



Ethanol Extracts of *Cornus alba* Improve Benign Prostatic Hyperplasia by Inhibiting Prostate Cell Proliferation through Modulating 5 Alpha-Reductase/Androgen Receptor Axis-Mediated Signaling

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Purpose: The aim of this study was to investigate the efficacy of ethanol extracts of *Cornus alba* (ECA) against benign prostatic hyperplasia (BPH) *in vitro* and *in vivo*.

Materials and Methods: The prostate stromal cells (WPMY-1) and epithelial cells (RWPE-1) were used to examine the action mechanism of ECA in BPH *in vitro*. ECA efficacy was evaluated *in vivo* using a testosterone propionate (TP)-induced BPH rat model.

Results: Treatment with ECA inhibited the proliferation of prostate cells by inducing G1-phase cell cycle arrest through the regulation of positive and negative proteins. Treatment of prostate cells with ECA resulted in alterations in the mitogen-activated protein kinases and protein kinase B signaling pathways. The transcriptional binding activity of the NF- κ B motif was suppressed in both ECA-treated prostate cells. In addition, treatment with ECA altered the level of BPH-associated axis markers (5 α -reductase, fibroblast growth factor-2, androgen receptor, epidermal growth factor, Bcl-2, and Bax) in both cell lines. Finally, the administration of ECA attenuated the enlargement of prostatic tissues in the TP-induced BPH rat model, accompanied by histology, immunoblot, and serum dihydrotestosterone levels.

Conclusions: These results demonstrated that ECA exerted beneficial effects on BPH both *in vitro* and *in vivo* and might provide valuable information in the development of preventive or therapeutic agents for improving BPH.

Keywords: Benign prostatic hyperplasia; BPH rat model; Ethanol extracts of *Cornus alba*; RWPE-1; WPMY-1

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INTRODUCTION

Benign prostatic hyperplasia (BPH), which is the noncancerous enlargement of the prostate gland, is characterized by hyperplastic stromal muscle and glandular epithelial tissue growth in the prostate [1]. The results of BPH lead to lower urinary tract symptoms (LUTS), which comprise the leakage of urine, nocturia, residual urine, weak urinary stream, and frequent urination [2]. Medical and surgical treatment for BPH, such as transurethral resection and pharmacological treatments, is widely available [3-5]. However, surgical trials and drug therapies to treat BPH are accompanied by recurrence and side effects, including bleeding, urinary incontinence, serious allergic reactions, decreased libido, and abdominal pain [3-5]. Therefore, the development of effective, tolerable, safe, and novel natural phytotherapeutic agents for BPH is urgently required.

It is well-accepted that the abnormal proliferation of prostate cells relates to the development of BPH, which is deeply associated with the androgen signaling cascade induced by dihydrotestosterone (DHT) [1,6]. Testosterone is converted to DHT by 5 α -reductase (5AR) in prostate cells [6]. The binding of DHT to the androgen receptor (AR) induces its translocation to the nucleus, resulting in the modulation of genes that control growth factors and leading to the proliferation of prostate cells through a cascade of several molecular events [7-9]. During the BPH development, the prostate cells proliferate via the modulation of cell cycle proteins, apoptosis regulators, signaling pathways, and transcription factors by accumulating DHT [7-9]. Pathophysiological approaches have led to the development of 5AR inhibitors (finasteride and dutasteride), which are mainly used to treat BPH as pharmacological drugs [4,5]. However, these drugs have severe side effects [4,5]. Therefore, based on the potential targets of molecular events that occur during the progression of BPH, many investigations are being advanced to develop safe and novel therapeutic agents from phytochemical resources.

Plants in the *Cornaceae* family are usually trees or deciduous shrubs. *Cornus alba* is a species of *Cornaceae*, which is widely distributed mainly in northern China, Siberia, and Korea [10]. The *Cornaceae* species have been shown to possess antimicrobial, antioxidant, antidiabetic, and anticancer properties [10-12]. The

leaves and bark of *Cornus alba* have particularly been used in traditional herbal medicine and are considered potential treatments for hemolysis and inflammation [12]. A previous study reported that the ellagitannins isolated from *Cornus alba* bark showed antiproliferative effects on normal prostate RWPE-1 cells *in vitro* [13]. However, the physiological efficacy of *Cornus alba* extracts in BPH *in vitro* and *in vivo* has not yet been reported.

Thus, the aim of this study was to evaluate the effect and role of ethanol extracts of *Cornus alba* (ECA) in a model of BPH. Therefore, in this study, we investigated the action mechanism of ECA-mediated anti-BPH using prostate WPMY-1 and RWPE-1 cell lines. In addition, we evaluated the potential efficacy of ECA in an animal BPH model.

MATERIALS AND METHODS

1. Sample preparation and materials

Cornus alba was collected from the Korean National Arboretum (Pocheon, Korea) in June 2020. Detailed sample preparation and the other materials used in this study are described in the Supplement File.

2. Ethics statement

The ethics committee approved the use of laboratory animals in this study for animal care and experimentation at Chung-Ang University (approval number: 2020-00096).

3. Cell culture, viable cell counting, CCK-8 assay, cell cycle analysis, immunoblotting, and electrophoresis mobility shift assay

The human prostate WPMY-1 and RWPE-1 cells were utilized in this study. Detailed cell culture, viable cell counting, cell cycle analysis, immunoblotting, and electrophoresis mobility shift assay (EMSA) were performed as described in the Supplement File.

4. Animals, BPH animal model, hematoxylin and eosin staining, immunohistochemistry, and enzyme-linked immunosorbent assay

Male Sprague-Dawley (SD) rats (200 $\pm$ 20 g), aged 5 weeks old, were obtained from Dae-Han Experimental Animal Center (Dae-Han Biolink Co.). Detailed descriptions of animals, BPH animal model, hematoxylin and

eosin (H&E) staining, immunohistochemistry, and enzyme-linked immunosorbent assay (ELISA) can be found in the Supplement File.

5. Statistical analysis

Data are represented as the mean±standard deviation from at least three separate experiments. The statistical analysis was conducted using IBM SPSS Statistics version 26 (IBM Corp.). To compare the mean performance of the treatment and control groups, one-way ANOVA was implemented followed by Fisher's least significant difference test for post hoc analysis. A p-value was used to determine statistical significance ($p < 0.05$, $p < 0.01$, and $p < 0.001$).

RESULTS

1. Treatment with ECA inhibited WPMY-1 and RWPE-1 prostate cell proliferation

Cell counting assays were performed using WPMY-1 and RWPE-1 cells to investigate the inhibitory ability of ECA on the proliferation of prostate cells. Treatment with ECA significantly inhibited the proliferation of both WPMY-1 and RWPE-1 cells in a dose-dependent pattern (Fig. 1A, 1B). Treatment of WPMY-1 cells with ECA (400 $\mu\text{g/mL}$) resulted in about 50% inhibition after 24 hours of exposure, compared to the control cells (Fig. 1A, 1B). Treatment with 100 $\mu\text{g/mL}$ ECA exhibited an approximately 50% suppression in RWPE-1 proliferation (Fig. 1A, 1B). Reduced proliferation of WPMY-1 and RWPE-1 cells were confirmed by cellular morphology (Fig. 1C).

2. Exposure to ECA induced G1-phase cell cycle arrest in prostate cells

To determine whether the cell cycle regulation was associated with the ECA-mediated reduction in prostate cell proliferation, FACS analysis was performed after a 24 hours treatment of ECA. Compared to the control group, ECA treatment exhibited an increase in the proportion of WPMY-1 cells in the G1-phase and a decline in the proportion of those in the G2/M-phase (Fig. 2A). Treatment of RWPE-1 cells with ECA induced the accumulation of G1-phase cells and a reduction in the population of G2/M-phase cells (Fig. 2B). However, the proportion in S-phase did not significantly alter in either cell line (Fig. 2A, 2B). These results indicate that ECA suppressed the proliferation of prostate cells by

inducing G1-phase cell cycle arrest.

3. Cell cycle regulators at G1-phase are involved in the ECA-induced inhibition of prostate cells

To investigate whether ECA controls cell cycle regulation in prostate cells, we examined the expression level of cell cycle markers in G1, including positive regulators (cyclins and cyclin-dependent kinases [CDKs]) and negative regulators (p21, p53, and p27). Treatment with ECA decreased the expression levels of CDK4, cyclin D1, and cyclin E in both WPMY-1 and RWPE-1 cells (Fig. 3). However, CDK2 expression was not affected by ECA treatment (Fig. 3, Supplement Fig. 1). Analysis of the negative regulators indicated that ECA treatment induced an increase in p21 and p27 expression levels, while the level of p53 remained unchanged (Fig. 3, Supplement Fig. 1). Our results suggest that ECA can inhibit the proliferation of prostate cells by stimulating G1-phase cell cycle arrest through the regulation of cell cycle proteins.

4. Treatment with ECA regulates different AKT and MAPK signaling pathways in prostate cells

We investigated the expression levels of protein kinase B (AKT) and mitogen-activated protein kinase (MAPK) signaling molecules to evaluate the effect of ECA on these signaling pathways in prostate cells. ECA treatment decreased the phosphorylation levels of extracellular signal-regulated kinase (ERK) 1/2, c-Jun N-terminal kinase (JNK), and p38 in the WPMY-1 cells (Fig. 4A). In addition, the level of AKT phosphorylation was increased in the presence of ECA (Fig. 4A). As shown in Fig. 4B, ECA treatment to the RWPE-1 cells induced ERK1/2 and JNK phosphorylation. Whereas the phosphorylated level of p38 was reduced in response to ECA (Fig. 4B). Treatment with ECA upregulated AKT phosphorylation in the RWPE-1 cells (Fig. 4B). These data show that ECA can regulate prostate cell responses through different signaling pathways.

5. The binding ability of the NF- κ B motif is reduced by ECA treatment in WPMY-1 and RWPE-1 cells

NF- κ B signaling is a key factor in the proliferation, cell cycle regulation, and signaling pathways of prostate cells [14,15]. Next, we performed an EMSA experi-

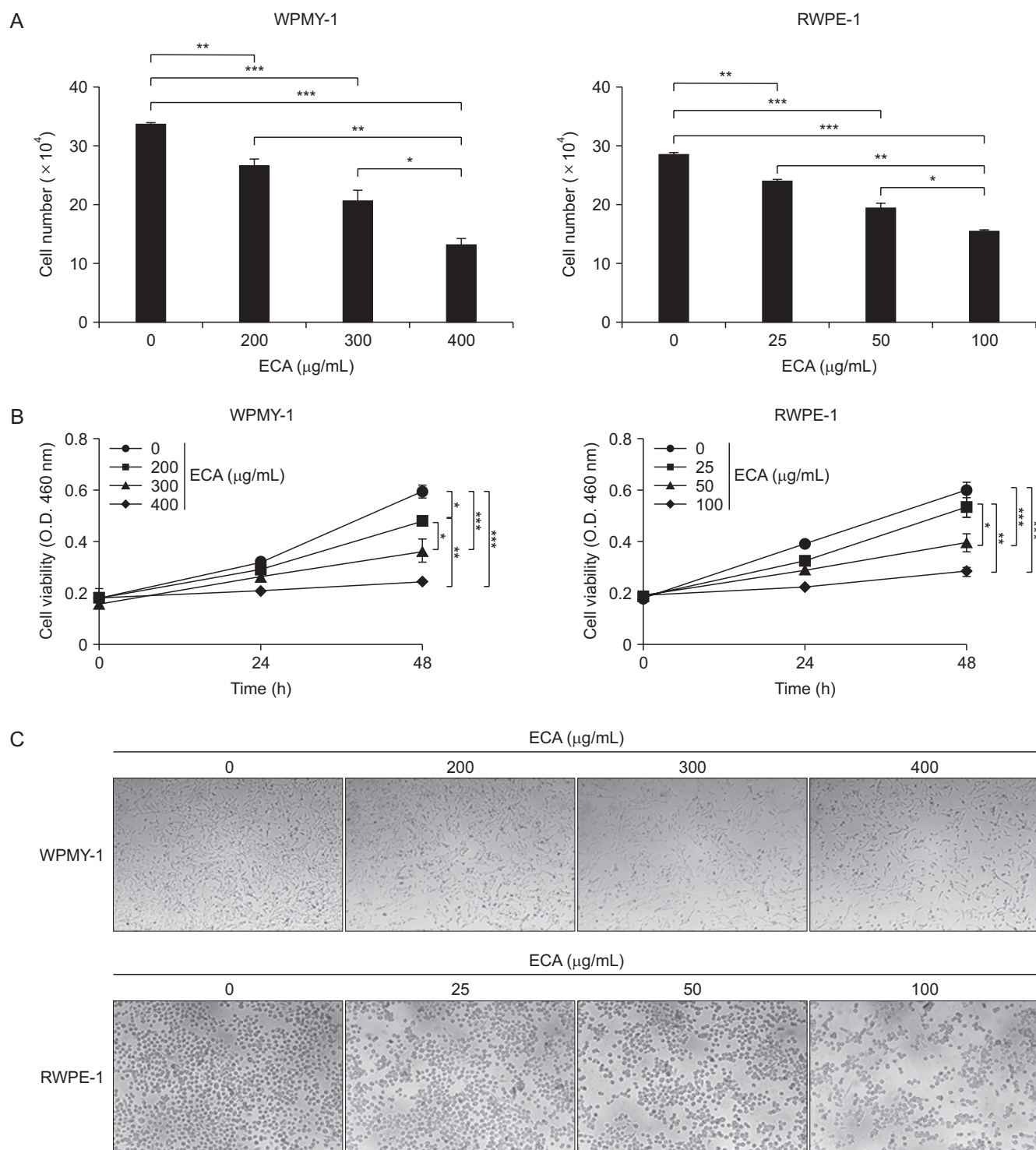


Fig. 1. The proliferation of prostate cells was inhibited by ECA treatment. Both WPMY-1 and RWPE-1 cells were treated with various concentrations of ECA for 24 hours. (A) A cell counting assay was performed to examine the proliferation of prostate cells. (B) The cell viability was measured using cell counting kit-8 assay. (C) Cellular morphology was observed in ECA-treated prostate cells under a light microscope. Images are at ×1,000 magnification. All data are represented as the mean±standard deviation of three independent experiments (*p<0.05, **p<0.01, and ***p<0.001). ECA: extracts of *Cornus alba*.

ment using nuclear extracts to investigate whether ECA can regulate the binding activity of NF-κB in prostate cells. Both prostate cells were incubated with

ECA for 24 hours, and nuclear extracts were collected from cell lysates. Treatment with ECA suppressed the binding ability of the NF-κB motif in WPMY-1 cells

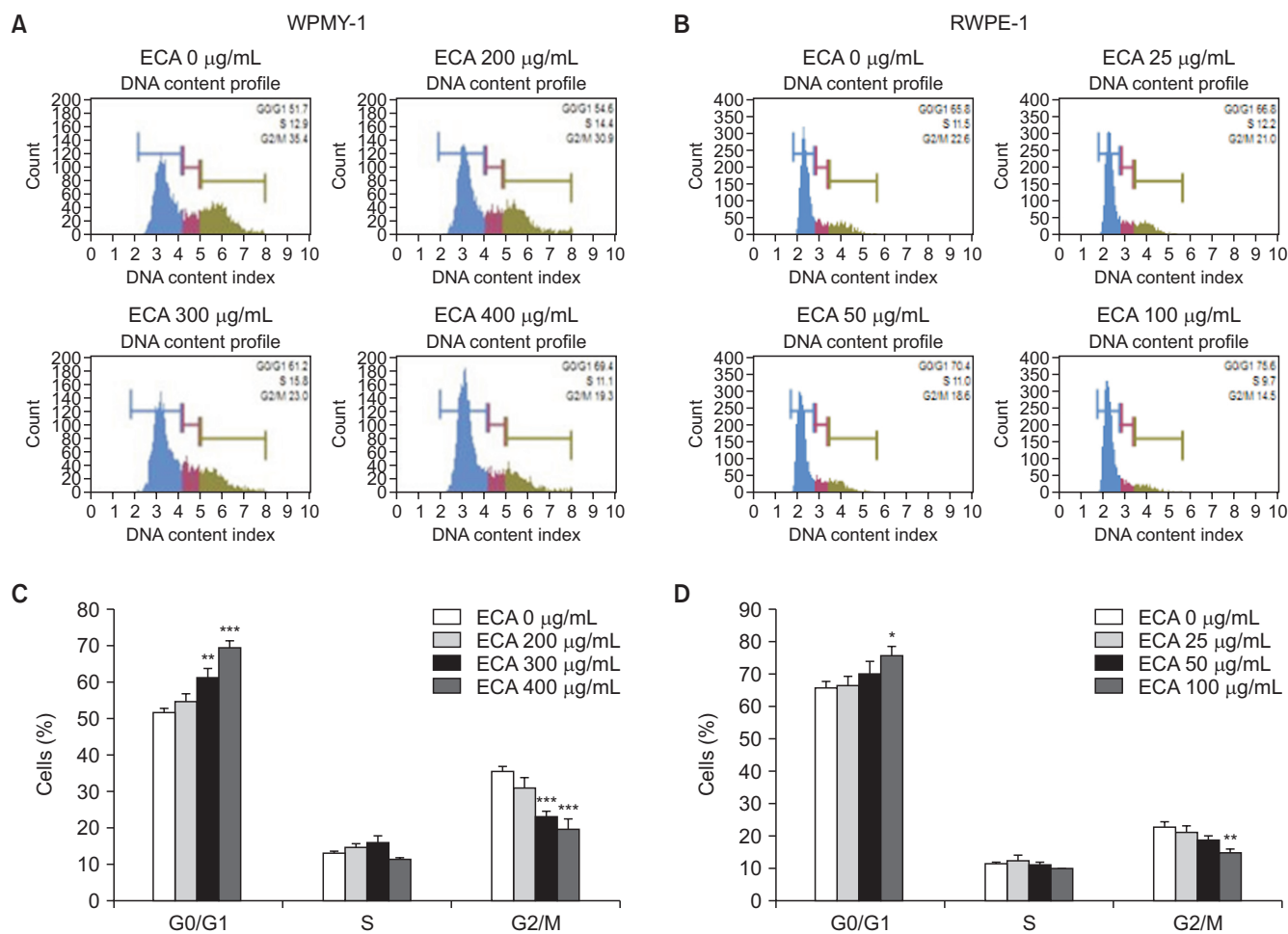


Fig. 2. Induction of cell cycle arrest at the G1-phase in ECA-treated prostate cells. (A, B) Both cells were treated with indicated concentrations of ECA for 24 hours, followed by flow cytometry analysis to estimate the cell cycle distribution. (C, D) The ratio of cell cycle populations in G1-, S-, and G2/M phases. All results are expressed as the mean±standard deviation of three independent experiments (*p<0.05, **p<0.01, and ***p<0.001). ECA: extracts of *Cornus alba*.

(Fig. 5). In addition, treatment of RWPE-1 cells with ECA attenuated the NF-κB binding affinity (Fig. 5). These findings demonstrate that the decline in transcriptional activation by the NF-κB motif is partially involved in the ECA-mediated reduction in prostate cell proliferation.

6. Effect of ECA in BPH-associated axis markers in prostate cells

Next, we investigated whether ECA can alleviate the protein expression of BPH-associated axis markers in prostate cells. As shown in Fig. 6A, immunoblot results indicate that ECA treatment decreased the expression levels of BPH-associated regulatory markers, such as 5AR, fibroblast growth factor (FGF)-2, AR, and epidermal growth factor (EGF) proteins in WPMY-1 and RWPE-1 cells. In addition, we examined the apoptosis

signaling markers in ECA-treated prostate cells. As a result, the level of Bcl-2, an anti-apoptosis protein, was downregulated following ECA treatment in both cell lines (Fig. 6B). Treatment with ECA upregulated the expression level of an apoptotic protein marker Bax in both cell types (Fig. 6B). These data indicate that ECA might suppress prostate cell proliferation by altering the BPH-associated axis markers.

7. Administration of ECA attenuated prostatic enlargement in a testosterone propionate-induced BPH rat model

Subsequently, we applied a BPH rat model, which had been induced by testosterone propionate (TP) for 4 weeks, to evaluate whether ECA could reduce prostate hyperplasia. Finasteride (Fi) was utilized as a positive control. No significant change was observed in body

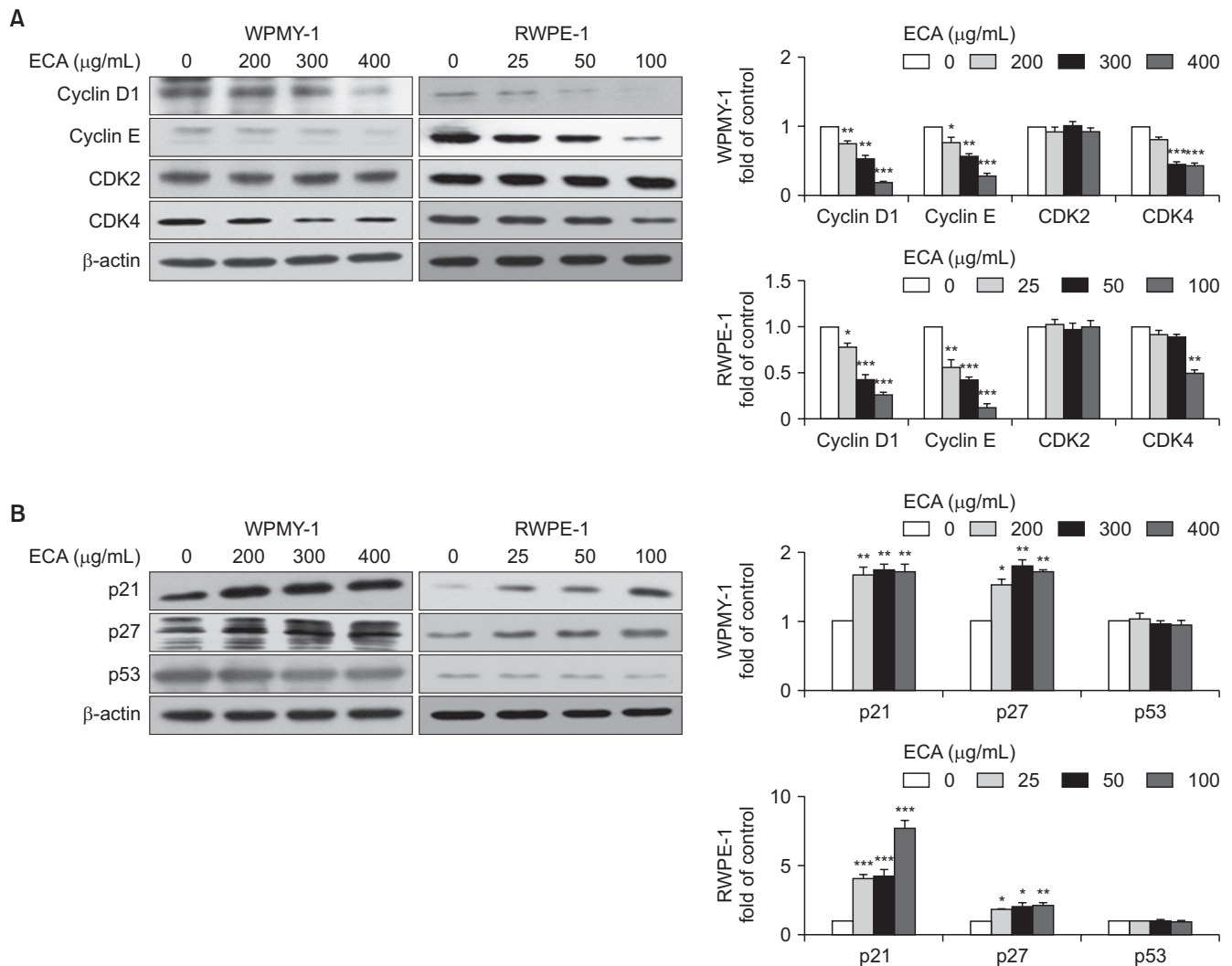


Fig. 3. Treatment with ECA induced G1-phase cell cycle arrest in prostate cells by mediating positive- and negative-cell cycle regulators. Cells were incubated with indicated ECA concentrations for 24 hours. (A) Protein levels of cyclin D1, cyclin E, cyclin-dependent kinase (CDK) 2, and CDK4 were analyzed by immunoblotting using total cell lysates. (B) An immunoblot assay was performed to assess the alterations in p21, p27, and p53 levels. β-actin was used as the internal control. Values are expressed as the mean±standard deviation of three independent experiments (*p<0.05, **p<0.01, and ***p<0.001). ECA: extracts of *Cornus alba*.

weight between the TP- and ECA-treated groups (Fig. 7A). The ECA-treated groups (100 mg/kg) revealed an approximately 31% reduction in the PW/BW index compared to the TP-treated groups (Fig. 7B, 7C). The ECA-treated groups (100 mg/kg) exhibited comparable efficacy to the groups treated with finasteride (Fig. 7B, 7C). Serum was collected from the rats and the level of DHT was measured, which is a critical regulator of prostate enlargement. It was determined that serum DHT levels were decreased in the rats administered with ECA or finasteride (Fig. 7D). To inspect the histological examination of prostate tissues in the animal experiment, H&E and Ki-67 staining were performed. The results showed that the TP-stimulated histological

observation in the hyperplastic prostatic tissues was alleviated in the ECA-treated groups, compared to rats treated with TP (Fig. 7E, 7G). The histological effect of ECA in prostatic change was equivalent to the efficacy of finasteride (Fig. 7E, 7G). The assessment of prostatic hyperplasia was confirmed by quantifying the thickness of the epithelial tissue in the ECA-treated groups (Fig. 7F). Finally, the expression levels of AR signaling markers (5AR, AR, and FGF) and apoptosis-associated molecules (Bcl-2 and Bax) were investigated in the prostate tissues from ECA-treated rats and compared to those from the TP-treated rats. The immunoblot experiment revealed similar results between the *in vivo* prostate tissues and *in vitro* data from the ECA-treated

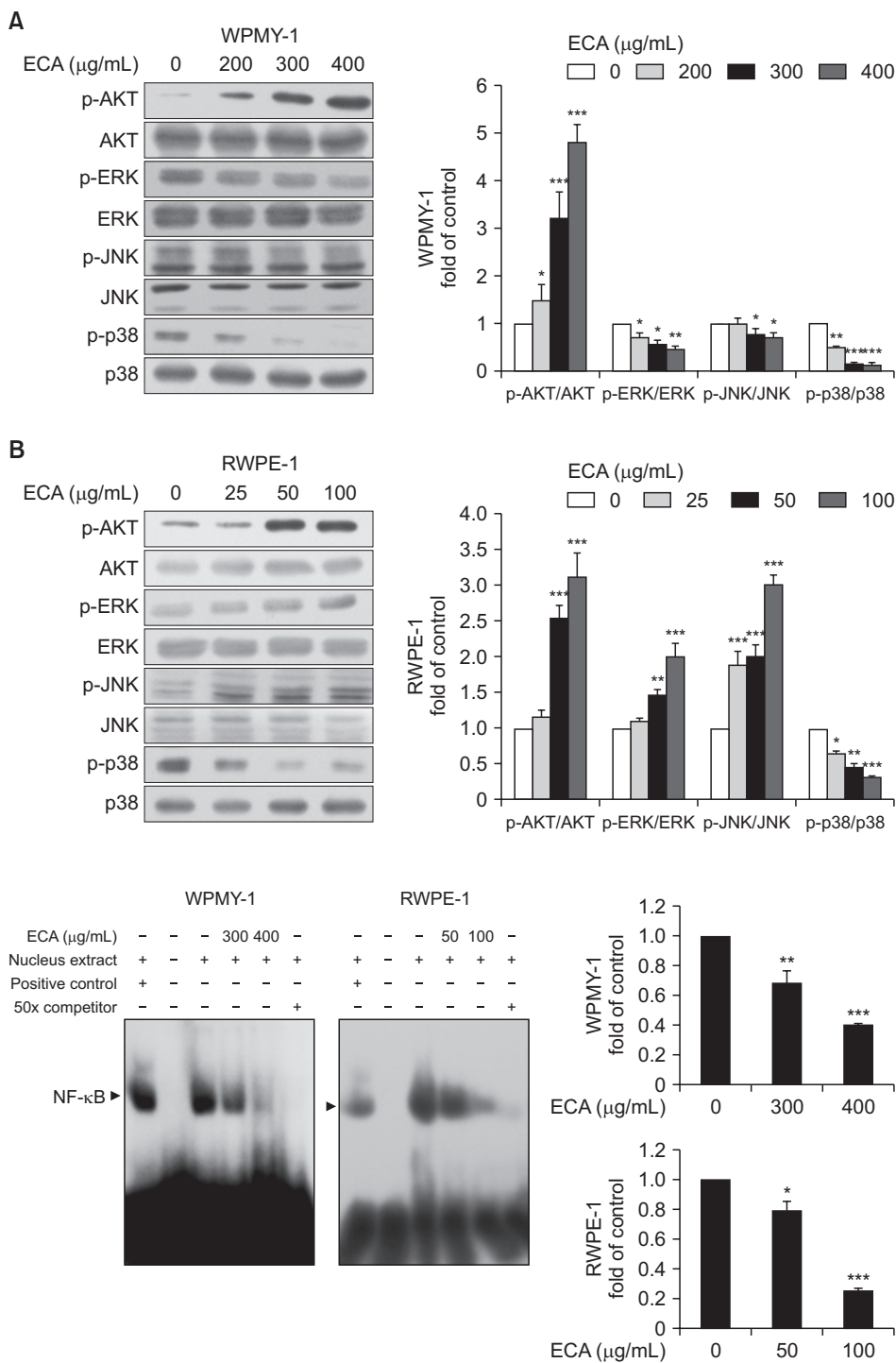


Fig. 4. Altered levels of MAPKs and AKT signaling molecules in ECA-treated prostate cells. WPMY-1 and RWPE-1 cells were treated with indicated concentrations of ECA for 24 hours. Changes in the phosphorylation level of ERK1/2, JNK, p38 (A), and AKT (B) were evaluated by immunoblotting. The levels of non-phosphorylated signaling molecules served to normalize the levels of phosphorylated signaling molecules. Values are expressed as the mean±standard deviation of three independent experiments (* $p<0.05$, ** $p<0.01$, and *** $p<0.001$). MAPK: mitogen-activated protein kinase, AKT: protein kinase B, ECA: extracts of *Cornus alba*, ERK: extracellular signal-regulated kinase, JNK: c-Jun N-terminal kinase.

Fig. 5. Exposure to ECA decreased the binding activity of NF-κB in both normal prostatic cell lines. After treatment of prostate cells with ECA for 24 hours, nuclear proteins were obtained, followed by EMSA to examine the binding ability of the NF-κB motif using radiolabeled oligonucleotide probes. Values are represented as the mean±standard deviation of three independent experiments (* $p<0.05$, ** $p<0.01$, and *** $p<0.001$). ECA: extracts of *Cornus alba*, EMSA: electrophoresis mobility shift assay.

groups (Fig. 8). These results suggest that ECA impeded the enlargement of the prostate in TP-stimulated BPH *in vivo*.

DISCUSSION

It has been suggested that *Cornus alba* is rich in flavonoids and hydrolyzable tannins [10,11,16]. *Cornus alba*

has been demonstrated to possess various physiological effects, such as antiphlogistic, hemostatic, and diuretic effects [12,15,17]. However, the efficacy of *Cornus alba* against BPH remains to be elucidated. Therefore, this study aimed to determine the effect of ECA against BPH using *in vitro* and *in vivo* models.

Proliferation in the prostate is normally regulated by maintaining a balance between cell death and

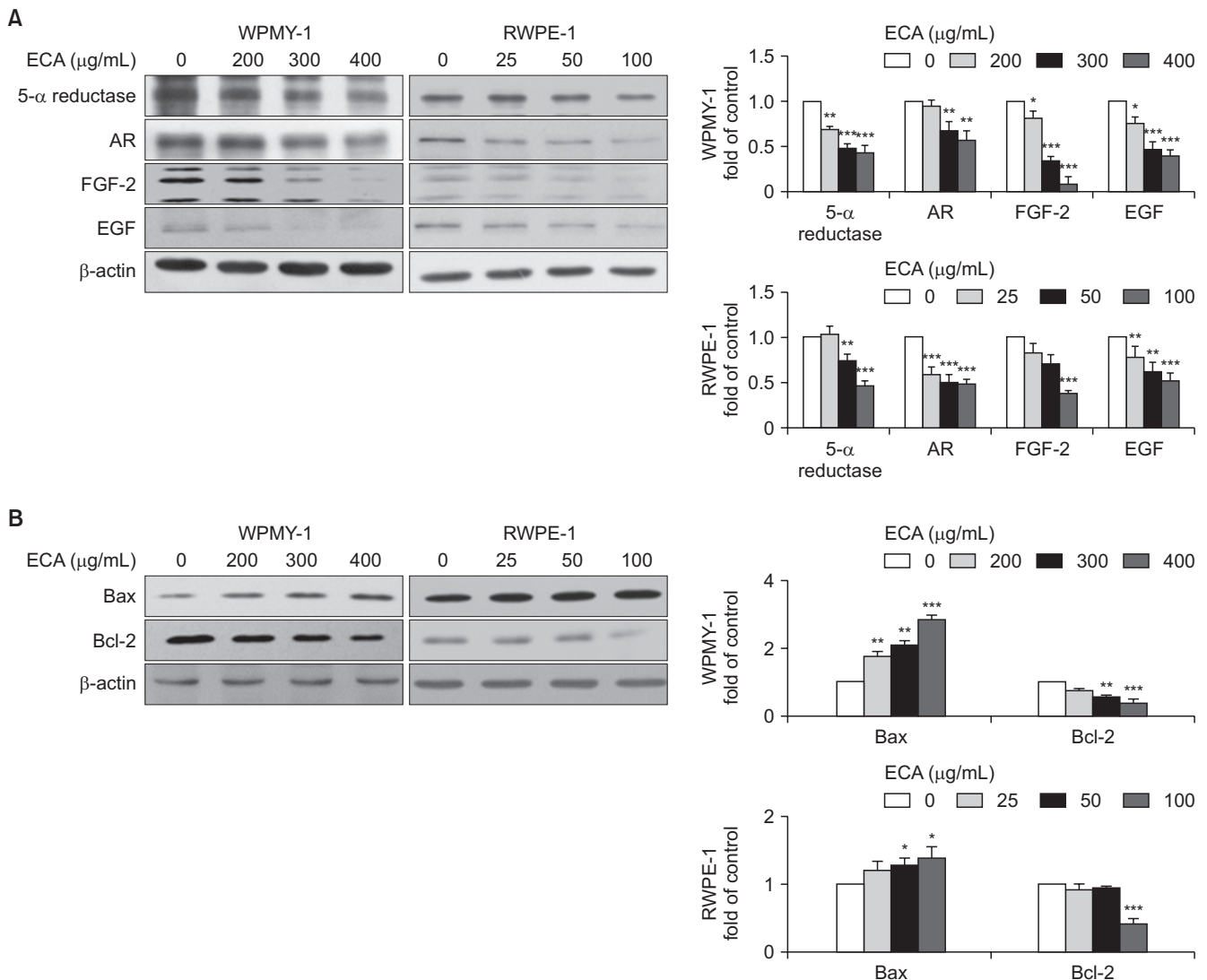


Fig. 6. Effects of ECA in benign prostatic hyperplasia (BPH)-associated axis markers in prostate cells. Cells were incubated with or without ECA treatment for 24 h in both prostate cells. (A, B) Immunoblot was performed to investigate the protein levels of BPH-associated axis markers (5 α -reductase, FGF-2, AR, EGF, Bcl-2, and Bax). β -actin was used as the internal control. Values are represented as the mean $\pm$ standard deviation of three independent experiments (* p <0.05, ** p <0.01, and *** p <0.001). ECA: extracts of *Cornus alba*, FGF: fibroblast growth factor, AR: androgen receptor, EGF: epidermal growth factor.

cell growth [1,6]. A disruption in balance triggers the abnormal proliferation of prostate cells, such as stromal cells and epithelial cells, leading to the androgen signaling-dependent pathological development of BPH [1,6]. In the present study, ECA impeded the proliferation of both WPMY-1 and RWPE-1 prostate cells, as determined by a cell counting assay and cell viability assay. The abnormal cell proliferation is a preferential phenotype, which is characterized by the progression of cell cycle checkpoints, such as G1-, S-, and G2/M-phases [9,18-20]. Cell cycle progression at the G1-phase is tightly controlled by positive regulators, such as cyclin D1, cyclin E, CDK4, and CDK2, and CDK inhibi-

tors, such as p27, p21, and p53 [9,18-20]. The CDK-cyclin complexes (CDK2-cyclin E and CDK4-cyclin D1) are predominant regulators in the progression of G1- to S-phase [9,18-20]. In addition, CDK inhibitors suppress the formation of CDK-cyclin complexes, which leads to G1-phase cell cycle arrest [18,19]. Therefore, we examined whether ECA-mediated prostate cell proliferation inhibition was involved in cell cycle regulation. In our study, ECA treatment induced G1-phase cell cycle arrest in both prostate cell lines. The expression levels of CDK4, CDK2, and cyclin D1 in prostate cells were decreased in the presence of ECA. However, the level of cyclin E was not affected by ECA treatment. Moreover,

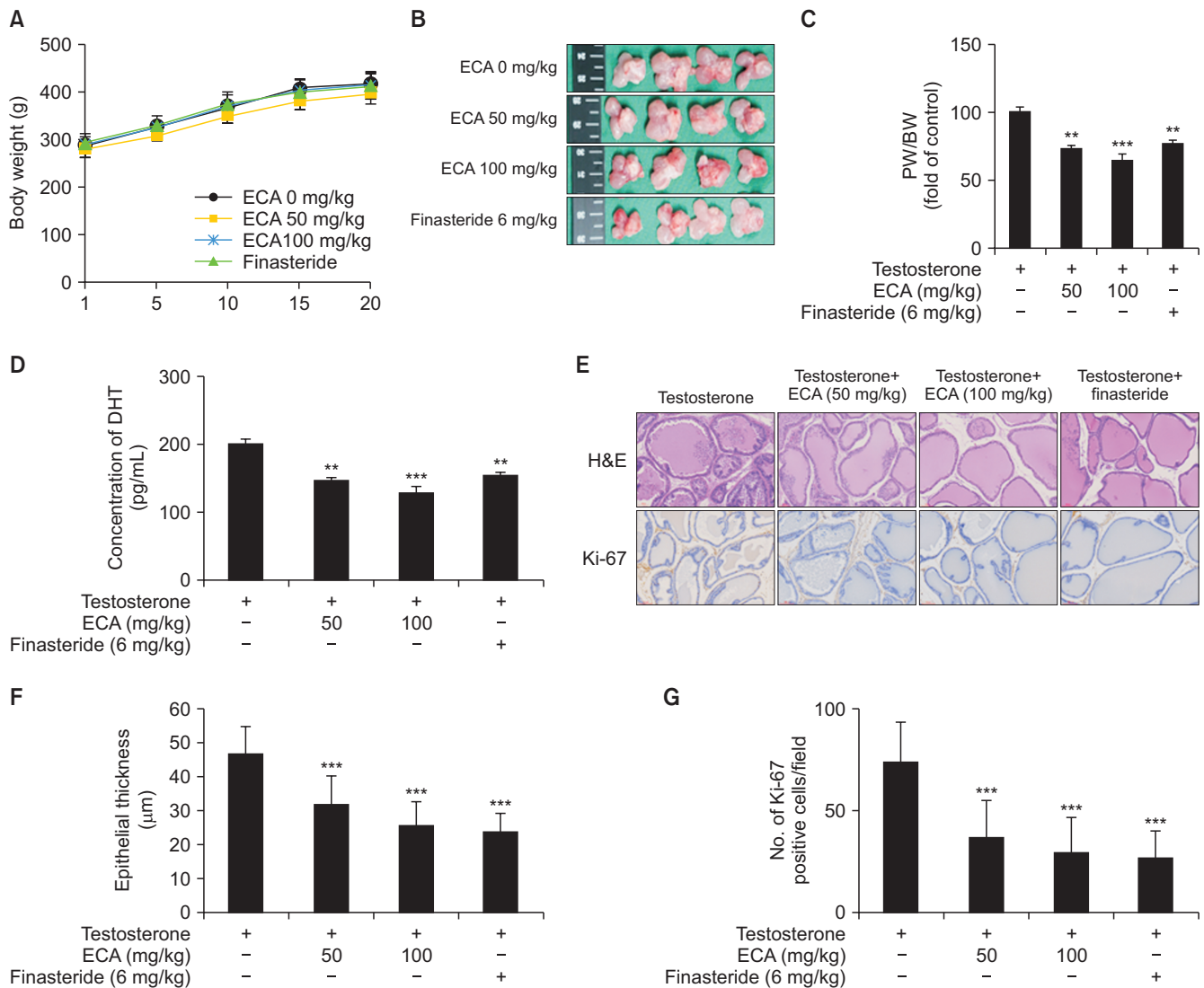


Fig. 7. The administration of ECA effectively reduced TE-induced prostate growth. (A) Body weight changes in TE-induced rat models were monitored every 5 days throughout the experimental period. (B) Representative photographs and (C) PW/BW from the dissected prostates of 5 groups. (D) The DHT levels were analyzed using an ELISA kit. (E) Prostatic tissue slides were stained with H&E and Ki-67 and observed at 40× magnification. (F) The thickness of the prostatic epithelium was measured in the tissue samples. (G) Quantitative analysis of positive Ki-67 expression. The results are expressed as the mean±standard deviation (n=7). ECA: extracts of *Cornus alba*, PW/BW: prostate weight-to-body weight ratio, TE: testosterone enanthate, DHT: dihydrotestosterone, ELISA: enzyme-linked immunosorbent assay. *p<0.05, **p<0.01, and ***p<0.001 compared to the testosterone group.

the treatment of prostate cells with ECA stimulated an increase in both p21 and p27 levels, although no alteration was observed in the level of p53. Our results demonstrate that the ECA-mediated suppression of prostate cell proliferation was attributed to the G1-phase cell cycle arrest via the downregulation of the CDK4/cyclin D1 complex by increasing the level of the CDK inhibitors (p21 and p27).

Signaling molecules, such as MAPK and AKT, are that have been considered critical regulators of cell proliferation, apoptosis, and cell cycle [21-26]. The MAPKs

signaling molecules contain ERK1/2, p38MAPK, and JNK in many types of cells [25]. Therefore, we investigated the phosphorylation levels of ERK1/2, p38MAPK, JNK, and AKT in ECA-treated prostate cells. ECA treatment increased AKT phosphorylation in WPMY-1 cells, whereas the phosphorylation of three types of MAPKs (ERK1/2, p38MAPK, and JNK) was decreased in the WPMY-1 cells. In the RWPE-1 cells, the levels of AKT, ERK1/2, and JNK phosphorylation significantly increased following ECA treatment. However, the treatment of RWPE-1 with ECA resulted in reduced

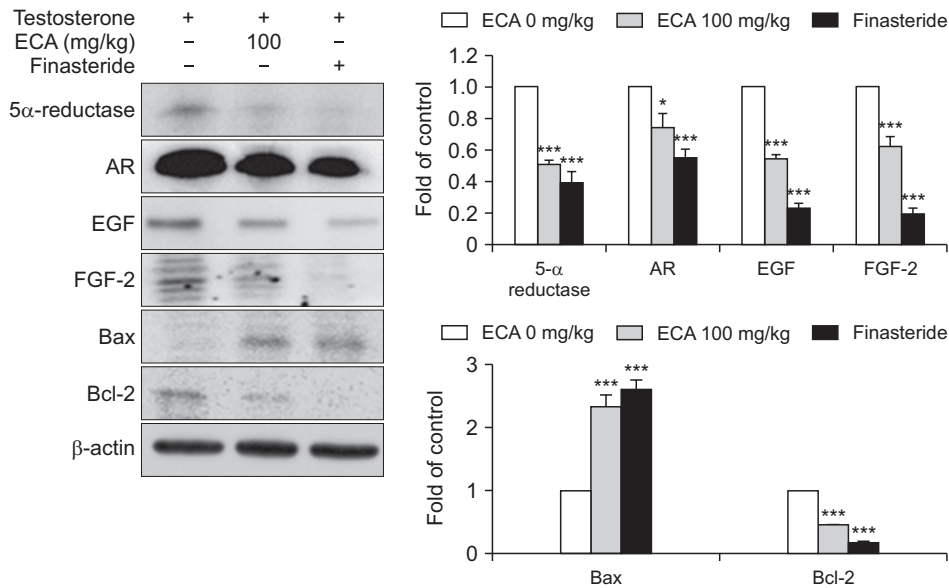


Fig. 8. The oral dose of ECA reduced protein levels of BPH-associated axis markers in the TP-induced BPH rat model. The expression levels of BPH-associated axis markers (5 α -reductase, FGF-2, AR, EGF, Bcl-2, and Bax) were investigated in prostate tissues from TP-induced BPH rats using immunoblotting. The finasteride group was used as the positive control and β -actin was used as the internal control. All data are represented as the mean $\pm$ standard deviation; $n=7$, * $p<0.05$ and *** $p<0.001$, compared to the testosterone group. ECA: extracts of *Cornus alba*, BPH: benign prostatic hyperplasia, TP: testosterone propionate, FGF: fibroblast growth factor, AR: androgen receptor, EGF: epidermal growth factor.

p38MAPK phosphorylation. These results indicate that ECA can mediate different signaling pathways, depending on the type of prostate cells. In addition, another key transcription factor NF- κ B operates in various cellular functions that regulate cell growth and the cell cycle [14]. Furthermore, heightened activation of canonical NF- κ B was observed in both epithelial and stromal cells of human BPH, particularly in advanced stages of the disease, and was associated with AR expression [8]. Cumulated studies have demonstrated that transcription factors, such as NF- κ B, AP-1, STAT3, and E2F-1, are critical in the proliferation of prostate cells [27-29]. Our study showed that ECA treatment impeded the ability of NF- κ B transcriptional binding activity in both prostate cell lines. These results suggest that NF- κ B may act as a regulator in the ECA-induced inhibition of prostate cell proliferation.

Although the pathological mechanisms of BPH remain to be elucidated, extensive studies have suggested that the levels of the 5AR-AR axis, DHT, FGF, EGF, Bcl-2, and Bax execute as pivotal mediators of BPH development [1,6-9]. The binding of DHT to AR sends the signal to induce several growth factors, such as FGF and EGF, resulting in the regulation of prostate cell growth and cell cycle progression [6-9]. In addition, elevated levels of DHT via the 5AR-AR axis control the proliferation and apoptosis of prostate cells to maintain homeostasis [6-9]. Therefore, we investigated the expression levels of the molecular markers required for BPH development *in vitro*, in ECA-treated prostate cells. In our study, ECA treatment reduced the levels of

5AR, AR, DHT, FGF, EGF, and Bcl-2 in prostate cells, whereas the level of Bax was increased. Additionally, we used a TP-induced BPH rat model to examine the efficacy of ECA on the pathophysiological change and hormonal regulation of prostate tissues *in vivo*. Oral administration of ECA (100 mg/kg) alleviated the prostatic growth and pathological alterations by decreasing both the size and weight of the prostate tissues without promoting significant death signs, compared to the BPH groups injected with TP. Furthermore, ECA decreased the prostate tissue thicknesses and reduced the levels of testosterone and DHT in the serum, compared to the TP-induced BPH rat group. The inhibitory effect of ECA on the TP-stimulated proliferation of prostate cells in BPH rats was confirmed via the use of Ki-67 and H&E histology. Here, the efficacy of ECA against TP-induced BPH rats was almost equivalent to finasteride. Finally, the levels of molecular biomarkers (5AR, AR, EGF, FGF, Bcl-2, and Bax) involved in the TP-induced prostatic pathological growth were verified in prostate tissues obtained from BPH rats administered ECA. Our study indicated that ECA might attenuate the DHT production by reducing the activation of the 5AR-AR axis, thereby resulting in a reduction in prostate size, leading to the disturbance of BPH enlargement. Collectively, our findings demonstrate that ECA could exert repressive effects on BPH via the regulation of prostatic growth by controlling the 5AR-AR-DHT axis, apoptosis-related molecules, and growth factors.

Many studies have demonstrated the physiologically

effective nature of the *Cornaceae* family against various pathological conditions [10-12]. A previous study from our group identified the 13 main physiologically effective constituents of *Cornus alba* [12]. It has been suggested that ellagitannins (cornusiin A and camptothin B) isolated from *Cornus alba* inhibited the proliferation of prostate cells by suppressing 5AR activity [13]. It is possible that ellagitannins have potential uses as biological markers in the development of novel preventive agents against anti-BPH. In addition, results from our group showed no adverse effects in *Cornus alba* leaves through various safety tests [30]. Therefore, we subsequently investigated whether ECA containing ellagitannins could demonstrate anti-BPH efficacy. In the present study, we found a therapeutic effect against BPH *in vitro* and *in vivo* via the use of ellagitannins-rich ECA. Although ECA might not outperform established treatments like finasteride, its potential lies in the lower possibility of side effects and improved tolerance [30]. In addition, it is prospective that the cornusiin A and camptothin B in the leaves of *Cornus alba* might play a critical role in the anti-BPH efficacy observed in our present *in vitro* and animal studies. Further studies are needed to examine the mechanism of action related to the anti-BPH effect of cornusiin A and camptothin B in both *in vitro* and *in vivo* models.

CONCLUSIONS

In summary, ECA suppressed hyperplastic growth *in vitro* via a reduction in prostate cells by regulating CDK inhibitors to induce G1-phase cell cycle arrest, alteration of signaling pathways, decreased ability of NF- κ B binding motif, and the dysregulated levels of BPH-associated molecular markers. Moreover, in the animal model using TP-stimulated BPH rats, oral administration of ECA alleviated the pathological progression of prostatic hypertrophy. Overall, our findings provide data highlighting the potential efficacy of ECA as a therapeutic resource to treat or prevent BPH. A clinical study will need to be performed in the future to confirm these data.

Conflict of Interest

The authors have no conflicts to disclose.

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None.

Author Contribution

Conceptualization: MWL, WJK, SKM. Data curation: BH, HK. Formal analysis: HJC, HK. Funding acquisition: HJC, MWL, SKM. Investigation: BH, JK. Methodology: SCM, SKM. Project administration: SKM, JWK, MWL. Resources: SCM, MWL. Software: BH, JK, HK. Supervision: SCM, MWL, SKM. Validation: SP, HJC. Visualization: BH, SP. Writing – original draft: BH, MWL, SKM. Writing – review & editing: YHC, WJK, SCM, TBJ, KMK, JCJ.

Supplementary Materials

Supplementary materials can be found via <https://doi.org/10.5534/wjmh.230200>.

Data Sharing Statement

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

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