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•Research article•

The extract of *Celtis choseniana* Nakai alleviates testosterone-induced benign prostatic hyperplasia through inhibiting 5*α* reductase type 2 and the Akt/NF-*κ*B/AR pathway

HONG Geum-Lan[Δ], KIM Tae-Won[Δ], LEE Hui-Ju, KIM Yae-Ji, KIM Kyung-Hyun, JUNG Ju-Young[*]

Department of Veterinary Medicine & Institute of Veterinary Science, Chungnam National University, Daejeon 34134, Republic of Korea

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[ABSTRACT] Benign prostatic hyperplasia (BPH) is a chronic male disease characterized by the enlarged prostate. *Celtis choseniana* Nakai (*C. choseniana*) is medicinally used to alleviate pain, gastric disease, and lung abscess. In this study, the effect of *C. choseniana* extract on BPH was investigated using testosterone-induced rats. Sprague Dawley rats were divided into five groups: control, BPH (testosterone 5 mg·kg^{-1}), Fina (finasteride 2 mg·kg^{-1}), and *C. choseniana* (50 and 100 mg·kg^{-1}). After four weeks of TP treatment with finasteride or *C. choseniana*, prostate weights and DHT levels were measured. In addition, the prostates were histopathologically examined and measured for protein kinase B (Akt)/nuclear factor-*κ*B (NF-*κ*B)/AR signaling, proliferation, apoptosis, and autophagy. Prostate weight and epithelial thickness were reduced in the *C. choseniana* groups compared with that in the BPH group. The extract of *C. choseniana* acted as a 5*α* reductase inhibitor, reducing DHT levels in the prostate. Furthermore, the extract of *C. choseniana* blocked the activation of p-Akt, nuclear NF-*κ*B activation and reduced the expression of AR and PSA compared with BPH. Moreover, the expression of Bax, PARP-1, and p53 increased, while the expression of bcl-2 decreased. The present study demonstrated that *C. choseniana* extract alleviated testosterone-induced BPH by suppressing 5*α* reductase and Akt/NF-*κ*B activation, reducing AR signaling and inducing apoptosis and autophagy in the prostate. These results suggested that *C. choseniana* probably contain potential herbal agents to alleviate BPH.

[KEY WORDS] Apoptosis; Androgen receptor; Benign prostate hyperplasia; *Celtis choseniana* Nakai; 5*α*-Reductase type 2; NF-*κ*B
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Introduction

Benign prostate hyperplasia (BPH) is a chronic male disease that affects more than 50% of men over 60 years of age [1, 2]. Patients with BPH present prostate enlargement that results in a series of uncomfortable symptoms, such as lower urinary tract symptoms and erectile dysfunction [3]. Although BPH is not a life-threatening disease, increased awareness of patients' decreased quality of life sparked interest in the management of prostate hyperplasia and hypertrophy.

An imbalance between proliferation and apoptosis leads to BPH or prostate cancer [4]. Androgen is considered a risk factor for prostate enlargement. Dihydrotestosterone (DHT), which is converted from testosterone *via* 5*α*-reductase type 2, bound to the androgen receptor (AR), activating prostate hyperplasia of stromal and glandular epithelial cells in the prostate [5]. On the other hand, low testosterone levels induced cell death in the prostate by activating apoptosis [6]. Inhibiting the conversion from testosterone to DHT facilitated a reduction in prostate size, alleviating BPH symptoms in animal and clinical studies [7, 8].

Celtis choseniana Nakai is an endemic herbal plant that belongs to the family Ulmaceae [9]. Traditionally, the genus *Celtis* has been used as a medicinal plant in China, Korea, and Japan. Its leaves and bark can be used to treat pain, gastric disease and lung abscess [10, 11]. In addition, *C. choseniana* extract exhibited anti-inflammatory activity during macrophage-mediated inflammatory responses [12]. The main phytomedicinal constituents of *C. choseniana* extract are quercetin, luteolin, and kaempferol, which are anti-inflammatory flavonoids [13, 14]. Among them, luteolin exhibited the highest anti-inflammatory effects [12]. However, there is not much research concerning the phytomedicinal activity of the extract of *C. choseniana*. Therefore, in this study, the effect of *C. choseniana* extract on BPH was investigated

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[*Corresponding author] Tel: 82-42-821-8899-7902, Fax: 82-42-821-7926, E-mail: jyjung@cnu.ac.kr
[Δ]These authors contributed equally to this work.
These authors have no conflict of interest to declare.

using a testosterone-induced rat model of BPH *in vivo*.

Material and Methods

Plant material

The plant, *Celtis choseniana* Nakai is an accepted name on the plant list (http://www.theplantlist.org). The 001−011 (3rd) used in this research was obtained from Korea Plant Extract Bank in Korea Research Institute of Bioscience and Biotechnology (Daejeon, Korea). The plant was collected from Ulleung-gun, Gyeongsangbuk-do, Korea, in 2000. A voucher specimen (KRIP 0004939) was kept in the herbarium of Korea Research Institute of Bioscience and Biotechnology. The plant (96 g) dried in the shade and powdered was added to 1 L of 99.9% methyl alcohol (HPLC grade) and extracted through 30 cycles (40 kHz, 1500 W, 15 min, ultrasonication-120 min. standing per cycle) at room temperature using an ultrasonic extractor (SDN-900H, SD-ULTRASONIC Co., Ltd., Seoul, Korea). After filtration and drying under reduced pressure, *C. choseniana* extract (10.3 g) was obtained.

HPLC analysis of luteolin in C. choseniana

The constituents of *Celtis choseniana* Nakai were analyzed by high performance liquid chromatography (HPLC) with a 1260 Infinity II system (Agilent, CA, USA) equipped with a UV detector. Luteolin (Sigma, St. Louis, USA) was used as the reference compound [12]. The conditions are described in Table 1.

Experimental animals

The experimental protocols were approved (201906A-CNU-116) by the Institutional Animal Ethics Committee of Veterinary Medicine, Chungnam National University. Sprague-Dawley (SD) 6-week-old-male rats were purchased from Orient Bio (Korea). Before experiments, the animals were acclimatized under stable conditions (22 ± 2 °C, 30%−35% relative humidity, 12 h light/dark cycle) for one week, with assess to standard food and water *ad libitum*. This research was carried out according to the Guide for the Care and Use of Laboratory Animals (8th edition).

A total of 25 SD rats were randomly divided into five groups (*n* = 5). Except for the control rats, all rats were subcutaneously (SC) injected with testosterone propionate (TP, 10 mg·kg^{-1}, Tokyo Chemical Ins. Co., Tokyo, Japan) for four weeks daily. All rats were treated with their corresponding compound for four weeks as follows: a control group (10 mg·kg^{-1} PBS, p.o.), a BPH group (10 mg·kg^{-1} TP alone, s.c.), a Fina group (TP, s.c. + finasteride 10 mg·kg^{-1}, p.o.), a 50 group (TP, s.c. + *C. choseniana* 50 mg·kg^{-1}, p.o.), and a 100 group (TP, s.c. + *C. choseniana* 100 mg·kg^{-1}, p.o.). At the end of the experiment, the rats were fasted overnight and anesthetized with CO_2 in a chamber. The blood samples obtained from the heart were centrifuged at 3000 *g* for 15 min, and the serum was stored at −80 °C until later use. The dissected prostate tissues and body weight of SD rats were measured at the end of the experimental period. The relative prostate weights were calculated by dividing the prostate weights by the body weights. Half of the prostate gland was formalin-fixed and subjected to paraffin embedment and sectioning. The remaining prostate samples were frozen in liquid nitrogen and stored at −80 °C for Western blot analysis.

Table 1 Instrument and working conditions for analysis of *C. choseniana* extract by high performance liquid chromatography (HPLC)

HPLC analysis condition		
Column	Gemini C_{18}, 250 mm × 4.6 mm, 5 μm	
Detector	UV detector, 370 nm	
Mobile phase	A: 0.1% TFA in DW; B: 0.1% TFA in ACN	
Flow rate	1.0 mL·min^{-1}	
Injection volume	10 μL	
Gradient condition/min	A/%	B/%
0	100	0
50	50	50
60	0	100
70	100	0

Measurement of DHT levels

DHT levels in serum and prostate tissue samples were measured using a commercial enzyme-linked immunosorbent assay kit (MyBiosource, California, USA) according to the manufacturer's instructions.

Western blot

The collected cells and frozen prostate tissue samples were lysed and quantified as previously described [15]. For cytosolic and nuclear fractions, the prostates were homogenized in an extraction kit (Abcam, Cambridge, UK) according to the manufacturer's instruments. Protein concentrations were determined by a bicinchoninic acid assay (BCA, Thermo Fisher Scientific, Massachusetts, USA). Western blot was performed as previously described [15]. The primary antibodies used are shown in Table S1. Proteins were visualized with an enhanced chemiluminescence detection kit (Amersham Pharmacia Biotech, Buckinghamshire, UK) and quantified using Image J software (Image J v1.46a; NIH, USA).

Histological study

The prostate was fixed in 10% neutral buffered formalin phosphate solution. Embedded in paraffin, the blocks were cut into 5-μm sections. Hematoxylin and eosin (H&E) staining and immunohistochemistry were performed as previously described [15] using primary antibodies as follow; anti-5α-reductase type 2 (1 : 500, Santa Cruz). All stained sections were visualized under a light microscope (Nikon eclipse 80i, Nikon Corporation, Tokyo, Japan) at 200 × and 400 × magnification and images were captured with DP Controller software. Ten randomly selected images were acquired for each section, and the epithelial thickness was measured using Image J software.

TUNEL staining

TUNEL staining was performed using a peroxidase in situ apoptosis detection kit (Merck, Darmstadt, Germany) according to the manufacturer's instructions. The slides were deparaffinized and dehydrated according to our laboratory protocol. Color was developed using a 3,3′-diaminobenzid-

ine substrate, and Mayer's hematoxylin was used for counterstaining. The apoptosis-positive area in the stained sections was visualized as brown under a light microscope (Nikon eclipse 80i) at 400 × magnification. Ten randomly selected images were acquired for each section, and apoptosis-positive cells were expressed as a number per 100 prostate cells.

Statistical analysis

All experiments were conducted in a double-blind manner. The results were randomly selected and are expressed as the mean ± SEM of double experiments. GraphPad Prism version 5.0 (GraphPad Software, La Jolla, CA, USA) was used for data analysis. For comparisons, the Mann-Whitney *U* test was used to determine the statistical differences for nonparameteric data between groups. A post hoc Tukey test was used for comparisons among multiple groups when relevant. A P value < 0.05 was considered statistically significant.

Results

HPLC analysis of the extract of C. choseniana

HPLC analysis for the quality control of *C. choseniana* extract was performed using standard anti-inflammatory flavonoid luteolin [12]. Luteolin in *C. choseniana* Nasaki extract was detected at 40.1 min. The extract of *C. choseniana* Nasaki was standardized with luteolin. Representative chromatograms of the reference standard (luteolin) and *C. choseniana* Nasaki extract are shown in Fig. 1 and Table S2. The luteolin content in *C. choseniana* extract was 0.14%, and luteolin was used as a quality control for further study.

Effects of C. choseniana extract on prostate weights and histopathological changes

To induce the BPH model of rats, the animals were treated with both the extract of *C. choseniana* and testosterone. As shown in Table 2, the body weights of the BPH group increased compared with those of the control group. However, prostate weights significantly (P < 0.001) increased both absolutely and relatively. The Fina group showed a significant reduction in prostate weights (P < 0.01) compared with the BPH group. The prostate weights of the *C. choseniana*-treated groups also decreased, without statistical difference. Fig. 2 reflects the histopathological changes of the prostate. The difference in prostate tissue was pronounced as shown in Fig. 2B. The tubular glands of the control group were lined with cuboidal epithelium supported by stromal tissue. In contrast, the BPH group showed a columnar glandular epithelium with hyperplastic processes in multiple layers and a decrease in lumen volume. However, the groups treated with finasteride and *C. choseniana* showed reduced hyperplastic processes and increased lumen volume compared with the BPH group. The thickness of the prostate epithelium also significantly increased (P < 0.001) in the BPH group compared with those in the control group. However, the thickness of the prostate epithelium in the Fina and *C. choseniana* groups significantly decreased (P < 0.001, Fig. 2C).

Effects of C. choseniana extract on 5α-reductase in prostatic tissue and serum and prostatic DHT levels

The expression of 5α-reductase and DHT level were determined to evaluate the effects of *C. choseniana* extract. The BPH group presented significant increases in the % area of the 5α-reductase positive stained prostate (P < 0.001) compared with the control group. However, in both of the *C. choseniana* groups, the positive stained area decreased, compared with that in the BPH group. In particular, the % area of the positive stained prostate was more obvious in the 50 mg·kg^{-1} *C. choseniana* group. In RWPE cells, the 5α-re-

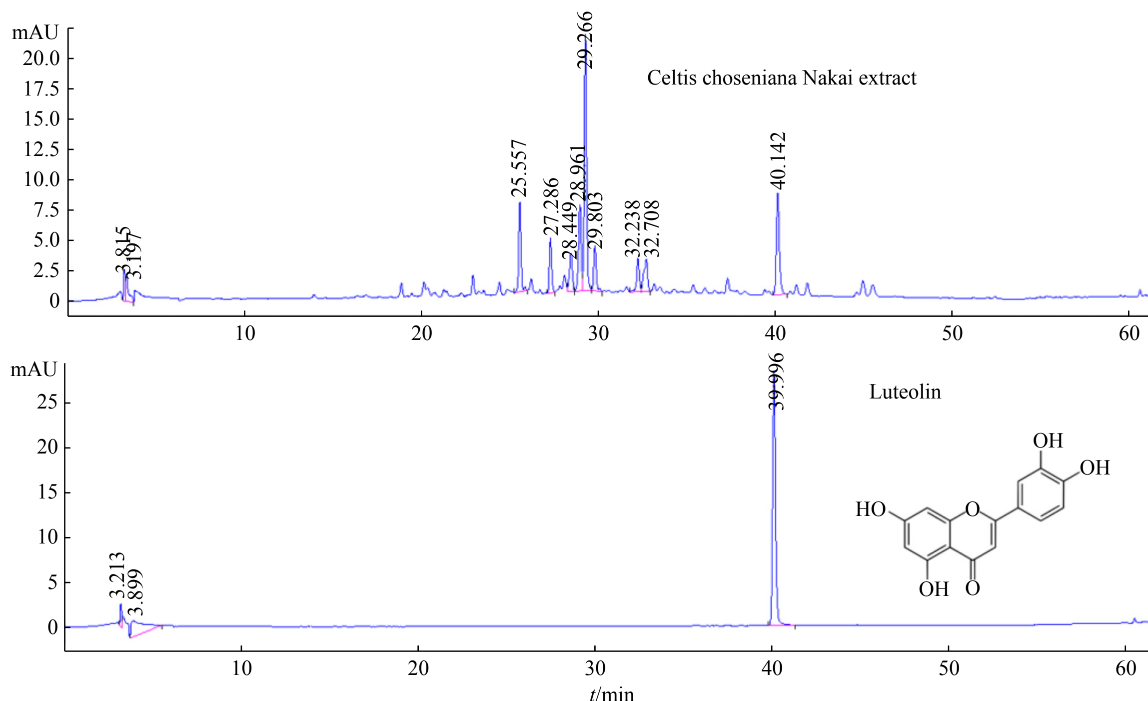

Fig. 1 Representative high performance liquid chromatography (HPLC) chromatograms of *C. choseniana* extract

Table 2 Effects of *C. choseniana* extract on body weight and prostate weight (mean ± SEM, *n* = 5). $^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$ *vs* control; $^{*}P < 0.05$ *vs* BPH

	Body weight/g		Prostate weight/g	
	Initial	Final	Absolute	Relative
Control	216.63 ± 7.183	397.1 ± 12.207	0.816 ± 0.0116	0.207 ± 0.008
BPH	215.81 ± 8.315	345.60 ± 14.01$^{\#}$	1.644 ± 0.0433$^{\#\#\#}$	0.478 ± 0.0188$^{\#\#\#}$
Fina	216.03 ± 6.354	341.9 ± 9.105	1.35 ± 0.0581*	0.397 ± 0.0229*
C. choseniana 50 mg·kg^{-1}	216.51 ± 8.197	375.04 ± 5.747	1.517 ± 0.0848	0.0405 ± 0.0249
C. choseniana 100 mg·kg^{-1}	216.46 ± 6.321	349.4 ± 10.784	1.496 ± .00653	0.428 ± 0.0138

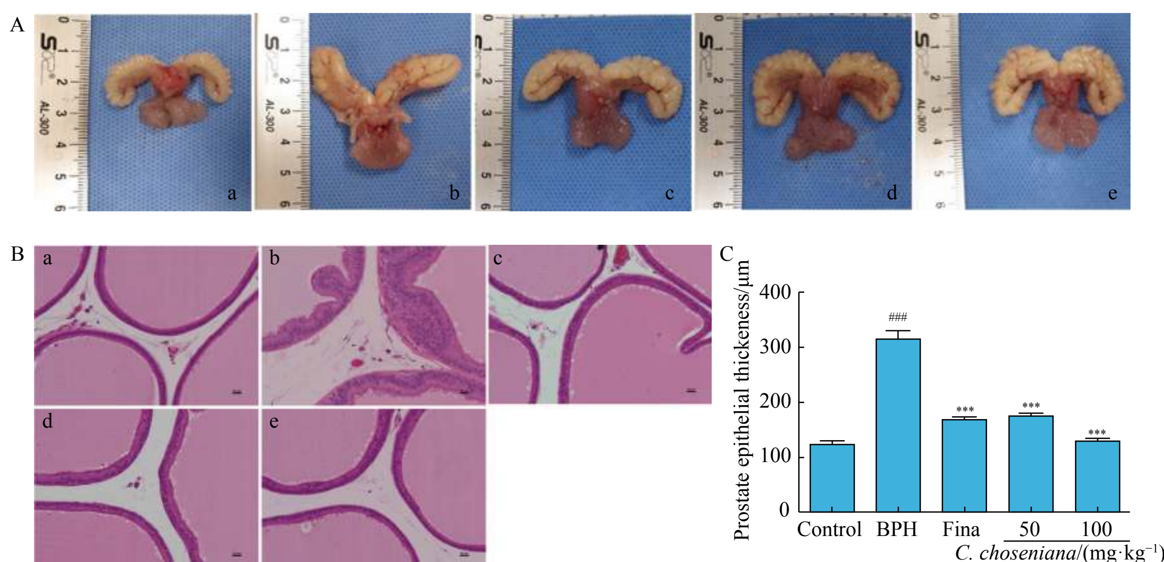

Fig. 2 Effects of *C. choseniana* extract on prostate size and histopathological changes according to hematoxylin and eosin (H&E) staining. Scale bar = 20 μm. BPH in rats was generated through daily subcutaneous injection of testosterone propionate (TP, 5 mg·kg^{-1}) for 28 days. A. The prostate. (a) The Control group: PBS; (b) The BPH group: TP; (c) The Fina group: TP + Finasteride (10 mg·kg^{-1}, p.o.); (d) The 50 group: TP + *C. choseniana* (50 mg·kg^{-1}, p.o.); and (e) The 100 group: TP + *C. choseniana* (100 mg·kg^{-1}, p.o.). B. Histopathological changes. C. The thickness of prostatic epithelial cells increased in the BPH group and decreased in the Fina and *C. choseniana*-treated groups. Data are presented as mean ± SEM (*n* = 5). $^{\#\#\#}P < 0.001$ *vs* the control group; $^{***}P < 0.001$ *vs* the BPH group

ductase concentration increased in testosterone-treated RWPE cells. However, the level of 5*α*-reductase was significantly reduced ($P < 0.001$) in *C. choseniana* and testosterone-cotreated RWPE cells (Fig. S1). The results of 5*α*-reductase expression were accompanied by DHT levels, as shown in Fig. 3. The BPH group demonstrated significantly ($P < 0.001$) increased levels of DHT in serum and prostatic tissue compared with the control group. In both the 50 and 100 mg·kg^{-1} groups, DHT levels in serum and prostatic tissue significantly decreased ($P < 0.05$) compared with those in the BPH group.

C. choseniana extract inhibited the NF-κB signaling in BPH tissues

As shown in Fig. 4, protein kinase b (AKT) phosphorylation increased in the BPH group compared with that in the control group. Interestingly, in the Fina and *C. choseniana* groups, AKT phosphorylation decreased compared with that in the BPH group. Furthermore, the expression of the nuclear factor kappa light chain enhancer of activated B cell (NF-*κ*B) and the I-kappa B kinase (I-*κ*B) was investigated in the prostatic nuclei and cytosols. The expression of cytosolic I-*κ*B significantly increased in the BPH group compared with that in the control group. However, the expression of I-*κ*B decreased in the Fina and *C. choseniana* groups, especially the expression of I-*κ*B in the 100 mg·kg^{-1} *C. choseniana* group. Similarly, the expression of NF-*κ*B in the prostatic nuclei significantly increased in the BPH group compared with that in the control group. However, the expression of NF-*κ*B decreased in the Fina and *C. choseniana* groups. (Fig. 4).

Effects of C. choseniana extract on BPH proliferation in prostate tissues

As shown in Fig. 5, the expression of AR significantly increased ($P < 0.001$) in the BPH group compared with that in the control group. However, the expression of AR in the Fina group decreased. In particular, the *C. choseniana* extract groups showed significant decreases ($P < 0.001$) in AR expression compared with the control group. Unlike the changes in AR, estrogen receptor (ER)-*α* did not decrease in

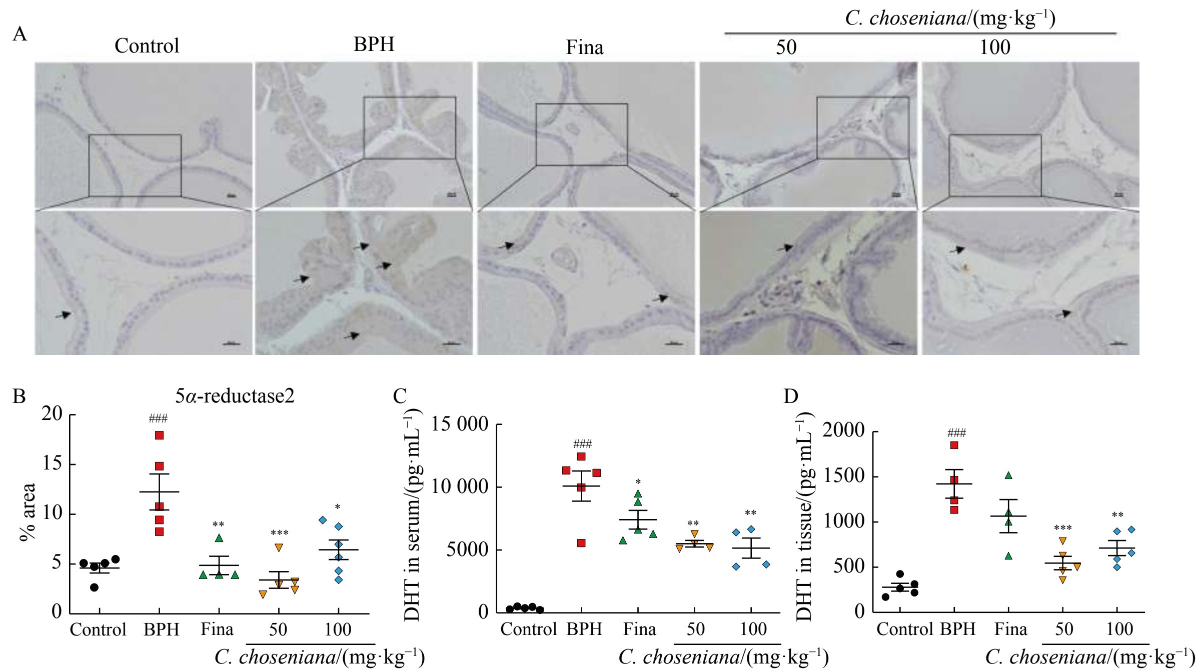

Fig. 3 Effects of *C. Choseniana* extract on DHT in serum and prostate tissue and 5α-reductase in prostate tissue. Scale bar = 20 μm. A and B, Immunohistochemistry of 5α-reductase type 2 antibody with % area of 5α-reductase tyep2-positive pictures. C and D, Concentrations of DHT in rat serum and prostatic tissue. BPH in rats was generated through daily subcutaneous injection of TP for 28 days. Control: PBS; BPH: TP; Fina: TP + Finasteride (10 mg·kg^{-1}, p.o.); the 50 group: TP + *C. choseniana* (50 mg·kg^{-1}, p.o.); and the 100 group: TP + *C. choseniana* (100 mg·kg^{-1}, p.o.). Arrows indicate 5α-reductase type 2-positive stained prostate epithelial cells. Data are presented as mean ± SEM (n = 5). $^{###}P < 0.001$ *vs* the control group; $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ *vs* the BPH group

the Fina and *C. choseniana* groups. In addition, there was no significant difference in the expression of ER-β. Along with the AR expression results, the BPH group demonstrated a markedly increased level of prostatic prostate specific antigen (PSA) compared with the control group. However, the Fina group and both *C. choseniana* groups exhibited significant decreases (P < 0.001) in PSA level compared with the BPH group. Similarly, the proportion of proliferating cell nuclear antigen (PCNA) significantly increased (P < 0.001) in the BPH group compared with that in the control group, but was reduced in the Fina and *C. choseniana* groups, as shown in Fig. 5.

Effects of C. choseniana extract on apoptosis in BPH tissues

To evaluate apoptosis, the levels of the apoptosis-related proteins, B cell lymphoma associated X (Bax), B cell lymphoma 2 (Bcl-2), and poly (ADP-ribose) polymerase (PARP-1) were examined. As shown in Fig. 6, the ratio of Bax to Bcl-2 significantly decreased (P < 0.005) in the BPH group. However, this ratio increased in the Fina and *C. choseniana* groups and significantly increased in the 50 mg·kg^{-1} *C. choseniana* group. According to TUNEL staining, the number of positive cells decreased in the BPH group but increased in the Fina and *C. choseniana* groups.

Effects of C. choseniana extract on autophagy in BPH tissues

As shown in Fig. 7, AMP-activated protein kinase (AMPK) phosphorylation was reduced in the BPH group compared with that in the control group. However, the phosphorylation of AMPK significantly increased (P < 0.001) in the Fina and *C. choseniana* extract-treated groups. To evaluate autophagy, the expression of the microtubule-associated protein 1A/1B light chain (LC3) and sequenstrosome-1 (p62) was examined by Western blot. The conversion of LC3-I to LC3-II decreased in the BPH group, while the expression of p62 in the BPH group significantly increased (P < 0.001) compared with those in the control group. However, in the groups treated with Fina or *C. choseniana* extract, LC3-II conversion increased, and p62 expression was significantly reduced.

Discussion

BPH is the most common disease characterized by glandular and stromal tissue hyperplasia. Androgen signaling and apoptotic processes are the major factors related to the development and progression of BPH [16, 17]. Although there are many therapeutic agents, such as 5α-reductase inhibitors or α-adrenergic blockers, research is being carried out on novel therapeutic substances due to drug side effects, such as loss of libido, erectile dysfunction, and upper respiratory tract infection [18]. In the present study, we evaluated the therapeutic effects of *C. choseniana* on testosterone-induced BPH development. Luteolin is one of the phytomedicinal constituents of *C. choseniana* and exerted anti-inflammatory activity [12]. When prostate inflammation develops into chronic disease, an imbalance between proliferation and apoptosis occurs in prostate tissue, leading to a prostate volume increase and a higher international prostate symptom score [19, 20]. The

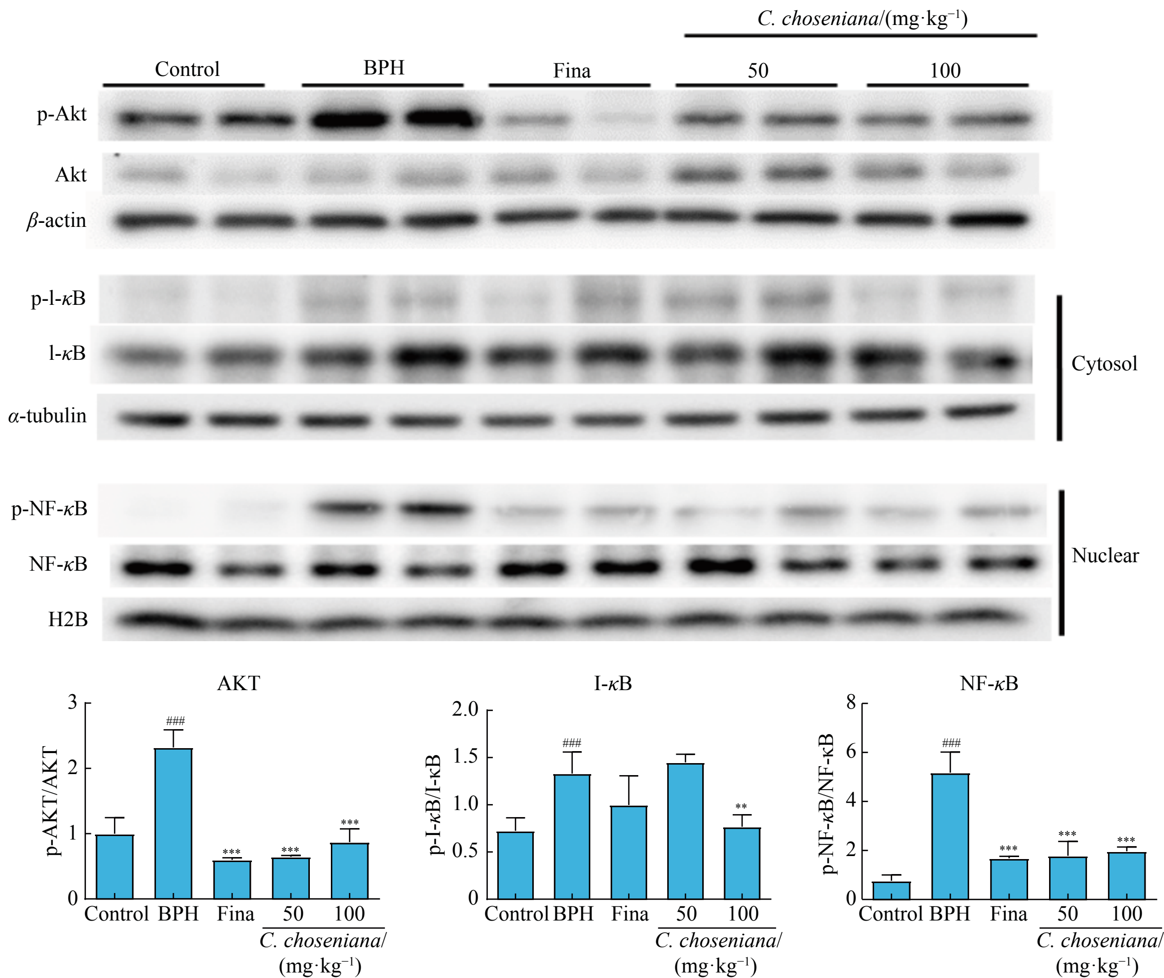

Fig. 4 Effects of *C. Choseniana* extract on Akt/NF-κB signals in prostate tissue. Western blot analysis of the expression of p-Akt, NF-κB and I-κB in rat prostate tissue. BPH in rats was generated through daily subcutaneous injection of TP for 28 days. Control: PBS; BPH: TP; Fina: TP + Finasteride (10 mg·kg^{-1}, p.o.); the 50 group: TP + *C. choseniana* (50 mg·kg^{-1}, p.o.); and the 100 group: TP + *C. choseniana* (100 mg·kg^{-1}, p.o.). Data are presented as mean ± SEM (n = 5). $^{\#\#\#}P$ < 0.001 *vs* the control group; $^{**}P$ < 0.01, $^{***}P$ < 0.001 *vs* the BPH group

effects of *C. choseniana* on BPH have yet to be elucidated. In this study, we investigated the effects of *C. choseniana* on BPH, which was related to the decline of androgen receptor signals.

Enlarged prostate and proliferation of the stromal and epithelium are important markers of BPH [21]. In the present study, prostate size and weight increased in testosterone-induced BPH rats. In addition, histopathological BPH showed alterations in the prostate, with increased epithelial thickness and stromal cells and reduced lumens. However, the Fina and *C. choseniana* groups presented reduced relative prostate weights and epithelium thickness.

The balance of testosterone and DHT hormones is the major factor during the development of BPH. Testosterone is converted to DHT by 5α-reductase, and DHT promotes prostate cell proliferation and survival by binding AR [2, 22, 23]. Recently, estrogen was implicated as a factor in the development of BPH [24]. Although the association between androgen and estrogen in BPH was not clearly elucidated, both androgen and estrogen played key roles in the development and progression of BPH [25]. Among the two estrogen receptors, ERα and ERβ, ERα mediates cell proliferation, whereas ERβ mediates cell apoptosis in prostate cells [24]. Our study showed reduced 5α-reductase expression in the prostate tissue of the Fina and *C. choseniana* groups compared with those in the BPH group. Furthermore, an increase in DHT level in serum and prostate tissue was found in the BPH group. In contrast, a remarkable reduction in serum and prostatic DHT level were seen in both *C. choseniana* groups. It was supported by the result that 5α reductase was reduced in cells treated with *C. choseniana* and testosterone compared with those treated with testosterone alone. Moreover, AR was also markedly reduced in the *C. choseniana* groups compared with that in the BPH group. Therefore, the extract of *C. choseniana* suppressed 5α reductase activity, resulting in a reduction in DHT level and AR. Unlike changes in the AR expression, there were no significant difference in ERs. The shrinkage rate of prostate volume was limited to just 20% or so after 5α-reductase inhibition [26]. Despite changes in androgen receptors, prostate weight did not significantly decrease as the expression of

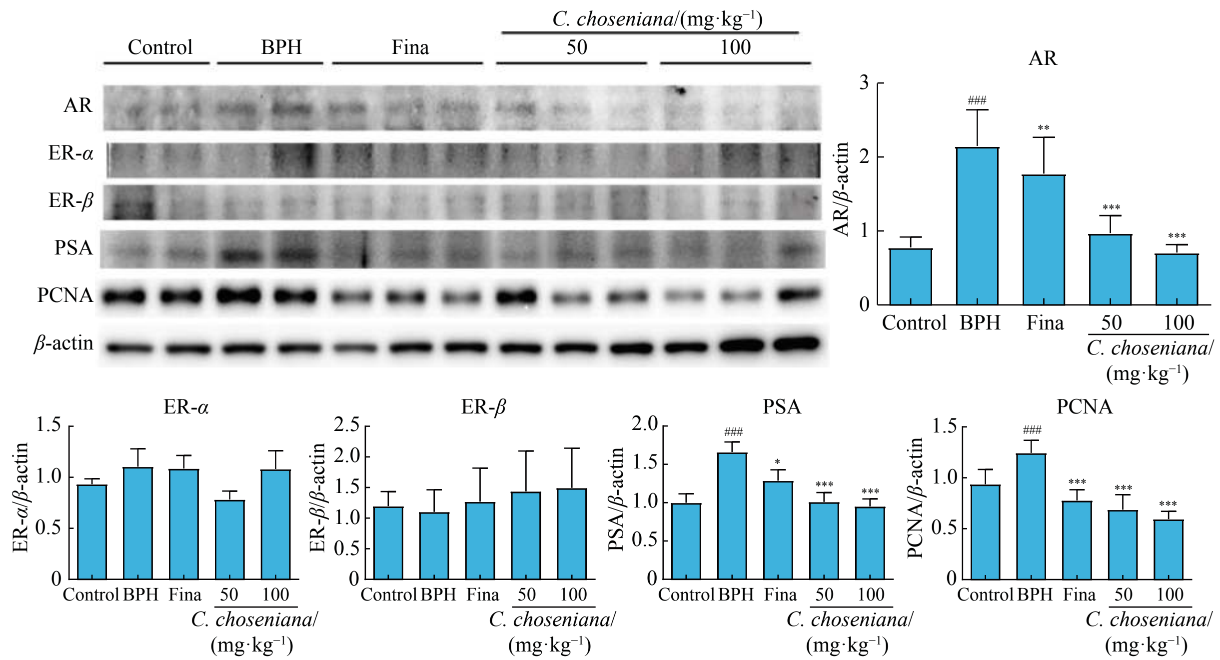

Fig. 5 Effects of *C. Choseniana* extract on the expression of AR, ER-*α*, ER-*β*, PSA, and PCNA in prostate tissue. Western blotting analysis of AR, ER-*α*, ER-*β*, PSA, and PCNA and *β*-actin in rat prostate tissue samples. BPH in rats was generated through daily subcutaneous injection of TP for 28 days. Control: PBS; BPH: TP; Fina: TP + Finasteride (10 mg·kg^{-1}, p.o.); the 50 group: TP + *C. choseniana* (50 mg·kg^{-1}, p.o.); and the 100 group: TP + *C. choseniana* (100 mg·kg^{-1}, p.o.). Data are presented as mean ± SEM (*n* = 5). $^{###}P < 0.001$ *vs* the control group; $^{***}P < 0.001$ $^{**}P < 0.01$ and $^{*}P < 0.05$ *vs* the BPH group

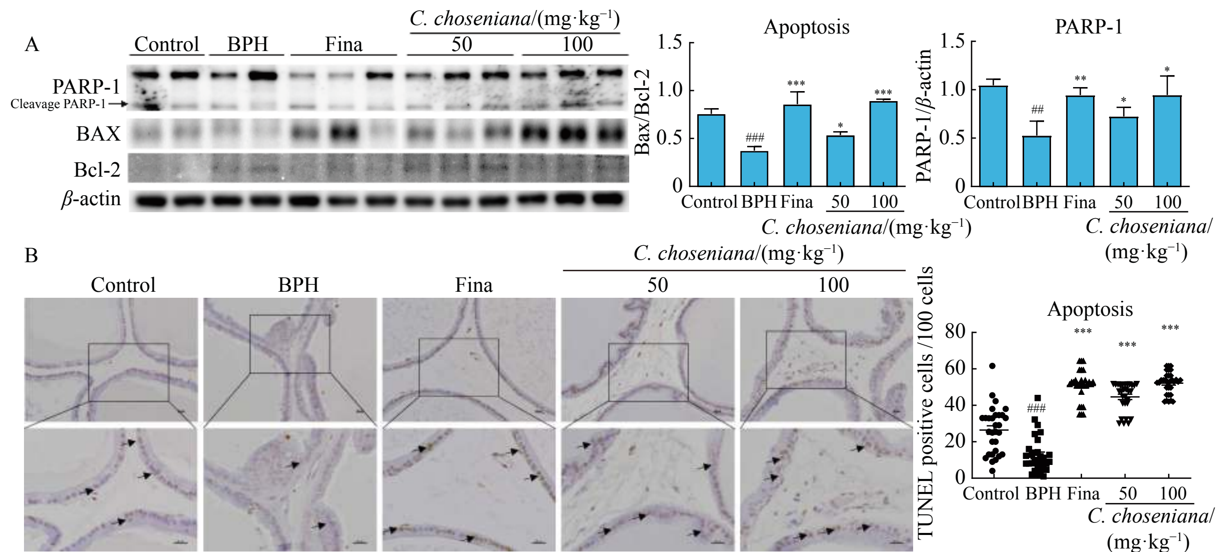

Fig. 6 Effects of *C. Choseniana* extract on apoptosis in prostate tissue. A. Western blotting of PARP-1, Bax, Bcl-2, and *β*-actin and Bax/Bcl-2 ratio in rat prostate tissue. B. TUNEL staining and positive cells per 100 cells in prostate tissue. Arrows indicate positive-stained cell in the prostate epithelial cells. BPH in rats was generated through daily subcutaneous injection of TP for 28 days. Control: PBS; BPH: TP; Fina: TP + Finasteride (10 mg·kg^{-1}, p.o.); the 50 group: TP + *C. choseniana* (50 mg·kg^{-1}, p.o.); and the 100 group: TP + *C. choseniana* (100 mg·kg^{-1}, p.o.). Data are presented as mean ± SEM (*n* = 5). $^{###}P < 0.001$ *vs* the control group; $^{**}P < 0.01$ and $^{*}P < 0.05$ *vs* the BPH group

ER*α* was not reduced, and the expression of ER*β* did not significantly increase.

Akt and NF-*κ*B were up-regulated during BPH progression and development [27], but relatively low in the normal prostate [28]. Activation of the Akt pathway resulted in I-*κ*B phosphorylation, degradation of I-*κ*B and liberated NF-*κ*B accompanied by nuclear translocation [29]. Activation of nuclear NF-*κ*B was related to AR transcriptional activity [30]. *In vivo* and *in vitro*, continuous activation of NF-*κ*B maintained high nuclear AR levels, resulting in AR signaling. Additionally, NF-*κ*B was shown to activate the transcription regulatory element of the *PSA* gene, and bind the sites located in

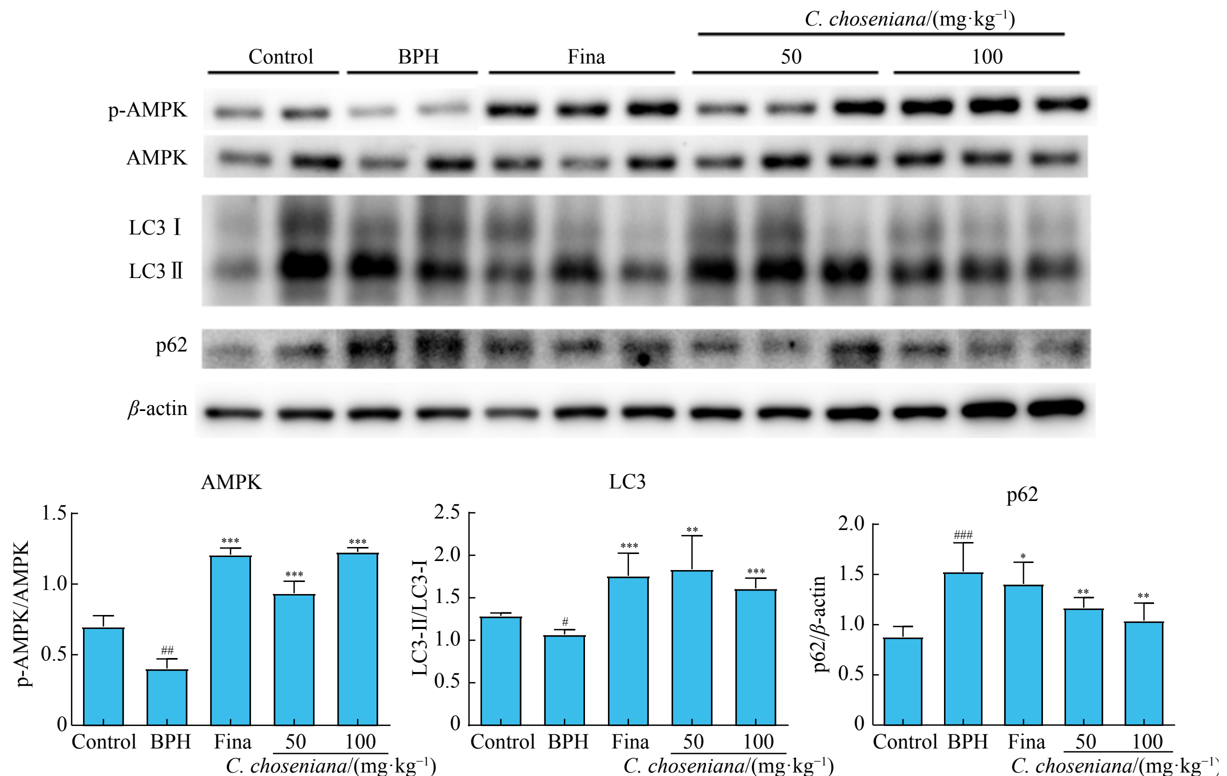

Fig. 7 Effects of *C. Choseniana* extract on autophagy in prostate tissue. Western blotting of LC3 and p62 in prostate tissue. BPH in rats was generated through daily subcutaneous injection of TP for 28 days. Control: PBS; BPH: TP; Fina: TP + Finasteride (10 mg·kg^{-1}, p.o.); the 50 group: TP + *C. choseniana* (50 mg·kg^{-1}, p.o.); and the 100 group: TP + *C. choseniana* (100 mg·kg^{-1}, p.o.). Data are presented as mean ± SEM (n = 5). $^{###}P < 0.001$ *vs* the control group; $^{***}P < 0.01$ and $^{*}P < 0.05$ *vs* the BPH group

the PSA core enhancer [27, 31]. PSA is a key marker for the diagnosis of BPH, which is an acidic nuclear protein mainly expressed in the *S* phase of the cell cycle using a cell proliferation marker. The levels of PSA and PCNA increased in BPH and prostatic cancer [32, 33].

In our study, BPH activated phospho-Akt and nuclear NF-*κ*B after dissociation from I-*κ*B, increasing the expression of AR, PSA and PCNA compared with the control. In particular, the expression of phospho-Akt, nuclear NF-*κ*B, and cytosolic I-*κ*B was reduced in the *C. choseniana*-treated group. Following NF-*κ*B activation, the *C. choseniana* groups showed significant decreases in PSA and PCNA levels compared with the BPH group. The reason for reducing AR signaling in *C. choseniana* is that *C. choseniana* suppresses the activation of Akt phosphorylation and nuclear NF-*κ*B.

Moreover, NF-*κ*B promoted cell survival by inducing antiapoptotic molecules, including Bcl-2 [34]. Catz and Johnson [35] reported that NF-*κ*B/p65 and p50 complexes bound to the Bcl-2 promotor and induced the expression of Bcl-2 in LNCaP cells. In fact, in our study, parallel activation of NF-*κ*B increased the expression of pro-apoptotic markers, including Bax, and decreased the expression of anti-apoptotic markers, including Bcl-2. Furthermore, the Bax/Bcl-2 ratio increased in the *C. choseniana* groups, indicating the induction of apoptosis in the *C. choseniana* groups. TUNEL staining supported our Western blot results.

Furthermore, recent studies have showed that AR played a differential role in various cell death signaling pathways, inhibiting apoptosis, necrosis, and autophagy [36, 37]. Androgen deprivation and blocking AR induced AMPK activation, promoting autophagy [38]. During the autophagy process, LC3, which is present on the inner membrane of autophagosomes, binds to p62 to form autophagosome, and is then preferentially degraded during autophagy. Generally, an increase in LC3 and a decrease in p62 were characterized by autophagy flux [39, 40]. Our results showed that AMPK phosphorylation increased in the *C. chonsenia*-treated groups inducing autophagy. According to the AMPK phosphorylation, the expression of LC3 increased and the expression of p62 decreased, indicating autophagic flux induction in the *C. choseniana*-treated groups. In summary, *C. choseniana* induces apoptosis and autophagy by suppressing AR activation.

Conclusions

In conclusion, the present study demonstrates that *C. choseniana* extract alleviates testosterone-induced BPH, by suppressing 5*α* reductase and Akt/NF-*κ*B/AR signaling and inducing apoptosis and autophagy. These results suggest that *C. choseniana* probably contain potential phytomedicinal herbal agents to alleviate BPH.

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