bladder function. Discovering the pharmacological effect of antioxidant compound on detrusor may present protective strategies that enhances bladder contractility and alleviates voiding dysfunction.
2. Materials and Methods

2.1. Chemical Reagents. High purity AS-IV (≥98%, #A111275) and sodium carboxymethyl cellulose (CMC-Na) were purchased from the Aladdin Company. Compound C was purchased from the MedChemExpress (#HY-13418A). For animal studies, 1% CMC-Na was used as solubilizer to prepare 4% and 2% (g/ml) AS-IV suspension. 25 or 50 mg/kg body weight of AS-IV was garaged daily according to the experiment schedule. The control group and pBOO group were gavage by equal volume of 1% CMC-Na. For studies, AS-IV was prepared as a 10 mM compound C was added to cell culture for 10-second duration) were measured. Data were calculated as mean ± SEM.

2.2. Creation of pBOO Model. Female Sprague-Dawley rats (200-250 g), purchased from the Gepharmatech (Nanjing, China), had access to food and water ad libitum and were subjected to 12 hour light/dark cycles; they were anesthetized with isoflurane and underwent microsurgical creation of pBOO essentially as described [12]. Briefly, anesthetized rats underwent a lower abdominal incision to expose the bladder. A 22 g polypropylene catheter was placed adjacent to the bladder outlet, and a sterile surgical ligature was placed snugly around the bladder outlet and catheter. The catheter was then removed, and the abdominal incision was closed with sterile silk (muscular layer) and sterile surgical clamps (skin). Another group of rats received a sham-operation that consisted of surgically exposing the lower rat abdomen and briefly compressing the bladder outlet with sterile forceps.

All animal studies were performed according to guidelines established by the Research Animal Care Committee of Nanjing Medical University, China (Permit Number: IACUC-NJMU 1404075).

2.3. Cystometric Evaluation. Rats were anesthetized with urethane (1.5 g/kg). The bladder were exposed via a midline abdominal incision followed by removal of pubic symphysis and the medial portion of the pubic bones. A three-way valve-connected catheter (Becton, Dickinson and Company, Franklin Lakes, NJ, United States) was inserted into the top of bladder, the other two ends of the pipeline were connected to a peristaltic pump (Chonry Co., Nanjing, China) and a pressure transducer (Chengyi Co., Chengdu, China). Cystometric measurement was performed by infusing warm saline at a rate of 0.2 ml/min. The transduced pressure were recorded by a signal processing system (RM6240C; Chengyi Co.).

2.4. Tissue Harvest. After 4 weeks gavage, the rats were euthanized, and bladders for contractility testing were immediately immersed in ice-cold Krebs’ solution (120 mM NaCl, 5.9 mM KCl, 25 mM NaHCO₃, 1.2 mM Na₂HPO₄, 1.2 mM MgCl₂, 6H₂O, 2.5 mM CaCl₂, and 11.5 mM dextrose) with minimal handling. Tissues used for molecular analysis were immediately snap-frozen in liquid nitrogen, while tissues used for morphological staining were fixed with 4% paraformaldehyde.

2.5. Contractility Testing. Functional analysis of muscle strips was performed as described previously [13]. Briefly, bladder SM strips were prepared by removing the mucosa under stereomicroscope vision. Bladder tissue was attached to a force transducer (Grass Technologies), suspended in Krebs solution in an organ bath, and maintained at 37°C to equilibrate for 1 hour bubbled with a mixture of 95% O₂ and 5% CO₂ under a force of 0.5 grams. Contractile responses to carbachol (1 nM to 10 μM), 120 mM KCl, 10 μM α, β-methyl-ATP, and to EFS (1 to 64 Hz, 40 V, 0.5 ms pulse width, 10-second duration) were measured. Data were calculated as force (millinewtons) normalized by tissue cross-sectional area and are expressed as means ± SEM.

2.6. Primary Detrusor Cell Separation. Primary detrusor smooth muscle cells from each model group were separated as previously described [14]. Briefly, isolated bladder tissue from the sacrificed rats were cut to small pieces, the muscle layer were cleared from the mucosa, epithelium, and other attached tissue under a dissection microscope. Separated muscle tissue were cut into fine sand like particles and digested with 10 U/ml elastase (type III), 150 U/ml collagenase (type I), and 2.0 mg/ml bovine serum albumin (all purchased from Sigma). After incubation at 37°C for 90 min (mix every 5 min), the cells were filtered through a 100 μm cell strainer and centrifuged at 3000 rpm for 5 min. The obtained smooth muscle cells were resuspended in DMEM medium supplemented with 10% fetal bovine serum (HyClone) and seeded into indicated dish. Once the cells adhere to the wall, they were harvested, measured, or immune-stained.

2.7. Immunoblot. For isolation of protein in bladder tissue, a commercial kit was used following its protocol (Beyotime). The primary antibodies were as follows (dilution 1:1000): rabbit anti-Pink1 (#23274-1-AP, Proteintech), rabbit anti-
Prkn (#14060-1-AP, Proteintech), rabbit anti-LC3 (#14600-1-AP, Proteintech), rabbit anti- P62 (#18420-1-AP, Proteintech), mouse anti-Phospho-AMPKα (Thr12) (#50081, Cell Signaling), rabbit anti-AMPKα (#2532, Cell Signaling), rabbit anti-Phospho-Ulk1(Ser555) (#5869, Cell Signaling), and rabbit anti-Ulk1 (#8054, Cell Signaling). Secondary antibodies were anti-mouse IgG and anti-rabbit IgG (Jackson Laboratory, West Grove, PA, USA). All blots were repeated three times using tissue lysates merged from six rats’ samples in each group.

2.8. Immunostaining. Classic H&E and TUNEL staining were performed by the Servicebio Company (Wuhan, China). For immunohistochemistry staining, the bladder sections were immersed in citrate buffer (0.01 M, pH = 6.0) at 95°C for 5 min to perform heat-induced antigen retrieval. The primary antibodies used were rabbit anti-aSMA (#19245, Cell Signaling), rabbit anti-Col I (#72026, Cell Signaling), mouse anti-Col III (#sc-271249, Santa Cruz), mouse anti mACHR M2 (#sc-37312, Santa Cruz), and mouse anti mACHR M3 (#sc-518107, Santa Cruz). DAB kits (Gene Tech, Shanghai, China) were then used to show the immunohistochemical staining, and the nuclei were stained with haematoxylin. Images were obtained with a DP70 camera (Olympus, Tokyo, Japan).

2.9. Fluorescence and Confocal Imaging. Primary detrusor cells were plated on glass bottom imaging dishes, and stained with each dyes as indicated. Nuclei were marked with 500 nM Hoechst 33342; lysosomes were labeled with LysoTracker Green (100 nM); mitochondria were marked with 100 nM Tomm20 Red, according to the manufacturer’s instructions. Cells were imaged using Zeiss LSM710 confocal microscope.

2.10. Mitochondrial Respiratory Function Assay. A Seahorse XF96e Analyzer (Seahorse Bioscience—Agilent, Santa Clara, CA, USA) was used to measure the mitochondrial respiratory function. In this study, 5 × 10^4 primary detrusor cells were plated in each well, followed by indicated treatment. Before the assay, the growth medium was replaced with the Seahorse XF assay media (Eagle’s modified Dulbecco’s medium, w/o glucose, pH = 7.4; Agilent Seahorse) to which 1 mM pyruvate, 10 mM glucose, and 2 mM L-glutamine were supplemented. Cells were incubated without CO2 for 1 h and followed by measuring the baseline and stimulated oxygen consumption rate (OCR) (pmol/min). Three reagents were followed by measuring the baseline and stimulated oxygen consumption rate (OCR) (pmol/min). Three reagents were followed by measuring the baseline and stimulated oxygen consumption rate (OCR) (pmol/min). Three reagents were followed by measuring the baseline and stimulated oxygen consumption rate (OCR) (pmol/min). Three reagents were followed by measuring the baseline and stimulated oxygen consumption rate (OCR) (pmol/min).

2.11. Measure of Inflammatory Cytokines. For the detection of bladder tissue inflammatory cytokines, 200 μg bladder tissue were dissected, and the whole layer tissue was homogenized in ice-cold normal saline and followed by centrifuged at 1,500 ×g for 15 min at 4°C. The supernatant were analyzed by each corresponding kit, IL-1β: #H002; IL-6: #H007-1-1; IL-10: #H024; and TNFα: #H052-1. All four kits were purchased from the Nanjing Jiancheng Bioengineering Institute.

2.12. Measure of Oxidative Stress. For testing the accumulation of reactive oxygen species (ROS), malondialdehyde (MDA), the activities of glutathione peroxidase (GSH), and superoxide dismutase (SOD), primary detrusor cells were separated and homogenized in ice-cold normal saline and followed by centrifuged at 1,500 ×g for 15 min at 4°C. The supernatant were analyzed by each corresponding kit, ROS: #E004-1-1; SOD: #A001-3-2; GSH: #A005-1-2; and MDA: #A005-1-2. All four kits were purchased from the Nanjing Jiancheng Bioengineering Institute.

2.13. Real-Time PCR. Total RNA was extracted from bladder tissue with TRIzol (Invitrogen, #12183555), followed by precipitation with isopropanol and ethanol. Complimentary DNA (cDNA) was obtained by reverse transcription of 0.5 μg RNA using reverse transcription and cDNA synthesis kit (Takara, # 639506). Quantitative real-time PCR analysis was performed with SYBR Green Master Mix (Vazyme, Q111-02) and analyzed using a Roche Real-Time PCR System. Expression levels were determined using the relative standard curve method and normalized to the housekeeping gene Gapdh. Primer sequences for qPCR were list below. Gapdh: forward, TGTCATGGGAGTAGACGAG, reverse, CAGCCAGTTGGTGTAACGG. Drp1: forward, CAGGAATGTGTACGTTCCCTAA, reverse, CCTGAATTACTTGTCCGTGTA. Mfn1: forward, CCTACTGCTCTCCTTACCCCA, reverse, AGGGACGCCATCTCTGTTGA. Mfn2: forward, AGAAGTGGACCGGTATAC, reverse, CACTTGCGTGTACCCCTGA. Opal1: forward, TGGAAAAATGGTTCGAGGTCG, reverse, CATTCCGGTCCTTAGGAAAGG. Pink1: forward, TTCCTCGGCCGACTCGGTA, reverse, CTGCGTCTCCTGACGCC. Prkn: forward, GAGGTGATTCTGTCAAGGA, reverse, CCGGCAAAAATCAGCACGAG.

2.14. Statistical Analysis. In vivo assay was repeated two times, with the number of mice included in each group indicated. Significant differences were assessed by using one-way ANOVA followed by the Student-Newman-Keuls (SNK) test. P < 0.05 was considered statistically significant.

3. Result

3.1. Experiment Design and General Information. The pharmacological effect of purified AS-IV (Figure 1(a)) on pBOO-induced detrusor dysfunction was analyzed following experiment schedule (Figure 1(b)). Rats either subjected to pBOO (n = 6) or not (sham, n = 6) get a 3-week recovery period, then followed by gavages of control/AS-IV (25 mg/kg, low, n = 6)/AS-IV (50 mg/kg, high, n = 6) for another 4 weeks. Weekly measurement of the body weight showed a decreasing body mass in the pBOO group from the 5th week to the end point, while AS-IV treatment reversed this declination (Figure 1(c)). Increased urine pressure of pBOO model leads to bladder compensatory hypertrophy, as reflected by the raised ratio of bladder weight/body weight, AS-IV exposure dose-dependently reduced this bladder enlargement.
3.2. AS-IV Treatment Ameliorates Bladder Wall Remodeling in pBOO Rats. To further determine the effect of AS-IV on pBOO-induced bladder wall remodeling, we take histological and morphometric analysis by H&E, αSMA, Tunel, and Col I/III. In the group of pBOO, the bladder wall presented significant reduced muscle mass on H&E, compared to shams, while AS-IV treatment reversed this effect (Figure 2(a)). Compared with the precise staining in sham/low/high group, the αSMA staining in pBOO group was dispersed and blurry, which indicated the apoptosis of muscle cells and the leakage of antigens into the bladder tissue (Figure 2(b)). TUNEL assay showed clustered positive staining in epithelium and detrusor in pBOO model group, while after AS-IV exposure, apoptosis with in the smooth muscle is reduced (Figure 2(c)). Col I and Col III staining indicated high levels of fibrotic lesions in the bladders from pBOO group compared to sham rats, and AS-IV also reduced this effect dose-dependently (Figure 2(d)).

3.3. AS-IV Treatment Enhances the Contractile Force of Detrusor in pBOO Rats. Pharmacological evaluation of AS-IV on contractile activity of bladder tissue strips were then analyzed. Electrical stimulation (Figure 3(a)), cholinergic drug (carbachol) (Figure 3(b)), ATP (Figure 3(c)), and potassium channel activator (KCl) (Figure 3(d)) were used...
to induce contraction. Detrusors from obstructed mice displayed decreased contractility with all agonists compared with those from shams, while AS-IV treatment reversed this effect dose-dependently (Figures 3(a)–3(d)). The protein levels of M2 and M3 receptors were then analyzed, using bladder tissue lysis that contains the whole layer of bladder wall. The M3 level was upregulated in BOO group, while M2 was not changed (Figure 3(e)). Both muscarinic receptors were not affected by AS-IV treatment (Figure 3(e)).

3.4. AS-IV Treatment Reduces the Inflammation and Oxidative Stress in pBOO Rats. Using the bladder tissue lysate, the inflammatory cytokines IL-6 (Figure 4(a)), IL-1β (Figure 4(b)), IL-10 (Figure 4(c)), and TNFα (Figure 4(d)) were determined by ELISA. Consistent with morphology results, the cytokine levels upregulated in pBOO model group, and AS-IV reversed these inflammation responses (Figures 4(a)–4(d)).

AS-IV reportedly reduces oxidative stress [8–10], which plays central role in pBOO-induced detrusor underactivity and damage [4]. We then analyzed the oxidative stress level in primary detrusor cells separated from the bladder wall of the four groups. Compared with the sham group, the levels of cellular ROS (Figure 4(e)) and MDA (Figure 4(f)) were markedly increased, while the activity of antioxidative GSH (Figure 4(g)) and SOD (Figure 4(h)) were reduced in pBOO group. AS-IV dose-dependently reversed the aggravated oxidative damage induced by pBOO (Figures 4(e)–4(h)).

3.5. AS-IV Treatment Recovers Mitochondria Function and Activates Mitophagy in Primary Detrusor Cells. Oxidation of metabolic intermediates of the electron transport chain

Figure 2: AS-IV treatment ameliorates bladder wall remodeling in pBOO rats. (a–d) Bladder wall sections of four groups were analyzed by morphology staining as indicated in panel. General structure (H&E) (a), muscle tissue (αSMA) (b), apoptosis (Tunel, black arrow pointed to parts of positive nuclear) (c), and fibrosis (Col I and Col III) (d) staining indicated that pBOO induced bladder wall remodeling, and AS-IV treatment alleviated this progress.
within mitochondria generates superoxide, and uncontrolled release of ROS will further damage mitochondria function [15]. We then analyzed the mitochondria function in primary detrusor cells separated from model rats (Figure 5(a)). Results of the sea-horse assay showed that AS-IV treatment raised both the baseline and maximal oxygen consumption rate (OCR) in smooth muscle cells of pBOO rats (Figures 5(b) and 5(c)). However, the mitochondria content were not affected by either pBOO operation or by AS-IV treatment (Figure 5(d)).

Mitochondria are highly dynamic organelles, which biology function is delicately modulated through fission, fusion, and mitophagy [16]. Through qPCR assay, the mRNA level of key regulators that govern mitochondria dynamic are profiled. Mitochondria fission, as reflected by the dynamin-related protein 1 (Drp1), and mitochondria fusion, as reflected by the mitofusin1 (Mfn1), Mfn2, and optic atrophy 1 (OPA1), were not affected after AS-IV exposure, while the expression level of mitophagy gene Pten-induced putative kinase 1 (Pink1) and Parkin (Prkn) were significantly decreased in pBOO group and upregulated after AS-IV treatment (Figure 5(e)). Protein level of Pink1, Prkn, and autophagic flux, as represented by LC3II and P62, showed same trend after AS-IV treatment (Figure 5(f)). The fluorescent staining results revealed that compared to the cells from pBOO rats, AS-IV treatment significantly increased the costaining of mitochondrial and lysosome, which represent mitophagy (Figure 5(g)).

3.6. AS-IV Activates Mitophagy through an AMPK-Dependent Way. AS-IV reportedly enhances AMPK activity [17], a master regulator of autophagy/mitophagy. Once activated, AMPK phosphorylates unc-51-like kinase 1 (Ulk1) and starts a signal pathway that initiates autophagosome formation [18]. Using separated detrusor cells from normal rats, the underlying mechanisms of AS-IV on activating mitophagy were then examined. Western blots results suggested that protein level of Pink1, Prkn, and autophagic flux were upregulated after AS-IV treatment, while blocking AMPK with its inhibitor compound C completely reversed these effects (Figure 6(a)). Measurement of the cellular ROS accumulation (Figure 6(b)) and SOD activity
Underactive bladder (UAB)-related BOO were evident due to contractility in voiding dysfunction patients. Although bladder remodels and ultimately leading to a loss of detrusor function when the demand outstrips the adaptive capability, the bladder hypertrophy to overcome the increased outlet resistance is adaptive, involving a compensatory phase of smooth muscle hypertrophy. Following BOO, the bladder contracts against an obstructed outlet, including impaired mitochondria function. During detrusor contraction, the obstructed bladder should increase adenosine monophosphate (AMP)/adenosine triphosphate (ATP) ratio, together with a reduced activity of mitochondria function. The damaged mitochondria contribute produced energy and generates more peroxides; both of which weakens detrusor contractility. Interestingly, our data and previous study showed that even though the AMP/ATP ratio increased in BOO animals, the activities of AMPK, energy sensor that governs the homeostasis status of the cell, were blocked. This inconsistency could partly be due to the reduced phosphorylation of AMPK upstream kinase, such as Ca2+/calmodulin-dependent protein kinase (CaMKKβ) [28]. Further research into the acknowledged gaps in this area is needed in the future. Direct measurements of such residual volumes were not performed in this study. We cannot rule out the possibility of detrusor contractility as the bladder decompensates [22]. In the well-studied rodent model, the contractile forces in response to electrical field stimulation, carbachol, and KCl in 4-weeks BOO rats were 30.7–51.7% of those in control rats [23]. Similar results were observed in several mouse-based BOO studies [24, 25]. In our study, tissue samples were collected in 7 weeks BOO model, a more severe decompensating stage with detrusor underactivity. Using multiple morphology and ELISA method, our data suggested that AS-IV dose-dependently reduced bladder tissue apoptosis, fibrosis, and inflammation, as well as increased the contractile capacity. Though AS-IV showed protective effect in BOO model at the time point of 7 weeks, short-term study are still needed to fully delineate the contribution of AS-IV to the development of BOO induced bladder dysfunction at different time points.

Many factors contribute to the low detrusor contractility following BOO, including impaired mitochondria function. During detrusor contraction, the obstructed bladder should increase adenosine monophosphate (AMP)/adenosine triphosphate (ATP) ratio [26], together with a reduced activity of mitochondria function [27]. The damaged mitochondria contribute produced energy and generates more peroxides; both of which weakens detrusor contractility. Interestingly, our data and previous study showed that even though the AMP/ATP ratio increased in BOO animals, the activities of AMPK, energy sensor that governs the homeostasis status of the cell, were blocked. This inconsistency could partly be due to the reduced phosphorylation of AMPK upstream kinase, such as Ca2+/calmodulin-dependent protein kinase (CaMKKβ) [28].

**4. Discussion**

In the present study, we determined the protect effect of AS-IV on pBOO rats—an experimental model for detrusor dysfunction and bladder wall remodeling. Currently, no effective pharmacotherapy exists to restore detrusor contractility in voiding dysfunction patients. Although pharmacological treatments, such as alpha-blockers [19], for underactive bladder (UAB)-related BOO were effective, they showed no effect on detrusor contractility [20]. Parasympathomimetics can theoretically enhance detrusor contractility because they augment excitatory acetylcholine action between the synapses. However, muscarinic agonists or cholinesterase inhibitors have shown limited efficacy and adverse effects on restoration of detrusor contraction [21]. Hence, investigation of novel therapeutic strategies that is enhancing detrusor function is warranted. In our study, AS-IV enhanced the contractility of detrusor tissue, reduced the oxidative stress and activated mitophagy through an AMPK-ULK1-dependent way, and presented a promising pharmacological treatment.

Bladder wall remodeling, such as collagen deposition, fibrosis in submucosa, and bladder wall apoptosis, happens after pBOO. Theoretically, in conditions such as BPH or BOO, the bladder contracts against an obstructed outlet and went through a two-stage change. The initial response is adaptive, involving a compensatory phase of smooth muscle hypertrophy to overcome the increased outlet resistance. When the demand outstrips the adaptive capability, the bladder remodels and ultimately leading to a loss of detrusor contractility as the bladder decompensates [22]. In the well-studied rodent model, the contractile forces in response to electrical field stimulation, carbachol, and KCl in 4-weeks BOO rats were 30.7–51.7% of those in control rats [23]. Similar results were observed in several mouse-based BOO studies [24, 25]. In our study, tissue samples were collected in 7 weeks BOO model, a more severe decompensating stage with detrusor underactivity. Using multiple morphology staining and ELISA method, our data suggested that AS-IV dose-dependently reduced bladder tissue apoptosis, fibrosis, and inflammation, as well as increased the contractile capacity. Though AS-IV showed protective effect in BOO model at the time point of 7 weeks, short-term study are still needed to fully delineate the contribution of AS-IV to the development of BOO induced bladder dysfunction at different time points.

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Figure 5: AS-IV treatment recovers mitochondria function and activates mitophagy in primary detrusor cells. (a) Experiment schedule: SMCs were separated from detrusor of each model group via an enzyme-based method. Once the cells attached to the wall, they were used for the studies below. (b, c) Analysis of mitochondrial respiration by OCR analysis. (d) Analysis of mitochondrial DNA levels. (e) Expression profile of marker genes regulating mitochondria fission, fusion, and mitophagy, using qPCR assay. (f) Western blot testing the protein level of Pink1, Prkn, Lc3, and P62. (g) Fluorescent staining of mitochondria (Tomm20, red) and lysosome (Lyso-Tracker, green). Error bars show ±SEM. *P < 0.05, **P < 0.001, pBOO vs. sham; #P < 0.05, ## P < 0.01, ### P < 0.001, AS-IV-treated groups vs. pBOO. Error bars show ±SEM.
that a diverse postvoiding residual volume may contribute to the changed cystometric data. But consistent results were also observed in contractility testing using separated muscle strip in vitro, which was certainly not affected by the residual urine volume. Therefore, our data showed that AS IV enhanced the contractile capacity. However, the expression of muscarinic receptors in bladder wall were not changed after AS-IV treatment. It seems AS-IV enhanced contractility not by restoring the expression of muscarinic receptors, but by protecting the detrusor from oxidative stress and apoptosis. What is more, the AS-IV was systemic administrated by gavage in this study; its biofunction on the central nervous system cannot be ignored and should be studied in the future via patch clamp-based methods.

Through targeting multiple genes and downstream pathways such as AMPK [29], Hif-1α [30], and TLR4 [31], AS-IV demonstrated potential cardio-protective and immunological enhancement activities through experimentation in vitro and in vivo [32]. In this research, pBOO suppressed the AMPK-Ulk1 signaling, while AS-IV treatment reversed this effect. The AMPK-Ulk1 pathway plays central role in regulating mitophagy. The AMPK complex senses mitochondrial damages and activates Ser/Thr kinase ULK1, which represents the most upstream activator of the autophagic pathway [33]. Regarding mitophagy, ULK1 is phosphorylated at Ser555 by AMPK, which triggers ULK1 translocation to mitochondria and mitophagy [34, 35]. In our study, the phosphorylated AMPK/Ulk1 were downregulated in pBOO group, while AS-IV reversed this effect dose-dependently. Hence, the role of AS-IV in promoting mitophagy likely acts through the AMPK-Ulk1 pathway. Using separated primary detrusor SMCs, blocking AMPK reversed the antioxidant and mitophagy promoting effect of AS-IV; however, limited by the lack of in vitro model of pBOO, whether blocking the AMPK-Ulk1 pathway could reverse AS-IV’s effect on detrusor contractility is still unknown and needs further study.

In conclusion, our results suggest that AS-IV, key ingredient of Astragali Radix, is a detrusor protector that restore muscle contractility and ameliorates bladder wall remodeling in pBOO model, via activating mitophagy and restoring mitochondria function through AMPK-Ulk1 pathway.

Data Availability

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.
Authors’ Contributions

Xiaoyu Zhu is responsible for conceptualization, investigation, and writing—original draft; Fangze Tao for investigation, validation, and visualization; Li Zhang for formal analysis and investigation; Yajie Yu for investigation and writing—review and editing; Yanchao Xu for investigation and resources; Gang Yang for investigation and writing—review and editing; Zhifeng Wei for investigation and validation; Yidong Cheng for investigation and resources; Xuelai Yin for writing—review and editing and visualization; Xinyuan Zhang for resources; Wu Wei for resources, conceptualization, and writing—review and editing; and Anxi Wang for conceptualization, supervision, writing—original draft, and writing—review and editing. Xiaoyu Zhu, Fangze Tao, and Li Zhang contributed equally to this work.

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References


