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Asparagi radix alleviates testosterone-induced benign prostatic hyperplasia by inhibiting 5α-reductase activity and androgen receptor signaling pathway

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ABSTRACT

BACKGROUND/OBJECTIVES: Recently, herbal medicines have gained attention for the treatment of benign prostatic hyperplasia (BPH), a common disease in elderly men. In this study, we aimed to determine the effect of ethanol extract of *Asparagi radix* (EAR), which is traditionally used to treat various diseases, on BPH development using a testosterone-induced BPH model.

MATERIALS/METHODS: Testosterone propionate (TP)-treated Sprague–Dawley rats were used to establish a BPH model *in vivo*. EAR was orally administered along with TP, and finasteride was used as a positive control. All rats were sacrificed at the end of the experiment, and pathological changes in the prostate tissue and levels of key biomarkers associated with BPH pathogenesis were assessed.

RESULTS: Oral administration of EAR significantly inhibited TP-induced BPH by reducing the prostate weight, lumen size, and epithelial thickness in a concentration-dependent manner. EAR also significantly abrogated the expression of 5α-reductase type 2 (SRD5A2), proliferating cell nuclear antigen, and prostate-specific antigen (PSA) induced by TP. Additionally, serum levels of testosterone, dihydrotestosterone, and PSA were elevated in the TP-induced group but decreased in the EAR-treated group. EAR also decreased the expression levels of the androgen receptor (AR) and its coactivators in TP-induced BPH model rats.

CONCLUSION: Our findings revealed that EAR protected against BPH by inhibiting 5α-reductase activity and AR signaling pathway, suggesting its potential for BPH treatment.

Keywords: *Asparagus*; benign prostatic hyperplasia; androgen receptor; SRD5A2; prostate-specific antigen

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Conflict of Interest

The authors declare no potential conflicts of interests.

Author Contributions

Conceptualization: Hwangbo H, Cha HJ, Kim GY, Choi YH; Data curation: Kim TH, Kim HS, Moon SK; Formal analysis: Hwangbo H, Kim HS, Moon SK, Kim GY; Investigation: Kim MY, Ji SY, Kim DH, Noh JS, Kim TH; Methodology: Hwangbo H, Kim MY, Ji SY, Kim DH, Noh JS; Project administration: Kim GY, Choi YH; Supervision: Choi YH; Writing - original draft: Hwangbo H, Kim GY, Choi YH; Writing - review & editing: Hwangbo H, Kim GY, Choi YH.

INTRODUCTION

Benign prostatic hyperplasia (BPH), which is primarily characterized by prostate enlargement and urinary tract dysfunction, is a common age-related disease among elderly men worldwide [1,2]. Although many studies have investigated the causes of BPH, with androgen signaling being the most popular, its exact molecular pathogenesis remains unclear. In a normal prostate, the androgen receptor (AR) is typically saturated with low levels of androgens, testosterone, dihydrotestosterone (DHT), and AR-dependent products, such as prostate-specific antigen (PSA) [3,4]. DHT, converted from testosterone by 5α-reductase (SRD5A2), has a much higher affinity for AR than testosterone and preferentially binds to DHT in the stromal and glandular epithelial cells of the prostate. AR bound to DHT subsequently translocates to the nucleus, forms a complex with its co-activators, and stimulates the transcriptional activity of genes involved in cell proliferation, thereby accelerating prostatic hypertrophy [5,6]. Transurethral resection, involving the removal of a part of the prostate, is an effective treatment for BPH. However, it leads to various side effects, such as urinary incontinence and bleeding, in elderly patients [7]. Therefore, 5α-reductase inhibitors, which block the conversion of testosterone to DHT, and AR blockers, which relax the smooth muscles of the prostate or reduce muscle tension in the bladder to facilitate the flow of urine, are widely used [5,8]. Androgen-targeting drugs are no longer used to treat BPH due to adverse liver reactions resulting in severe hepatotoxicity [9]. The 5α-reductase inhibitors also exert various side effects, such as sexual dysfunction [10,11]. Therefore, herbal medicines are currently being explored as effective alternatives for BPH treatment.

Many studies have investigated the biological effects, including antioxidant, anti-inflammatory, anti-diabetic, neuroprotective, and anti-aging effects, of *Asparagi radix*, the root of *Asparagus cochinchinensis* (Lour.) Merr., and its components [12-16]. Xiong *et al.* [17] demonstrated that the aqueous extract and polysaccharides of *A. radix* significantly improve the superoxide dismutase activity and spleen index but reduce the malondialdehyde content, thereby delaying aging. Lei *et al.* [18,19] showed that the water extract of *A. cochinchinensis* shoots and roots exhibits potent radical scavenging activity and protects against age-specific ultrastructural alterations in the brain, kidneys, liver, heart, and lungs in a D-galactose-induced aging mouse model. Kim *et al.* [20] revealed that the hot water extract of *A. radix* decreases the serum inflammatory cytokine and corticosterone levels, reduce depression-like behavior, and alleviate anxiety symptoms an *in vivo* menopausal depression model established after oophorectomy by regulating the brain-derived neurotrophic factor–tropomyosin receptor kinase B signaling pathway. Furthermore, Lee *et al.* [21] suggested that the *A. radix* aqueous extract delays the pathological progression of Alzheimer's disease (AD), a representative age-related cognitive impairment disease, by suppressing oxidative stress and increasing the nerve growth factor secretion in an amyloid β-induced AD model. However, whether *A. radix* alleviates BPH remains unknown. Therefore, in this study, as part of our ongoing research to discover beneficial herbs for BPH treatment, we demonstrated the inhibitory effects of the ethanol extract of *A. radix* (EAR) on testosterone propionate (TP)-induced BPH model Sprague–Dawley rats for the first time. Additionally, we assessed the therapeutic potential of *A. radix* for BPH.

MATERIALS AND METHODS

Preparation of EAR

Dried *A. radix* kindly donated by Dong-eui Korean Medical Center (Busan, Korea) was used to prepare EAR, as previously described [22]. Briefly, the dried materials were pulverized, powdered, soaked in 70% ethanol, and extracted at 50°C for 48 h. To remove the debris, the liquid extracts were filtered, concentrated using the Hei-VAP Rotary Evaporator (Heidolph, Schwabach, Germany), and freeze-dried using a freeze dryer (Vision Biotech, Kimpo, Korea). The lyophilized powder (EAR) was further diluted in sterilized water to appropriate concentrations and administered to Sprague–Dawley rats.

Animals and treatment

Male Sprague–Dawley rats (age, 6-week-old; weight, 220 ± 20 g; Samtako Bio Korea, Osan, Korea) were used to establish an *in vivo* BPH model. They were provided ad libitum access to water and food and kept in an animal facility under a controlled environment (23 ± 2°C temperature, 12/12 h light/dark cycle, and 55 ± 9% humidity). All animal experiments were conducted according to the regulations of the Animal Experiment Ethics Committee of Dong-eui University (approval number: R2023-026). TP-induced BPH model was established using Sprague–Dawley rats, as previously described [23]. After 1 week of adaptation before the start of the experiment, the rats were randomly divided into 5 groups, as previously reported [23]: 1) normal group (normal, n = 8), 2) 3 mg/kg TP (Tokyo Chemical Industry Co., Tokyo, Japan; BPH, n = 8), 3) 3 mg/kg TP and 200 mg/mL EAR (EAR1, n = 8), 4) 3 mg/kg TP and 400 mg/mL EAR (EAR2, n = 8), and 5) 3 mg/kg TP and 5 mg/kg finasteride (FINA; Sigma-Aldrich, St. Louis, MO, USA; n = 8) or positive control (PC) groups (**Table 1**). Animals were injected intraperitoneally with TP and FINA and administered orally with EAR. After 7 wk, blood samples were taken from the heart under anesthesia to analyze the serum levels of PSA, steroid 5α-reductase type 2 (SRD5A2), DHT, and testosterone via enzyme-linked immunosorbent assay (ELISA). During the experimental period, changes in the body weight of rats were weekly examined. After the experiment, weights of the prostate, liver, heart, spleen, kidneys, and lungs were measured. The prostate and testes were stored at –80°C for histological analysis and immunohistochemistry (IHC).

Histological examination

For histological analysis of the prostate and testicular tissues, hematoxylin and eosin (H&E) staining was performed, as previously described [24]. Briefly, the prostate and testicular tissues were fixed with 10% formalin solution (Junsei Chemical Co., Ltd., Tokyo, Japan) for more than 24 h at room temperature, washed with distilled water, and embedded with paraffin. Then, the tissues were cut into 4-μm thick slices, and paraffin was removed. Finally, the slices were stained with H&E (Sigma-Aldrich), and staining intensity was measured using the EVOS Cell Imaging Systems (Thermo Fisher Scientific, Waltham, MA, USA).

Table 1. Different BPH groups treated with the EAR

Groups	Injection	Administration	Duration
Normal	Corn oil	Sterilized water	8 wk
BPH	TP (3 mg/kg body weight/day)	Sterilized water	8 wk
EAR1	TP (3 mg/kg body weight/day)	EAR (200 mg/kg body weight/day)	8 wk
EAR2	TP (3 mg/kg body weight/day)	EAR (400 mg/kg body weight/day)	8 wk
PC	TP (3 mg/kg body weight/day)	FINA (5 mg/kg body weight/day)	8 wk

BPH, benign prostatic hyperplasia; EAR, ethanol extract of *Asparagi radix*; Normal group, corn oil injection and sterilized water fed group; BPH group, TP injection and sterilized water fed group; EAR1 group, TP injection and 200 mg/kg EAR fed group; EAR2 group, TP injection and 400 mg/kg EAR fed group; PC group, TP and FINA injection group; TP, testosterone propionate; PC, positive control; FINA, finasteride.

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IHC analysis

For immunostaining of the prostate tissue, deparaffinized and hydrated tissue sections were treated with a blocking solution for 20 min method to inhibit non-specific binding, as previously described [25]. The tissue sections were incubated with specific primary antibodies at 4°C overnight, followed by incubation with the secondary antibody (VectorLabs, Burlingame, CA, USA) for 30 min and then with the avidin-biotin conjugate reagent (Thermo Fisher Scientific) for 20 min. After mixing with 3,3'-diaminobenzidine tetrahydrochloride (DAB kit; Vector Biolabs, Malvern, PA, USA), images were acquired using the EVOS Cell Imaging System.

ELISA

To measure the levels of PSA, SRD5A2, DHT, and testosterone in the blood, serum samples were obtained from the heart of Sprague–Dawley rats. Then, the supernatant and serum samples were added to microtiter plates pre-coated with target-specific capture antibodies and analyzed using commercial ELISA kits (PSA: LifeSpan Biosciences, Inc., Seattle, WA, USA; DHT: MyBioSource Inc., San Diego, CA, USA; testosterone: BioVision Inc., Milpitas, CA, USA; SRD5A2: Cloud-Clone Corp., Katy, TX, USA), according to the manufacturers' recommendations. Then, absorbance of each sample was measured at 450 nm using a microplate reader (BioTek Instruments, Winooski, VT, USA) installed at TRCORE, Dong-eui University (Busan, Korea), and a standard curve was plotted.

Statistical analysis

Experimental results are expressed as the mean ± SD. Statistical significance was determined using one-way analysis of variance followed by Tukey's post-hoc test with the GraphPad Prism software (GraphPad Software, Inc., La Jolla, CA, USA). Statistical significance was set at $P < 0.05$.

RESULTS

EAR alleviates prostate hypertrophy in TP-induced BPH model rats

To determine whether EAR alleviates BPH, BPH was induced in Sprague–Dawley rats via subcutaneous injections of TP for 7 wk. The prostate was significantly enlarged after TP treatment (**Fig. 1A**). Moreover, prostate weight was approximately 2-fold higher in the BPH group than in the normal group (**Fig. 1B**), confirming the successful establishment of TP-induced BPH model *in vivo*. Although no significant differences were observed in the EAR1 group, strong inhibitory effect on prostatic hypertrophy was observed in the EAR2 group, which was lower than that in the PC group (**Fig. 1A and B**). Furthermore, the prostate index was significantly lower in the EAR2 group than in the BPH model rats (**Fig. 1C**). Body weight was measured weekly during the experimental period. Compared to the normal group, a slight weight loss was observed in all experimental groups. However, no significant differences were detected among the treatment groups (**Fig. 1D**). In addition, weights of organs, including the heart, liver, lungs, kidneys, and spleen, were measured at the end of the experiment, and no significant differences were observed between the normal and treatment groups (**Table 2**). These results suggest that EAR alleviates TP-induced prostate hypertrophy without significant toxicity.

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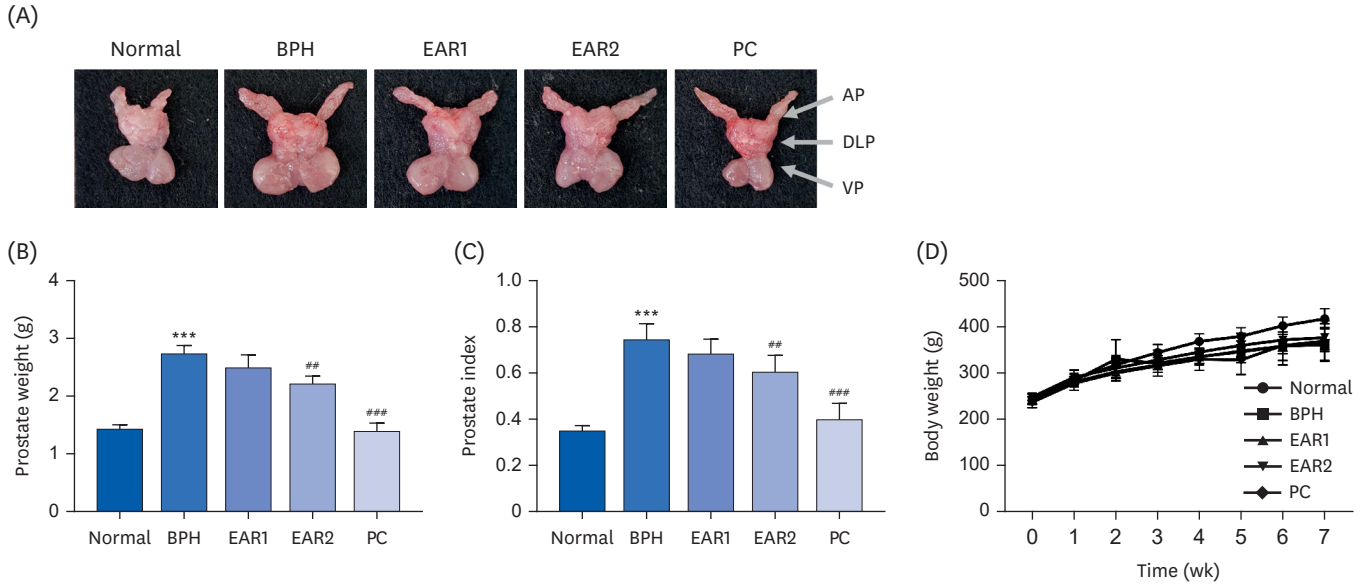

Fig. 1. Inhibitory effects of EAR on rats with TP-induced BPH. (A) Representative photographs showing the prostate size changes in each experimental group (VP, DLP, AP). (B, C) Changes in the prostate weight (B) and prostate index (C) of rats in 4 different experimental groups were assessed. (D) Body weight changes in rats during the experiment. Body weights of rats were recorded weekly.
Normal group, corn oil injection and sterilized water fed group; BPH group, TP injection and sterilized water fed group; EAR1 group, TP injection and 200 mg/kg EAR fed group; EAR2 group, TP injection and 400 mg/kg EAR fed group; PC group, TP and FINA injection group; BPH, benign prostatic hyperplasia; EAR, ethanol extract of *Asparagi radix*; TP, testosterone propionate; PC, positive control; AP, anterior prostate; DLP, dorsolateral prostate; VP, ventral prostate.
$^{***}P < 0.001$ vs. Normal; $^{\#\#}P < 0.01$, and $^{\#\#\#}P < 0.001$ vs. BPH (n = 6–8).

Table 2. Effects of EAR on the organ weights of TP-induced BPH model rats

Groups	Organ weights (g)									
	Heart	*P*-value	Lungs	*P*-value	Liver	*P*-value	Spleen	*P*-value	Kidneys	*P*-value
Normal	1.03 ± 0.04		1.51 ± 0.11		12.1 ± 1.2		0.67 ± 0.05		2.57 ± 0.21	
BPH	1.06 ± 0.05	0.8056	1.40 ± 0.16	0.601	10.8 ± 0.9	0.3268	0.61 ± 0.04	0.3115	2.66 ± 0.14	0.9147
EAR1	1.09 ± 0.06	0.1875	1.31 ± 0.19	0.127	11.3 ± 1.5	0.2167	0.60 ± 0.06	0.2444	2.70 ± 0.21	0.7917
EAR2	1.12 ± 0.05	0.0615	1.32 ± 0.16	0.153	10.8 ± 0.7	0.2586	0.64 ± 0.11	0.9438	2.60 ± 0.21	0.9986
PC	1.07 ± 0.05	0.5990	1.31 ± 0.22	0.080	11.4 ± 0.9	0.6378	0.58 ± 0.07	0.0697	2.68 ± 0.32	0.8555

Values are presented as mean ± SD.
EAR, ethanol extract of *Asparagi radix*; TP, testosterone propionate; BPH, benign prostatic hyperplasia; Normal group, corn oil injection and sterilized water fed group; BPH group, TP injection and sterilized water fed group; EAR1 group, TP injection and 200 mg/kg EAR fed group; EAR2 group, TP injection and 400 mg/kg EAR fed group; PC group, TP and FINA injection group; PC, positive control.

EAR inhibits the TP-induced histological changes in the prostate and testicular tissues of Sprague–Dawley rats

H&E staining was used to determine whether EAR inhibits the pathological signs of BPH. Compared to the normal group, BPH group showed the typical signs of BPH, including highly columnar and multilayered epithelia (**Fig. 2A**). EAR inhibited these pathological changes in a concentration-dependent manner, but did not completely abolish the changes as in the PC group. Decrease in the luminal area of the prostate tissue and increase in the thickness of prostate epithelial cells in the BPH group were significantly alleviated in the EAR1 and EAR2 groups (**Fig. 2B and C**). Moreover, compared to those in the normal group, the number of Sertoli cells and spermatogenesis within the lumen decreased in the BPH group. However, these phenomena partially recovered with EAR administration, and the recovery was more dominant in the EAR2 group than in the PC group (**Fig. 2D**). These data suggest that EAR not only suppresses the pathological damage to the prostate but also preserves the testis functions.

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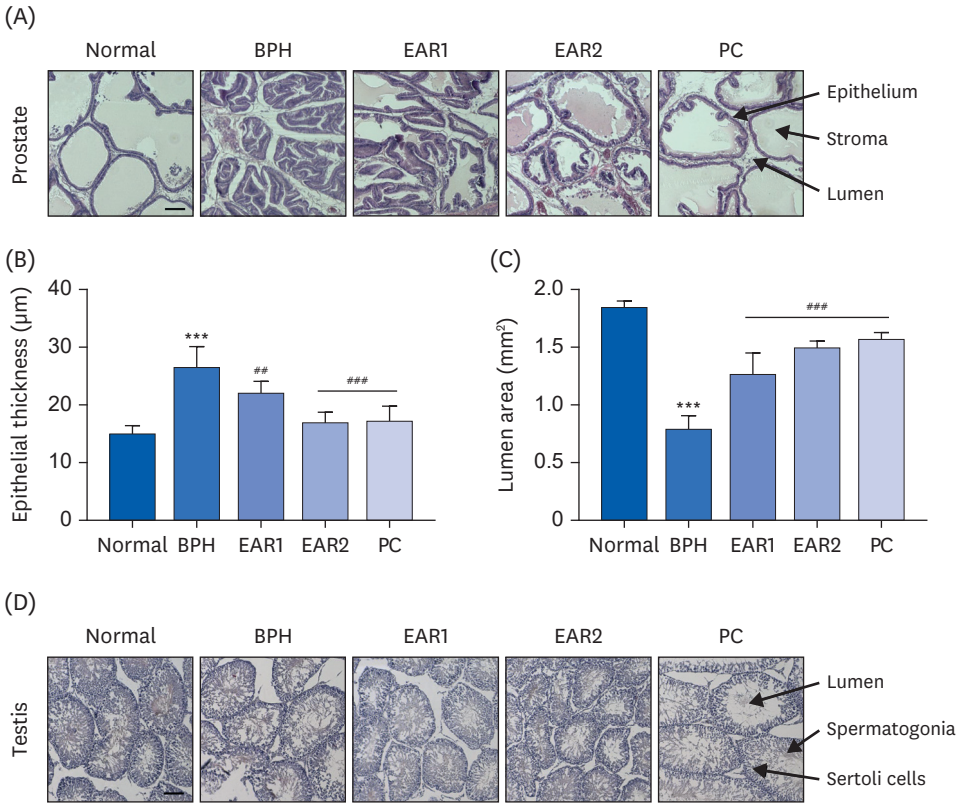

Fig. 2. EAR improves the histological changes in the prostate and testicular tissues of TP-induced BPH model rats. (A, E) Representative images of hematoxylin and eosin-stained prostate (A) and testicular tissues (D). Scale bar = 100 μm. (B, C) Measured epithelial thickness (B) and lumen area (C) of the prostate tissue.
Normal group, corn oil injection and sterilized water fed group; BPH group, TP injection and sterilized water fed group; EAR1 group, TP injection and 200 mg/kg EAR fed group; EAR2 group, TP injection and 400 mg/kg EAR fed group; PC group, TP and FINA injection group; BPH, benign prostatic hyperplasia; EAR, ethanol extract of *Asparagi radix*; TP, testosterone propionate; PC, positive control.
$^{***}P < 0.001$ vs. Normal; $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ vs. BPH (n = 6).

EAR decreases the expression levels of proliferating cell nuclear antigen (PCNA) and PSA in TP-induced BPH model rats

Next, we examined the levels of PCNA, a representative cell proliferation marker, to investigate whether the recovery of histomorphological changes in the prostate by EAR was due to the decreased proliferation of prostate cells. IHC revealed that expression levels of PCNA were significantly upregulated in the prostate of BPH-induced rats; however, EAR reversed this effect in a concentration-dependent manner. The expression levels of PCNA were maintained at the normal levels in the PC group (**Fig. 3A and C**). To further evaluate the role of EAR in the activation of androgen signaling by DHT, we examined the changes in PSA levels. As shown in **Fig. 3B and D**, increase in PSA expression induced by DHT was reduced in an EAR dose-dependent manner. Serum ELISA revealed that PSA levels decreased with EAR treatment (**Fig. 3E**). Overall, these results suggest that EAR alleviates BPH by inhibiting prostate cell hyperproliferation and androgen signaling.

EAR attenuates the androgen signaling pathway and 5α-reductase activity in TP-induced BPH model rats

To elucidate the mechanisms by which EAR improves BPH, the role of AR signaling was assessed. AR expression levels were higher in the prostate tissue of the BPH group than in the prostate tissue of the normal group (**Fig. 4A and C**). Moreover, SRD5A2, an AR target, was

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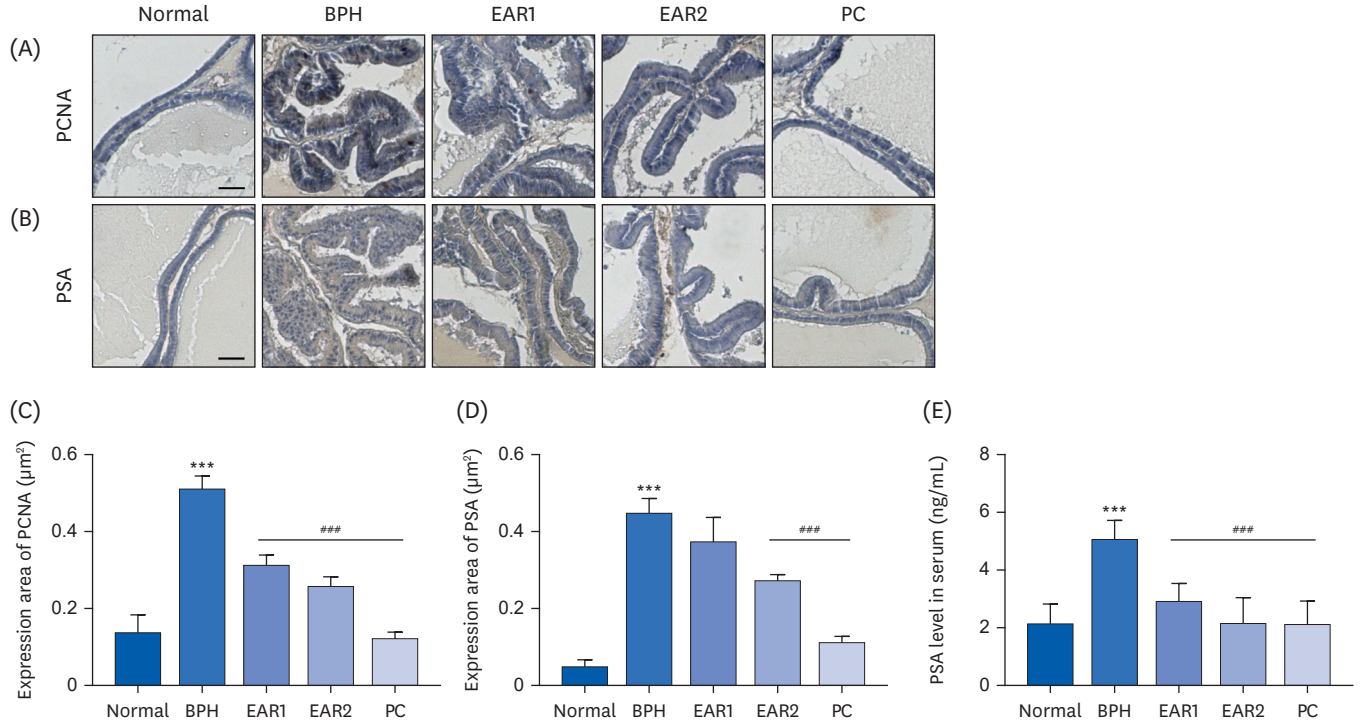

Fig. 3. Inhibitory effects of EAR on TP-induced increase in the expression levels of PCNA and PSA in the prostate of Sprague–Dawley rats. (A-D) Expression levels of PCNA and PSA in the prostate of each experimental group were examined via immunohistochemistry (A, B) and quantified (C, D). Scale bar = 50 μm. (E) PSA concentrations in the serum of each experimental group were quantified using a commercially available enzyme-linked immunosorbent assay kit.
Normal group, corn oil injection and sterilized water fed group; BPH group, TP injection and sterilized water fed group; EAR1 group, TP injection and 200 mg/kg EAR fed group; EAR2 group, TP injection and 400 mg/kg EAR fed group; PC group, TP and FINA injection group; BPH, benign prostatic hyperplasia; EAR, ethanol extract of *Asparagi radix*; TP, testosterone propionate; PC, positive control; PCNA, proliferating cell nuclear antigen; PSA, prostate specific antigen.
$^{***}P < 0.001$ vs. Normal; $^{###}P < 0.001$ vs. BPH (n = 6).

strongly expressed in the BPH group (**Fig. 4B and D**). TP injection also increased the serum levels of SRD5A2 (**Fig. 4E**). However, these changes were attenuated by EAR a concentration-dependent manner, suggesting that EAR blocks the TP-induced activation of androgen signaling. We investigated whether the levels of DHT and testosterone were proportional to the increase in SRD5A2 expression by TP. As shown in **Fig. 4F and G**, DHT and testosterone levels were markedly increased in the BPH group. Interestingly, inhibitory effect of EAR on the TP-induced increase in testosterone and DHT levels was comparable to that observed in the PC group. Therefore, EAR not only reduced the testosterone levels but also blocked the conversion of testosterone to DHT by inactivating 5α-reductase.

EAR decreases the expression levels of AR and its coactivators in TP-induced BPH model rats

As EAR inhibits the expression of AR and its downstream factor, PSA, we hypothesized that it inhibits AR transactivation by interfering with the functions of AR coactivators. To verify this, we investigated the expression levels of AR-associated protein 70 (ARA70) and steroid receptor coactivator-1 (SRC1), the major coactivators of AR. Notably, expression levels of both proteins were increased in the prostate of BPH model rats (**Fig. 5**). Although the levels were lower in the EAR groups than in the PC group, the expression levels of both proteins gradually decreased as the concentration of EAR increased. These results suggest that the decrease in the expression of AR coactivators, ARA70 and SRC1, by EAR is related to the decrease in AR expression.

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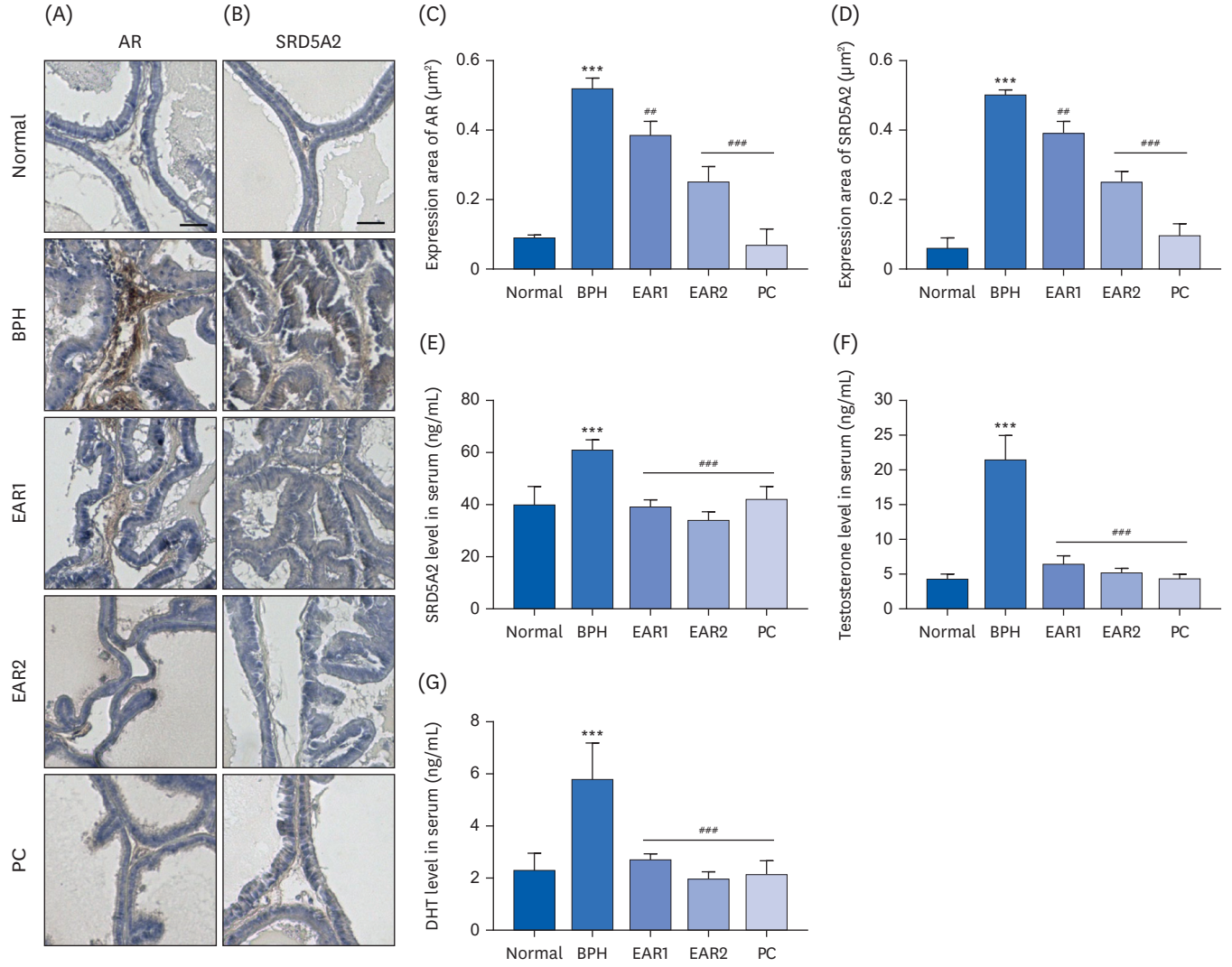

Fig. 4. Effects of EAR on the expression levels of AR and SRD5A2 in the prostate and SRD5A2, testosterone, and DHT levels in the serum of TP-induced BPH model rats. (A-D) Expression levels of AR and SRD5A2 in the prostate of each experimental group were examined via immunohistochemistry (A, B) and quantified (C, D). Scale bar = 50 μm. (E) Serum levels of SRD5A2, testosterone, and DHT in each experimental group were quantified using commercially available enzyme-linked immunosorbent assay kits.
AR, androgen receptor; SRD5A2, steroid 5α-reductase type 2; Normal group, corn oil injection and sterilized water fed group; BPH group, TP injection and sterilized water fed group; EAR1 group, TP injection and 200 mg/kg EAR fed group; EAR2 group, TP injection and 400 mg/kg EAR fed group; PC group, TP and FINA injection group; BPH, benign prostatic hyperplasia; EAR, ethanol extract of *Asparagi radix*; TP, testosterone propionate; PC, positive control; DHT, dihydrotestosterone.
$^{***}P < 0.001$ vs. Normal; $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ vs. BPH (n = 6).

DISCUSSION

The prostate exhibits male hormone-dependent proliferation and function. Excessive secretion of androgens promotes the proliferation of prostate epithelial and stromal cells, playing a crucial role in the development of BPH, a benign proliferative disorder [3,4]. However, the specific molecular mechanisms underlying BPH remain unclear. Blocking AR signaling may be beneficial for BPH treatment [3,8]. To evaluate the effect of EAR on BPH, we established a TP-induced BPH rat model and used FINA as aa PC in this study. This *in vivo* BPH model is widely used owing to its similarity to patients with BPH [26,27]. Compared with normal rats, the prostate size, weight, and prostate index were significantly increased

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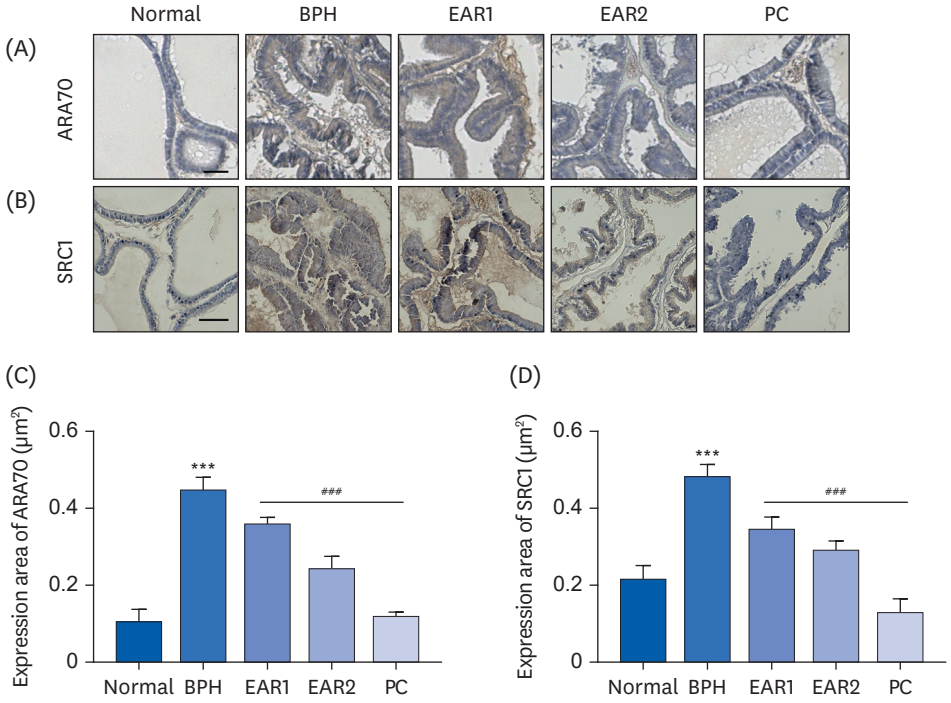

Fig. 5. Inhibitory effects of EAR on TP-induced increase in the expression levels of ARA70 and SRC1 in the prostate of Sprague–Dawley rats. Expression levels of ARA70 and SRC1 in the prostate of each experimental group were examined via immunohistochemistry (A, B) and quantified (C, D). Scale bar = 50 μm.
Normal group, corn oil injection and sterilized water fed group; BPH group, TP injection and sterilized water fed group; EAR1 group, TP injection and 200 mg/kg EAR fed group; EAR2 group, TP injection and 400 mg/kg EAR fed group; PC group, TP and FINA injection group; BPH, benign prostatic hyperplasia; EAR, ethanol extract of *Asparagi radix*; TP, testosterone propionate; PC, positive control; ARA70, androgen receptor-associated protein 70; SRC1, steroid receptor coactivator-1.
$^{***}P < 0.001$ vs. Normal; $^{###}P < 0.001$ vs. BPH (n = 6).

in the TP-induced BPH model rats, confirming the successful establishment of the BPH model. Consistent with our previous findings [23], histological analysis revealed that BPH model rats had a lower luminal area and higher epithelial cell thickness than the control rats. However, these BPH characteristics were markedly diminished in the EAR groups, albeit to a lesser extent than that in the PC group, highlighting the inhibitory effects of EAR on BPH. This may be because EAR suppresses the hyperproliferation of prostate cells in rats with BPH. Indeed, this was confirmed by the lower expression levels of PCNA in the EAR treatment group than in the BPH group. PCNA is a key indicator of cell proliferation in BPH prostate tissue. Decreased expression of this protein indicates BPH recovery [16,23]. Secretion of seminal plasma from the prostate is essential for normal sperm production; however, the testicular function is decreased in BPH [28,29]. Here, rats with TP-induced BPH exhibited other pathological findings, such as deterioration of spermatogenesis. Although 5α-reductase inhibitors, such as FINA, improve the symptoms of BPH, they cannot improve testicular damage and may even worsen it [5,11]. In this study, EAR improved the TP-induced pathological injury in testicular tissues. However, whether it alleviates the adverse side effects of FINA warrants further investigation.

Here, we investigated the mechanisms by which EAR suppressed BPH. We found that EAR significantly ameliorated the TP-induced expression of PSA in the prostate and reduced its levels in the serum. PSA is an androgen-targeting gene that serves as a key marker for the diagnosis of BPH and prostate cancer [30,31]. Therefore, our results suggest that EAR

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could inhibit BPH induced by TP by lowering the activity of AR signaling, and that EAR significantly reduced TP-induced expression of AR. Testosterone and DHT are involved in the development of male reproductive organs and play key roles in the hyperproliferation of prostate epithelial and stromal cells [3,4]. Therefore, increased serum levels of both hormones are closely associated with prostate growth and BPH development. However, compared to testosterone, DHT, which is converted from circulating testosterone by 5α-reductase, is detected at much higher levels in the serum of men with BPH of similar age than in healthy men [1,10]. Moreover, because DHT has a much higher affinity for AR than testosterone, 5α-reductase has been a potential target for the development of BPH inhibitors [5,6]. In this study, serum levels of testosterone and DHT were markedly higher in the TP-induced BPH model group than in the normal group. However, EAR significantly lowered the levels of both hormones, similar to FINA in the PC group. Furthermore, EAR reduced the SRD5A2 levels, which are elevated in BPH, to the levels observed in the normal and PC groups, confirming its 5α reductase inhibitory function similar to FINA. These results suggest EAR as a natural alternative to 5α reductase inhibitors, such as FINA and dutasteride, which are synthetic drugs causing serious side effects.

DHT–AR complex, formed with a high affinity for DHT, migrates to the nucleus and binds to androgen response elements (AREs), which are targeted by AR, activating genes encoding PSA and growth factors required for prostate cell proliferation [4,6]. In the nucleus, interactions of the DHT–AR complex with AREs to regulate gene expression require AR coactivators, such as ARA70 and SRC1, which do not directly bind to DNA [32,33]. ARA70, which was first identified as an AR-specific coregulator, increases the AR stability and expression and induces its nuclear translocation, thereby enhancing its transcriptional activity [34,35]; it also directly increases the activity of the PSA promoter [36]. SRC1 is the first phosphorylated compound known to regulate steroid receptors that serves as a coactivator and enhances the transcriptional activity of AR in a ligand-dependent manner [37,38]. It also significantly affects the transcriptional activity of AR signaling target genes, including PSA and growth factors, to promote BPH [3,5]. Therefore, inhibiting the expression of AR coactivators or inducing their dissociation from AR may suppress the development of not only BPH but also prostate cancer [37,39]. In this study, EAR suppressed the expression of SRC1 and ARA70 in rats with TP-induced BPH. Our results showing a decrease in AR and its coactivators by EAR suggest that it is related to the inactivation of the DHT-AR complex. However, further studies are needed to determine whether EAR can improve the efficacy of anti-androgen therapy for BPH by inhibiting the expression of these coactivators.

In summary, to the best of our knowledge, this study is the first to demonstrate the protective effects of *A. radix* against BPH. In rats with TP-induced BPH, EAR reduced the prostate size and weight and improved the histopathological deformations of the testes and prostate. Mechanistically, EAR is a viable alternative to 5α-reductase inhibitors and anti-androgens to control the DHT–AR axis that plays crucial roles in prostate development and growth (**Fig. 6**). However, further comprehensive analyses and safety studies are necessary to validate our findings. Nevertheless, our results highlight EAR as a promising therapeutic agent for BPH.

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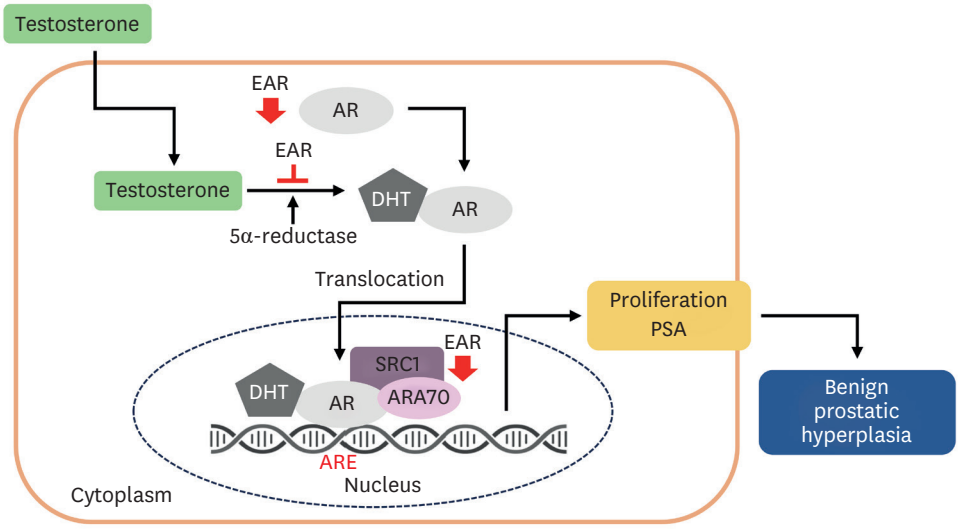

Fig. 6. Schematic of the mechanism underlying EAR-mediated alleviation of TP-induced BPH.
EAR, ethanol extract of *Asparagi radix*; AR, androgen receptor; DHT, dihydrotestosterone; ARA70, androgen receptor-associated protein 70; SRC1, steroid receptor coactivator-1; ARE, androgen response elements; PSA, prostate specific antigen.

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