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Hovenia dulcis Honey Suppresses Androgen-Induced Epithelial–Mesenchymal Transition in Benign Prostatic Hyperplasia

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Keywords: androgen receptor | benign prostatic hyperplasia | dihydrotestosterone | epithelial–mesenchymal transition | *Hovenia dulcis* honey | inflammation

ABSTRACT

Benign prostate hyperplasia (BPH) is characterized by abnormal prostate epithelial and stromal cell growth, which leads to bladder outlet obstruction (BOO) and lower urinary tract symptoms (LUTS). BPH pathogenesis involves key signaling pathways, including androgen/androgen receptor (AR) and transforming growth factor-beta (TGF-β)/Smad, which contribute to cell proliferation, transformation, and epithelial–mesenchymal transition (EMT). To date, the effect of *Hovenia dulcis* honey (HH) on BPH has not been reported. Herein, in vivo and in vitro BPH models were used to determine whether HH has therapeutic effects and its underlying mechanisms, if present. To evaluate the anti-BPH effect of HH in vivo, mice were treated with testosterone propionate (TP; 10 mg/kg, s.c.), finasteride (Fi; 10 mg/kg, i.p.), or HH (600 mg/kg, p.o.) for 4 weeks. Additionally, HH in vitro efficacy was evaluated using a dihydrotestosterone (DHT)-stimulated RWPE-1 prostate cell model. HH significantly reduced prostate size, epithelial thickness, and markers of AR signaling (prostate-specific antigen [PSA], proliferating cell nuclear antigen [PCNA], and DHT) as well as exhibited anti-inflammatory effects by lowering the expression of inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) and inhibited the EMT process by decreasing α-smooth muscle actin (α-SMA), neural cadherin (N-cadherin), and vimentin levels while restoring epithelial cadherin (E-cadherin) expression. These findings suggest that HH inhibits the androgen/AR and TGF-β/Smad signaling pathways and may offer a novel therapeutic approach for BPH treatment.

Abbreviations: 5ARIs, 5α-reductase inhibitors; AR, androgen receptor; BOO, bladder outlet obstruction; BPH, benign prostatic hyperplasia; BW, body weight ratio; COX-2, cyclooxygenase-2; DHT, dihydrotestosterone; EMT, epithelial–mesenchymal transition; HH, *Hovenia dulcis* honey; IL-6, interleukin-6; iNOS, inducible nitric oxide synthase; LUTS, lower urinary tract symptoms; PSA/KLK3, prostate-specific antigen/Kallikrein 3; PW, prostate weight; TGF-β, transforming growth factor-beta; TNF-α, tumor necrosis factor-α; TP, testosterone propionate.

Buyun Kim and Young-Eun Kim contributed equally to this work.

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1 | Introduction

Benign prostatic hyperplasia (BPH) is a common age-related disorder characterized by abnormal proliferation of epithelial and stromal cells in the prostate transition zone. This condition can lead to bladder outlet obstruction (BOO) and lower urinary tract symptoms (LUTS), significantly affecting the quality of life in aging men (Lee et al. 2017; Collaborators 2022). Current pharmacological treatments for BPH include high-affinity α1-receptor blockers and 5α-reductase inhibitors (5ARIs), such as terazosin, finasteride, and dutasteride (Xu et al. 2020). However, these medications primarily alleviate symptoms rather than targeting the underlying pathological mechanisms of BPH, such as fibrosis (Rodriguez-Nieves and Macoska 2013) and chronic inflammation (Inamura and Terada 2024). Furthermore, long-term use of these drugs is associated with side effects, including dizziness, decreased libido, and erectile dysfunction, which limit patient compliance and necessitate the development of alternative therapeutic approaches.

Androgen signaling is the dominant driver of BPH pathogenesis. The androgen receptor (AR) pathway plays a critical role in prostate growth and function, and its dysregulation contributes significantly to BPH progression. Dihydrotestosterone (DHT), a potent androgen converted from testosterone by 5α-reductase, binds to AR and promotes epithelial and stromal cell proliferation in the prostate (Carson and Rittmaster 2003; Ho and Habib 2011). Androgen signaling not only stimulates hyperplastic growth but also interacts with inflammatory and fibrotic pathways, exacerbating BPH progression (Abdel-Aziz et al. 2020). Given this, AR-targeting therapies, including 5ARIs and AR antagonists, remain key strategies for BPH management. However, the role of androgen signaling in epithelial–mesenchymal transition (EMT) and fibrosis, key contributors to BPH progression, requires further investigation.

BPH's exact pathogenesis remains unclear; however, recent studies highlight the significant role of chronic inflammation and EMT in disease progression. According to the epithelial–mesenchymal interaction theory, the prostatic stroma plays a crucial role in BPH development (Broster and Kyprianou 2015). Additionally, EMT has been implicated in the abnormal accumulation of mesenchymal-like cells derived from prostatic epithelial cells, contributing to fibrosis and prostate enlargement (Hu et al. 2014; Broster and Kyprianou 2015). EMT is a normal physiological process involved in tissue development, cancer progression, and fibrotic disorders (Rout-Pitt et al. 2018; Georgakopoulos-Soares et al. 2020). However, its aberrant activation in BPH contributes to pathological remodeling, resulting in fibrosis and increased prostate volume (Yang et al. 2022). Once fibrosis progresses, the prostate enlarges further, worsening BPH symptoms (Alonso-Magdalena et al. 2009; Broster and Kyprianou 2015).

Several key signaling pathways regulate EMT, including transforming growth factor-beta (TGF-β), Wnt/β-catenin, and Notch, which influence cellular structural changes and motility. For example, TGF-β activation leads to the loss of epithelial cadherin (E-cadherin) and an increase in mesenchymal markers such as vimentin and neural cadherin (N-cadherin) (Alonso-Magdalena et al. 2009). In BPH, EMT leads to epithelial cell mobility and adoption of mesenchymal characteristics, causing spreading or reorganizing within surrounding tissues, leading to increased prostate volume and functional changes (Odero-Marah et al. 2018). Given this, targeting EMT has emerged as a potential therapeutic strategy for BPH, and drugs modulating EMT-related pathways are currently under development.

Honey, a natural product with well-documented health benefits, contains various bioactive compounds, including sugars, minerals, vitamins, and phenolic compounds, which exhibit anti-inflammatory, antioxidant, and antimicrobial properties (Estevinho et al. 2008; Khalil et al. 2012; Samarghandian et al. 2017). Among them, *Hovenia dulcis* honey (HH) is known for its high polyphenol content and superior biological activity compared to acacia honey (Park et al. 2020; You et al. 2022). However, despite its well-documented pharmacological benefits, the direct effects of HH on key BPH-related mechanisms, including AR signaling, EMT, and fibrosis, have not been thoroughly investigated.

Honey from the oriental raisin tree (*H. dulcis*) was used for all experiments; samples were provided by the Korea Beekeeping Cooperative (Namyangju, Gyeonggi-do, Republic of Korea) and Rural Development Administration (Jeonju, Jeollabuk-do, Republic of Korea). This study aims to evaluate whether HH modulates TGF-β/Smad2-mediated EMT and AR signaling in a testosterone propionate (TP)-induced BPH animal model and a DHT-stimulated prostate cell model. Specifically, we hypothesize that HH may exert protective effects against BPH progression by inhibiting EMT, reducing inflammatory cytokine expression, and modulating AR-related pathways. By elucidating its mechanistic actions in both in vivo and in vitro models, our study provides new insights into the potential of HH as a natural therapeutic agent for BPH management.

2 | Materials and Methods

2.1 | Chemicals and Materials

Honey from the oriental raisin tree (*H. dulcis*) was used for all experiments; samples were provided by the Korea Beekeeping Cooperative (Namyangju, Gyeonggi-do, Republic of Korea) and Rural Development Administration (Jeonju, Jeollabuk-do, Republic of Korea). DHT and finasteride were purchased from Thermo Fisher Scientific, Co. Ltd. (USA); AR (PA1-110) was purchased from Invitrogen. Prostate-specific antigen/Kallikrein 3 (PSA/KLK3; #5356), β-actin (#4967), cyclooxygenase-2 (COX-2; #12282), inducible nitric oxide synthase (iNOS; #13120), tumor necrosis factor-α (TNF-α; #8184), interleukin-6 (IL-6; #12153), phospho-suppressor of Mothers against Decapentaplegic (p-Smad2; #18338), suppressor of Mothers against Decapentaplegic (Smad2; #5339), TGF-β (#3711), E-cadherin (#14472), N-cadherin (#14215), vimentin (#5741), and α-smooth muscle actin (α-SMA; #14968) were obtained from Cell Signaling Technology (Danvers, MA, USA). Secondary antibodies were purchased from Bio-Rad (Hercules, CA, USA).

TABLE 1 | Primer sequences used in this study.

Human gene	Forward (5′–3′)	Reverse (3′–5′)
AR	TCGTCCACGTGTAAGTTGCG	GAGCCAGGTGTAGTGTGTGC
PSA	CTGACCTCAAATACCTGGCCT	ACAAAAGGGTGATCTTGCTGG
SRC-1	TGGCGTTGCTTGTTGTGGTG	GCTGGTATCCTTCCTTAGTG
PCNA	AGGAAAGTCTAGCTGGTTTCG	TTAAACGGTTGCAGGCGTAG
TNF-α	CCCATCTATCTGGGAGGGGT	ATCCCAAAGTAGACCTGCCC
IL-6	CCGGGAACGAAAGAGAAGCT	AGGCGCTTGTGGAGAAGG
IL-1β	AGCTGATGGCCCTAAACAGA	TCGGAGATTTCGTAGCTGGAT
E-Cadherin	GGGTGTCGAGGGAAAAATAGG	CGAGAGCTACACGTTCACGG
N-Cadherin	ATGCACATCCTTCGATAAGACTG	TCAGGCGTCTGTAGAGGCTT
Vimentin	CTTTGTCGTTGGTTAGCTGGT	GACGCCATCAACACCGAGTT
α-SMA	TCAAATACCCCATTGAACACGG	GGTGCTCTTCAGGTGCTACA
β-Actin	CTTTGCGGATGTCCACGTCA	GGCCAGGTCATCACCATTGG

Abbreviations: AR, androgen receptor; E-cadherin, epithelial cadherin; IL-1β, interleukin-1 beta; IL-6, interleukin-6; N-cadherin, neural cadherin; PCNA, proliferating cell nuclear antigen; PSA, prostate-specific antigen; SRC-1, steroid receptor coactivator-1; TNF-α, tumor necrosis factor-α; α-SMA, α-smooth muscle actin.

TABLE 2 | Primer sequences used in this study.

Mouse gene	Forward (5′–3′)	Reverse (3′–5′)
AR	GGGCCACTGAACACTGAAGA	TAGCGGAGCTTTGTGACTGG
PCNA	ACTCTACAACAAGGGGCACA	AGAGCTTGGCAATGGGAACA
PSA	ATTGGGTTTAGCGCATGGGA	CAAAGACACTTGTGCGGGTG
TNF-α	TGGTGGTTTGTGAGTGTGAGG	AGGCACTCCCCCAAAAGATG
IL-6	ATAACGCACTAGGTTTGCCGA	AGTCCTTCCTACCCCAATTTCC
α-SMA	TATAGGTGGTTTCGTGGATGC	CCTTCGTGACTACTGCCGAG
β-Actin	ACTTTGGGGGATGTTTGTCC	ACTGAGCTGCGTTTTACACCC

Abbreviations: AR, androgen receptor; E-cadherin, epithelial cadherin; IL-1β, interleukin-1 beta; IL-6, interleukin-6; N-cadherin, neural cadherin; PCNA, proliferating cell nuclear antigen; PSA, prostate-specific antigen; SRC-1, steroid receptor coactivator-1; TNF-α, tumor necrosis factor-α; α-SMA, α-smooth muscle actin.

2.2 | Qualitative Analysis of HH Using UHPLC-Q-Exactive Orbitrap Mass Spectrometry

Chromatographic analysis was performed with Thermo Dionex UltiMate 3000 system, equipped with a binary pump, an autosampler, a column oven, and a diode array UV/VIS detector (Thermo Scientific Dionex, Sunnyvale, CA, USA). In brief, chromatographic separation of HH was performed using an Acquity BEH C_{18} analytical column ($100 \times 2.1\ mm^2$, 1.7 µm), with gradient elution, employing mobile phase A (0.1% formic acid [v/v] in water) and mobile phase B (acetonitrile). The flow rate was set to 0.3 mL/min, and the column temperature was maintained at 40°C. Phytochemical identification in HH was conducted using a quadrupole-Q-Exactive Orbitrap mass spectrometer equipped with a heated electrospray ionization interface (Thermo Scientific, Waltham, MA, USA). MS analysis of HH was performed in both positive and negative ionization modes using an HESI source for accurate MS spectral collection and molecular mass measurements of the analytes. The MS spectra were acquired at a normalized collision energy of 25 eV in full MS and ddMS2 scan modes, covering a scan range of 100–1500 m/z. The specific analytical conditions and mass spectrometry parameters are detailed in prior studies (Hwang and Ma 2018; Pak et al. 2022). Data acquisition and analysis were completed using Xcalibur v.4.2 and Tracefinder v.4.0 software (Thermo Fisher Scientific, Foster, CA, USA). MS-grade methanol, acetonitrile, water, and formic acid were sourced from Thermo Fisher Scientific (Pittsburgh, PA, USA). The analytical reference standards, each with a purity above 97%, were acquired from Sigma-Aldrich Co. (St. Louis, MO, USA), Targetmol (Wellesley Hills, MA, USA), and ChemFaces (Wuhan, China).

2.3 | RWPE-1 Cell Model and Culture Conditions

Human prostate epithelial cell line RWPE-1 was purchased from the American Type Culture Collection (Manassas, VA, United States). Untransformed RWPE-1 cells are derived from normal human prostate epithelial cells and are immortalized but non-tumorigenic (Benbrahim-Tallaa et al. 2005). RWPE-1 cells serve

as a representative model for studying BPH because they retain characteristics of normal prostate epithelial cells, allowing for the investigation of cellular responses to hormonal stimulation and growth factors. The suitability of RWPE-1 cells for studying EMT lies in their ability to undergo EMT when stimulated by androgens, making them an ideal model for examining EMT processes critical in BPH pathogenesis (Munoz-Moreno et al. 2022). This makes RWPE-1 cells particularly useful for examining AR signaling pathways and EMT processes, which are critical in the pathogenesis of BPH. RWPE-1 cells were grown in a CO_2 incubator at 37°C with 5% CO_2 in K-SFM containing 50 μg/mL BPE and 5 ng/mL EGF, supplemented with a 1% antibiotic/antimycotic mixture and streptomycin.

2.4 | Cell Viability and Proliferation Assay

Cell proliferation was quantified using CCK-8 kits (Dojindo Molecular Technologies Inc., Doc, USA). BPH-1 cells were first seeded into 96-well plates (1×10^5 cells/well) and cultured for 24 h. Next, cells were treated with HH (20 mg/mL) alone or with various combinations of HH (20 mg/mL) and DHT (10 nM) for 24 h, followed by incubation with CCK-8 solution for 4 h. Viable cell numbers were then determined by evaluating at 450 nm using an Epoch microplate reader (Biotek, Winooski, VT, USA).

2.5 | Animal Model and Treatment Protocol

C57BL/6J male mice (20–25 g; 5 weeks; $n = 7$) were purchased from Doo Yeol Biotech (Seoul, Korea). Mice were accommodated under 12 h light/dark cycle at a relative humidity of 50% ± 10% and a temperature of 22°C ± 2°C. All experiments were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee (IACUC) of the Korea Institute of Oriental Medicine, and the experimental protocol was approved under Approval No. 24-039.To eliminate the intrinsic effects of endogenous testosterone, mice in all experimental groups, except the control (CON) group and single-agent treatment groups (HH and Fi), underwent bilateral orchiectomy, followed by a 7-day recovery period. The CON group and the single-agent treatment groups received sham surgeries to maintain baseline testosterone levels for comparison. Mice were randomly assigned to one of six groups ($n = 7$ per group) and received specific treatments for 5 weeks as follows: (1) CON group ($n = 7$), (2) 10 mg/kg TP group (s.c., $n = 7$), (3) 10 mg/kg Finasteride group (Fi, i.p., $n = 7$), (4) 600 mg/kg *Hovenia* honey group (HH, p.o., $n = 7$), (5) TP + 600 mg/kg HH group (p.o., $n = 7$) and (6) TP + 10 mg/kg Fi group (i.p., $n = 7$). The dose of TP (10 mg/kg) was selected on the basis of previous studies that established its efficacy in inducing BPH-like conditions in rodent models, mimicking androgen levels observed in clinical BPH case. Similarly, the finasteride dose (10 mg/kg) reflects commonly used concentrations in preclinical models, corresponding to therapeutic doses effective in human BPH treatment (Kim et al. 2020). The duration of the treatment (5 weeks) was chosen to ensure sufficient time for BPH development and therapeutic evaluation of HH and finasteride. At the end of the treatment period, mice were euthanized, and prostates were carefully harvested, blotted dry, and immediately weighed. The relative prostate weight (PW) ratio was calculated by normalizing each prostate's weight to the average weight of the CON group to assess hypertrophic changes induced by TP and therapeutic interventions.

2.6 | Histological Examination and Prostate Morphometry

Prostate tissue was fixed in 4% formalin, embedded in paraffin, and sliced into 4-mm-thick sections. Next, sections were stained with hematoxylin and eosin (H&E), and images were acquired using a microscope (Leica DFC 295; Wetzlar, Germany). Epithelium thickness within the prostate was measured using the Leica Application Suite (v3.3.0; Leica Microsystems Inc., Buffalo Grove, IL, USA).

2.7 | DHT Enzyme-Linked Immunosorbent Assay (ELISA)

For serum ELISA, blood samples were collected from mice, and serum was separated by centrifugation at 1700 × *g* for 30 min at 20°C. Serum DHT concentration was then determined using a commercial ELISA kit (Cusabio Biotech Co. Ltd., Houston, TX, USA) with all procedures following the manufacturer's recommendations.

2.8 | Western Blot Analysis

Proteins were isolated using a commercial extraction reagent (iNtRON Biotechnology, Gyeonggi-do, Korea) and quantified using a BCA protein assay kit (#23225, Thermo Fisher). Extracted proteins were then separated via 4%–12% SDS-PAGE and electrically transferred to a PVDF membrane (Merck Millipore, Burlington, MA, USA). Membranes were then immunoblotted with primary and secondary antibodies. Primary antibodies against AR, PSA, COX-2, iNOS, TNF-α, IL-6, p-Smad2, Smad2, TGF-β, E-cadherin, N-cadherin, Vimentin, α-SMA, and β-actin were used.

2.9 | Quantitative Real-Time-Polymerase Chain Reaction (qRT-PCR)

For qRT-PCR, RNA was extracted from prostate tissue using QIAzol (Qiagen, Hilden, Germany). Purified RNA was then reverse-transcribed into cDNA using a Maxima First Strand cDNA Synthesis Kit (Thermo Fisher). Amplification by qPCR was performed in 10-μL reaction volumes containing primers and Power SYBR Green Master Mix (Thermo Fisher); signal detection used a C1000 Touch Thermal Cycler (Bio-Rad, Hercules, CA, USA). Samples were then compared using the relative CT method, and β-actin was used for normalization for quantification using the $2^{-}\Delta\Delta$Cq method. All primer sequences are listed in Tables 1 and 2.

2.10 | Statistical Analysis

All experiments were conducted in triplicate, and results are presented as mean ± standard error of the mean (SEM) from

three independent experiments. Statistical analyses and data visualization were performed using GraphPad Prism v8.0.

Data normality was assessed using the Shapiro–Wilk test, and all datasets followed a normal distribution. Therefore, for comparisons among multiple groups, one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) post hoc test was applied. A p value less than 0.05 was considered statistically significant. For comparisons with the BPH group, statistical significance was indicated using asterisks: $p < 0.05$*, $p < 0.01$**, and $p < 0.001$***. For comparisons with the CON group, significance was denoted using hash symbols: $p < 0.05$#, $p < 0.01$##, and $p < 0.001$###.

3 | Results

3.1 | Identification of Phytochemicals in HH Using UHPLC-Q-Exactive Orbitrap MS

The phytochemical analysis of HH using UHPLC-Q-Exactive Orbitrap MS identified five flavonoid components: myricetin, ampelopsin (dihydromyricetin), pinocembrin (dihydrochrysin), quercetin, and taxifolin. Honey, including HH, serves as a natural source of flavonoids, phenolic acids, and their derivatives. Previous studies suggest that the composition and concentration of these components in honey are critical in defining its biological properties, such as antioxidant potential (Meda et al. 2005; Khalil and Sulaiman 2010; Chan et al. 2013). Moreover, among the various techniques available for identifying phenolic and flavonoid components in honey, the most commonly used is the combination of MS and HPLC. Five flavonoid components, including myricetin, ampelopsin, pinocembrin, quercetin, and taxifolin, identified in HH were analyzed by comparing the retention time (t_R), measured precursor ions, and MS/MS fragments with those of reference standard substances. The results of the analysis confirmed that the negative ionization mode provides the most suitable conditions for analyzing these compounds. This was confirmed by the electrospray ionization properties in the negative ion mode of flavonoids known from previous studies (Cuyckens and Claeys 2004). Accordingly, we present that the details of the flavonoids inherent in HH in negative ionization conditions are summarized in Table 3. Therefore, we aimed to conduct an experiment on the improvement effect of HH, which contains these flavonoids, on BPH.

Common honey is well-known for its rich content of phenolic compounds, which include flavonoids such as quercetin, kaempferol, apigenin, and chrysin (Valverde et al. 2022). These bioactive compounds contribute significantly to the antioxidant, anti-inflammatory, and antimicrobial properties of honey. However, in honey derived from HH, the predominant phenolic constituents shift toward ampelopsin, taxifolin, and myricetin. Ampelopsin, a flavonoid known for its potent antioxidant and anti-inflammatory effects, along with taxifolin and myricetin, which are both recognized for their potential therapeutic properties, may play a crucial role in managing conditions such as BPH. Given the known involvement of oxidative stress and inflammation in the pathogenesis of BPH, these bioactive compounds could contribute to alleviating symptoms and potentially slowing the progression of the condition through their antioxidative and anti-inflammatory mechanisms. The distinctive phenolic profile

TABLE 3 | Phytochemical composition of *Hovenia dulcis* honey identified by UHPLC-Q-exactive orbitrap MS.

No	t_R (min)	Elemental composition	Adduct	Estimated (m/z)	Calculated (m/z)	Error (ppm)	Identification
1	5.88	$C_{15}H_{12}O_8$	$[M-H]^-$	319.0459	319.0462	0.7306	Ampelopsin
2	7.08	$C_{15}H_{10}O_8$	$[M-H]^-$	303.0510	303.0511	0.3876	Taxifolin
3	8.03	$C_{42}H_{62}O_{16}$	$[M-H]^-$	317.0303	317.0305	0.7178	Myricetin
4	9.66	$C_{15}H_{10}O_7$	$[M-H]^-$	301.0354	301.0357	1.0815	Quercetin
5	14.46	$C_{15}H_{12}O_4$	$[M-H]^-$	255.0663	255.0665	1.0334	Pinocembrin

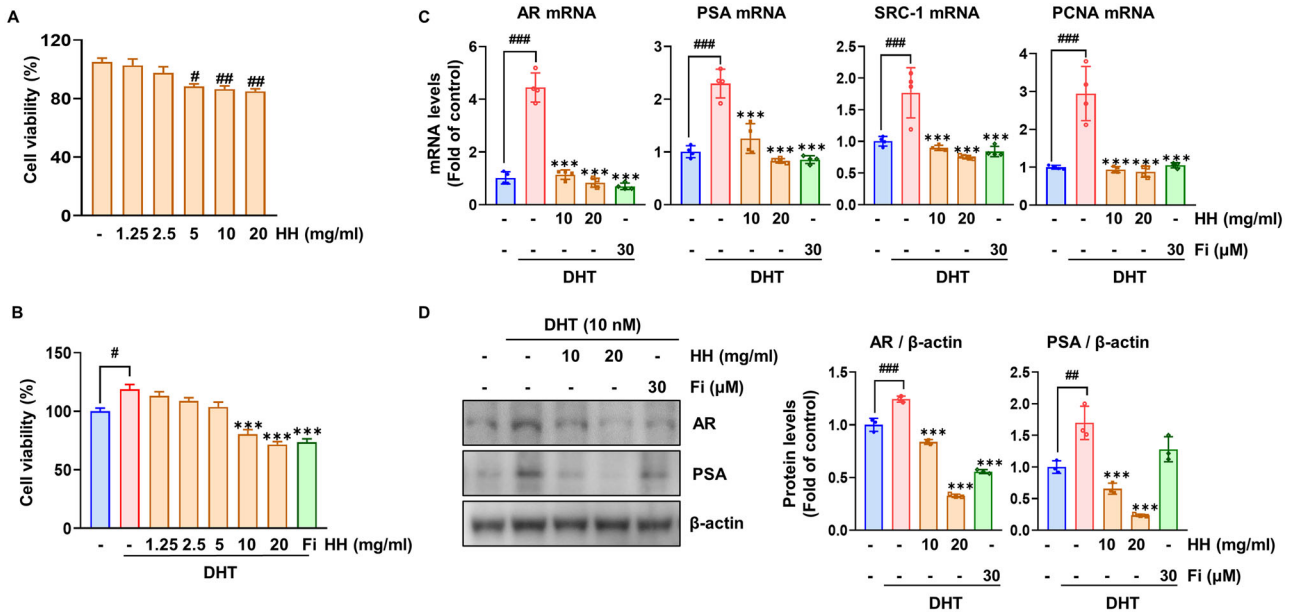


FIGURE 1 | **Evaluation of cell viability and regulation of androgen receptor (AR) signaling**. (A) Cell viability was measured after treatment of RWPE-1 cells with *Hovenia dulcis* honey (HH) for 24 h. (B) Cell viability was measured after treatment of RWPE-1 cells with *H. dulcis* honey and dihydrotestosterone (DHT) for 24 h. (C) mRNA expression levels of AR, prostate-specific antigen (PSA), steroid receptor coactivator-1 (SRC-1), and proliferating cell nuclear antigen (PCNA) as assessed by quantitative real-time PCR (qRT-PCR). (D) Protein expression levels of AR and PSA confirmed by Western blotting. All statistical analyses were performed using one-way ANOVA, and values are presented as the mean ± standard deviation (SD) of three replicates. ##$p < 0.01$, ###$p < 0.001$, ####$p < 0.0001$ compared to the control. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$ compared to the DHT.

of honeysuckle honey suggests that its specific constituents may offer targeted therapeutic benefits in the treatment of BPH.

3.2 | HH Inhibits Cell Proliferation by AR Signaling in DHT-Induced RWPE-1 Cells

To examine the antiproliferative effect of HH, RWPE-1 (normal) prostate epithelial cells were used to establish an in vitro model of BPH via DHT induction. Our experimental results evaluated cell viability according to the ISO 10993-5 standard (ISO 2009). No cytotoxic effect was observed in RWPE-1 cells at any concentration of HH (Figure 1A), and cell proliferation was significantly inhibited in DHT-stimulated RWPE-1 cells (Figure 1B), suggesting that HH counteracts DHT-induced proliferation. Subsequent examination of the expression of prostate biomarkers (e.g., AR, PSA, steroid receptor coactivator-1 [SRC-1], and proliferating cell nuclear antigen [PCNA]) revealed that HH significantly reduced the expression of AR, which is induced by androgen response to DHT, PSA, a prostate-specific androgen-sensitive biomarker, SRC-1, a coactivator of nuclear receptors including AR, and PCNA, a key marker of cell proliferation (Figure 1C). Decreased PCNA expression following HH treatment directly supports an inhibitory effect on cell proliferation. Moreover, HH significantly reduced AR and PSA protein expression (Figure 1D). We conclude that HH inhibits cell proliferation via regulating androgen/AR signaling.

3.3 | HH Inhibits Inflammatory Responses in DHT-Induced RWPE-1 Cells

Inflammation promotes cell proliferation in prostate tissue, thereby causing BPH (Gandaglia et al. 2013). Patients with BPH and inflammation exhibit high levels of histological and molecular BPH markers, suggesting that inflammation accompanies worsening BPH symptoms (Chughtai et al. 2011). Therefore, we investigated whether HH exhibits anti-inflammatory effects. First, we examined the inflammatory response enzymes COX-2 and iNOS (Wu et al. 2007; Abdel-Aziz et al. 2020). We observed that COX-2 and iNOS activity induced by DHT was reduced by HH (Figure 2A). However, COX-2 produces PGE2, iNOS produces NO, and both promote inflammatory cytokine expression (e.g., IL-6 and TNF-α). We then investigated whether DHT-induced inflammatory cytokine expression in RWPE-1 cells was inhibited by HH, and observed that DHT induction increased IL-6 and TNF-α protein expression, while HH reduced them (Figure 2B). Furthermore, IL-6, TNF-α, and interleukin-1 beta (IL-1β) mRNA levels upregulated by DHT were downregulated by HH (Figure 2C). These results indicate that HH effectively suppresses inflammatory responses in DHT-induced RWPE-1 cells by inhibiting the expression of pro-inflammatory cytokines, suggesting its potential as an anti-inflammatory agent in BPH treatment.

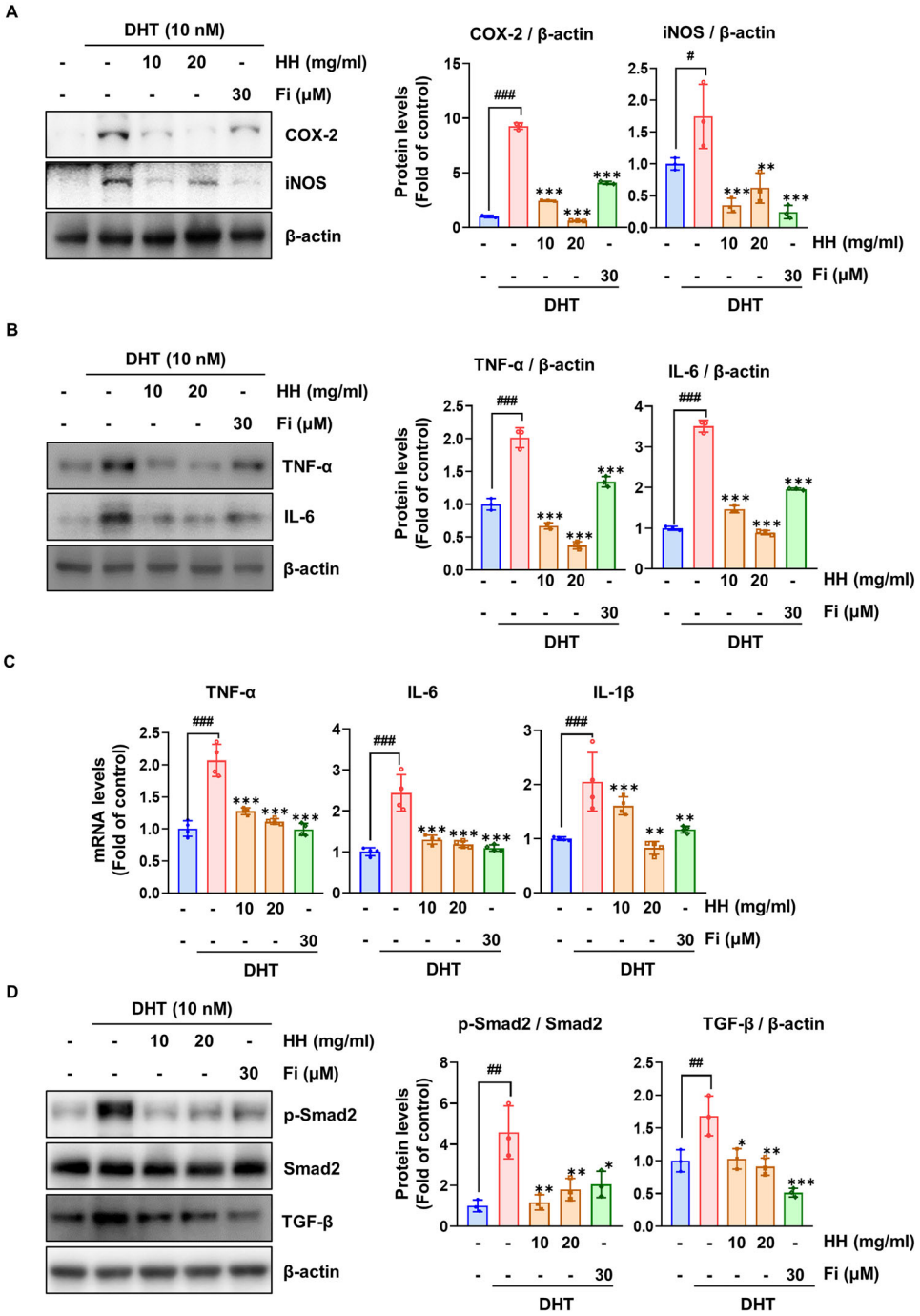


FIGURE 2 | **Effects of *Hovenia dulcis* honey on inflammatory responses and regulation of transforming growth factor-beta (TGF-β)/Smad signaling**. (A and B) Protein expression levels of cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), tumor necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6) confirmed by Western blotting. (C) mRNA expression levels of TNF-α, IL-6, and interleukin-1 beta (IL-1β) as assessed by qRT-PCR. (D) Protein expression levels of phosphorylated Smad2 (p-Smad2), Smad2, and TGF-β confirmed by Western blotting. All statistical analyses were performed using one-way ANOVA, and values are presented as the mean ± standard deviation (SD) of three replicates. #$p < 0.05$, ##$p < 0.01$, ###$p < 0.001$, ####$p < 0.0001$ compared with control. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$ compared to the DHT. DHT, dihydrotestosterone.

3.4 | HH Regulates Activation of TGF-β/Smad2 Signaling in DHT-Induced RWPE-1 Cells

In BPH, TGF-β/Smad signaling pathway regulates cell growth, differentiation, and apoptosis (Alonso-Magdalena et al. 2009; Kim et al. 2023; Li et al. 2024), and its abnormal activation leads to fibrosis and stromal cell proliferation, causing prostate enlargement (Giarratana et al. 2024). We then tested whether HH inhibits EMT via activation of the TGF-β/Smad2 signaling pathway by assessing marker expression. High TGF-β and p-Smad2 proteins levels in DHT-stimulated RWPE-1 cells were significantly reduced by HH (Figure 2D). We then confirmed the expression of EMT-related markers and found that HH treatment significantly increased E-cadherin expression, whereas

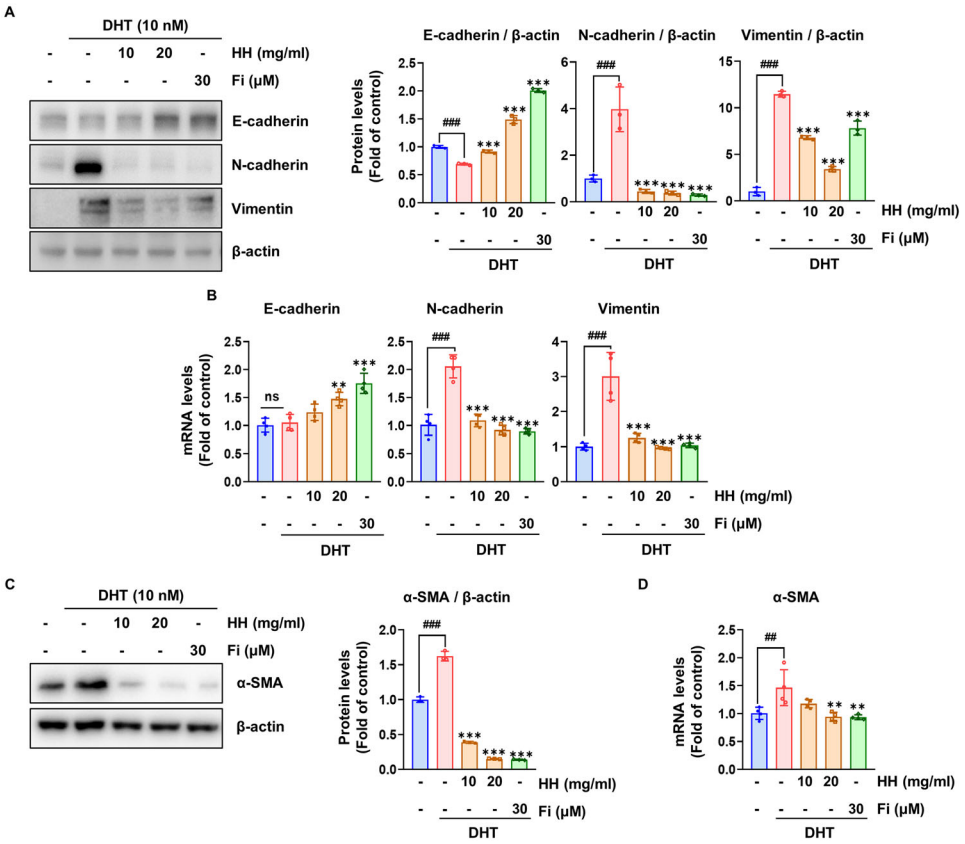


FIGURE 3 | **Inhibitory effect of *Hovenia dulcis* honey on epithelial–mesenchymal transition (EMT).** (A) Protein expression levels of E-cadherin, N-cadherin, and vimentin confirmed by Western blotting. (B) mRNA expression levels of E-cadherin, N-cadherin, and vimentin confirmed by qRT-PCR. (C) Protein expression levels of alpha-smooth muscle actin (α-SMA) confirmed by Western blotting. (D) mRNA expression levels of α-SMA confirmed by qRT-PCR. All statistical analyses were performed using one-way ANOVA, and values are presented as the mean ± standard deviation (SD) of three replicates. ##$p < 0.01$, ###$p < 0.001$, ####$p < 0.0001$ compared with control. **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$ compared to the DHT. DHT, dihydrotestosterone; E-cadherin, epithelial cadherin; N-cadherin, neural cadherin.

protein (Figure 3A) and mRNA (Figure 3B) levels of N-cadherin and vimentin decreased. Additionally, α-SMA expression mRNA and protein were significantly reduced by HH (Figure 3C,D). These findings suggest that HH inhibits EMT in DHT-induced RWPE-1 cells by downregulating the TGF-β/Smad2 signaling pathway, thereby reducing fibrosis-related markers and promoting epithelial characteristics. This highlights HH's potential role in modulating key fibrotic processes in BPH.

3.5 | HH Prevents BPH in TP-Induced BPH Mice

Prostate tissue from TP-induced BPH mice was analyzed to assess whether HH reduced prostate hypertrophy. Prostates enlarged due to TP injection reduced in size following HH and Fi treatment (Figure 4B). PW and PW to body weight ratio (PW/BW) were significantly higher in the TP than the CON group but were significantly reduced following HH and Fi treatment (Figure 4C,D). DHT levels in mice were determined through serum ELISA. Serum DHT levels were significantly elevated by TP injection but were significantly reduced by HH and Fi (Figure 4E). H&E staining was then used to identify histological changes in prostate tissue samples from each group. Relative to CON group, TP group exhibited BPH symptoms, including a multilayered epithelium. However, these symptoms were lessened in the HH and Fi groups (Figure 5F), with a specifically notable therapeutic effect on the thickness of the prostate epithelium (Figure 5G). The data demonstrate that HH effectively reduces prostate enlargement, DHT levels, and histological abnormalities associated with BPH in TP-induced mice. These results suggest that HH has protective effects against prostate hypertrophy, making it a promising candidate for BPH management.

3.6 | HH Exhibits AR Signaling and Anti-Inflammatory Effects in TP-Induced Mice

Effect of HH treatment on AR signaling markers and inflammatory factors in TP-induced BPH mice was investigated. We found that AR, PCNA, and PSA mRNA were upregulated in TP-induced BPH mice but decreased following HH and Fi administration (Figure 5A). PSA protein expression followed a similar pattern (Figure 5B), as did the expression levels of inflammatory mediators, such as COX-2 and iNOS proteins (Figure 5C), and inflammatory cytokine proteins IL-6 and TNF-α (Figure 5D). TNF-α and IL-6 mRNA expression also increased in the TP group but was lower in the HH and Fi groups (Figure 5E). These results indicate that HH not only suppresses AR signaling but also reduces inflammation in TP-induced BPH mice. This dual effect suggests that HH may provide therapeutic benefits by targeting

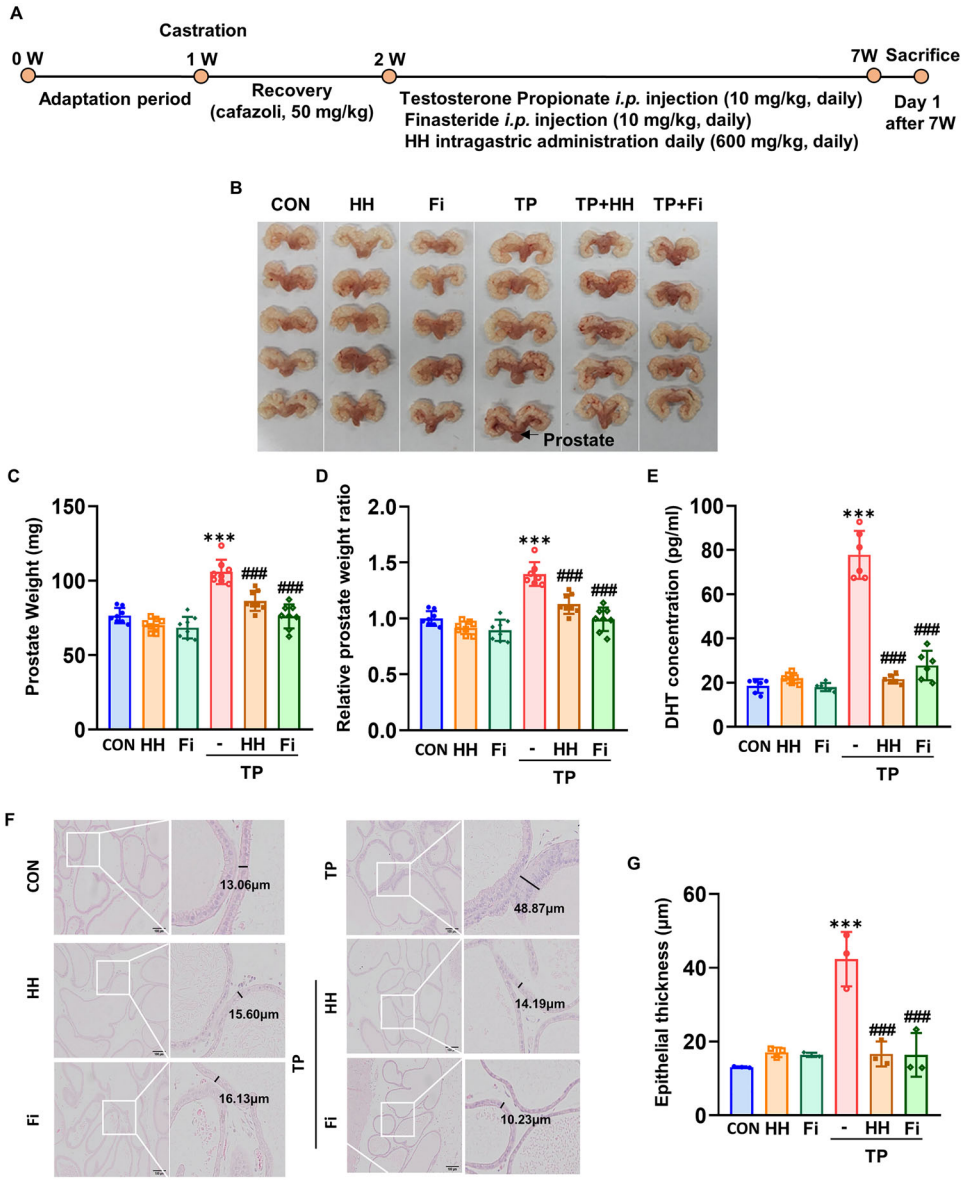


FIGURE 4 | Effect of *Hovenia dulcis* honey on prostate hypertrophy in testosterone propionate (TP)-induced benign prostatic hyperplasia (BPH) mice. (A) Schematic representation of the BPH animal experiment. (B) Representative images of excised prostate tissues from each experimental group ($n = 5$ per group). (C and D) Measurements of prostate weight (PW), relative PW ratio, and PW/body weight (PW/BW) ratio ($n = 7$ per group). (E) Dihydrotestosterone (DHT) levels in mouse serum quantified via ELISA ($n = 7$ per group). (F and G) Prostate tissue sections stained with hematoxylin and eosin (H&E) and examined under a microscope, including epithelial thickness measurements ($n = 3$ per group). All statistical analyses were performed using one-way ANOVA, and values are presented as the mean ± standard deviation (SD) of three replicates. ****$p < 0.0001$ compared with CON group. ####$p < 0.0001$ compared with TP group.

both hormonal and inflammatory pathways involved in BPH progression.

3.7 | HH Inhibits EMT Activity via Modulating the TGF-β/Smad Signaling Pathway

In BPH, expression of TGF-β induces EMT (He et al. 2016). In the TP group, we found that protein expression of the epithelial marker E-cadherin decreased, whereas expression of the mesenchymal markers N-cadherin and vimentin increased. However, in the HH and Fi groups, EMT was inhibited via upregulating E-cadherin and downregulating N-cadherin and vimentin (Figure 6A). In BPH tissue, TGFBR2 is overexpressed, activating TGF-β/Smad signaling via Smad2/3 phosphorylation, and the abnormal activation of this pathway may promote BPH progression. Results reveal that it induced EMT in the TP group and activated TGF-β/Smad signaling, whereas the HH and Fi groups showed decreased expression of TGF-β and p-Smad2 (Figure 6B). During EMT progression, α-SMA is expressed as an indicator of the mesenchymal transition and plays an important role in physiological or pathological conditions such as tissue fibrosis and wound healing (Yang et al. 2017). For example, EMT and α-SMA expression may be upregulated in BPH and cancer cells (Speca et al. 2012; Hu et al. 2014). Our results show that α-SMA protein and mRNA expression were significantly higher

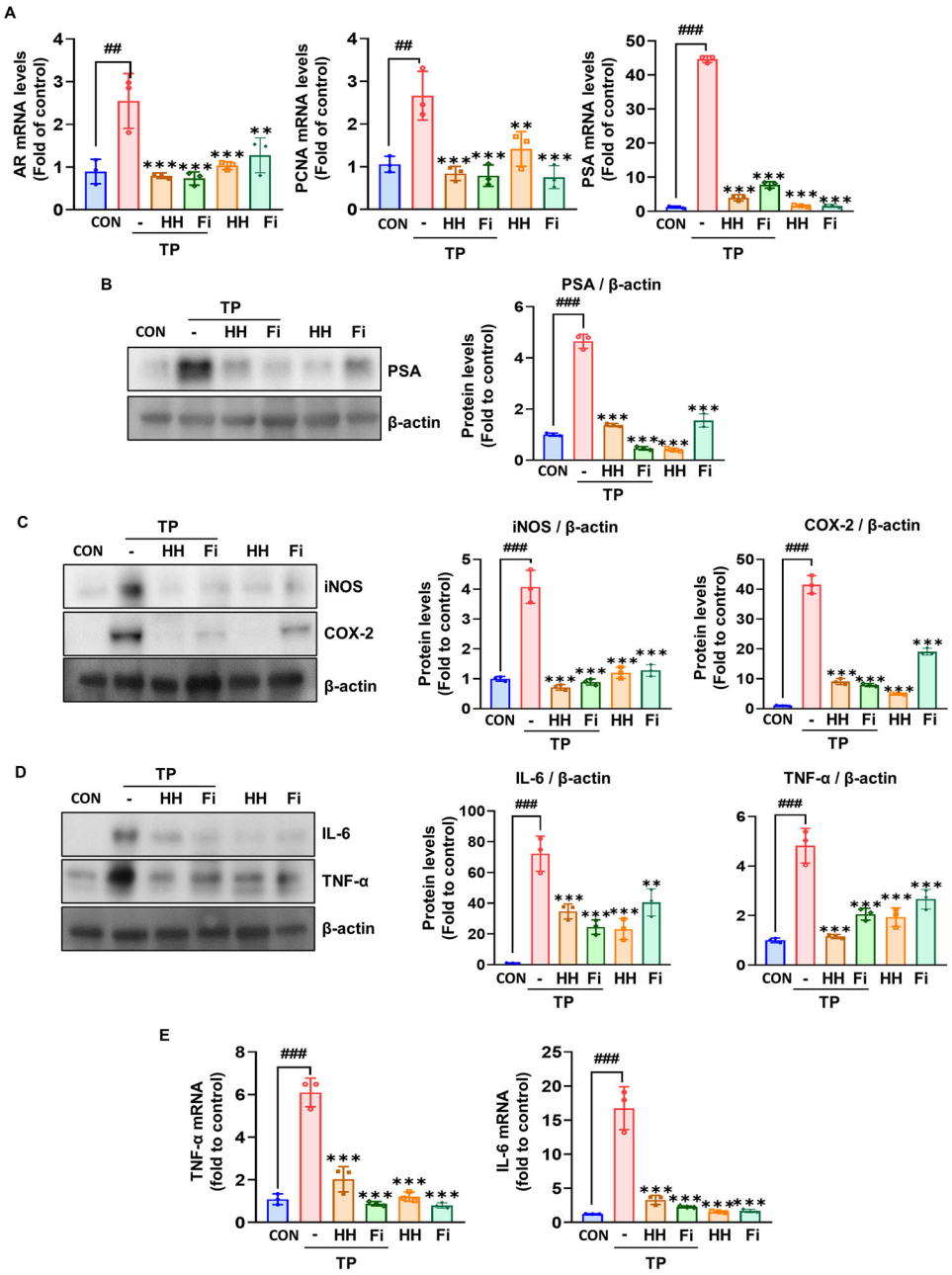


FIGURE 5 | **Effect of *Hovenia dulcis* honey on inflammatory responses in TP-induced BPH mice**. (A) mRNA expression levels of AR, proliferating cell nuclear antigen (PCNA), and prostate-specific antigen (PSA) confirmed by qRT-PCR. (B–D) Protein expression levels of PSA, inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) confirmed by Western blotting. (E) mRNA expression levels of TNF-α and IL-6 confirmed by qRT-PCR. All statistical analyses were performed using one-way ANOVA, and values are presented as the mean ± standard deviation (SD) of three replicates. ##$p < 0.01$, ###$p < 0.001$, ####$p < 0.0001$ compared with CON group. **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$ compared to the TP group. TP, testosterone propionate.

in the TP group than the control but were downregulated in the HH and Fi groups (Figure 6C,D). Our results therefore suggest that TP-induced BPH can be prevented and treated by regulating TGF-β/Smad signaling and EMT by HH.

4 | Discussion

We investigated the effect of HH on AR signaling and TGF-β/Smad2, an EMT-related signaling pathway related to BPH. In BPH, androgen/AR signaling promotes prostate epithelial cell proliferation, which supports the growth of stromal cells during BPH development (Silva et al. 2001). Therefore, abnormal activation of androgen/AR signaling can lead to BPH or prostate cancer (Izumi et al. 2013). We used a TP-induced BPH mouse model to investigate the therapeutic effect of HH and found that HH significantly reduced prostate size, epithelial thickness, serum DHT levels, and AR, PCNA, and PSA mRNA levels. Additional in vitro experiments showed that HH treatment downregulated DHT-induced AR, PSA, SRC-1, and PCNA mRNA

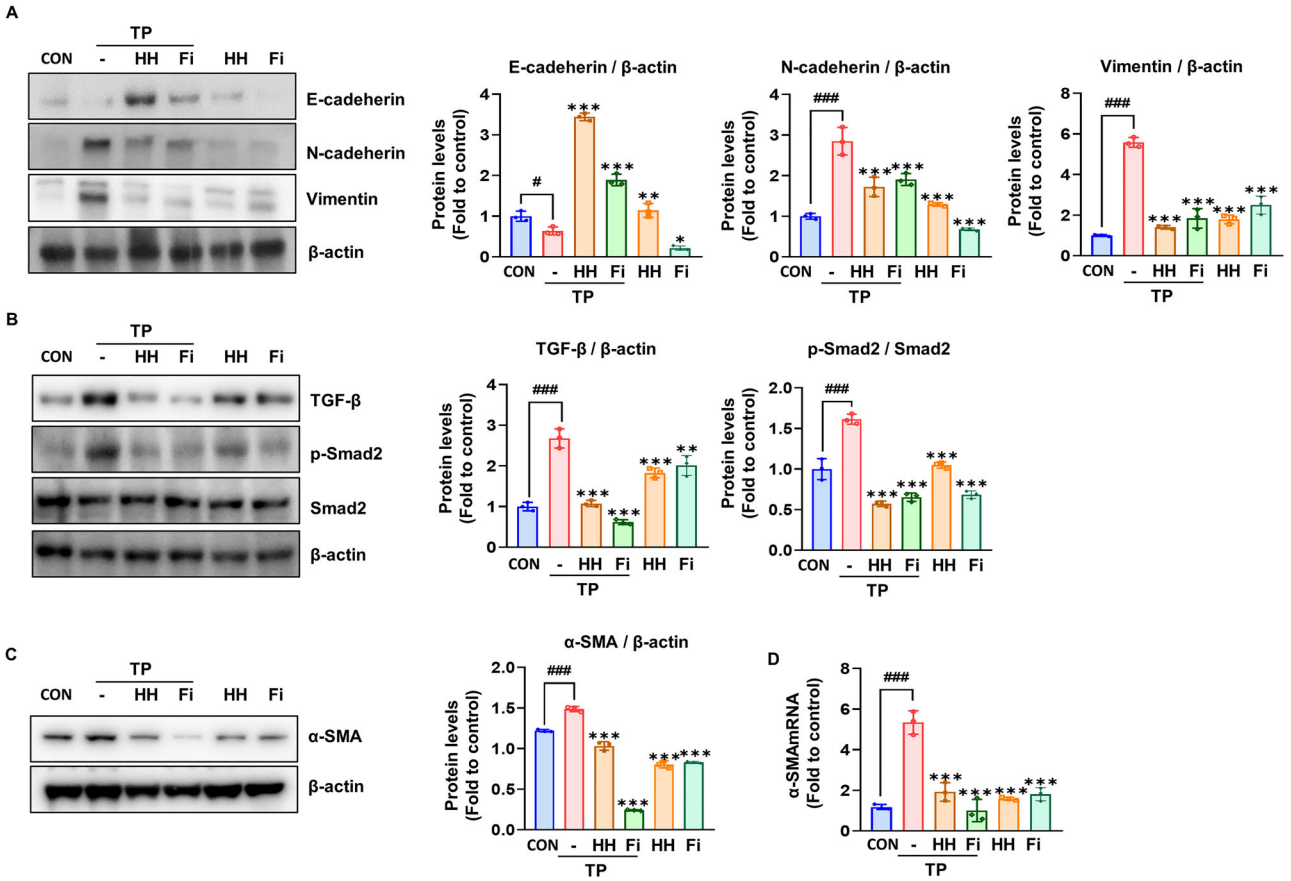


FIGURE 6 | **Prevention of epithelial–mesenchymal transition (EMT) via regulation of the TGF-β/Smad pathway in TP-induced BPH mice**. (A–C) Protein expression levels of E-cadherin, N-cadherin, vimentin, TGF-β, phosphorylated Smad2 (p-Smad2), Smad2, and alpha-smooth muscle actin (α-SMA) confirmed by western blotting. (D) mRNA expression levels of α-SMA as assessed by qRT-PCR. All statistical analyses were performed using one-way ANOVA, and values are presented as the mean ± standard deviation (SD) of three replicates. #$p < 0.05$, ##$p < 0.01$, ###$p < 0.001$, ####$p < 0.0001$ compared with CON group. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$ compared to the TP group. TP, testosterone propionate.

expression. Previous studies have shown that AR activation by androgens affects neutrophils and macrophages, thereby promoting the inflammatory response (Pace and Werz 2020). Here, we confirmed that iNOS, COX-2, IL-6, and TNF-α expression were suppressed by HH treatment in TP-induced BPH mouse and DHT-induced RWPE-1 cells. These results suggest that HH may confer therapeutic benefits via inhibiting AR signaling during BPH and by managing inflammation and disease progression.

HH not only reduces AR signaling but also exerts its therapeutic effects by modulating the EMT pathway. Existing BPH research often focuses on how androgen and TGF-β signaling regulate cell proliferation and transformation. For example, one study showed that TGF-β signaling is crucial for various cellular processes, including EMT in fibrosis and cancer (Wang et al. 2023; Giarratana et al. 2024). DHT and other androgens enhance TGF-β signaling, which may activate Smad pathways and promote EMT (Kang et al. 2001). This is significant for BPH progression and prostate cancer, as these pathways are involved in pathological cell proliferation and tissue remodeling (Alonso-Magdalena et al. 2009). Here, we demonstrated that TP treatment induced EMT in a BPH animal model, whereas treatment with HH and Fi reversed this effect. Our results highlight the role of α-SMA in BPH progression, with TP treatment significantly increasing α-SMA expression, and HH and Fi treatment suppressing α-SMA levels, suggesting that it may prevent fibrosis and abnormal stromal growth. Specifically, HH was shown to downregulate the expression of AR, PSA, and SRC-1, while simultaneously regulating EMT-related markers—increasing E-cadherin and decreasing N-cadherin, vimentin, and α-SMA levels. This suggests that HH inhibits EMT through the suppression of the TGF-β/Smad2 signaling pathway, which is crucial in BPH progression. Furthermore, HH reduced the expression of pro-inflammatory cytokines such as IL-6, TNF-α, COX-2, and iNOS, indicating its potential to mitigate inflammation-associated prostate enlargement. This integrated regulation of hormonal signaling, EMT progression, and inflammation highlights HH as a promising multi-target therapeutic candidate for BPH management.

Although we did not conduct specific experiments to isolate the effects of individual components in HH, existing literature suggests that certain phenolic compounds present in HH, such as ampelopsin, taxifolin, and myricetin, may play critical roles in its therapeutic effects. Ampelopsin is known for its strong antioxidant (Zhang et al. 2021) and anti-inflammatory properties (Qi et al. 2012), which could contribute to the suppression of pro-inflammatory cytokines observed in this study. Taxifolin and myricetin have been reported to influence cell proliferation and apoptosis pathways (Zhu et al. 2020; Butt et al. 2021), potentially affecting AR signaling and EMT processes (Das et al. 2021; Yuan

et al. 2024). The synergistic action of these bioactive compounds may underlie the observed anti-inflammatory, anti-fibrotic, and antiproliferative effects of HH in BPH models. Further studies are needed to isolate and characterize the specific contributions of these components to HH's therapeutic effects.

5 | Conclusion

In conclusion, our study demonstrates that HH exerts significant therapeutic effects on BPH by targeting multiple pathological pathways. HH effectively reduces prostate enlargement, suppresses AR signaling, and inhibits the TGF-β/Smad2-mediated EMT process. Additionally, HH exhibits potent anti-inflammatory properties, as evidenced by the downregulation of pro-inflammatory cytokines and enzymes such as IL-6, TNF-α, COX-2, and iNOS. The combined effects of HH on androgen signaling, fibrosis, and inflammation suggest its potential as a natural therapeutic agent for managing BPH. Although the exact bioactive components responsible for these effects remain to be fully elucidated, compounds such as ampelopsin, taxifolin, and myricetin are likely contributors. Further studies focusing on the specific mechanisms and clinical applications of HH are warranted to validate its efficacy in BPH treatment.

Author Contributions

Buyun Kim: writing–conceptualization, methodology, validation, investigation, data curation, visualization, writing–original draft, writing–review and editing. **Young-Eun Kim**: conceptualization, methodology, validation, investigation, visualization, writing – original draft. **Eun-Bin Kwon**: methodology, investigation, data curation. **Wei Li** and **Hye Jin Yang**: resources, formal analysis, data curation. **Sung-Joon Na**, **Sang Mi Han**, **Soon Ok Woo**, **Hong Min Choi**, **Siwon Moon**: methodology, investigation. **Young Soo Kim** and **Jang-Gi Choi**: writing – review and editing, resources, funding acquisition, conceptualization.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data will be made available on request.

Ethics Statement

Animal experiments on benign prostatic hyperplasia followed the guidelines of the Animal Care and Use Committee of the Korea Institute of Oriental Medicine (approval number 24-039).

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