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ORIGINAL RESEARCH

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Euodia rutaecarpa fruit attenuates testosterone-induced benign prostatic hyperplasia in rats by inhibiting 5α-reductase activity and androgen receptor signaling pathway

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Abstract

In this study, effect of an ethanol extract of *Euodia rutaecarpa* fruit (EER), known to have various pharmacological effects, on benign prostatic hyperplasia (BPH) was evaluated. To induce BPH in an *in vivo* animal model, testosterone propionate (TP) was injected to rats. EER was administered orally with TP injection. Finasteride, a 5α-reductase inhibitor, was used as a positive control. After all mice were sacrificed at the end of the experiment, pathological changes in prostate tissues and levels of key biomarkers involved in BPH development were assessed. Oral administration of EER significantly suppressed TP-induced BPH by diminishing prostate weight, lumen size and epithelial thickness. EER also abrogated the expression of prostate-specific antigen, proliferating cell nuclear antigen, and 5α-reductase type 2 induced by TP. In addition, serum levels of testosterone, dihydrotestosterone (DHT) and prostate specific antigen were elevated in TP challenged rats but decreased in EER-administered rats. Moreover, the improvement effect of EER on TP-induced BPH was associated with decreased expression of androgen receptor (AR) and its coactivators. The current findings show that EER can protect against BPH by attenuating the activation of 5α-reductase and inhibiting the AR signaling pathway, suggesting that EER has great potential in blocking BPH pathogenesis.

Keywords

Euodia rutaecarpa fruit; Benign prostatic hyperplasia; Androgen receptor; Dihydrotestosterone; 5α-reductase

1. Introduction

Evodia rutaecarpa (Juss.) Benth., a plant belonging to the Rutaceae family, is widely used as one of the major medicinal herbs in traditional medicine in East Asia, including Korea. The fruit of this plant (*Evodia fructus*) has been prescribed to promote blood purification, relieve pain and aid gastrointestinal function [1, 2]. Recent studies have also suggested that extracts and bioactive components of this fruit possess pharmacological properties against various diseases, including anti-inflammatory, antioxidant and anticancer effects [3–7]. These beneficial effects are due to the action of chemical components found in *Evodia fructus*, including alkaloids, terpenoids, flavonoids, essential oils and so on [1, 2, 8]. Importantly, there is growing evidence that extracts of *Evodia fructus* and their constituents can significantly reduce the risk of a variety of age-related diseases, including neurodegenerative diseases, metabolic disorders, cardiovascular abnormalities, osteoarthritis, musculoskeletal disorders and immunosuppression [9–13]. For example, using an animal model of Alzheimer's disease, it has been demonstrated that neuroprotective effects of evodiamine, the major alkaloid of *Evodia fructus*, are associated with inhibition of cellular senescence and its potent anti-inflammatory and antioxidant properties [14–16].

Benign prostatic hyperplasia (BPH) is characterized by excessive enlargement of the prostate and urinary tract dysfunction. It is a prevalent age-related disease in men worldwide [17, 18]. Although the exact molecular pathogenesis of BPH remains unclear, one of the most widely known mechanism is a change in androgen signaling due to hormone imbalance. Of two major androgens, dihydrotestosterone (DHT) and testosterone, DHT converted from testosterone by two 5α-reductase isoenzymes (5α-reductase type 1 and 5α-reductase type 2) has a much higher affinity for binding to the androgen receptor (AR) than testosterone. Of the two 5α-reductase isoenzymes, 5α-reductase type 2 is a predominant isoenzyme that is more expressed in epithelial and stromal cells of the prostate than 5α-reductase type 1 [19, 20]. Type 2 5α-reductase is activated more in the prostate of BPH patients than in the prostate of healthy people. DHT levels are also increased in the prostate of BPH patients compared to those in healthy controls. These two

factors are recognized as strong biomarkers of BPH [21, 22]. AR, which binds to DHT and moves to the nucleus, forms a complex with a coactivator and then stimulates the transcriptional activity of genes including prostate specific antigen (PSA, a representative AR-dependent product) and proliferating cell nuclear antigen (PCNA, a biomarker of cell proliferation), thereby promoting prostatic hyperplasia [22, 23]. To date, the most effective treatment for BPH is transurethral resection, which removes part of the prostate. However, side effects such as urinary incontinence and bleeding may occur and the risk is high for elderly patients [24]. As an alternative to surgical treatment, α-adrenergic receptor blockers, which can relax smooth muscles of the prostate or reduce muscle tension in the bladder to facilitate urine flow, and 5α-reductase inhibitors, which can block the conversion of testosterone to DHT, have been used recently [23, 25]. However, 5α-reductase inhibitors also have various side effects, including sexual dysfunction. Anti-androgen targeting drugs are no longer used to treat BPH due to adverse liver reactions resulting in severe hepatotoxicity [26–28]. Therefore, there are increasing attempts to discover treatments for BPH from herbal medicines that have been used to treat various ailments for a long time. The potential of *E. rutaecarpa* for treating BPH was first reported by Park *et al.* [29]. Although their results were insufficient to provide evidence that the proliferation of BPH epithelial cells was inhibited by ethanol extract of *Evodia fructus*, which might be helpful in treating BPH, the fact that the activity of 5α-reductase *in vitro* was inhibited was a meaningful result. However, no studies have been conducted to provide direct evidence of whether *E. rutaecarpa* could alleviate BPH. Thus, the objective of this study was to investigate the efficacy of ethanol extract of *E. rutaecarpa* fruit (EER) in treating BPH for the first time. A testosterone propionate (TP)-induced BPH in Sprague-Dawley (SD) rat model was established. Finasteride (FINA), a 5α-reductase inhibitor, was used as a control.

2. Materials and methods

2.1 Chemicals and reagents

Male SD rats and all materials needed for animal rearing were purchased from Samtako Bio Korea (Osan, Republic of Korea). TP used in this study was obtained from Tokyo Chemical Industry Co. (#57-85-2, Tokyo, Japan). FINA was manufactured by Sigma-Aldrich (#98319-26-7, St. Louis, MO, USA). Formalin and hematoxylin-eosin (H&E) staining solutions for tissue staining were purchased from Junsei Chemical Co. Ltd. (#50-00-0, Tokyo, Japan) and Sigma-Aldrich (#517-28-2, St. Louis, MO, USA), respectively. Primary antibodies, secondary antibody (VECTASTAIN Elite ABC HRP Kit), avidin-biotin conjugate (ABC) reagent, and 3,3′-diaminobenzidine tetrahydrochloride hydrate (DAB Kit) used for immunohistochemistry (IHC) were provided by Santa Cruz Biotechnology, Inc. (PCNA, #sc-25280; PSA, #sc-7316; AR, #sc-7305; steroid 5α-reductase type 2 (SRD5A2), #sc-20659; AR-associated protein 70 (ARA70), #sc-373739; steroid receptor co-activator-1 (SRC1), #sc-136077, Santa Cruz, CA, USA), Vector Biolabs (#PK-6100, Burlingame, CA, USA), Thermo Fisher Scientific (#32020, Waltham, MA, USA) and VectorLabs (#SK-4100, Malvern, PA, USA), respectively. To measure amounts of PSA, testosterone, SRD5A2, and DHT in serum samples, enzyme-linked immunosorbent assay (ELISA) kits manufactured by LifeSpan Biosciences, Inc. (#LS-F3383, Seattle, WA, USA), Sigma-Aldrich (#SE120120, St. Louis, MO, USA), Cloud-Clone Corp. (#SEM285Ra, Katy, TX, USA), and Mybiosource Inc. (#MBS2502409, San Diego, CA, USA) were used.

2.2 EER preparation

Dried fruits of *E. rutaecarpa* used to prepare EER were kindly donated by Dong-Eui Korean Medical Center (Busan, Republic of Korea). In brief, to prepare EER according to a modified method [30], dried materials were pulverized, powdered, soaked in 70% ethanol, and extracted at 50 °C for 48 h. To remove debris, liquid extracts were filtered, concentrated using a Hei-VAP Rotary Evaporator (P/N: 571-01300-00, Heidolph, Schwabach, BY, Germany), and then freeze-dried using a freeze dryer (VS-4150ND, Vision Biotech, Kimpo, Republic of Korea). The lyophilized powder of EER was diluted in sterilized water to appropriate concentrations and administered to SD rats.

2.3 Animals and treatment

Male SD rats (6-week-old, weighing 220 ± 20 g, n = 30) were used in this study to derive an *in vivo* BPH model. They were provided *ad libitum* access to water and food in an animal facility with a temperature (23 ± 2 °C), light/dark cycle (12/12 h), and humidity (55 ± 9%) controlled environment. The TP-induced BPH model using SD rats was set up as previously described [31]. After one week of adaptation, rats were randomly divided into five groups by referring to a previous method [31]: ① control group (control), ② 3 mg/kg TP administration group (BPH), ③ 3 mg/kg TP and 200 mg/kg EER administration group (EER2), ④ 3 mg/kg TP and 400 mg/kg EER administration group (EER4), and ⑤ 3 mg/kg TP and 5 mg/kg FINA administration group (FINA) (Table 1). Each group consisted for six animals. The control group was treated with corn oil by subcutaneous injection and sterilized water by oral administration. The BPH group was administered with TP by subcutaneous injection. The treatment groups, EER2, EER4 and FINA, were administered with each treatment by oral with TP by subcutaneous injection. After 8 weeks, blood was taken from the heart and sacrificed under anesthesia using CO_2 gas inhalation to analyze serum levels of PSA, SRD5A2, DHT and testosterone by ELISA (Fig. 1A). During the experiment period, changes in rat's body weight were examined once a week. After the experiment was completed, weights of the prostate, heart, lung, liver, spleen and kidney were measured. Isolated prostates were stored at −80 °C for histological analysis or IHC.

2.4 Histological examination

For histological analysis of prostate tissue, H&E staining was performed with reference to a previous method [32]. Briefly, prostate tissues were fixed in 10% formalin solution for 24

TABLE 1. Group design for BPH treatment using EER.

Groups	Injection	Administration	Duration
Control	Corn oil	Sterilized water	8 wk
BPH	TP (3 mg/kg body weight/d)	Sterilized water	8 wk
EER2	TP (3 mg/kg body weight/d)	EER (200 mg/kg body weight/d)	8 wk
EER4	TP (3 mg/kg body weight/d)	EER (400 mg/kg body weight/d)	8 wk
FINA	TP (3 mg/kg body weight/d)	FINA (5 mg/kg body weight/d)	8 wk

BPH: benign prostatic hyperplasia; EER: Euodia rutaecarpa fruit; FINA: finasteride; TP: testosterone propionate.

h, washed with distilled water, and then embedded through dehydration, transparency and paraffin infiltration processes. Paraffin blocks were then made. Afterwards, the tissue was cut at a thickness of 4 μm using. After paraffin was removed, tissue sections were stained with H&E. The staining intensity was compared using a Cell Imaging System (EVOS M7000, Thermo Fisher Scientific).

2.5 IHC analysis

For immunostaining of prostate tissue, deparaffinized and hydrated tissue sections were treated with a blocking solution for 20 min to inhibit non-specific binding according to a previous method [33]. Primary antibodies corresponding to proteins to be detected were incubated with tissue sections at 4 ℃ overnight. Afterwards, tissue sections were incubated with a secondary antibody at room temperature for 30 min and then reacted with ABC reagent at room temperature for 20 min. Afterwards, stained tissues were developed with a DAB kit. Images were acquired using the EVOS Cell Imaging System.

2.6 ELISA

To measure amounts of PSA, SRD5A2, DHT and testosterone in the blood, serum separated from the heart of each SD rat was used. In brief, supernatants and serum prepared were added to microtiter plates precoated with target-specific capture antibodies to be analyzed using commercial ELISA kits according the manufacturer's recommendations. The absorbance of each sample was measured at 450 nm using an ELISA reader. The value for each sample was calculated using a standard curve.

2.7 Statistical analysis

Experimental results are expressed as mean ± standard deviation. Statistical significance between multiple treatment groups was determined with one-way analysis of variance followed by Tukey's *post-hoc* test using GraphPad Prism software version 8.4.2 (GraphPad Software, Inc., La Jolla, CA, USA) confirmed through testing. Statistical analysis was conducted to analyze differences in TP-induced BPH and effects of EER2, EER4 and FINA. Statistical significance was considered when the *p*-value was 0.05 or less.

3. Results

3.1 EER reduces prostate hypertrophy in rats with TP-induced BPH

To investigate whether EER could inhibit BPH, BPH was induced in SD rats through subcutaneous injection of TP. As shown in Fig. 1B, visual inspection revealed that TP resulted in significant enlargement of the prostate. In fact, the prostate weight was increased approximately 1.8-fold more in the BPH group compared to that in the control group (Fig. 1C), meaning that the BPH model was well established with TP injection. Although there was no significant inhibitory effect on prostate hypertrophy in the EER2 group, a significant inhibitory effect on prostate hypertrophy was observed in the EER4 group. This inhibitory effect was somewhat lower than that in the FINA group (Fig. 1C). The prostate index, which was increased in rats with TP-induced BPH, was significantly lowered in the EER4 group (Fig. 1D). Body weight was measured every week during the experimental period. Compared with the control group, all experimental groups showed a slight weight loss. However, no significant change in weight was detected in treatment groups (Table 2). In addition, weights of organs including the heart, lung, liver, spleen and kidney were measured at the end of the experiment. It was found that they were slightly decreased in the BPH group. However, they were not significantly changed in treatment groups (data not shown). These results indicate that EER can reduce TP-induced prostate hypertrophy without significant toxicity.

3.2 EER diminishes TP-induced histological changes in prostate tissues of SD rats

H&E staining was performed to examine whether EER could reduce pathological signs in prostates of rats with BPH. As shown in Fig. 2A, the prostate in the BPH group showed typical signs of BPH, including higher columnar epithelium and multilayered epithelial cells compared to the control group. However, these pathological changes were suppressed when the concentration of EER administered was increased, although the suppression was not as completely as shown in the FINA group. Additionally, the decrease in prostate lumen area and the increase in prostate epithelial cell thickness seen in the BPH group were significantly alleviated in both EER2 and EER4 groups (Fig. 2B,C).

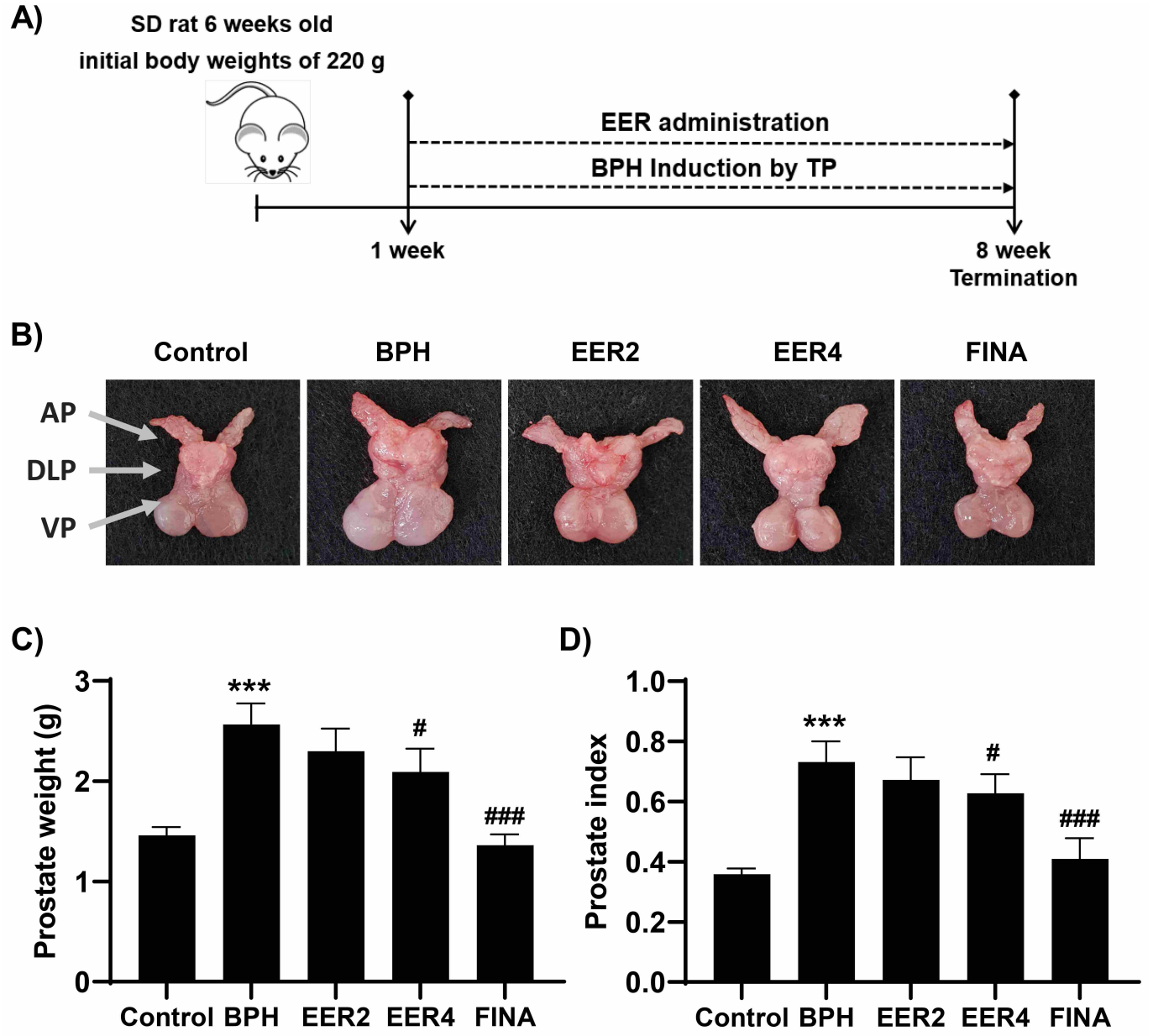

FIGURE 1. Effects of EER on prostatic hypertrophy in SD rats with TP-induced BPH. (A) Schematic diagram of the experimental procedure. (B) Representative photographs showing changes in prostate size in each experimental group (VP: ventral prostate; DLP: dorsolateral prostate; AP: anterior prostate). (C,D) Changes in prostate weight (C) and prostate index (D) (***$p < 0.001$ *vs.* Control; $^{\#}p < 0.05$ and $^{\#\#\#}p < 0.001$ *vs.* BPH). SD: Sprague-Dawley; EER: *Euodia rutaecarpa* fruit; BPH: benign prostatic hyperplasia; TP: testosterone propionate; FINA: finasteride.

TABLE 2. Changes in body weight, prostate weight and index.

Week		Control	BPH	EER2	EER4	FINA
Body	0	246.9 ± 8.1	242.3 ± 8.5	245.6 ± 9.7	240.5 ± 7.6	238.5 ± 11.5
Weight (g)	7	418.2 ± 21.7	362.6 ± 10.7**	364.0 ± 35.5	377.8 ± 29.9	370.2 ± 43.8
Prostate weight (g)		1.5 ± 0.1	2.6 ± 0.2***	2.3 ± 0.2	2.1 ± 0.2$^{\#}$	1.4 ± 0.1$^{\#\#\#}$
Prostate index		0.4 ± 0.02	0.7 ± 0.07***	0.7 ± 0.08	0.6 ± 0.06$^{\#}$	0.4 ± 0.07$^{\#\#\#}$

$^{**}p < 0.01$ and $^{***}p < 0.001$ vs. Control; $^{\#}p < 0.05$ and $^{\#\#\#}p < 0.001$ vs. BPH. BPH: benign prostatic hyperplasia; EER: Euodia rutaecarpa fruit; FINA: finasteride.

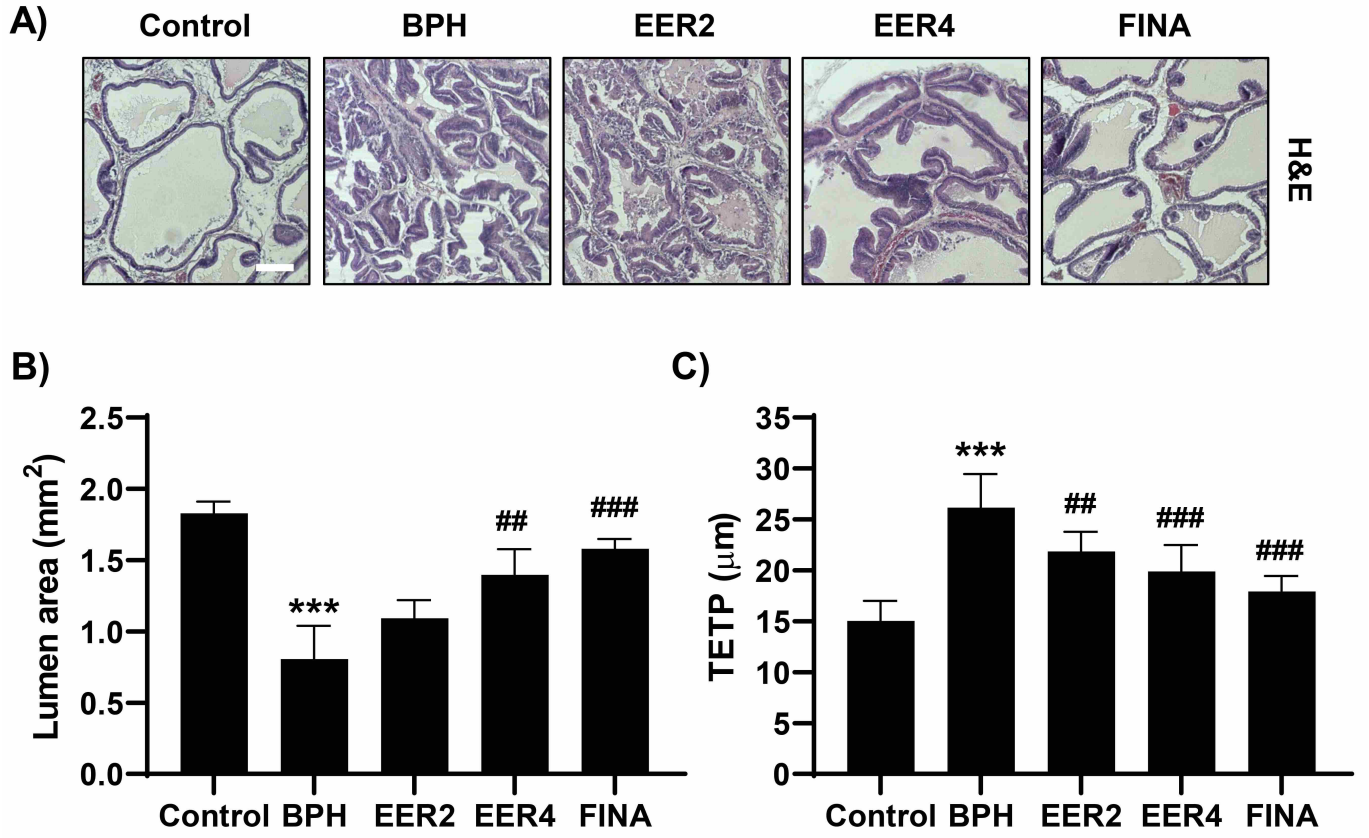

FIGURE 2. Attenuation of TP-induced histological changes in prostates of BPH rats induced by EER. (A) Representative images of H&E stained prostate. (B,C) Measured lumen area (B) and thickness of epithelium tissue from prostate (TETP, C) of prostate tissue (***$p < 0.001$ *vs.* Control; $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$ *vs.* BPH). BPH: benign prostatic hyperplasia; EER: *Euodia rutaecarpa* fruit; FINA: finasteride; H&E: hematoxylin-eosin.

3.3 EER lowers expression levels of PCNA and PSA increased in TP-treated SD rats

To determine whether the recovery of pathological changes in the prostate caused by EER was due to inhibition of proliferation of prostate cells, the expression of PCNA, a representative cell proliferation marker, was confirmed. IHC results indicated that the expression of this protein was significantly upregulated in prostates of BPH-induced rats (Fig. 3A,B), but significantly downregulated by EER at increased concentration and maintained at the control level in the FINA group. To further evaluate the role of EER in the activation of androgen signaling by DHT, the expression of PSA was examined. As shown in Fig. 3C,D, the increase in PSA expression by DHT was significantly restored by EER administration. Furthermore, ELISA results using serum also showed that PSA levels tended to decrease with EER treatment (Fig. 3E). These findings indicated that the inhibitory effect of BPH by EER was due to inhibition of hyperproliferation of prostate cells together with interference with androgen signaling.

3.4 EER weakens activation of androgen signaling and 5α-reductase in TP-induced BPH rats

To clarify the mechanisms by which EER could improve BPH, the role of AR signaling was assessed. As expected, levels of AR and SRD5A2 expression were elevated approximately 3.8- and 4.5-fold, respectively, in prostate tissues of the BPH group compared to those in the control group (Fig. 4A–C). TP injection also increased serum levels of SRD5A2 (Fig. 4D). However, these changes were attenuated by EER in a concentration-dependent manner in rats through feeding, suggesting that EER could block the activation of TP-induced androgen signaling. It was further investigated whether the activation of SRD5A2 by TP was attributable to changes in testosterone and DHT levels. As shown in Fig. 4E,F, their levels in the sera of the BPH group were significantly elevated. Interestingly, the inhibitory effect of EER on TP-induced increases in testosterone and DHT levels was similar to that in the FINA group. Therefore, EER not only could reduce testosterone levels, but also could inhibit the activity of 5α-reductase and block the conversion of testosterone to DHT.

3.5 EER attenuates expression of co-activators of AR in TP-induced BPH rats

Because EER repressed the expression of AR signaling, it is likely that EER could suppress AR transactivation by interfering with the function of AR co-activators. Therefore, we investigated alterations in expression of ARA70 and SRC1, the major co-activators of AR. We found that their expression levels were increased in the prostates of BPH rats (Fig. 5). Although the inhibitory effect of EER was somewhat lower than that of FINA, expression levels of both proteins were

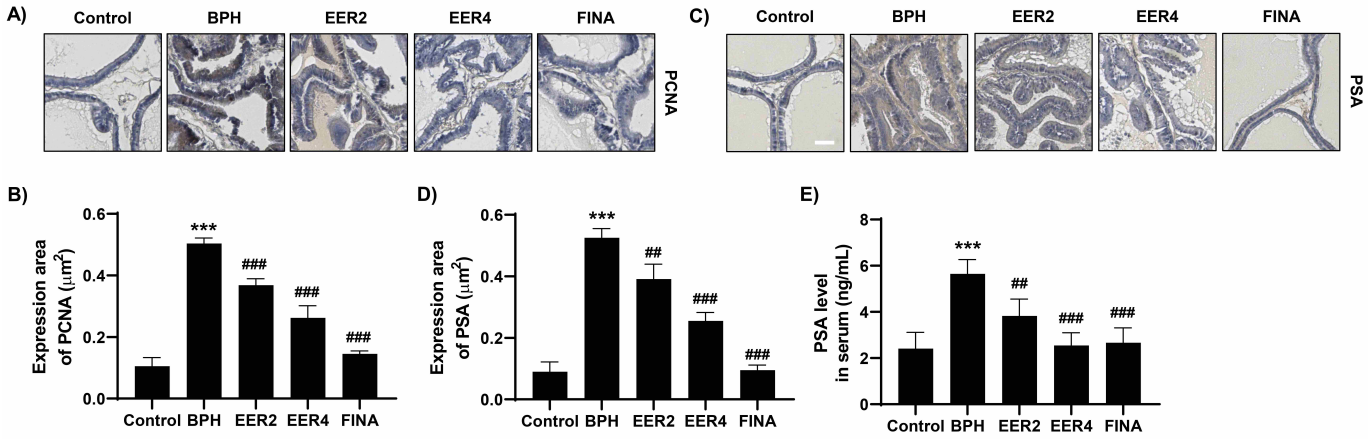

FIGURE 3. Inhibition of PCNA and PSA expression in prostates of TP-induced BPH rats increased by EER. (A–D) Expression levels of PCNA and PSA in prostate tissues of each experimental group examined through IHC (A,B) with their expression quantified (C,D, ***$p < 0.001$ *vs.* Control; $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$ *vs.* BPH). (E) Serum levels of PSA in each experimental group were quantified using a PSA ELISA kit (***$p < 0.001$ *vs.* Control; $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$ *vs.* BPH). BPH: benign prostatic hyperplasia; EER: *Euodia rutaecarpa* fruit; FINA: finasteride; PSA: prostate-specific antigen; PCNA: proliferating cell nuclear antigen.

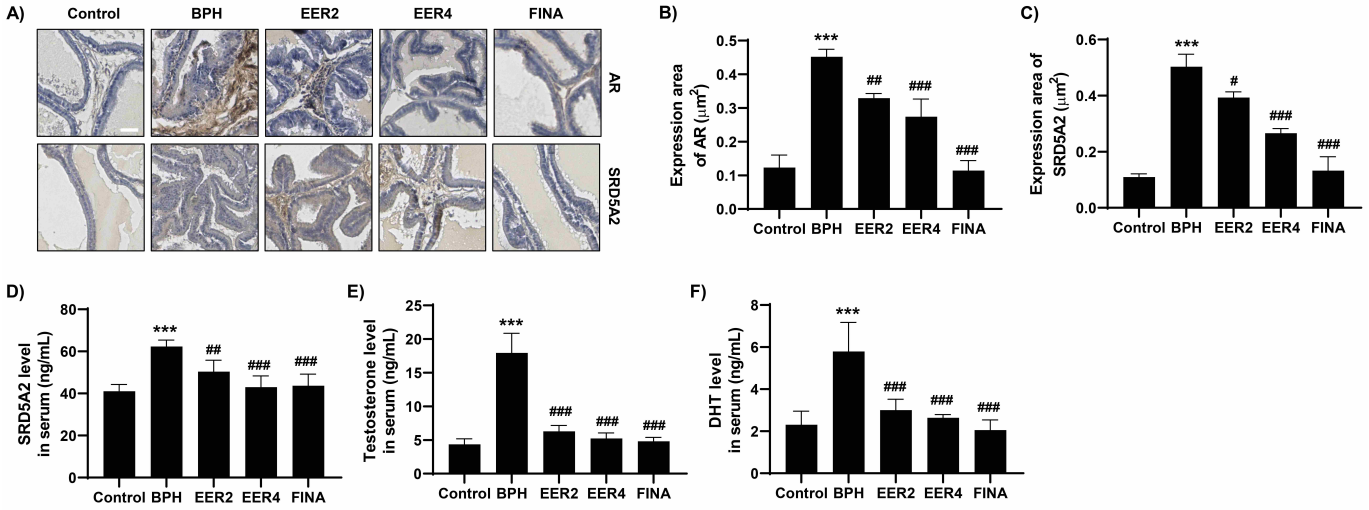

FIGURE 4. Inhibitory effect of EER on expression of AR and SRD5A2 in the prostate and serum levels of SRD5A2, testosterone, and HDT in TP-induced BPH rats. (A–C) Expression levels of AR and SRD5A2 in prostate tissues of each experimental group were examined through IHC (A) and their expression levels were quantified (B,C, ***$p < 0.001$ *vs.* Control; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$ *vs.* BPH). (D–F) Serum concentrations of SRD5A2 (D), testosterone (E), and HDT (F) in each experimental group were quantified using commercially available ELISA kits (***$p < 0.001$ *vs.* Control; $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$ *vs.* BPH). BPH: benign prostatic hyperplasia; EER: *Euodia rutaecarpa* fruit; FINA: finasteride; AR: androgen receptor; SRD5A2: steroid 5α-reductase type 2; DHT: dihydrotestosterone.

gradually decreased when the concentration of EER administered was increased, indicating that EER could interfere with TP-mediated transactivation of AR-dependent genes by inhibiting the expression of AR co-activators.

4. Discussion

Although the molecular mechanism of BPH induction remains unclear, excessive secretion of androgens is known to be able to enhance the proliferation of epithelial and stromal cells in the prostate, acting as a critical factor for the development of BPH [21, 22]. Thus, blocking AR signaling might be a crucial strategy for treating BPH [21, 25]. To evaluate the effect of EER on BPH, we established a TP-induced BPH animal model. This BPH animal model is widely used as an *in vivo* BPH model because it shows clinically similar pathological phenomena to BPH patients. In good agreement with previous findings, present results showed that prostate size, prostate weight and prostate index of rats mimicking the BPH environment with TP injection were significantly increased compared to those of control rats. Histological analysis showed that rats with BPH had lower luminal area and higher epithelial cell thickness, typical features of BPH, than control rats. However, these BPH characteristics were

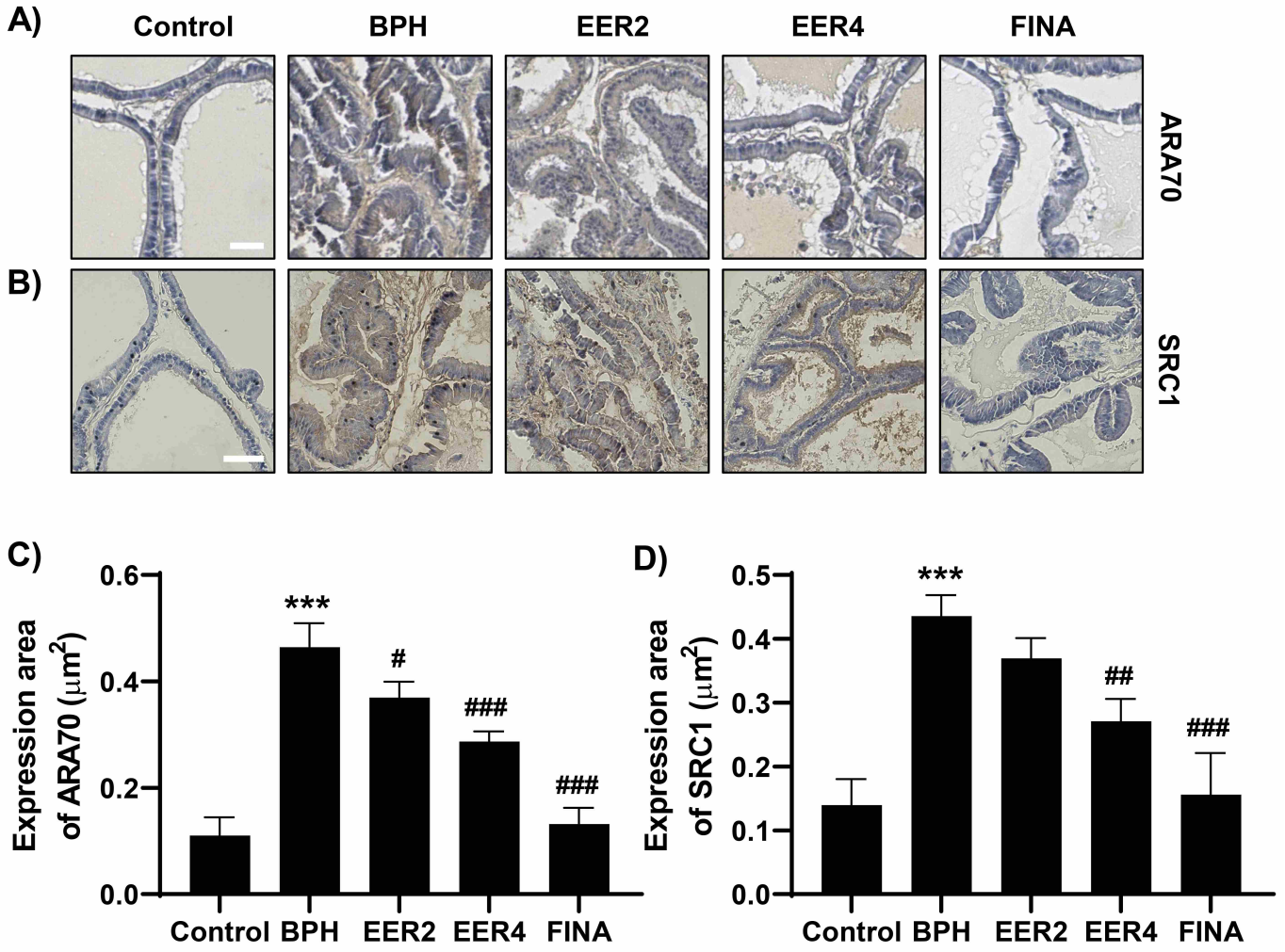

FIGURE 5. EER attenuates expression of ARA70 and SRC1 increased in prostates of TP-induced BPH rats. Expression levels of ARA70 and SRC1 in prostate tissues of each experimental group were examined through IHC (A,C) and quantified (B,D, ***$p < 0.001$ *vs*. Control; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ *vs*. BPH). BPH: benign prostatic hyperplasia; EER: *Euodia rutaecarpa* fruit; FINA: finasteride; ARA70: AR-associated protein 70; SRC1: steroid receptor co-activator-1.

markedly diminished in the EER administration group, albeit to a lesser extent than those in the FINA group, highlighting the effect of EER on prostatic hyperplasia. This might be because EER inhibited the hyperproliferation of prostate cells in rats with BPH. This was proven by the fact that the expression of PCNA induced in the prostate tissue of BPH rats was decreased in EER groups. Because PCNA is a key indicator of cell proliferation in BPH prostate tissues, decreased expression of this protein is widely considered evidence of BPH recovery [31, 34].

Meanwhile, PSA is an androgen target gene that serves as a key marker for diagnosing BPH and prostate cancer [35, 36]. In follow-up experiments conducted to unravel how EER suppressed BPH, we found that EER administration significantly ameliorated the increase of PSA expression in prostate tissues and sera of TP-induced BPH rats. These data support that EER could improve TP-induced BPH by lowering the activity of AR signaling. We found that EER significantly reduced TP-induced expression and secretion of AR. Androgens such as testosterone and DHT are involved in the development and function of male reproductive organs. They contribute to hyperproliferation of epithelial and stromal cells in the prostate [21, 22]. Therefore, increased levels of both testosterone and DHT in the serum are closely associated with prostate growth and development of BPH. However, compared to testosterone, DHT, which is converted from circulating testosterone by 5α-reductases, is detected at much higher levels in the sera of men with BPH of similar age than in healthy men without BPH [18, 26]. In addition, because the affinity of DHT for AR is much higher than that of testosterone, 5α-reductase has been a potential target for the development of BPH inhibitors [20, 23]. In the current study, levels of testosterone and DHT were markedly increased in the BPH group compared to those in the control group. However, EER administration significantly lowered TP-induced levels of both testosterone and DHT hormones, similar to results obtained with FINA used as a positive control (FINA group). Moreover, in EER groups, the level of SRD5A2 increased in the BPH group was maintained at a level similar to that in the control group and the FINA group, confirming that EER had a 5α-reductase 2 inhibitor function similar to FINA. These results suggest that EER has potential as a natural candidate for treating BPH to

replace 5α-reductase 2 inhibitors such as FINA or dutasteride, which are synthetic drugs that cause serious side effects.

The DHT-AR complex formed through high affinity for DHT can migrate into the nucleus to bind to androgen response elements (AREs), which are targeted by AR, activating genes encoding PSA and growth factors required for prostate cell proliferation [20, 22]. Subsequently, the interaction of the DHT-AR complex with the ARE to regulate gene expression in the nucleus requires the recruitment of AR coactivators such as ARA70 and SRC1 [37, 38]. ARA70 first identified as an AR-specific coregulator is involved in increasing AR expression, stability, and inducing nuclear translocation, thereby promoting AR transcriptional activity [39, 40]. SRC1 is known to be the first phosphorylated member to regulate steroid receptors. It is a coactivator that can enhance the transcriptional activity of AR in a ligand-dependent manner [41, 42]. SRC1 also significantly intervenes in the transcriptional activity of AR signaling target genes, including PSA, and growth factors to promote BPH [21, 23]. These findings suggest that suppressing the expression of AR coactivators or inducing their dissociation from AR could be a therapeutic strategy that not only can inhibit BPH, but also can inhibit the development of prostate cancer [41, 43]. In this study, EER suppressed the expression of both SRC1 and ARA70 in prostates of rats with TP-induced BPH, suggesting that EER might be able to alleviate the development of BPH by interfering with the binding of the DHT-AR complex to ARE. However, further studies are needed to explore how EER can inhibit the expression of these coactivators to improve the effectiveness of anti-androgen therapy for BPH.

This study was conducted with the aim to discover a new treatment for BPH from traditional herbal medicine. EER, an ethanol extract of *E. rutaecarpa* fruit (EER), was confirmed to be able to attenuate TP-induced prostatic hypertrophy and pathological changes. The current results imply that suppression of BPH development by EER was mediated through inactivation of 5α-reductase 2 and decreased expression of AR and its coactivators (Fig. 6). However, before applying EER to clinical trials for BPH, identification of major bioactive substances contained in EER and analysis of their mechanisms of action must be preceded.

5. Conclusions

In conclusion, EER administration reduced the increase in prostate size and weight and improved the histopathological changes of the prostate in TP-induced BPH rats, providing the first evidence that *E. rutaecarpa* fruit extract might be effective in the treatment of BPH. Mechanistically, EER is a viable alternative to anti-androgen activators and 5α-reductase 2 inhibitors for modulating the DHT-AR axis, which plays a critical role in the development and growth of the prostate. Although further clinical trials based on component analysis and safety studies of EER are needed, the results of this study suggest that EER has the potential to attenuate the initiation and progression of BPH.

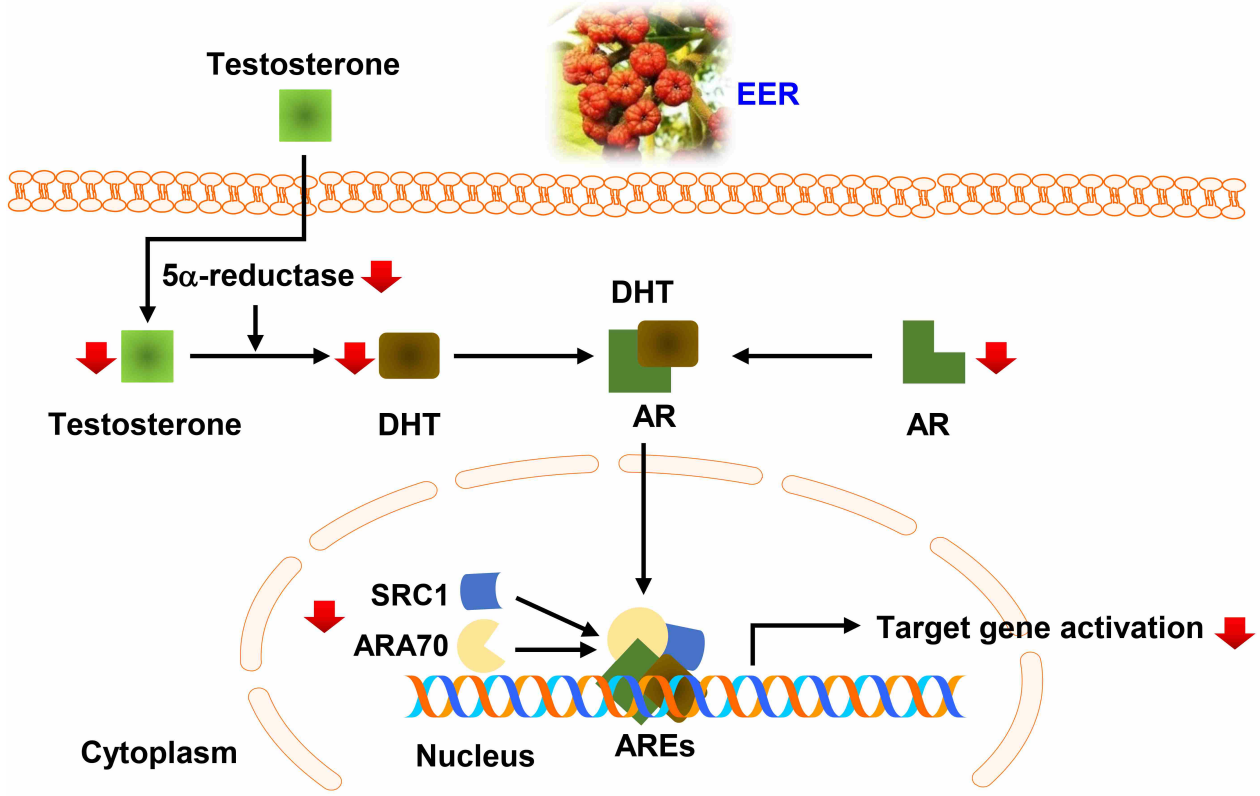

FIGURE 6. Schematic diagram showing that EER improves TP-induced BPH. EER: *Euodia rutaecarpa* fruit; DHT: dihydrotestosterone; AR: androgen receptor; SRC1: steroid receptor co-activator-1; ARA70: AR-associated protein 70; ARE: androgen response element.

AVAILABILITY OF DATA AND MATERIALS

The data presented in this study are available on reasonable request from the corresponding author.

AUTHOR CONTRIBUTIONS

YHC—designed the study and carried them out; supervised the data collection, analyzed the data, interpreted the data; prepared the manuscript for publication and reviewed the draft of the manuscript; has read and approved the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Animal care and experiments were performed in compliance with regulations of the Animal Experiment Ethics Committee of Dong-Eui University (Approval number: R2023-026).

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CONFLICT OF INTEREST

The author declares no conflict of interest.

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