https://doi.org/10.31925/farmacia.2025.6.19 **ORIGINAL ARTICLE**

THE MITIGATING EFFECT OF AZILSARTAN ON INDUCED BENIGN PROSTATIC HYPERPLASIA IN RATS THROUGH WNT/β-CATENIN/ PPAR-γ AND ANGIOTENSIN-II AXIS

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Abstract

Benign prostatic hyperplasia (BPH) is a common and disruptive health problem among elderly men. This study investigated the effect of azilsartan on BPH and its mechanisms. This study investigated the effectiveness of azilsartan medoxomil in preventing testosterone propionate (TP)-induced benign prostatic hyperplasia in rats. The experiment included 40 male Wistar rats. Animals were randomly assigned to four groups. The healthy (control) group received the vehicle orally and subcutaneously daily. The Induced (TP) group was given only 3 mg/kg testosterone propionate SC daily for 28 days. The therapy groups (TP + FIN and TP + AZ) were given testosterone propionate, finasteride 5 mg/kg/day, and azilsartan medoxomil 5 mg/kg/day orally. Prostate tissue samples were collected from euthanised animals on day 30 to evaluate prostatic index, histology, hyperplastic markers, inflammatory markers and PPAR-γ gene expression. Azilsartan significantly lowers prostate index compared to the induced (TP) group ($P < 0.0001$). The treatment normalised BPH markers, including 5α-reductase type 2 enzyme, dihydrotestosterone, TGF-β, VEGF, IL-6 and PCNA ($P < 0.0001$), while downregulating Ang II receptors and upregulating PPAR γ receptors. The agent reduced β-catenin levels in the tissue. AZ can prevent BPH in rats by decreasing cellular proliferation through activation of the PPAR-γ pathway. It also interferes with renin-angiotensin, prostatic androgen and Wnt pathways.

Rezumat

Acest studiu a evaluat efectul azilsartanului asupra BPH și mecanismele implicate. A fost investigată eficacitatea azilsartanului medoxomil în prevenirea hiperplaziei benigne de prostată induse de propionatul de testosteron (TP) la șobolani. Experimentul a inclus 40 de șobolani Wistar masculi, repartizați în patru grupuri. Grupul martor sănătos a primit vehiculul pe cale orală și subcutanată zilnic. Grupul TP a primit doar propionat de testosteron 3 mg/kg SC zilnic timp de 28 de zile. Grupurile terapeutice (TP + FIN și TP + AZ) au primit propionat de testosteron, finasteridă 5 mg/kg/zi și, respectiv, azilsartan medoxomil 5 mg/kg/zi administrate oral. În ziua 30 au fost recoltate probe de țesut prostatic de la animalele eutanasiate, pentru evaluarea indexului prostatic, histologiei, markerilor hiperplazici și inflamatori, precum și expresiei genei PPAR-γ. Azilsartanul a redus semnificativ indexul prostatic comparativ cu grupul TP ($P < 0.0001$). Tratamentul a normalizat markerii specifici BPH, precum 5α-reductaza tip 2, dihidrotestosteronul, TGF-β, VEGF, IL-6 și PCNA ($P < 0.0001$), concomitent cu inhibarea receptorilor angiotensinei II și creșterea expresiei receptorilor PPAR-γ. De asemenea, azilsartanul a redus nivelurile de β-catenină în țesutul prostatic. Azilsartanul poate preveni BPH la șobolani prin diminuarea proliferării celulare prin activarea căii PPAR-γ. Acesta interferează suplimentar cu sistemul renină–angiotensină, cu semnalizarea androgenică prostatică și cu calea Wnt.

Keywords: benign prostatic hyperplasia, testosterone propionate, PPAR-γ, azilsartan medoxomil, beta-catenin, angII-1a receptor

Introduction

Benign prostatic hyperplasia (BPH) is a prevalent androgen-related disease in elderly men. The illness is characterised by the proliferation of epithelial and stromal muscle cells, which causes symptoms in the lower urinary tract [1]. Moreover, 5α-reductase is linked to benign prostatic hyperplasia, where androgens like testosterone and dihydrotestosterone (DHT) promote prostate growth. An imbalance between apoptotic cell death and cell-cycle progression can increase cell numbers, potentially leading to uncontrolled prostatic development. Inflammation is thought to contribute to the development of benign prostatic hyperplasia. Infections can elicit a localised inflammatory response by releasing cytokines and chemokines that promote inflammation. This increases the proliferation of stromal and epithelial prostatic cells [2]. Abnormal prostate growth causes bladder outlet obstruction, resulting in obstructive lower urinary tract symptoms (LUTS).

Several mechanisms, including tissue remodelling, age-related changes in hormone levels, the participation of numerous growth factors, and disruptions in metabolic balance, have been hypothesised to influence the aetiology of BPH [3]. For most patients, pharmacotherapy is the primary therapeutic option. Alpha-blockers and 5α-reductase inhibitors (5-ARIs) are typical drugs used to treat benign prostatic hyperplasia. Alpha-blockers, such as doxazosin, terazosin, tamsulosin and alfuzosin, reduce the dynamic component of prostatic obstruction by relaxing smooth muscle fibres in the prostate. Finasteride and other α-reductase inhibitors are used to treat BPH. They prevent testosterone conversion to dihydrotestosterone, resulting in lower DHT levels and reducing hyperplastic development of the prostate, resulting in a 20 - 30% reduction in prostate size [1].

The Wnt signalling cascade is a conserved mechanism that regulates multiple cellular activities during the embryogenesis and in adult tissue homeostasis, including stem cell function, proliferation, motility, survival and differentiation [4]. Activation of the canonical Wnt signalling pathway causes aberrant growth and cellular proliferation in the prostate gland, leading to elevated β-catenin levels in hyperplastic tissues [5]. Canonical Wnt signalling starts with Wnt ligands interacting with particular receptors. This leads to the buildup of cytosolic β-catenin, which then translocates to the nucleus and activates downstream proteins. As a result, it may trigger various events, including differentiation, cell proliferation, apoptosis, fibrosis, inflammation and epithelial-mesenchymal transition. The majority of human illnesses, including benign prostatic hyperplasia (BPH), have been linked to dysregulation of the traditional Wnt pathway [5].

Peroxisome proliferator-activated receptor-γ (PPAR-γ) is a nuclear receptor that regulates metabolic pathways and inflammatory responses [6]. PPAR-γ is highly expressed in the ventral prostate of rats, human prostate tissues and cultured human prostate cell lines [7]. PPAR-γ activation may suppress Wnt/β-catenin signalling. Activation of the Wnt/β-catenin pathway can inactivate PPAR-γ in many tissues [8].

Benign prostatic hyperplasia is caused by aberrant transforming growth factor-beta (TGF-β) signalling, which results in extracellular matrix production, stromal proliferation, trans-differentiation and epithelial-mesenchymal transition [9]. Angiogenesis, or the development of new blood vessels from existing vasculature, plays a crucial role in the pathogenesis of benign prostatic hyperplasia. Elevated vascular endothelial growth factor (VEGF) expression is linked to BPH development, as VEGF is the primary regulator of angiogenesis [10].

The presence of angiotensin II (ATII) receptors across various organs suggests that the renin-angiotensin system (RAS) is involved in diverse physiological processes, prompting researchers to focus on it [11]. The RAS is a local, circulating hormonal system that mediates both autocrine/paracrine actions and systemic effects [12]. The significance of the RAS in the prostate remains mainly unknown. Local RAS overactivity may contribute to the development of BPH by encouraging cellular proliferation and increasing prostatic smooth muscle tone [13]. Angiotensin II receptor blockers (ARBs) can be evaluated across a variety of disease models owing to their pleiotropic effects. These include anti-inflammatory characteristics, antioxidant benefits, PPAR-γ activity and unclear mechanisms [11]. Azilsartan medoxomil (AZ) is the most recently approved angiotensin receptor blocker for the treatment of hypertension. This molecule acts as a prodrug, rapidly hydrolysing to produce the active component, azilsartan. AZ is a potent and highly selective ATII receptor antagonist, with high binding affinity and a modest dissociation rate compared with other angiotensin receptor blockers [14].

This study hypothesised that activation of PPAR-γ receptors could suppress prostatic stromal-epithelial proliferation and differentiation by downregulating both the Wnt signalling pathway and RAS activity. PPAR-γ activation reduces the expression of pro-inflammatory mediators, such as TGF-β and interleukin-6 (IL-6), thereby contributing to its anti-inflammatory effects. The study aimed to evaluate the protective effect of azilsartan medoxomil in testosterone propionate (TP)-induced BPH. Specifically, it focused on assessing changes in key BPH markers, including the proliferation marker proliferating cellular nuclear antigen (PCNA), inflammatory cytokines (IL-6 and TGF-β) and PPAR-γ gene expression. Additionally, the study aimed to investigate the interplay among androgen signalling, the Wnt/β-catenin pathway, PPAR-γ and the RAS pathway.

Materials and Methods

Chemicals and analysis Kits

All chemicals used in the study were of analytical grade and of the highest purity. Chemicals and analytical kits were purchased from different sources. Powder of azilsartan medoxomil and finasteride (HyperChem™, Hangzhou, China), dimethyl sulfoxide (Romil, UK), testosterone propionate injection (TestoRapid® 100 mg/mL, Alpha-Pharma, India), 0.9% sodium chloride 100 mL vial (Pioneer Pharmaceutical, Irbil, Iraq). The analytical ELISA kits of rat 5-α reductase, dihydrotestosterone, β-catenin, angiotensin-II type 1A, transforming growth factor-β, vascular endothelial growth factor, interleukin-6 and PCNA were purchased from BioString (Pennsylvania, USA).

Study setting, housing and husbandry

Albino Wistar male rats were purchased from the National Centre of Drug Control and Research in Baghdad, Iraq. Animals were allocated in the animal house of the College of Veterinary Medicine, University

of Baghdad, Baghdad, Iraq. The animals were left to acclimatise to the new environment for 10 days. The housing conditions were maintained to meet the requirements for laboratory animals, with good ventilation and air recirculation, a 12/12-hour light/dark cycle, a temperature of 25 ± 2°C, and a humidity of 45 - 60%. Animals were kept in transparent cages with unrestricted access to a standard diet of pelleted food and clean water; sterilised wood shavings were used as cage bedding. Throughout the acclimatisation period, animals were monitored daily for signs of declining health or overall weakness.

The study design and experimental protocol were prepared in compliance with the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) and the ARRIVE 2.0 guidelines [15]. The Institutional Review Board (IRB) of Al-Nahrain University/College of Medicine authorised the study (Approval number: UNCOMIRB20250144, Date: 26 October 2023).

Study design and grouping

A total of 40 male Wistar rats aged 16 - 18 weeks and weighing an average of 200 ± 50 g were randomly allocated to four study groups (n = 10) using the minimisation randomisation, with animal weight and treatment assignment used to minimise loss to follow-up during the experiment. Animal distribution between groups was based primarily on weight and was conducted by a professional animal handler blinded to the study groups. The study groups included: the control group, which received only the vehicle for the induction agent; and the treatment groups administered *via* subcutaneous (SC) and oral (PO) routes. The induced (TP) group, which received a daily SC dose of 3 mg/kg of testosterone propionate (TP) [5]. The treatment groups (TP + FIN) and (TP + AZ), in which each group received a daily dose of finasteride (FIN) 5 mg/kg, PO, the standard agent, and azilsartan (AZ) 5 mg/kg, PO [16], the intended test agent, as shown in Figure 1.

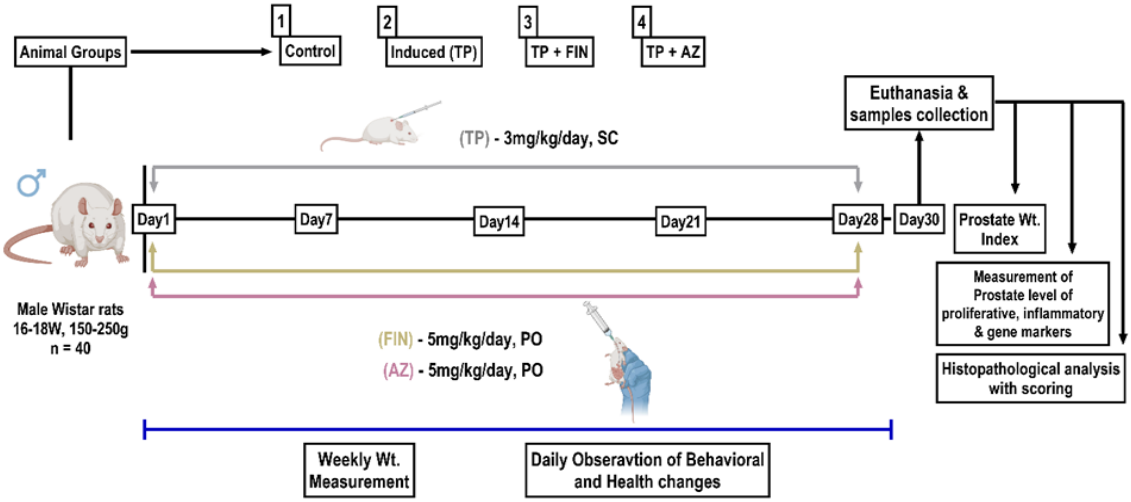

Figure 1.

Flow chart of the study design

PO = orally, SC = subcutaneous, Wt = weight, TP = testosterone propionate, FIN = finasteride, AZ = azilsartan

Induction of benign prostatic hyperplasia and treatments

The chronic hormonal model of BPH was selected as the model of interest for the current study because it can mimic certain aspects of human BPH pathology [17]. This model was achieved by daily subcutaneous injection of testosterone propionate (TP) at 3 mg/kg for 28 days [18]. It is important to highlight that the injection site was alternated daily to prevent lipo-dystrophy. A stock solution of TP injection was freshly prepared daily by mixing 1 mL of TestoRapid® (100 mg/mL) with corn oil to a final volume of 10 mL, yielding a concentration of 10 mg/mL. The administration of FIN 5 mg/kg and AZ 5 mg/kg was initiated on the first day of the induction and continued once daily for 28 days. A stock solution of both FN and AZ was daily prepared by adding 10 mg of the powder to 0.5 mL of DMSO, followed by completing the volume to 10 mL by slow addition of distilled water facilitated by a sonicated water bath at 37°C to achieve a stock solution with a final concentration of both agents of 1 mg/mL and 5% of DMSO. All interventions were performed by the same animal handler, who was blinded to the study groups and treatments.

Dose calculation of test agents

The correct dose and quantity were calculated for each animal and adjusted weekly based on changes in body weight. The following formula was used:

$$Quantitiy\ (mL) = \frac{Animal\ Wt.(kg)\ x\ Dose\ Rate\ (\frac{mg}{kg})}{Preparation\ Concentration\ (\frac{mg}{mL})}.$$

Animal euthanasia and sample collection

Two days had elapsed following the last dose of TP and treatments before euthanasia was performed. The euthanasia was done on day 30 by intraperitoneal

administration of xylazine 10 mg/kg (XylaMed™, Bimeda, USA) and ketamine 80 mg/kg (Mylan, Italy), followed by cervical dislocation [19]. Prostate tissue was dissected and cleared from the surrounding adipose tissue, then weighed. One part of the prostate was fixed in 10% buffered formalin for histopathological analysis, the other part was minced into small pieces, followed by addition of 1 mL of tissue lysis buffer (Thermo Scientific Fisher, USA) to each of 100mg of the minced tissues and homogenised using Polytron homogenizer (Thomas Scientific, USA) with an ice bucket to keep samples cold during the process. The tissue homogenate samples were centrifuged at 10,000 rpm for 15 minutes (refrigerated centrifuge, Hettich, Germany), and the supernatant was then collected into 2 mL Eppendorf tubes and stored at -20°C for later analysis [20].

Prostate weight index

The rat's body weight was recorded immediately before euthanasia, followed by recording prostate weight and calculating the prostate weight index using the formula [21]: Prostate Wt. Index = [(Prostate Wt. (mg)/(Rat Body Wt. (g)] * 100

Histopathology and scoring

Prostate tissues were fixed in 10% formalin for 48 hours, followed by the preparation of paraffin blocks. Each block was sectioned at 5 µm with a microtome. Slides for each section were prepared by treating the section with ethanol at different concentrations. Sections were then stained with haematoxylin and eosin (H&E), and xylene was added to fix the slide. Each group section was examined under a light microscope (Olympus, Japan) for structural and cellular alterations [22]. A semi-quantitative histological scoring system was used to determine the stage of hyperplasia, with 1 = mild, 2 = moderate, 3 = moderately severe and 4 = severe [23]. Each section was photographed with a camera (MU1403, AmScope, USA). The histopathological analysis was performed by two pathologists blinded to the study groups.

Enzyme-linked immunosorbent assay (ELISA)

The supernatant samples from prostate tissue homogenate were thawed at room temperature prior to analysis of the intended biomarkers by ELISA. Biomarkers implicated in the pathophysiology of BPH, including DHT, 5α-reductase type 2, β-catenin, AngII-1A, TGF-β, VEGF, IL-6 and PCNA, were analysed in prostate tissue homogenates. All analytical kits used a sandwich-type antigen-antibody ELISA for the detection of the target protein. The sample concentration was calculated using the standard curve provided by each manufacturing kit. A microplate reader (HumaReader®, USA) was used to measure absorbance at 450 nm.

Analysis of gene expression by RT-qPCR

A 30 - 50 mg sample of prostate tissue was preserved in TRIzol® reagent (Thermo Fisher Scientific, USA) for subsequent RNA extraction, following the kit's instructions. A Quantus fluorometer (Promega, USA) was used to quantify the extracted RNA. The relative mRNA expression of PPAR-γ in rat prostate tissue was quantified using the SYBR Green kit and a one-step real-time qPCR system (Sacace, Italy). All results were normalised to the housekeeping gene (GAPDH) using the delta–delta cycle threshold ($2^{-\Delta\Delta Ct}$) method [10]. Primers required for the investigation were designed using NCBI Primer-BLAST and subsequently purchased from Macrogen (Korea). Primers for the PPAR-γ gene are TGCTGGTGATCAGAAGGCTG (forward) and CACAGAGAGGTCCACAGAGC (reverse), while primers for the GAPDH gene are CGGGTTCCTA-TAAATACGGACTG (forward) and CCAATACG-GCCAAATCCGTTC (reverse).

Statistical analysis

The sample size for the study was determined using G*Power 3.1.9.4, with an F-family test and post hoc ANOVA, assuming an effect size of 0.55 and an alpha level of 0.05. Results are presented as mean ± standard deviation (SD), except for histopathology scoring, which is reported as median. The data were subjected to normality analysis using the Shapiro-Wilk and Kolmogorov-Smirnov tests. All data were normally distributed except for the histopathological analysis. Normally distributed data was analysed using One-way ANOVA, followed by post-hoc Tukey analysis, whilst histopathological scoring was evaluated using non-parametric Kruskal-Wallis analysis, followed by Dunnett post-hoc for-group comparison. P-values < 0.05 were considered statistically significant. GraphPad Prism 10.4.1 (GraphPad Software, Boston, Massachusetts, USA) was used for statistical analysis and figure generation.

Results and Discussion

The study involved daily monitoring of the animals' behavioural abnormalities and overall health. Throughout the experiment, the animals in the healthy (control) group showed only slight alterations in their health status. By the end of week 2, the animals in the induced-only group (TP) started to show signs of aggression. Certain animals in the induced (TP) group displayed aggression toward other inmates; however, it did not escalate to a degree that necessitated their removal from the experiment. The aggressive animals were housed in separate cages. In contrast, the injured individuals received immediate treatment with a 10% iodine antiseptic solution, followed by application of 2% fusidic acid cream (Leo Pharma, Denmark) to prevent opportunistic infections. The other animals in the treated groups (TP + FIN) and (TP + AZ) showed fewer signs compared to the induced (TP) group, and the same procedure was applied to the injured animals. The TP-induced BPH group had considerably higher prostate weight than the control group ($P < 0.0001$) (Figure 2A). However, treatment with FIN and AZ dramatically reduced prostate weight relative to untreated

BPH rats (Figure 2A). In TP-induced BPH rats, the prostate weight/body weight ratio (prostate index) was considerably higher ($P < 0.0001$) compared to BPH rats treated with FIN and AZ (Figure 2B).

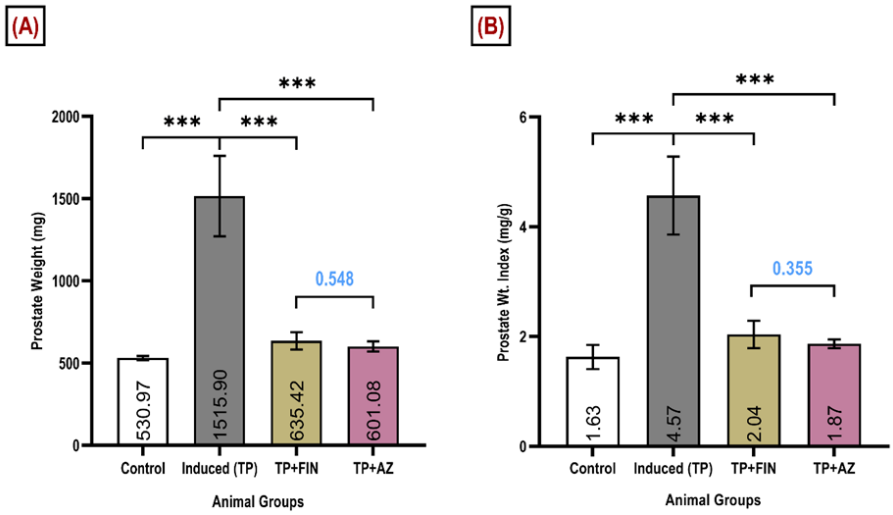

Figure 2.

Prostate weight and weight index among experimental groups

Results are expressed as mean ± SD, n = 10, and the significance level was set at ($P < 0.05$)

* represents $P < 0.01$, ** represents $P < 0.001$ and *** represents $P < 0.0001$ or lower

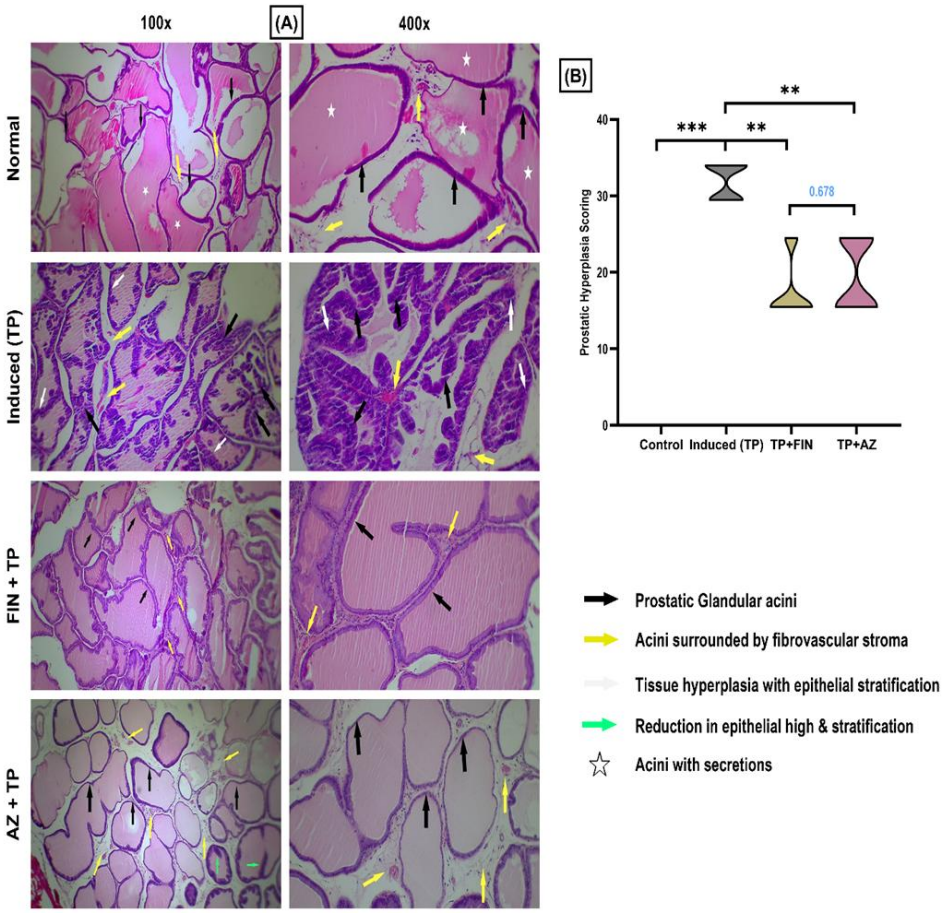

Figure 3.

Changes in histopathological analysis and scoring of the prostate tissue among animal groups. (A) The histo-pathological representation of the prostate tissue sections and (B) changes in the prostatic hyperplasia scoring level

Results are presented as median/rank. N = 9, the significant level was set at ($P < 0.05$)

* Represents $P < 0.01$, ** represents $P < 0.001$ and *** represents $P < 0.0001$ or lower. H&E, 100x and 400x

These findings were supported by the results of the histopathological analysis, where the sections of TP-induced only animals showed prominent tissue hyperplasia with irregular acinar shape and size, epithelial stratification and intra-luminal papillary projections, along with increased fibrosis and vascularity of the stroma when compared to the normal (control) animals, in which the section showed normal architecture of the prostatic glands with regular size and shape (Figure 3A). Finasteride and azilsartan, on the other hand, both reduced glandular complexity and hyperplasia, and prostatic acini showed primarily thinner epithelial thickness, reduced stratification and intraluminal papillary projections, with no significant difference between the agents, as indicated by the scoring analysis (Figure 3B). DHT is a key factor in the development of prostatic epithelial cells. ELISA experiments showed that TP-induced rats had significantly higher prostatic tissue levels of DHT ($P < 0.0001$) than control rats that received only vehicle (Figure 4B). FIN and AZ therapy significantly lowered prostatic tissue DHT levels ($P < 0.0001$). ELISA experiments showed that 5AR-2 concentrations in the TP-induced group were significantly greater ($P < 0.0001$) than in the normal control group (Figure 4A). Treatment with FIN and AZ significantly inhibited 5AR-2 expression in TP-treated rats ($P < 0.0001$).

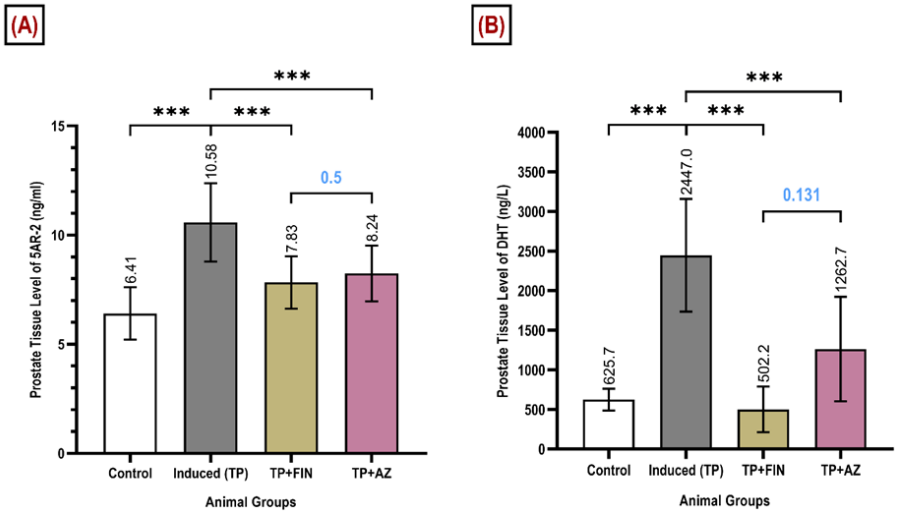

Figure 4.

Prostate tissue homogenate level of (A) 5α-reductase type-2 and (B) DHT among experimental groups

Results are expressed as mean ± SD, n = 10, and the significance level was set at ($P < 0.05$). * represents $P < 0.01$, ** represents $P < 0.001$ and *** represents $P < 0.0001$ or lower. 5AR-2 = 5α-reductase type-2, DHT = dihydrotestosterone

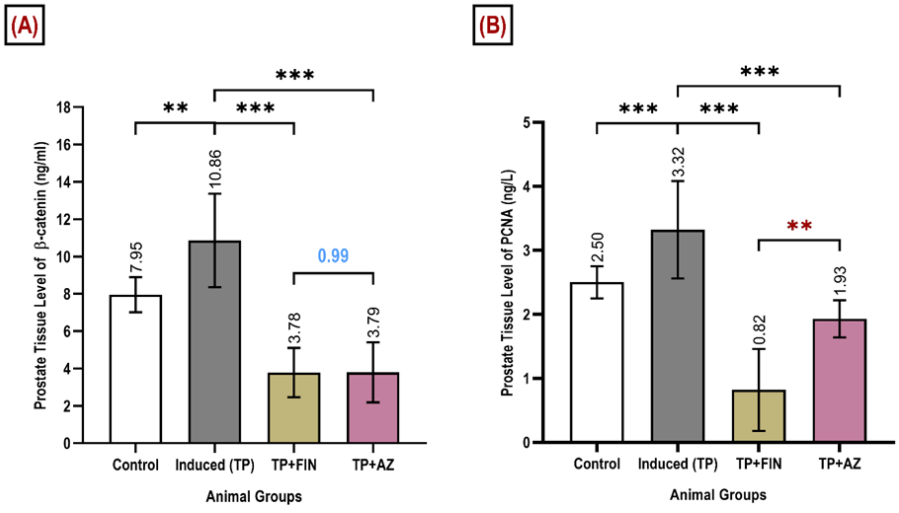

Figure 5.

Prostate tissue homogenate level of (A) β-catenin and (B) PCNA among experimental groups

Results are expressed as mean ± SD, n = 10, and the significance level was set at ($P < 0.05$). * represents $P < 0.01$, ** represents $P < 0.001$ and *** represents $P < 0.0001$ or lower. PCNA = proliferating cell nuclear antigen.

The Wnt/β-catenin pathway is a crucial signalling pathway with numerous downstream effects. The ELISA assay of tissue homogenate revealed a substantial elevation in β-catenin concentration ($P < 0.0001$) in the TP-only treated group (10.86 ± 2.50) relative to the control group (7.95 ± 0.94). AZ (3.79 ± 1.61) and FIN (3.78 ± 1.32) significantly reduced the protein levels of β-catenin in prostatic tissue homogenate ($P < 0.0001$) (Figure 5A).

The examination of tissue homogenates revealed a substantial elevation in PCNA levels in the TP-only treatment group ($P < 0.0001$) (3.72 ± 0.37 ng/L) compared to the control group (2.51 ± 0.25 ng/L) (Figure 5B). In the TP + Fin (0.82 ± 0.64 ng/L) and TP + AZ (1.93 ± 0.29 ng/L) groups, the concentration of PCNA was considerably reduced compared to the TP-only treated group ($P < 0.0001$). The results demonstrated that AZ can diminish the prostatic proliferation marker in the prostate tissues of BPH rats.

Prostatic RAS activates multiple signalling pathways, including angiogenesis, inflammation and proliferation, which contribute to the initiation and progression of BPH [24]. The examination of tissue homogenates revealed a substantial increase in AngII receptor type 1A levels in the TP-only treatment group ($P < 0.0001$) (15.27 ± 2.51 ng/L) compared to the control group (12.24 ± 0.96 ng/L) (Figure 6A). The concentration of AngII receptor type 1A in the TP + Fin (11.33 ± 1.09 ng/L) and TP + AZ (10.51 ± 1.19 ng/L) groups was considerably reduced compared to the TP-only treated group ($P < 0.0001$). The results demonstrated that AZ can diminish the prostatic proliferation marker in the prostate tissues of BPH rats.

ELISA studies indicated that prostatic tissue VEGF levels were significantly elevated ($P < 0.0001$) in TP-induced rats (694.6 ± 238.4) compared with control rats (307.6 ± 53.48) that received only vehicle. The intervention with FIN and AZ (440.8 ± 125.6) (472.3 ± 146.3) considerably reduced prostatic VEGF levels in comparison to the induced group (Figure 6B).

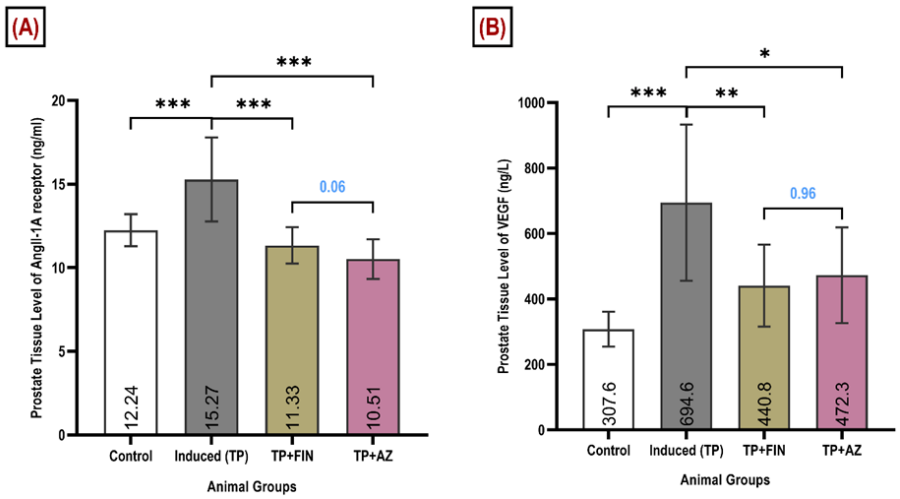

Figure 6.

Prostate tissue homogenate level of (A) AngII-1A receptor and (B) VEGF among experimental groups

Results are expressed as mean ± SD, n = 10, and the significance level was set at ($P < 0.05$)

* represents $P < 0.01$, ** represents $P < 0.001$ and *** represents $P < 0.0001$ or lower

VEGF = vascular endothelial growth factor, AngII-1A = Angiotensin II receptor type 1A

The examination of tissue homogenates revealed a substantial elevation in TGF-β levels in the TP-only treated group ($P < 0.0001$) (535.4 ± 54.81 ng/L) relative to the control group (354.51 ± 23.4 ng/L) (Figure 7A). The TGF-β concentration in the TP + Fin (262.4 ± 95.46 ng/L) and TP + AZ (325.1 ± 49.88 ng/L) groups was considerably lower than in the TP-only treated group ($P < 0.0001$).

ELISA experiments indicated that IL-6 levels in prostatic tissue were significantly higher in TP-induced rats (14.19 ± 1.29) than in control rats (7.26 ± 0.86) that received only vehicle ($P < 0.0001$). The intervention with FIN and AZ (7.12 ± 1.05) and (7.62 ± 1.187) significantly reduced prostatic IL-6 levels compared to the induced group (Figure 7B).

The amount of PPAR-γ receptor in prostate tissue homogenate was notably lower in the TP-only treated group (2.85 ± 0.82) when compared to the control group (4.12 ± 0.91). Furthermore, the concentration of PPAR-γ receptors in the AZ (3.8 ± 0.40) group was significantly higher ($P < 0.0001$) than in the TP-only-treated group (Figure 7B). The FIN-treated group showed no statistically significant difference (3.34 ± 0.75) ($P = 0.47$) when compared to the induced group. The rise in PPAR-γ was statistically significant ($P < 0.05$) in the AZ-treated group when compared to the FIN-treated group.

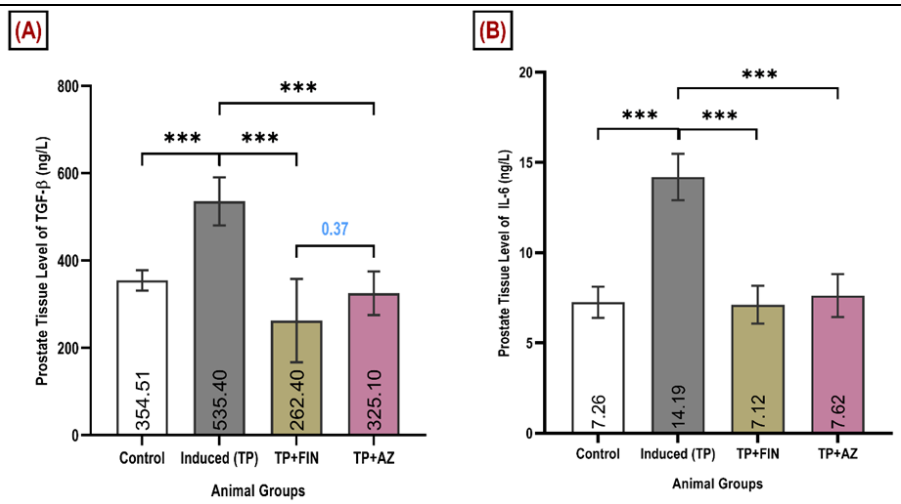

Figure 7.

Prostate tissue homogenate level of (A) TGF-β and (B) IL-6 among experimental groups

Results are expressed as mean ± SD, n = 10, and the significance level was set at ($P < 0.05$)

* represents $P < 0.01$, ** represents $P < 0.001$ and *** represents $P < 0.0001$ or lower. TGF-β = transforming growth factor-β

The receptor protein's expression level correlated with its genetic expression in prostate tissue. Seven samples (n = 7) from each group were analysed after an optimisation run to confirm the integrity of the constructed primers. Some samples were excluded due to contamination during tissue excision. The RT-qPCR results for gene expression analysis showed that PPAR-γ expression in prostate tissue samples from the TP-only induced group (0.68 ± 0.58) was lower compared to that in normal tissues (1.02 ± 0.21). Nonetheless, this reduction did not reach statistical significance ($P = 0.71$), as shown in Figure 8A. The reduction is associated with a minor decline in PPAR-γ tissue protein levels, as indicated by ELISA (Figure 8B). In contrast, animals treated with FIN and AZ exhibited a statistically significant increase ($P < 0.0001$) in PPAR-γ gene expression, with fold changes of 2.55 and 4.97, respectively, exceeding those observed in the control group. Gene expression in the treated groups increased significantly; however, tissue PPAR-γ protein levels remained comparable to those in the control group ($P < 0.05$) (Figure 7B). Tissue protein and gene expression analyses revealed notable benefits for AZ compared with FIN ($P < 0.05$).

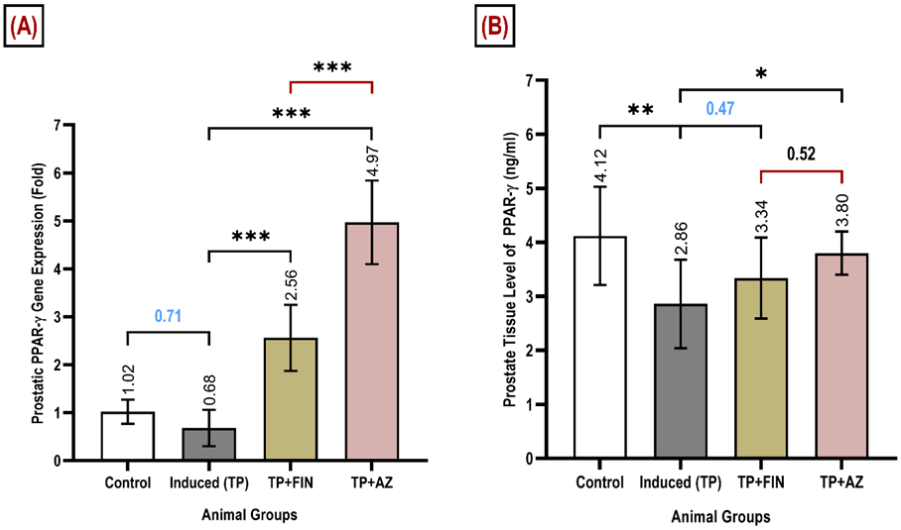

Figure 8.

Prostate tissue level of mRNA and protein level of PPAR-γ among experimental groups

(A) represents the fold expression of mRNA of PPAR-γ and (B) represents the tissue homogenate level of PPAR-γ

Results are expressed as mean ± SD. n = 7 for gene expression and n = 10 for ELISA. A significant level was set at ($P < 0.05$).

* represents $P < 0.01$, ** represents $P < 0.001$ and *** represents $P < 0.0001$ or lower

mRNA = messenger, ribonucleic acid, PPAR-γ = peroxisome proliferator-activated receptor-gamma

Benign prostatic hyperplasia is defined by the abnormal growth of epithelial and stromal elements within the prostate. This research examines the effects of finasteride and the angiotensin receptor blocker azilsartan on TP-induced experimental BPH in rats, focusing on prostate weight, histological alterations and molecular markers of cell proliferation, including PCNA. Animals administered TP subcutaneously at 3 mg/kg/day exhibited a significant increase in prostatic weight, prostate index, stromal and epithelial proliferation and differentiation, and induction of prostatic hyperplasia, thereby confirming the occurrence of prostatic hyperplasia. The findings are consistent with previous studies indicating that testosterone and its active metabolite dihydrotestosterone induce hyperplastic alterations in the prostate [21, 25]. Oral administration of FIN and AZ resulted in a significant reduction in prostate weight and weight index compared with the TP-induced group, with no difference between the two agents. Results for the FIN group were consistent with previous studies, which have shown that oral administration of finasteride reduces prostatic hyperplasia and differentiation [9, 25, 26]. In the AZ-treated group, studies have reported that angiotensin II (Ang II), acting through AT1 receptors, can stimulate prostate cell proliferation and increase sympathetic nerve activity, which may exacerbate urinary symptoms associated with BPH, thereby contributing to the disorder's pathophysiology by enhancing local sympathetic tone and promoting cell growth [26]. Another study has indicated that ARBs can lead to a decrease in prostate weight and size, reporting that losartan, an AT1 receptor antagonist, has been shown to inhibit the effects of Ang II on noradrenaline release from sympathetic nerves in the prostate, indicating that prostatic hyperplasia may be lessened by inhibiting this route [27, 28].

Earlier research indicated that testosterone propionate led to benign prostate hyperplasia in rats, resulting in a notable increase in prostatic tissue DHT levels [5, 29], and promoted the overexpression of the 5α-reductase type 2 enzyme [30]. Finasteride, a 5α-reductase type II inhibitor, significantly lowers DHT levels, attenuating androgen receptor activation. This suppression results in decreased expression of proliferative markers like PCNA and β-catenin, reduced pro-angiogenic (VEGF) and pro-fibrotic (TGF-β) factors and dampened the inflammatory cytokine IL-6 [9, 21]. AZ showed a significant reduction in 5-AR2 expression and DHT synthesis, inhibition of IL-6 and suppression of fibrotic and angiogenic factors *via* TGF-β and VEGF pathways. Furthermore, AZ's blockade of AT1R appears to diminish AR expression and disrupt the positive feed-back loop between the RAS and androgen signalling. Additionally, by counteracting oxidative stress and modulating the WNT/β-catenin axis, AZ may indirectly restore PPAR-γ expression, contributing to anti-proliferative and anti-inflammatory effects [26].

Proliferating cell nuclear antigen (PCNA) is a highly conserved eukaryotic protein that plays a crucial role in several DNA-related processes, including chromatin assembly, DNA replication, repair and post-replication modification [31]. Moreover, it has been demonstrated that PCNA levels are substantially correlated with the proliferative status of several organs, including the prostate. Additionally, rats with BPH produced by testosterone propionate showed a significant increase in PCNA expression [21]. AZ reduced PCNA levels to a degree comparable to that of the standard drug FIN, with statistical significance compared with the TP-induced group. Angiotensin receptor blockers (ARBs) have the potential to lower PCNA levels by inhibiting essential signalling pathways triggered by Angiotensin II, such as the phosphoinositide 3-kinase (PI3K)/Akt and mitogen-activated protein kinase (MAPK) pathways. The pathways play a vital role in controlling the proliferative effects of Ang II, and when ARBs block them, the transcription of genes linked to cell cycle progression, such as those encoding PCNA, decreases [32].

In BPH, testosterone elevates β-catenin levels through complex interactions between the Wnt/β-catenin pathway and androgen signalling. Androgens can boost the transcriptional activity of β-catenin signalling. A study indicates that AR enhances β-catenin binding to the promoter regions of target genes, such as c-Myc and Cyclin D1, which are associated with cell survival and proliferation [33]. Activation of this pathway was reduced by finasteride, an inhibitor of DHT formation. This results in modified tissue growth patterns and reduced cell proliferation [34]. The AZ-treated group exhibits a significant reduction in β-catenin levels. Ang II promotes prostate inflammation, enhancing β-catenin signalling and RAS activity, which creates a feedback loop that maintains hyperplasia [35]. The anti-proliferative effects of ARABs in prostate tissues may be linked to reduced AR activity and expression, mediated by their suppression of AT1R [36]. The regulation of RAS/Wnt/β-catenin signalling by GSK3β-mediated phosphorylation is the central mechanism of interaction between the Wnt/β-catenin and the RAS-ERK pathways. Under Wnt-stimulated conditions, β-catenin accumulates and is stabilised by escaping degradation through dissociation of the β-catenin destruction complex. This stabilisation also indirectly stabilises RAS proteins by masking their phosphorylation sites, which are necessary for their degradation [37].

Results of tissue homogenate analysis demonstrated that TGF-β levels were significantly increased in the TP-only treated group. A recent study showed that TGF-β and p-Smad2/3 were upregulated in TP-induced BPH mouse prostate [38]. Another study reported that testosterone-treated male monocyte-derived macrophages significantly upregulate TGF-β gene expression and TGF-β protein production [39]. FIN & AZ significantly

reduced TGF-β levels in prostate tissue compared with the TP-induced group. This reduction demonstrated by FIN is supported by previous studies, in which one reported that testosterone therapy dramatically increased TGF-β levels in human scalp dermal fibroblast experiments, and that pretreatment with finasteride reduced this induction by approximately 30.4%. This implies that finasteride may prevent the elevation of TGF-β typically triggered by androgens by lowering DHT levels [40]. Another study suggests the interaction between androgens and AR leads to TGF-β production, which plays an important role in epithelial-mesenchymal transition (EMT) that accompanies fibrosis in the prostate [41]. TGF-β expression and activity are stimulated by angiotensin II, particularly in vascular and renal tissues. AZ reduces downstream signalling that increases TGF-β production by blocking AT1R [41]. Other studies revealed that AZ can lessen the effects of angiotensin II on TGF-β signalling in renal tissues, reducing inflammation and fibrosis linked to hypertension and chronic kidney disease [42, 43].

The current study showed a substantial increase in VEGF levels in the prostatic tissue homogenate of the testosterone-induced group. These results are consistent with previous studies [44, 45]. On the other hand, treatment with finasteride resulted in a significant reduction in prostatic VEGF levels compared to the induced group. A clinical study demonstrated that individuals who received finasteride treatment for two to four weeks before transurethral resection of the prostate (TURP) had lower levels of VEGF than those in the control group [46]. This can be explained by the indirect effect of finasteride on VEGF through its ability to reduce the active form of testosterone, and thus androgen receptor activation and translocation in the nucleus, finally modulating the expression of different genes involved in cellular proliferation and even angiogenesis, including hypoxia inducible factor-1alpha (HIF-1α), which is necessary for VEGF signalling in regulating cell proliferation in a BPH stromal cell line [47]. The reduction in prostatic VEGF levels with azilsartan is attributable to ARBs that block AT1R, which prevent ERK1/2 phosphorylation, a process essential for Ang II-mediated VEGF production and the ensuing angiogenic responses [48].

Testosterone-induced BPH rats showed significantly increased IL-6 expression compared to controls. Studies have revealed the important role of cytokines in BPH, particularly interleukin-6, which is secreted by various prostate compartments: prostate cells, periprostatic fat cells, prostate microvascular endothelial cells and inflammatory and immune cells engaged in the assaulted tissue microenvironment [49, 50]. Research suggests intraprostatic DHT may activate inflammatory pathways, including IL-6 and IL-8, through genomic and non-genomic mechanisms [51]. Chronic inflammation in BPH tissues has been linked to elevated IL-6 levels, which contribute to stromal and epithelial proliferation [52]. This may explain the effect of FIN in reducing IL-6 tissue levels, mediated by reduced DHT levels due to 5α-reductase inhibition. It has been demonstrated that angiotensin II increases the IL-6 expression and synthesis across multiple organs, thereby contributing to oxidative stress, endothelial dysfunction and VEGF overexpression. Crosstalk among DHT, VEGF and Ang II has been found to influence vascular remodelling and cellular proliferation in the prostate [53]. Still, there is limited literature that explicitly explains this. This explains the results for AZ observed with IL-6 and Ang II, in which the agent reduced IL-6 and Ang II receptor levels in prostate tissue homogenate compared with the induced group.

PPAR-γ is a nuclear receptor that oversees various metabolic pathways and inflammatory responses [6]. PPAR-γ appears to play a crucial role in the growth and differentiation of the normal prostate. Jiang *et al*. demonstrated that low-grade prostatic intraepithelial neoplasia arises when prostatic epithelial cells in mice have PPAR-γ conditionally knocked out [54]. It is well established that PPAR-γ influences the development of BPH by regulating inflammatory pathways and cellular proliferation. Overexpression of PPAR-γ reduces prostatic inflammation, favouring the production of anti-inflammatory cytokines and downregulating pro-inflammatory ones, thereby contributing to stromal and epithelial cell regulation and affecting prostate enlargement [55]. As observed in the current study, TP-treated animals showed a significant reduction in PPAR-γ gene expression and protein levels, findings supported by recent research showing that exogenous TP-induced DHT elevations affect PPAR-γ expression and BPH development [56]. This effect was mitigated by the administration of either finasteride or azilsartan, as both agents restored PPAR-γ levels to near-normal values. The 5α-reductase inhibitor finasteride can indirectly influence PPAR-γ expression through its effects on androgen signalling [57]. The increase in PPAR-γ was statistically significant in the AZ-treated group. Recent studies indicate a connection among the RAS cascade, PPAR-γ expression and the canonical Wnt/β-catenin pathway. In the kidney, cells of the juxtaglomerular apparatus secrete renin, which is activated by the pro-renin receptor (PRR). PRR activates the canonical Wnt/β-catenin pathway, thereby stimulating angiotensin, renin, ACE and AT1 receptor secretion. Simultaneously, the activation of the Wnt/β-catenin pathway leads to a downregulation of PPAR-γ expression [58].

Angiotensin II acts primarily *via* the AT1 receptor, thereby activating MAPK and PI3K/Akt signalling and promoting VEGF expression in prostate cells. On the other hand, Ang II affects PPAR-γ in two ways, according to a previous study. The early and rapid phase was characterised by the export of PPAR-γ from the nucleus to the cytoplasm, whereas a decrease in PPAR-γ protein levels characterised the later phase.

Concomitantly, the two actions of the Ang II were accompanied by suppression of PPAR-γ DNA-binding activity and the reduction in PPAR-γ protein was coupled to inhibition of PPAR-γ target genes. Therefore, the downregulation of nuclear PPAR-γ protein and activity represents the overall effect of Ang II on PPAR-γ; thus, azilsartan acts by blocking the Ang II receptor and potentially suppresses the MAPK and PI3K/Akt activity, mitigating the VEGF production and upregulating PPAR-γ levels in the nucleus and increasing the PPAR-γ-DNA binding activity, which eventually further interferes with other signalling pathways like NF-κB. This can lead to reduced VEGF expression and production [59, 60].

Conclusions

Both finasteride (FIN) and azilsartan (AZ) mitigated TP-induced BPH by modulating distinct pathways, primarily through androgen suppression for FIN and RAS inhibition for AZ. AZ showed anti-inflammatory and anti-proliferative effects in hyperplastic prostate tissue, almost comparable to those of FIN, through reductions in IL-6, TGF-β and VEGF, mediated by the Ang II-1A and Wnt/β-catenin/PPAR-γ axis. Their complementary actions suggest promising potential for combined or sequential therapy to manage BPH more effectively, warranting further clinical investigation.

Conflict of interest

The authors declare no conflict of interest.

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