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Capsaicin exerts anti-benign prostatic hyperplasia effects via inhibiting androgen receptor signaling pathway

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Abstract: Background: Benign prostatic hyperplasia (BPH) is a common condition in middle-aged and elderly men. Enlargement of the prostate causes lower urinary tract symptoms. Capsaicin is a phytochemical extracted from chili peppers and exerts many pharmacological actions, such as anti-tumor and anti-inflammatory effects. **Methods:** Our study investigated the effect of capsaicin *in vitro* and in a mouse model *in vivo*. A prostatic stromal myofibroblast cell line (WPMY-1) was co-incubated with testosterone (1 μM) and different concentrations of capsaicin (10–100 μM) for 24 and 48 h. Capsaicin (10–100 μM) significantly inhibited testosterone-treated WPMY-1 cell growth at 48 h by MTT assay. The testosterone propionate (7.5 mg/kg)-induced BPH mouse model was used to examine the anti-proliferative effect of capsaicin. Treatment with capsaicin (10 mg/kg) for 14 days significantly attenuated prostatic hyperplasia. Finasteride was used as a positive control. **Results:** Capsaicin significantly decreased prostate weight and prostate index (prostate/body weight ratio) in BPH mice. The expression of 5α-reductase type II, androgen receptor (AR) and prostate specific antigen (PSA) protein expression and PSA serum were all significantly reduced in capsaicin-treated BPH mice. In addition, capsaicin also activated transient receptor potential vanilloid 1 mediated apoptosis and autophagy in BPH mice. **Conclusion:** These results demonstrate multiple positive effects of capsaicin in controlling prostate growth and suggest its therapeutic potential in the treatment of BPH.

Introduction

Benign prostatic hyperplasia (BPH) is a common condition in men over 50 years of age and is accompanied by lower urinary tract symptoms (LUTS) (Foo, 2019; Lerner *et al.*, 2021). BPH is directly related to a high circulating level of testosterone (Cleutjens *et al.*, 1996). Testosterone and dihydrotestosterone (DHT) are very important in regulating prostatic growth and the progress of BPH. The enzyme 5α-reductase converts testosterone into DHT. DHT induces signal pathways and prostate growth by binding to AR, which has a higher binding affinity than testosterone in the prostate. Two medications mainly used for BPH are α-blockers and 5α-reductase type II inhibitors (Plochocki and King, 2022). α-blockers improve urinary flow rates and decrease smooth muscle tone. Finasteride significantly reduces prostate volume and improves symptoms (Füllhase and Schneider, 2016).

Regular consumption of chili peppers has been reported to prevent cardiovascular diseases and cancers (Sanati *et al.*, 2018). Capsaicin is a pungent ingredient extracted from chili peppers which has been used for treating many diseases, including pain, the common cold, and rheumatism (Srinivasan, 2016). Capsaicin has been investigated for anti-inflammatory, analgesic, anesthetic, detoxification, anti-obesity, and anti-cancer effects (Barkin, 2013; Zheng *et al.*, 2017; Qin *et al.*, 2019; Persson *et al.*, 2018; Chen *et al.*, 2021b). Previous studies have reported that capsaicin induces apoptosis and autophagy in prostate cancer cells (Sánchez *et al.*, 2007; Ramos-Torres *et al.*, 2016). Capsaicin induced apoptosis, generated ROS production, and downregulated androgen receptor (AR) and prostate specific antigen (PSA) in LNCaP cells (Mori *et al.*, 2006). Another study also showed restoration of miR-449a by capsaicin leading to inhibition of AR signaling (Zheng *et al.*, 2015).

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Apoptosis and autophagy regulate cell growth and death in a cooperative manner. Autophagy deactivation was observed in prostatic inflammation samples from patients with BPH and LUTS (de Nunzio *et al*., 2017; de Nunzio *et al*., 2021). Anti-apoptosis effects were increased in the BPH rat model (Choi *et al*., 2021). Many phytochemicals are able to suppress prostatic cell growth by inducing autophagy, apoptosis and are used in the treatment of prostate cancer and BPH (Sachan *et al*., 2018; Kang *et al*., 2021; Ashrafizadeh *et al*., 2022). We have found little research on the pharmacological mechanisms of action of capsaicin in relieving BPH. Whether capsaicin can inhibit prostate growth and downregulate AR signaling pathway activation in BPH remains unknown. Our study aimed to evaluate the effects of capsaicin on BPH *in vitro* and *in vivo* in an animal model.

Materials and Methods

Chemical and reagent

The capsaicin (98% purity; cat. no. BP0312) was obtained from Chengdu Biopurify Phytochemicals, Ltd. (Chengdu, China). Dulbecco's Modified Eagle Medium (DMEM) was supplemented with 10% fetal bovine serum (FBS), and both were obtained from Cellmax (Beijing, China). The antibiotics penicillin (100 U/mL)-streptomycin (100 μg/mL) were purchased from Beyotime Institute of Biotechnology (Haimen, China). Testosterone (cat. no. T102169) and testosterone propionate (cat. no. T101368) were purchased from Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). PSA ELISA kits (cat. no. JM-02752M1) were purchased from Jiangsu Jingmei Biotechnology Co., Ltd. (Yancheng, China). Anti-β-actin antibodies were obtained from MilliporeSigma (cat. no. A5441; Massachusetts, USA). Anti-beclin-1 (cat. no. AB3219), anti-microtubule-associated proteins 1A/1B light chain 3B (anti-LC3B; cat. no. CY5992), anti-AR (cat. no. CY5030), and anti-5α-reductase type II (cat. no. CY8576) antibodies were purchased from Abways Technology (Shanghai, China). Primary antibodies against PSA (cat. no. ab76113) were purchased from Abcam (Shanghai, China). Primary antibodies against poly ADP-ribose polymerase (PARP) (cat. no. 9542) and caspase-3 (cat. no. 14220) were purchased from Cell Signaling Technology (Shanghai, China). Anti-transient receptor potential vanilloid 1 (TRPV1; cat. no. DF10320) was obtained from Affinity Biosciences (Changzhou, China).

Cell line and cell culture

Human prostatic stromal myofibroblast cell line (WPMY-1) was purchased from Shanghai Fuheng Biotechnology Co., Ltd. (Shanghai, China). Cells were cultured in DMEM medium supplemented with 1% penicillin-streptomycin and 10% FBS at 37°C in a humidified atmosphere with 5% CO_2 in an incubator.

Cell viability assay

The cells were seeded in 96-well plates at a density of 1×10^4 per well. The cells were treated with different concentrations of capsaicin (1–100 μM), with or without testosterone (1 μM), for 24 h or 48 h. Cell survival rate was measured by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) assay. After the each timepoint, 10 μL of MTT (5 mg/mL) was added to fresh medium (100 μL/well), and cells were incubated at room temperature for 4 h. Formazan crystals were dissolved in dimethyl sulfoxide (100 μL/well) with incubation at 37°C for 20 min. Absorbance was measured by a microplate reader at a wavelength of 570 nm.

Animal experiment

Seven-week-old male ICR (Institute of Cancer Research) mice were obtained from Hunan SJA Laboratory Animal Co., Ltd. (Changsha, Hunan, China). All experiments were approved and conducted according to the guidelines of the Animal Care and Ethics Committee of Yichun University (Approval No. 2022026). Mice were maintained at 22 ± 2°C under 12-h light/dark cycle conditions. Capsaicin (10 mg/kg) and testosterone propionate (TP; 7.5 mg/kg) were dissolved in corn oil. The BPH induction model was established in mice by subcutaneous injection of testosterone propionate once daily. Capsaicin was given by intragastric administration once daily. Mice were randomly categorized into three groups (n = 6 per group): (A) control group given vehicle alone, (B) testosterone propionate-induced BPH group (TP, 7.5 mg/kg), (C) TP + capsaicin (10 mg/kg). The mice were weighed weekly over the duration of the experiment, which was 14 days and then weighed and sacrificed. Prostate/body weight ratio was calculated as the prostate index. Before sacrificing the animals, serum was collected for determination of PSA concentration using an ELISA kit, according to the manufacturer's instructions.

Western blot analysis

Protein lysis buffer (T-PER) (cat. no. 78510; Thermo Fisher Scientific, Inc., Waltham, USA) was used to extract total prostatic proteins. Bradford protein assay was used to determine the protein concentration. Twenty micrograms of total protein were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. After electrophoresis, proteins were transferred to polyvinylidene difluoride membranes which were blocked with 5% non-fat dried milk in Tris-buffered saline with 0.1% Tween 20 at room temperature for 1 h. After washing at room temperature for 30 min, the membranes were mixed with primary antibody to 5α-reductase type II (1: 1000), AR (1: 1000), PSA (1: 1000), PARP (1: 1000), caspase-3 (1: 1000), LC3B (1: 1000), beclin-1(1: 1000), TRPV1 (1: 1000), β-actin (1: 8000) and incubated overnight at 4°C. Subsequently, the membranes were washed and incubated with horseradish peroxidase-coupled secondary antibody (1:1000) at room temperature for 1 h. Protein expression was determined using an enhanced chemiluminescent kit (Shanghai Epizyme Biomedical Technology Co., Ltd., Shanghai, China). Protein bands were visualized by ChemiScope 3300 Mini equipment and software (Shanghai, China). The densitometry of protein expression was determined by ImageJ 1.52a software (National Institutes of Health, Bethesda, USA).

Statistical analysis

All data are presented as the mean ± S.D. (standard deviation) and using one way ANOVA, followed by Tukey's *post hoc* test. $p < 0.05$ indicates a statistically significant difference.

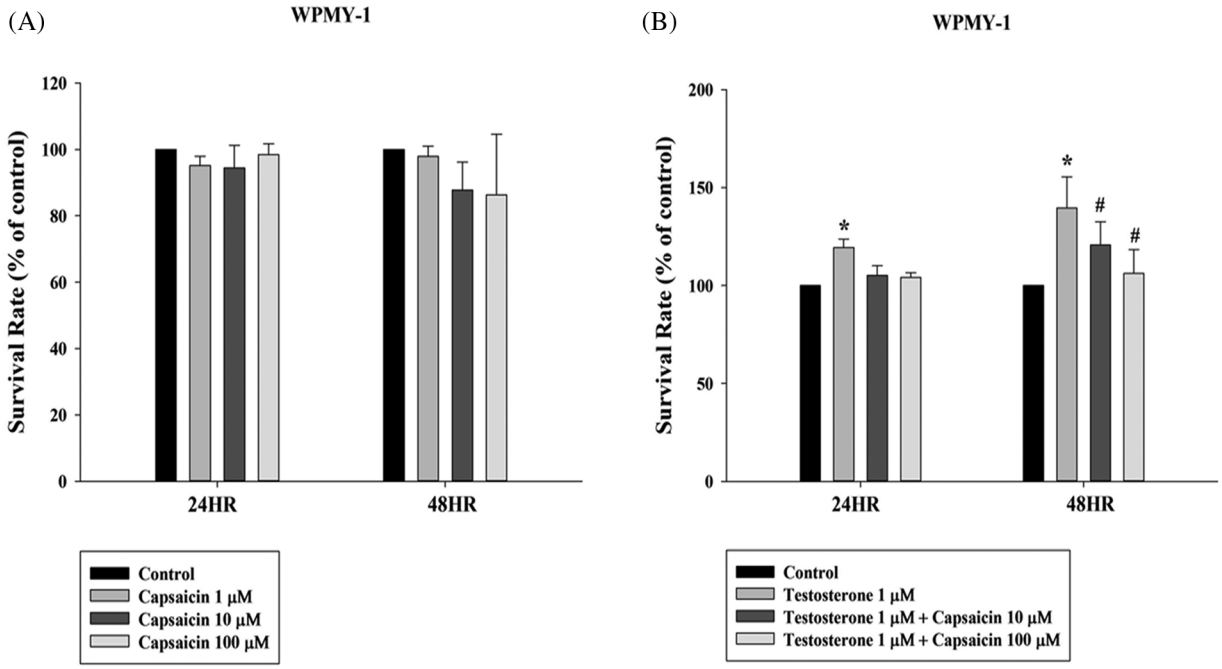

FIGURE 1. Capsaicin-mediated inhibition of testosterone-stimulated growth of WPMY-1 cells. Survival rate of WPMY-1 cells on incubation with capsaicin (1–100 μM) and testosterone (1 μM) for 24 and 48 h. All the values are represented as mean ± SD for three separate experiments (*$p < 0.05$ compared with control group, #$p < 0.05$ compared with testosterone group).

Results

Effect of capsaicin on the viability of WPMY-1 cells

The effects of capsaicin on WPMY-1 cell viability were determined by MTT assay. The effect of capsaicin (100 μM) in inducing cell death in WPMY-1 cells at 24 and 48 h was not so significant (Fig. 1A). Testosterone (1 μM) significantly induced cell growth at 24 and 48 h. The cell survival rate with testosterone (1 μM) at 48 h was 139.69 ± 9.15% compared with the control. However, capsaicin significantly inhibited cell growth in testosterone-treated WPMY-1 cells with a survival rate of 106.21 ± 7.07% compared with the control at 48 h (Fig. 1B).

Capsaicin attenuated prostatic hyperplasia in testosterone propionate-induced benign prostatic hyperplasia

The body weight of mice did not differ between the groups (Fig. 2A). In the testosterone-induced mouse model of BPH, prostate weight and prostate index of the capsaicin-treated group (10 mg/kg) were lower than those in BPH group (Figs. 2B and 2C). Treatment with capsaicin (10 mg/kg) and finasteride (10 mg/kg) both significantly suppressed the increased prostate weight by 68% and 54%, respectively, and the prostate index by 64% and 35%, respectively. Furthermore, serum PSA levels in the BPH group were higher than in the normal (vehicle) group but were significantly decreased in the capsaicin- and finasteride-treated groups (10 mg/kg) (Fig. 2D).

Capsaicin regulated the 5α-reductase-androgen receptor axis in testosterone propionate-induced benign prostatic hyperplasia mice

Normally DHT binds to the AR, and 5α-reductase type II then reduces the levels of testosterone. In BPH mice protein expression of 5α-reductase type II, AR and PSA were all significantly upregulated compared to the normal group (Fig. 3A). Furthermore, protein expression levels of 5α-reductase type II, AR, and PSA were significantly decreased by capsaicin treatment compared to the BPH group (Fig. 3A). This indicated that capsaicin is able to inhibit prostate growth by regulating the 5α-reductase-AR signaling pathway.

Capsaicin-induced autophagy and apoptosis by regulating transient receptor potential vanilloid 1 (TRPV1) expression

We determined the effect of capsaicin on apoptosis and autophagy, in testosterone propionate-treated BPH mice, by measuring the protein expression of (uncleaved) PARP, (pro-) caspase-3, LC3B, and beclin-1. The expression levels of (uncleaved) PARP and (pro-) caspase-3 were significantly increased in the BPH group (Fig. 3B). Capsaicin treatment significantly inhibited (uncleaved) PARP and (pro-) caspase-3 expression (Fig. 3B). The expression of beclin-1 protein decreased significantly in the BPH group, and this effect was reversed by capsaicin treatment (Fig. 3B). These results demonstrated that capsaicin was able to induce apoptosis and autophagy in BPH mice and TRPV1 expression was significantly increased in the capsaicin treatment group (Fig. 3C). This is consistent with previous reports demonstrating that capsaicin elicits TRPV1 channel activation (Yang and Zheng, 2017).

Discussion

BPH is a condition causing LUTS and bladder outlet obstruction that affects the quality of life in elderly men. Treatment involving 5α-reductase therapy is mainly used to reduce prostate volume and progressive hypertrophy. The

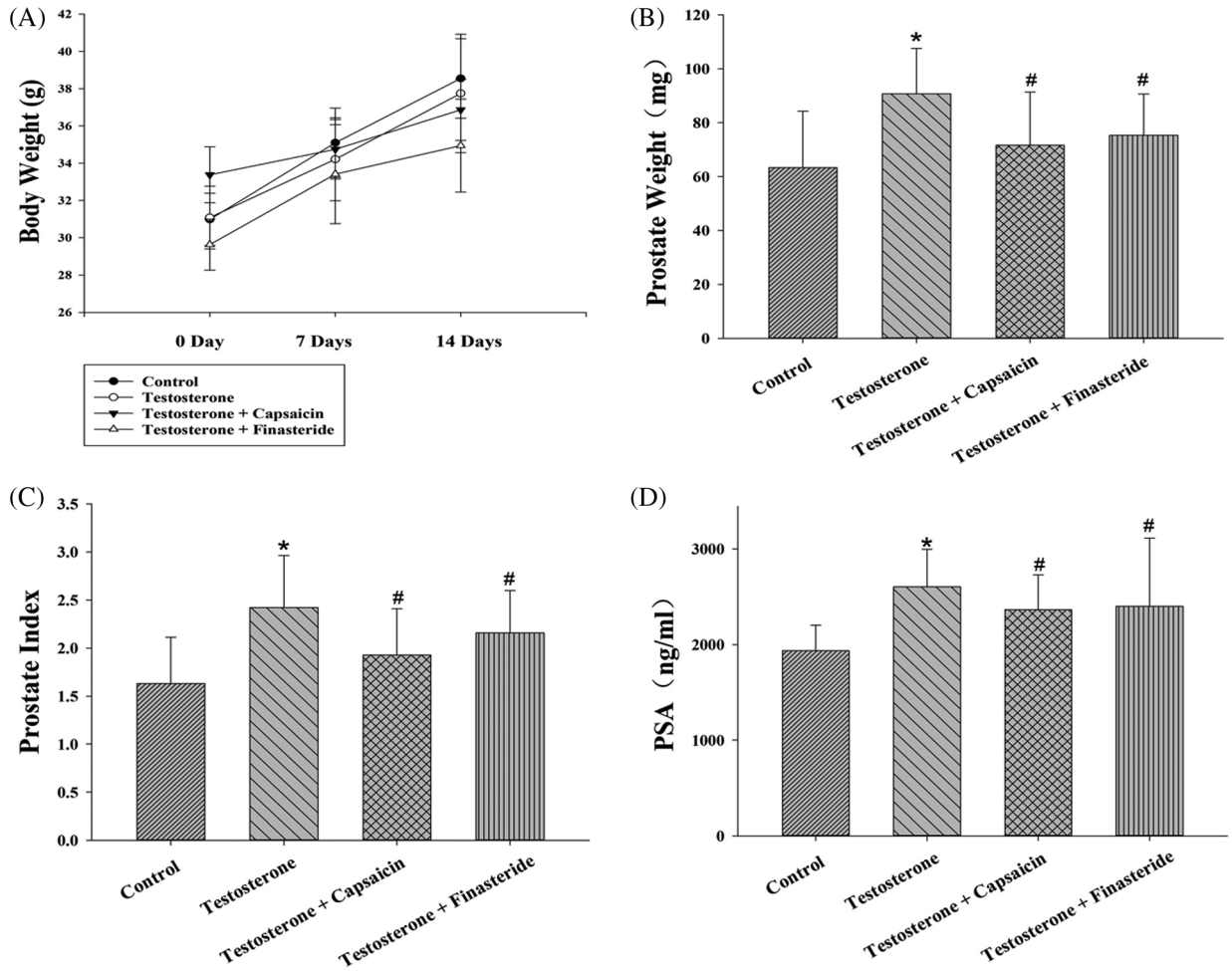

FIGURE 2. Effects of capsaicin (10 mg/kg) and finasteride (10 mg/kg) on prostate/body weight (A–B), prostate index (C) and serum prostate-specific antigen (PSA) level (D) in testosterone propionate (7.5 mg/kg)-induced benign prostatic hyperplasia (BPH) mice. Data are represented as mean ± SD (n = 6) (*$p < 0.05$ compared with control group, #$p < 0.05$ compared with testosterone group).

5α-reductase-AR signaling is considered a pivotal pathway in the treatment of BPH (Ilic and Misso, 2012). Discovering new, safe, effective therapies with few adverse effects would be important. Many researchers have focused on natural plant products. Previous studies have demonstrated that lycopene and curcumin displayed promising effects in the treatment of BPH (Alexandrov *et al.*, 2021; Qiao *et al.*, 2021).

Capsaicin is an active ingredient extracted from *Capsicum annum* (chili pepper). Its beneficial effects have been demonstrated in obesity, in inhibiting platelet aggregation, for analgesia, in inflammation, and in reducing hyperactive bladder symptoms by TRPV1-dependent or -independent pathways (Sharma *et al.*, 2013). Previous studies have shown the chemotherapeutic effects of capsaicin in prostate cancer (Díaz-Laviada, 2010; Sánchez *et al.*, 2019, 2022). The TP-induced BPH animal model employed in this study, has been widely studied (Dai *et al.*, 2017; Zou *et al.*, 2017). Testosterone can induce prostate cell growth. Several studies have indicated that testosterone (1–10 μM) *in vitro* can stimulate epithelial or stromal cellular growth (Hong *et al.*, 2020; Karunasagara *et al.*, 2020; Chen *et al.*, 2021a; Baek *et al.*, 2022). In this study, capsaicin treatment significantly suppressed TP-induced BPH and also inhibited testosterone-induced WPMY-1 prostate cell growth *in vitro*.

Previous studies have demonstrated that downregulation of the 5α-reductase-AR axis can improve BPH symptoms (Füllhase and Schneider, 2016). Our results demonstrated that capsaicin reduced the protein expression of AR, 5α-reductase type II, and PSA in TP-induced BPH mice. Furthermore, capsaicin also decreased serum PSA levels. These results suggest that the inhibitory effect of capsaicin on the androgen pathway affects the progression of BPH. In addition, capsaicin induced the expression of TRPV1 protein in TP-induced BPH mice suggesting its involvement in the inhibitory effects.

Apoptosis is a key event controlling cell survival and death. Apoptosis signaling involves an intrinsic pathway (Bcl-2-related cascade) and an extrinsic pathway (receptor-mediated). The 5α-reductase type II inhibitors induce prostate cell apoptosis in the treatment of BPH (Karunasagara *et al.*, 2020; Park *et al.*, 2019). Caspases are a family of protease enzymes that regulate apoptosis. Under stress or stimulation by phytochemicals, PARP, caspase-9, caspase-7, and caspase-3 are activated (Elkady, 2019; Qiu *et al.*, 2019). In the current study, the protein expression of

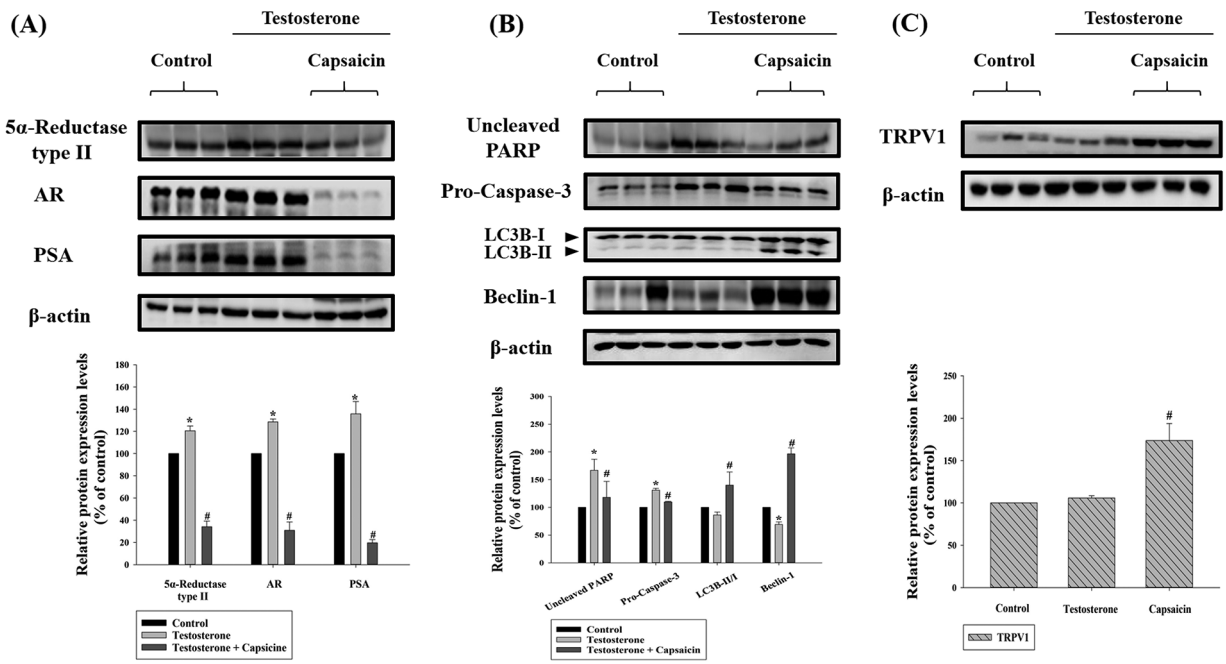

FIGURE 3. Effects of capsaicin (10 mg/kg) on the protein expression of 5α-reductase type II and androgen receptor signaling pathway (A), apoptosis, autophagy (B), and transient receptor potential vanilloid 1 (TRPV1) (C) in testosterone propionate (7.5 mg/kg)-induced benign prostatic hyperplasia (BPH) mouse model. Western blot analysis of protein expression of 5α-reductase type II, androgen receptor, prostate-specific antigen (PSA), (uncleaved) poly ADP-ribose polymerase (PARP), (pro-) caspase-3, microtubule-associated proteins 1A/1B light chain 3B (LC3B), and beclin-1. Data are represented as mean ± SD (*$p < 0.05$ compared with control group, #$p < 0.05$ compared with testosterone group).

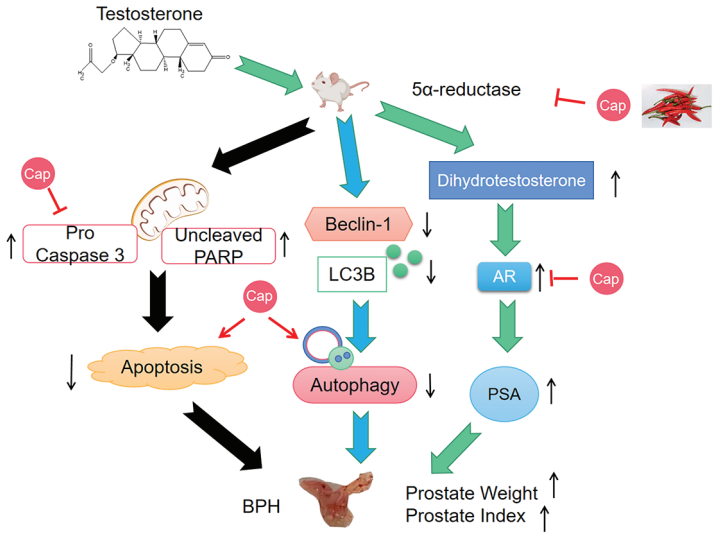

FIGURE 4. Diagram demonstrates that capsaicin (Cap) inhibits prostate growth in testosterone propionate-induced benign prostatic hyperplasia (BPH) mouse model. (1) Capsaicin inhibits prostate growth by interfering in the androgen receptor signaling pathway; (2) Capsaicin induces apoptosis and autophagy.

(pro-) caspase-3 and (uncleaved) PARP were upregulated in the BPH mice and expression of both was attenuated by capsaicin. The apoptotic effects of capsaicin were documented in the current study. Beclin-1 regulates autophagy, and the relationship between autophagy and apoptosis in cell death is complex (Xu and Qin, 2019). One study suggested that caspase-mediated cleavage of beclin-1 promoted crosstalk between apoptosis and autophagy (Djavaheri-Mergny *et al.*, 2010). LC3 is a soluble protein that is converted to LC3-II, which is recruited to autophagosomal membranes and acts as an autophagy-specific marker (Tanida *et al.*, 2008). In the current study, autophagy inhibition was observed in TP-induced BPH mice. Protein expression of LC3B-II and beclin-1 was decreased in the BPH mice, and capsaicin could induce autophagy by upregulating their expression.

In clinical practice, topical application of capsaicin can reduce pain and is particularly indicated in diabetic neuropathy and arthritis. Although the antioxidant and anti-inflammatory effects of capsaicin are well documented,

its irritative characteristics limit its clinical use. Capsaicin derivatives, with retained potency and no irritating adverse effects, need to be developed.

In summary, capsaicin significantly reduced the levels of 5α-reductase type II, AR, and PSA in BPH mice and reduced the prostate index. It reduced WPMY-1 cell growth *in vitro* and mediated cell death by inducing apoptosis and autophagy *in vivo* (Fig. 4). This study represents novel but relatively small studies investigating various aspects of prostatic cells. However, we did not carry out a detailed histological examination of the prostate. We also studied a single prostatic (myofibroblast) cell line; in the future, it would be useful to repeat the experiments with other cell lines or even human prostatic samples. Stromal and epithelial cells play a vital role in BPH development and the effects of capsaicin require future study. Taken together, these findings strongly suggest the therapeutic potential of capsaicin in BPH.

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Author Contributions: Study conception and design: CYC and CML; experiments performed: ZCS, HWC, XZC, HS; data collection: MQS, YDL; analysis and interpretation of results: BHT and ZTW; draft manuscript preparation: ZCS, CYC, CML. All authors reviewed the results and approved the final version of the manuscript.

Availability of Data and Materials: The data generated during the current study are available from the corresponding author on reasonable request.

Ethics Approval: All experimental procedures were approved and supervised by the Animal Care and Ethics Committee of Yichun University (Approval No. 2022026).

Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

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