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The Prostate

ORIGINAL ARTICLE

Probenecid and Eugenol Mitigate Testosterone Induced Benign Prostatic Hyperplasia by Targeting Uric Acid-Induced Inflammation and Pro-Survival Pathways

Nibrass Taher Abdali[1] | Yasmin S. Mohamed[2] | Hala F. Zaki[2] | Laila A. A. Ramadan[3] | Hassan Afify[3]

[1]Department of Pharmacology and Toxicology, College of Pharmacy, Al-Farahidi University, Baghdad, Iraq | [2]Department of Pharmacology, Faculty of Pharmacy, Cairo University, Cairo, Egypt | [3]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Egyptian Russian University, Cairo, Egypt

Correspondence: Hassan Afify (Hassan-afify@eru.edu.eg)

Received: 4 March 2025 | **Revised:** 6 June 2025 | **Accepted:** 24 July 2025

Funding: The authors received no specific funding for this work.

Keywords: benign prostatic hyperplasia | eugenol | inflammation | probenecid | testosterone

ABSTRACT

Background: Benign prostatic hyperplasia (BPH) is a prevalent condition among aging males, significantly impacting their quality of life. Emerging evidence links elevated uric acid (UA) levels to prostatic inflammation and hyperplasia, potentially through oxidative stress and activation of proliferative pathways. However, the role of UA in BPH pathogenesis remains poorly defined. The study aims to investigate the potential protective effects of a natural phenolic compound, eugenol, and/or probenecid against testosterone-BPH in rats, along with the possible underlying mechanisms.

Methods: BPH was induced by administering testosterone (3 mg/kg; s.c.) for 2 weeks, either alone or following a 1-week pretreatment with probenecid (200 mg/kg/day; i.p.), eugenol (10 mg/kg/day; p.o.), or their combination.

Results: The results demonstrated a significant increase in prostate index, cell survival markers such as cyclin D1 expression, and histopathological features indicative of BPH following testosterone administration. Furthermore, testosterone led to elevated uric acid levels, oxidative stress, inflammatory markers, and activation of pro-survival pathways including PI3-K/Akt/mTOR and NFκB. However, treatment with probenecid, eugenol, or their combination effectively attenuated these alterations, demonstrating their anti-inflammatory, antioxidative, and anti-hyperproliferative effects. Notably, combination therapy exhibited superior efficacy compared to individual treatments. These findings suggest that probenecid and eugenol may mitigate testosterone-induced BPH by targeting the uric acid-induced inflammation and pro-survival pathways.

Conclusions: This study provides novel insights into the therapeutic potential of probenecid and eugenol as adjunctive treatments for BPH, offering promising avenues for further clinical investigation.

1 | Introduction

Benign prostatic hyperplasia (BPH) is a prevalent factor contributing to lower urinary tract symptoms (LUTS) in elderly males. It comprises a rapid proliferation of prostate cells surrounding the urethra, resulting in bladder outlet obstruction and consequent urine incontinence. BPH rarely causes mortality; however, greatly affects the health and quality of life of elderly men. There is no defined limit for an enlarged prostate, and the diagnosis of BPH is primarily based on symptom severity and not solely on prostate volume, and BPH does not appear to significantly increase the risk of prostate cancer [1–4]. BPH is a widespread condition that impacts 50% of men at age 60 and 90% of men over 80. It causes troublesome LUTS that create a significant healthcare expense of almost $4 billion each year in the United States. LUTS greatly impair patient

quality of life, affecting more than 35 million males over the age of 30 who have had at least moderate symptoms. With the increasing aging population and rising incidence of BPH, there is a crucial need for safe, efficient, and easily accessible treatment choices for both patients and the urology field [5, 6].

While pathophysiology is yet unresolved, the androgen system, particularly the androgen receptor, plays a significant role. The 5α-reductase enzyme synthesizes dihydrotestosterone (DHT), which activates androgen receptors and leads to BPH development [7]. Furthermore, prostatic inflammation and metabolic factors are becoming identified for their role in benign prostate enlargement and LUTS [8, 9]. The use of testosterone to treat late-onset hypogonadism in males has grown, but worries regarding its detrimental impact on the prostate, including the possibility of prostate cancer, persist [10]. There is strong evidence from rat models of BPH that testosterone plays a key role in its development [11–13]. There is a discrepancy, however, as testosterone levels fall in men as they age, even while BPH rates rise with age [10]. The specific mechanism by which testosterone impacts BPH is unknown. Although circulating testosterone declines with age, intraprostatic DHT levels remain stable or may even increase due to heightened 5α-reductase activity and decreased metabolic clearance, thereby maintaining androgenic stimulation of the prostate [9, 10, 14]. According to one suggested mechanism, testosterone′s anabolic impact may cause muscle regeneration leading to increased nucleic acid and purine metabolism and, consequently, rising uric acid (UA). This, along with increased ATP consumption as muscle mass grows, may lead to raised UA levels, which have been related to BPH [8, 15].

Elevated UA levels can trigger inflammation by activating pro-inflammatory pathways and oxidative stress, which damages cells and tissues [16, 17]. While UA was formerly thought to have antioxidant capabilities, recent evidence suggests that hyperuricemia causes more damage to vascular endothelial cells than antioxidants, emphasizing its involvement in inflammation-induced damage [18]. UA irritation triggered by testosterone has been linked to the initiation of inflammation in prostate tissue. Irritation stimulates lymphocytes, particularly B and T cells, leading to excessive production of cytokines including IL-6, IL-1β, IL-2, and TNF-α, along with other growth factors [19, 20]. The IL-6 receptor serves as a tyrosine kinase-linked receptor that activates nuclear factor of kappa B (NFκB), phosphatidylinositol 3-kinase (PI3-K), and Akt upon activation. Akt, a serine/threonine protein kinase, is initially inactive; however, could be activated by growth factors and cytokines. Akt activation activates mammalian target of rapamycin (mTOR), causing it to translocate into the nucleus and increase the production of pro-survival genes such as B-cell lymphoma-2 and extra-large (Bcl-2 and Bcl-x). These pro-survival genes aim to evade apoptosis and increase prostatic tissue development [21–24].

Probenecid has long been used to reduce blood UA levels and hence prevent gout episodes. It does this by inhibiting anion transporters and blocking UA reabsorption in the kidneys [25]. In a previous study, probenecid demonstrated potential as a therapeutic agent for BPH by reducing PGE2 synthesis from COX-2, thereby inhibiting the EGFR/ERK1/2 signaling cascade. Additionally, probenecid has been shown to decrease NF-κB levels, promote apoptosis, and alleviate oxidative stress in normal tissue [26–29].

Eugenol, a phenolic compound, is present in a multitude of botanical specimens, including clove, rosemary, cinnamon, and nutmeg [30, 31]. In addition to being utilized in cosmetics and baked foods, beverages, sweets, and frozen dairy products, it is frequently employed as a flavoring agent [32]. Eugenol is widely recognized for its anti-inflammatory and antioxidant characteristics. Eugenol has been shown to have the capacity to mitigate the clastogenic effects of genotoxins and gamma radiation in vivo [33, 34]. Eugenol has demonstrated the ability to mitigate the testicular toxicity induced by acrylamide in rats through its modulation of the AMPK/p-AKT/mTOR signaling pathway and modification of the blood-testis barrier. Eugenol additionally induced apoptosis and autophagy in breast cancer cells via inhibition of the PI3K/AKT/FOXO3a pathway [35, 36].

The objective of the current study is to investigate the significance of the uric acid/AKT/mTOR signaling pathway in BPH induced by testosterone and to evaluate the potential therapeutic properties of eugenol and probenecid in mitigating BPH. By examining this pathway and the modulatory effects of eugenol and probenecid, the study aimed to discover novel therapeutic strategies for BPH.

2 | Materials and Methods

2.1 | Animals

The study involved male Wistar rats weighing 200–250 g, housed under controlled conditions with a temperature of 22 ± 2°C and a 12-h light/dark cycle. Rats were provided with a standard diet and unrestricted access to water throughout the experiment. The experimental protocol was approved by the Ethics Committee for Animal Experimentation at the Faculty of Pharmacy, Cairo University, and complied with the guidelines outlined in the Guide for Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 2011). The protocol approval code is (PT 1390).

2.2 | Chemicals

Testosterone and probenecid were obtained from Chemical Industries Development (CID) company, located in Giza, Egypt. Testosterone was prepared by dissolving it in pure olive oil, while probenecid was dissolved in isotonic saline immediately before oral gavage administration. Eugenol was acquired from Sigma Aldrich and mixed with olive oil for oral gavage.

2.3 | Experimental Design

Rats were randomly assigned to 7 groups ($n = 10$ per each). The rats of group I received i.p. injections of the vehicle mixture isotonic saline (1 mL/kg/d; i.p.), olive oil (1 mL/kg/d; s.c.), and (1 mL/kg/d; p.o.) for 3 weeks, to serve as the normal control

group. Group II (Eugenol): Rats received eugenol (10 mg/kg/day; p.o.) in olive oil for three consecutive weeks. Group III (Probenecid): Rats received probenecid (200 mg/kg/day; i.p.) in isotonic saline for three consecutive weeks. BPH was induced in Groups IV to VII by administering testosterone (3 mg/kg; s.c.) dissolved in olive oil for two consecutive weeks. Rats in Groups V and VI received eugenol and probenecid, respectively, starting 5 days before and continuing concurrently with testosterone administration as previously described. Group VII (Combination treated group): Rats were given a combination of eugenol and probenecid starting 5 days before and continuing concurrently with testosterone administration as previously described.

Seventy-two hours following the final testosterone injection, rats were anesthetized with pentobarbital sodium (100 mg/kg) (Wang & Wu, 2020), and blood samples were collected from the retro-orbital sinus using non-heparinized capillary tubes for serum separation. The blood samples were centrifuged, and the sera were collected and stored at −20°C for later evaluation of serum uric acid levels. Following blood collection, the rats were humanely killed by decapitation. Then the prostate tissues were promptly removed for morphological evaluation and weighing. The ventral lobes were preserved in 10% neutral buffered formalin and then embedded in paraffin for further histopathological and immunohistochemical evaluations. One portion of the prostate tissue was homogenized in ice-cold saline to create a 10% homogenate for biochemical and colorimetric assays. Another portion was placed in radio-immunoprecipitation assay (RIPA) buffer with a protease inhibitor cocktail for Western blot analysis. The remaining portion was preserved in RNA lysis buffer for subsequent qRT-PCR analysis.

2.4 | Assessment of Prostate Index

The rats were weighed while anesthetized. Prostate tissues were then removed and weighed individually. The prostate index was determined as the prostate weight divided by the rat's total body weight [37].

2.5 | Biochemical Analysis

2.5.1 | Assessment of Serum Uric Acid

The collected sera were subjected to analysis for serum uric acid levels using the colorimetric technique using Quanti Chrom™ uric acid assay kit (BioAssay Systems, USA), in accordance with the instructions provided by the manufacturer.

2.5.2 | Assessment of Oxidative Stress Parameters

The prostatic oxidative status was assessed via colorimetric assay of reduced glutathione (GSH), malondialdehyde (MDA), and catalase (CAT) enzyme activity. These assays were performed using Biodiagnostics Company kits, and the procedures were carried out in accordance with the manufacturer's instructions.

2.6 | Assessment of Inflammatory Markers

The prostatic contents of IL-6, IL-1β, and TNF-α were quantified using commercially available kits obtained from ABCAM; the procedures were carried out according to product instructions.

2.7 | Western Blot Analysis of Phosphorylated PI3K, Phosphorylated AKT, and Phosphorylated mTOR in Prostate Tissue

The radioimmunoprecipitation assay (RIPA) lysis buffer (Abcam) with added phosphatase and protease inhibitor mixture was used to prepare tissue lysate by sonication. The mixture (100 μg) of the samples and a constant amount of protein were added to the Wells for electrophoresis through a 10% SDS-PAGE gel. Then, the gel was transferred on a polyvinylidene difluoride (PVDF) membrane (Bio-Rad Laboratories). The process of blocking the membrane was carried out using a solution of 5% milk in Tris-buffered saline, Tween 20 (TBST). Then, the membrane was subjected to overnight incubation at 4°C with the primary antibody (anti p-PI3K (ser110α) (1:500, Catalog No. # PA5-87398), anti p-mTOR (ser2448) (1:500, Catalog No. #MA5-35832) anti p-AKT1 (ser473) (1:500, Catalog No. # PA5-85513); (Santa Cruz Biotechnology), after that the membrane was washed with TBST. Subsequently, the membranes were incubated with horse-radish-peroxidase conjugated secondary antibody for a duration of 1 h at room temperature, after that the membrane was washed with TBST. At last, the bands detection was achieved via improved chemiluminescence Western blotting kit (LI-COR®), then scanned with C-DIGIT® Blot Scanner (LI-COR Biosciences), and the bands intensities were then measured through Image LabTM Software (Bio-Rad). Subsequently, relative quantification was performed using the control protein β-actin.

2.8 | Reverse Transcription-Quantitative Polymerase Chain Reaction (RT-qPCR) Analysis of Gene Expressions for NFκB

The TRIzol® reagent (Invitrogen) was used to extract the whole ribonucleic acid (RNA) from the prostate tissue. Afterwards, the isolated RNA was subjected to reverse transcription to produce complementary deoxyribonucleic acid (cDNA). The primer sequences used for the β-actin and NFκB genes are found in Table 1. The RT-qPCR reactions were performed using Power SYBR® Green PCR Master Mix (Applied Biosystems) and carried out on a 7500 Real-Time PCR System (Applied Biosystems). The thermal cycles comprised an initial denaturation step at 95°C for 4 min, followed by 40 cycles of denaturation at 95°C for

TABLE 1 | Primers used for RT-qPCR analysis.

Gene	Sequence
β-actin	F: 5′ -AAGATCCTGACCGAGCGTGG-3′
	R: 5′ -CAGCACTGTGTTGGCATA GAGG-3′
NFκB	F: 5′ -TTACGGGAGATGTGAAGATG-3′
	R: 5′ -ATGATGGCTAAGTGTAGGAC-3′

10 s, annealing at 60°C for 30 s, and extension at 72°C for 10 s. The ΔΔCt method was utilized for data analysis [38], and the results were reported as relative fold changes in comparison to the gene expressions of the control group.

2.9 | Histopathological Examination

Dissected prostate tissue samples were flushed and fixed in 10% neutral buffered formalin for 72 h. Samples were trimmed and processed in serial grades of alcohols, cleared in Xylene, samples were infiltrated and embedded into Paraplast tissue embedding media. 4 μm thick tissue sections were cut by a rotatory microtome for demonstration of ventral lob of prostate gland. The sections were stained by Hematoxylin and Eosin as a general morphological examination staining method, then examined in blinded manner by an experienced histologist using light microscope. All standard procedures for samples fixation and staining according to Culling, C.F.A. (2013) [39]. The ventral prostate lobe was selected due to its high sensitivity to androgenic stimulation and its consistent histopathological response to testosterone administration. It is also the first lobe to exhibit morphological and inflammatory changes in rodent models of BPH, making it a reliable and widely accepted target for evaluating disease pathology and therapeutic responses [8, 26, 37, 40–42].

At least 6 nonoverlapping fields were randomly selected and scanned from each ventral lob tissue section of each sample for the determination of mean lining epithelial cells height in H&E-stained tissue sections [26].

2.10 | Immunohistochemical Staining of Cyclin D1

Five microns thick paraffin-embedded tissue sections were prepared. Immunohistochemical, according to the manufacturer's protocol. Deparaffinized retrieved tissue sections were treated by 0.3% H_2O_2 for 20 min. Then were incubated with anti-Cyclin D1 monoclonal antibody [EPR2241] –abcam– (1:100). 4°C overnight. Then washed out by PBS, followed by incubation with secondary antibody HRP Envision kit (DAKO) 20 min; washed out and incubated with diaminobenzidine (DAB) for 15 min. Washed by PBS, then counter-stained with hematoxylin, dehydrated and cleared in xylene, then coverslipped for microscopic examination.

At least six nonoverlapping fields were randomly selected and scanned from each ventral lob tissue section of each sample for the determination of mean percentage of immunohistochemical expression levels of Cyclin D1 in immunostained tissue sections. All light microscopic examination and morphometric data were obtained by using Leica Application module for histological analysis attached to Full HD microscopic imaging system (Leica Microsystems GmbH).

2.11 | Statistical Analysis

Statistical analysis was performed using GraphPad Prism version 9.5.1 (GraphPad Software). Data from all experimental groups (including biochemical parameters, gene expression levels, immunohistochemical staining (Cyclin D1 area %), and Western blot densitometry) were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. All data are expressed as mean ± standard deviation (SD), and a p-value of less than 0.05 was considered statistically significant. All graphs were constructed using GraphPad Prism software.

3 | Results

3.1 | Effect of Probenecid or Eugenol Either Alone or in Combination on Testosterone-Induced Increase in Prostate Index

The administration of testosterone resulted in a significant to reach 2.9-fold of the baseline value ($p < 0.0001$) increase in prostate index compared to the control group. Nevertheless, administration of probenecid, eugenol, or a combination of both led to a significant decrease in prostate index by 43.73%, 41.80%, and 56.39%, respectively, in comparison to the BPH group. Furthermore, the combination group showed a significant reduction in prostate index (by 22.50% and 25.07%) compared to either probenecid or eugenol-treated groups, respectively (Figure 1).

3.2 | Effect of Probenecid or Eugenol Either Alone or in Combination on Testosterone-Induced Increase in Cyclin D1

In the prostatic tissue of the BPH group, the area percentage of immunohistochemical expression of cyclin D1 significantly increased to reach 11.1-fold of the baseline value ($p < 0.0001$)

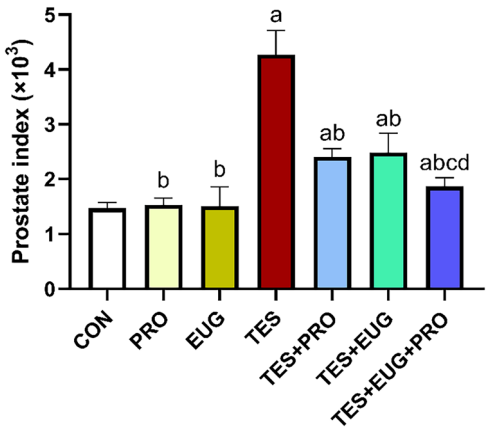

FIGURE 1 | Combined treatment with probenecid and eugenol attenuates testosterone-induced increase in prostate index. The data are expressed as mean ± SD and were analyzed using one-way ANOVA, followed by Tukey's multiple comparisons test. [a]Significantly different from the normal control group at $p < 0.05$, [b]Significantly different from BPH group at $p < 0.05$, [c]Significantly different from probenecid treated group at $p < 0.05$, [d]Significantly different from eugenol treated group at $p < 0.05$. CON, control; EUG, eugenol; PRO, probenecid; TES, testosterone (BPH). Ordered alphabetically. [Color figure can be viewed at wileyonlinelibrary.com]

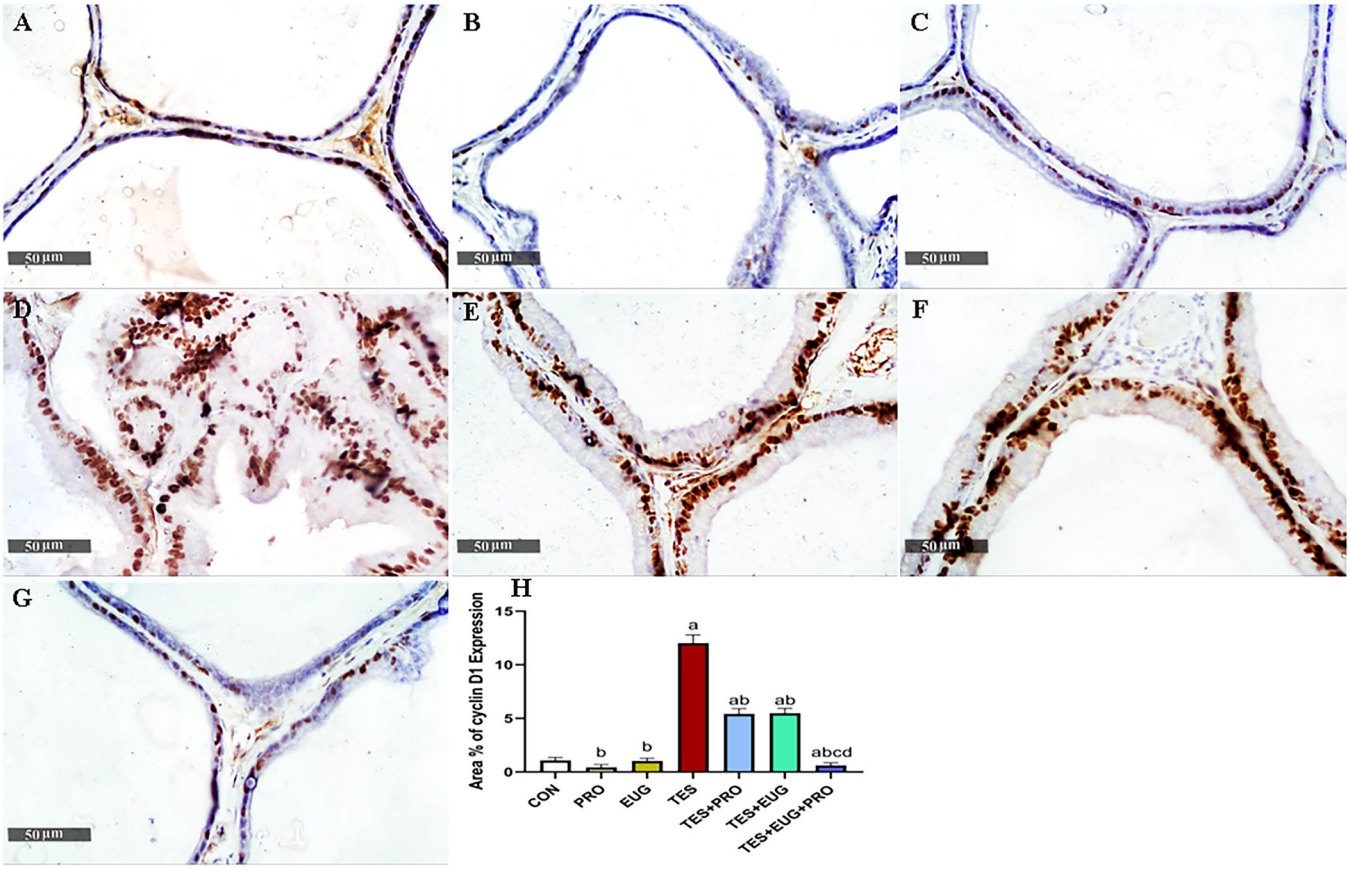

FIGURE 2 | Combined treatment with probenecid and eugenol attenuates testosterone-induced increase in area percentage of immunohistochemical expression of cyclin D1. (A) Normal control group, (B) probenecid control group, (C) Eugenol control group, (D) BPH group, (E) probenecid treated group, (F) Eugenol treated group, (G) combination treated group, and (H) graphical presentation of area percentage of immunohistochemical expression of cyclin D1. The data are expressed as mean ± SD and were analyzed using one-way ANOVA, followed by Tukey's multiple comparisons test. [a]Significantly different from the normal control group at $p < 0.05$, [b]Significantly different from BPH group at $p < 0.05$, [c]Significantly different from probenecid-treated group at $p < 0.05$, [d]Significantly different from eugenol-treated group at $p < 0.05$. CON, control; EUG, eugenol; PRO, probenecid; TES, testosterone (BPH). [Color figure can be viewed at wileyonlinelibrary.com]

compared to the normal control group. However, administration of probenecid, eugenol, or their combination resulted in significant reductions in cyclin D1 by 54.92%, 54.37%, and 94.87%, respectively, compared to the BPH group. Furthermore, the combination group exhibited a significant reduction in cyclin D1 by 88.62% and 88.75% compared to either probenecid or eugenol-treated groups, respectively (Figure 2).

3.3 | Effect of Probenecid or Eugenol Either Alone or in Combination on Testosterone-Induced Histopathological Features

As shown in Figure 3, the normal control group exhibited typical morphological features of rat prostate, with variably sized acini lined by intact cuboidal to low columnar epithelial cells, minimal stratification, intact subcellular details, homogenous pale eosinophilic luminal secretions, minimal inflammatory cell infiltrates, and normal vasculature. Both the probenecid and eugenol control groups showed the same histological characteristics as the normal control group without any abnormal changes. The BPH group, however, displayed a marked prevalence of proliferated acinar lining epithelium, focal stratification, papillary luminal projections, severely hyperemic stromal blood vessels, and perivascular inflammatory cell infiltrates. In the probenecid-treated group, there were moderate instances of hyperplastic acinar epithelium, moderately congested vasculature, and minimal inflammatory cell infiltrates. The eugenol-treated group showed similar features to the probenecid-treated group but with higher levels of inflammatory cell infiltrates. The combination-treated group demonstrated more organized histological features of prostatic tissue parenchyma, closely resembling the normal control samples. Epithelial thickness was significantly increased in the BPH group, showing a 3.9-fold elevation compared to the normal control group. Treatment with probenecid, eugenol, or their combination significantly reduced epithelial thickness by 12.57%, 12.02%, and 67.76%, respectively, relative to the BPH group. Notably, the combination treatment produced a more pronounced effect, with reductions of 63.13% and 63.35% compared to the probenecid- and eugenol-treated groups, respectively.

3.4 | Effect of Probenecid or Eugenol Either Alone or in Combination on Testosterone-Induced Increase in Uric Acid

When compared to the normal control group, testosterone administration significantly increased serum UA level to reach

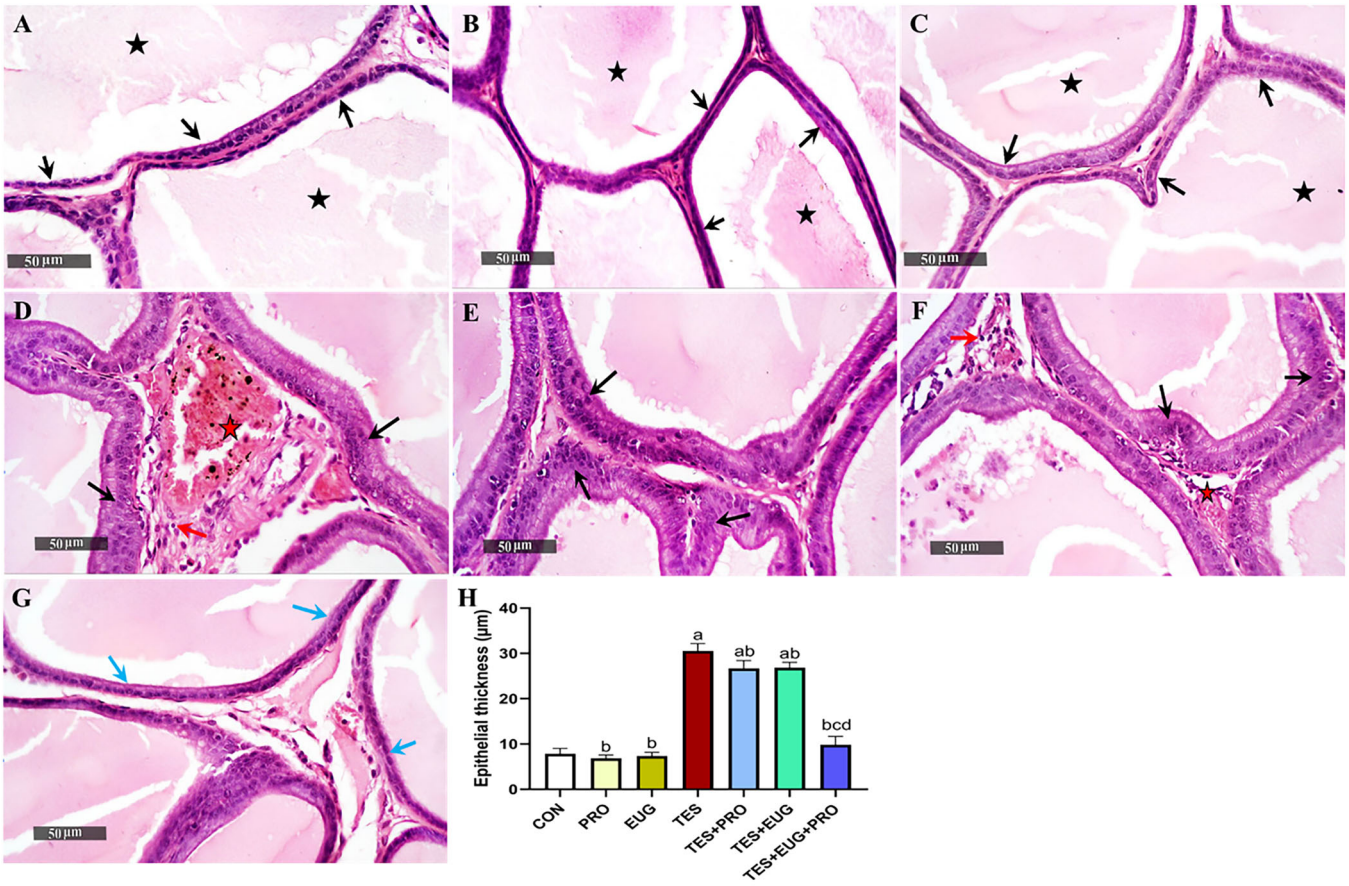

FIGURE 3 | Combined treatment with probenecid and eugenol attenuates testosterone-induced histopathological features in H&E staining. (A) Normal control group: demonstrated normal morphological features of rat prostate with many variable-sized acini lined by apparent intact cuboidal to low columnar epithelial cells with minimal stratification and showing intact subcellular details (black arrow), with homogenous, abundant pale eosinophilic luminal secretions (star) with minimal inflammatory cells infiltrates and normal vasculatures. (B) Probenecid control group: demonstrated the same records as normal control samples without abnormal histological changes records. (C) Eugenol control group: demonstrated the same records as normal control samples without abnormal histological changes records. (D) BPH group: marked higher prevalence of proliferated acinar lining epithelium with focal stratification and papillary luminal projections (black arrow) with severely hyperemic stromal Bvs (red star) accompanied with perivascular inflammatory cells infiltrates (red arrow). (E) Probenecid-treated group: demonstrated moderate persistent figures of hyperplastic acinar epithelium (black arrow) with moderate figures of congested vasculatures with minimal inflammatory cells infiltrates. (F) Eugenol-treated group: samples showed the same records as probenecid-treated group samples with added higher records of inflammatory cells infiltrates. (G) combination treated group: showed more organized histological features of prostatic tissue parenchyma resembling normal control samples. (H) Epithelial thickness (µm). The data are expressed as mean ± SD and were analyzed using one-way ANOVA, followed by Tukey's multiple comparisons test. [a]Significantly different from the normal control group at $p < 0.05$, [b]Significantly different from BPH group at $p < 0.05$, [c]Significantly different from probenecid-treated group at $p < 0.05$, [d]Significantly different from eugenol-treated group at $p < 0.05$. CON, control; EUG, eugenol; PRO, probenecid; TES, testosterone (BPH); UA, uric acid. [Color figure can be viewed at wileyonlinelibrary.com]

2.8 folds of the baseline value ($p < 0.0001$). In contrast, when compared to the BPH group, administration of probenecid, eugenol, or their combination led to significant reductions in UA levels of 35.41%, 43.4%, and 63.66%, respectively. In addition, the combination group showed a significant reduction in UA level (by 43.73% and 35.79%) compared to either probenecid or eugenol-treated groups, respectively (Figure 4).

3.5 | Effect of Probenecid or Eugenol Either Alone or in Combination on Testosterone-Induced Effects in Oxidative Stress Markers

In the BPH group, prostate tissue exhibited a significant reduction in tissue GSH content and CAT activity by 56.56% and 51.09%, respectively, compared to the normal control group. However, treatment with either probenecid, eugenol, or their combination resulted in a significant increase in tissue GSH content 1.93, 1.95, and 2.1 folds, respectively, and CAT activity by 1.7, 1.73, and 1.95 folds, respectively, compared to the BPH group. Additionally, the prostate tissue of the combination group exhibited a significant elevation in GSH content by 0.2 and 2.1 and CAT activity by 0.17 and 0.22 folds compared to the probenecid and eugenol treated groups, respectively (Figure 5).

Additionally, prostate tissue in the BPH group exhibited a significant increase in tissue MDA content to reach 3.4-fold compared to the normal control group. However, treatment with either probenecid, eugenol, or their combination resulted

in a significant reduction in MDA levels by 49.68%, 49.03%, and 66.96%, respectively, compared to the BPH group. Additionally, the prostate tissue of the combination group exhibited a significant reduction in MDA content by 34.35% and 35.19% compared to the probenecid and eugenol treated groups, respectively (Figure 5).

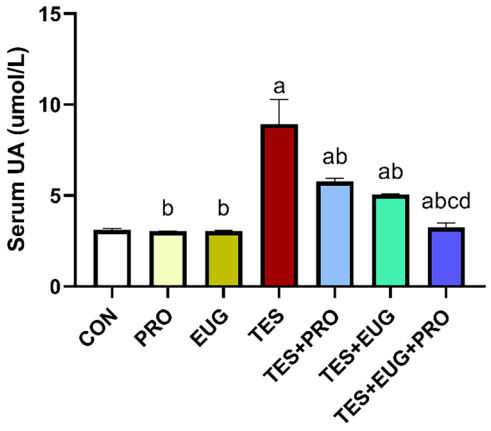

FIGURE 4 | Combined treatment with probenecid and eugenol attenuates testosterone-induced increase in UA. The data are expressed as mean ± SD and were analyzed using one-way ANOVA, followed by Tukey's multiple comparisons test. [a]Significantly different from the normal control group at $p < 0.05$, [b]Significantly different from BPH group at $p < 0.05$, [c]Significantly different from probenecid-treated group at $p < 0.05$, [d]Significantly different from eugenol-treated group at $p < 0.05$. CON, control; EUG, eugenol; PRO, probenecid; TES, testosterone (BPH); UA, uric acid. [Color figure can be viewed at wileyonlinelibrary.com]

3.6 | Effect of Probenecid or Eugenol Either Alone or in Combination on Testosterone-Induced Elevated Inflammatory Markers

Prostate tissue in the BPH group exhibited a significant elevation in tissue content of IL-6, IL-1β, and TNF-α to reach 3.3, 2.9, and 3.5 folds of the baseline value ($p < 0.0001$), respectively, compared to the normal control group. However, treatment with either probenecid, eugenol, or their combination resulted in a significant reduction in IL-6 (by 52.00%, 50.49%, and 61.17%, respectively), IL-1β (by 47.75%, 50.22%, and 59.94%, respectively), and TNF-α (by 38.91%, 38.9%, and 56.75%, respectively) compared to the BPH group. Furthermore, the prostate tissue in the combination group exhibited a significant reduction in IL-6 levels by 19.1% and 21.57%, IL-1β levels by 23.33% and 19.51%, and TNF-α levels by 29.2% and 29.21% compared to either probenecid or eugenol-treated groups, respectively (Figure 6).

3.7 | Effect of Probenecid or Eugenol Either Alone or in Combination Attenuates Testosterone-Induced Elevated NFκB, p-PI3K, p-AKT and p-mTOR

In the prostatic tissue of the BPH group, NFκB gene expression significantly increased to reach 6.5-fold of the baseline value

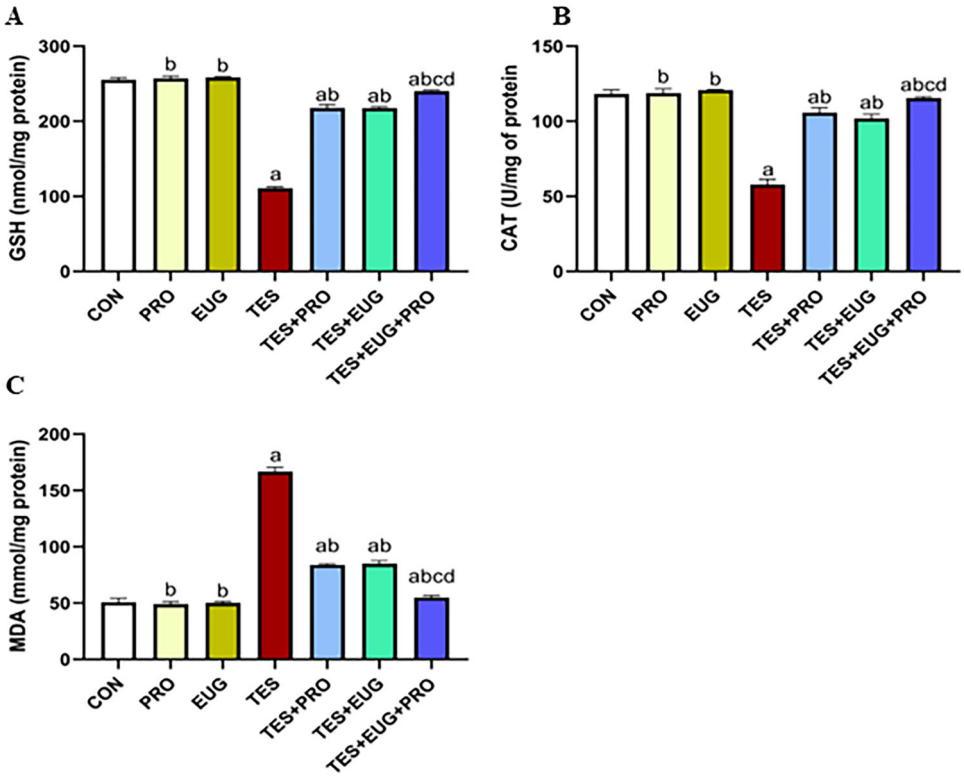

FIGURE 5 | Combined treatment with probenecid and eugenol attenuates testosterone induced effects in prostatic GSH (A), CAT (B), and MDA (C). The data are expressed as mean ± SD and were analyzed using one-way ANOVA, followed by Tukey's multiple comparisons test. [a]Significantly different from the normal control group at $p < 0.05$, [b]Significantly different from BPH group at $p < 0.05$, [c]Significantly different from probenecid-treated group at $p < 0.05$, [d]Significantly different from eugenol-treated group at $p < 0.05$. CAT, catalase; CON, control; EUG, eugenol; GSH, glutathione; MDA, malondialdehyde; PRO, probenecid; TES, testosterone (BPH). [Color figure can be viewed at wileyonlinelibrary.com]

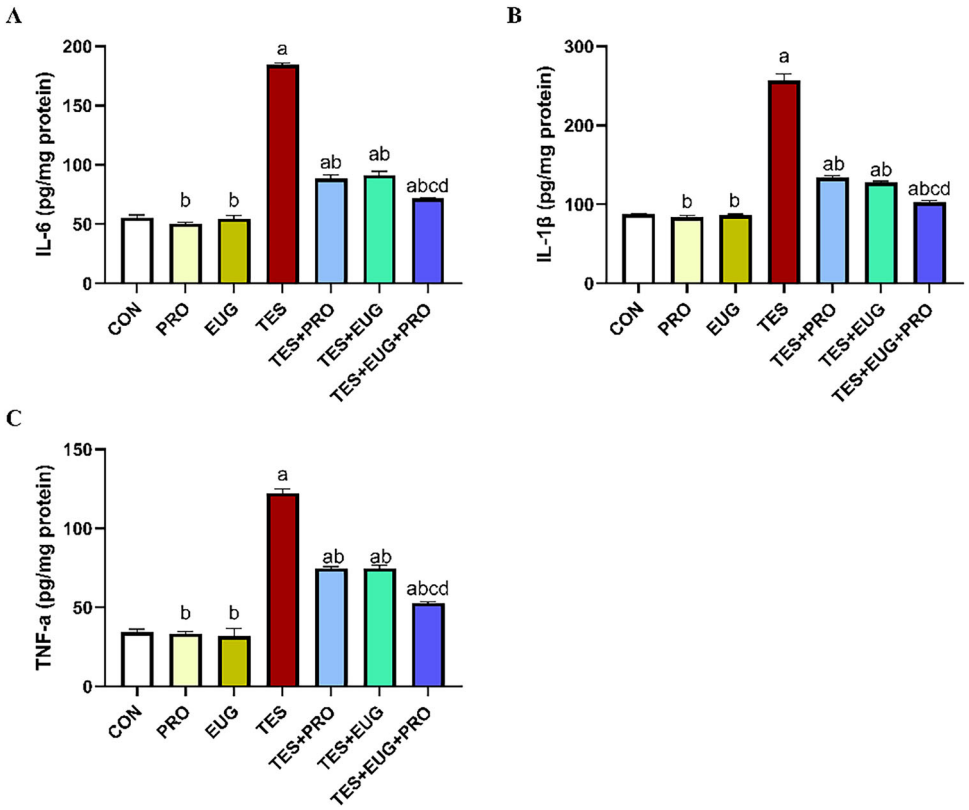

FIGURE 6 | Combined treatment with probenecid and eugenol attenuates testosterone-induced elevated inflammatory markers in prostatic tissue; IL-6 (A), IL-1β (B), and TNF-α (C). The data are expressed as mean ± SD and were analyzed using one-way ANOVA, followed by Tukey's multiple comparisons test. [a]Significantly different from the normal control group at $p < 0.05$, [b]Significantly different from BPH group at $p < 0.05$, [c]Significantly different from probenecid-treated group at $p < 0.05$, [d]Significantly different from eugenol-treated group at $p < 0.05$. CON, control; EUG, eugenol; IL, interleukin; PRO, probenecid; TNF-α, tumor necrosis factor alpha; TES, testosterone (BPH). [Color figure can be viewed at wileyonlinelibrary.com]

($p < 0.0001$) compared to the normal control group. However, administration of probenecid, eugenol, or their combination resulted in significant reductions in NFκB expression by 52.36%, 55.67%, and 63.57%, respectively, compared to the BPH group. Furthermore, the combination group exhibited a significant reduction in NFκB expression by 23.53% and 17.82% compared to either probenecid or eugenol-treated groups, respectively (Figure 7).

In the BPH group, prostate tissue displayed a significant increase in relative protein expression of p-PI3K, p-AKT, and p-mTOR to reach 6.5 folds of the baseline value ($p < 0.0001$ 712.50%, 347.56%, and 603.07%, respectively, compared to the normal control group. However, treatment with either probenecid, eugenol, or their combination led to a significant reduction in expression of p-PI3K (by 51.49%, 50.08%, and 59.58%, respectively), p-AKT (by 41.96%, 44.96%, and 65.94%, respectively), and p-mTOR (by 53.93%, 51.48%, and 66.14%, respectively) compared to the BPH group. Moreover, the combination group exhibited a significant reduction in p-PI3K relative protein expression (by 23.53% and 19.03%), p-AKT (by 41.31% and 38.12%), and p-mTOR (by 26.52% and 30.22%) compared to either probenecid or eugenol-treated groups, respectively (Figure 7).

4 | Discussion

BPH is a common contributor to LUTS in aging men, significantly impacting their health and well-being. These symptoms profoundly affect the quality of life of affected individuals. Given the growing elderly population and the rising prevalence of BPH, there is an urgent demand for accessible and effective treatment options to address this pressing health concern [1, 5]. The involvement of the androgen system, especially the androgen receptor, in BPH development is linked to dihydrotestosterone and subsequent prostatic inflammation. Despite its administration for late-onset hypogonadism, concerns remain regarding the impact of testosterone therapy on the prostate, given evidence from rat models highlighting its role in BPH despite decreasing levels in older men [8–10].

This study aimed to explore testosterone-induced BPH and the involvement of the uric acid/AKT/mTOR signaling pathway, alongside assessing the therapeutic potential of eugenol and probenecid in alleviating BPH.

This study elucidated the pathological and biochemical alterations associated with testosterone-induced BPH, corroborating findings from previous research [8, 10, 26, 37, 43]. Prostate hyperplasia was observed in the present study following testosterone injection, as evidenced by a significant increase in prostate index. The results of histopathological analysis supported these observations, revealing features that are typical of BPH. Nevertheless, these effects were mitigated by pretreatment with probenecid, eugenol, or their combination. This was initially selected to explore the potential protective capacity of probenecid and eugenol against the early pathogenic events of BPH.

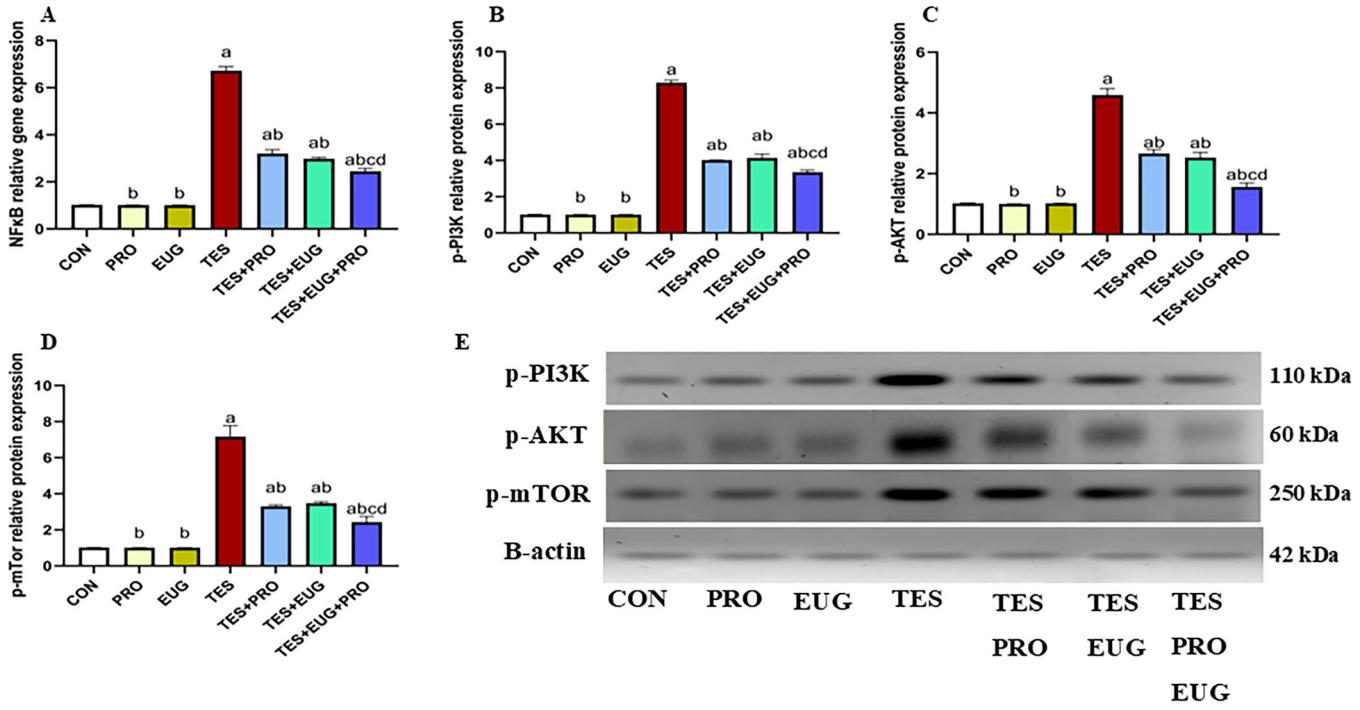

FIGURE 7 | Combined treatment with probenecid and eugenol attenuates testosterone-induced elevated NFκB (A), p-PI3K (B), p-AKT (C), p-mTOR (D), and (E) Representative Western blot bands for p-PI3K, p-AKT, p-mTOR, and β-actin. The data are expressed as mean ± SD and were analyzed using one-way ANOVA, followed by Tukey's multiple comparisons test. [a]Significantly different from the normal control group at $p < 0.05$, [b]Significantly different from BPH group at $p < 0.05$, [c]Significantly different from probenecid-treated group at $p < 0.05$, [d]Significantly different from eugenol-treated group at $p < 0.05$. CON, control; EUG, eugenol; PRO, probenecid; TES, testosterone (BPH). [Color figure can be viewed at wileyonlinelibrary.com]

Prostate hyperplasia was observed in the present study following testosterone injection, as evidenced by a significant increase in prostate index. The results of histopathological analysis supported these observations, revealing features that are typical of BPH. Nevertheless, these effects were mitigated by pretreatment with probenecid, eugenol, or their combination. Similar findings have been observed with testosterone in previous studies, and the mitigating effects were attributed to investigational protective drugs acting through different mechanistic pathways [8, 26, 37, 44, 45].

The immunohistochemical staining of cyclin D1 further corroborated these findings, revealing its overexpression induced by testosterone injection. Cyclin D1, a marker of cell proliferation, plays pivotal roles in regulating the cell cycle, ultimately leading to the division and duplication of prostatic stromal and epithelial cells [46, 47]. Furthermore, cyclin D1 serves as a critical regulator of cell cycle checkpoints, orchestrating the G1/S transition. In the same context, Ahmed et al. (2020) and Jang et al. (2023) reported the interplay between cyclin D1 and the proliferative state of prostatic cells in BPH [44, 48]. The overexpression of prostatic cyclin D1 was effectively counterbalanced by probenecid, eugenol, or their combination, demonstrating their antiproliferative potential against testosterone-induced BPH, which is consistent with previous findings [26, 49, 50].

The specific mechanism underlying testosterone's influence on BPH remains unclear and warrants further investigation in the current study. One proposed mechanism suggests that testosterone's anabolic effects may trigger muscle regeneration, resulting in increased nucleic acid and purine metabolism, consequently leading to elevated UA levels. Additionally, heightened ATP consumption accompanying muscle mass growth may further contribute to elevated UA levels, which have been linked to BPH [37]. The findings of the current study reveal that testosterone injection elevated prostatic UA levels, resulting in a significant increase in MDA and decreased prostatic content of CAT and GSH. These results align with previous studies attributing the role of testosterone replacement therapy and testosterone-injected animal models to induce asymptomatic hyperuricemia and profound increases in oxidative stress markers [26, 37, 51–53]. Probenecid, eugenol, or their combination significantly reduced prostatic levels of MDA and increased CAT and GSH levels compared to the BPH group. These findings suggest their antioxidative potential against testosterone-induced BPH, consistent with previous studies [26, 29, 37, 54, 55].

Elevated UA levels can induce inflammation by activating proinflammatory pathways and oxidative stress, resulting in cellular and tissue damage [18]. Testosterone-induced UA irritation has been associated with the initiation of inflammation in prostate tissue. This irritation prompts lymphocytes, particularly B and T cells, to produce excessive cytokines such as IL-6, IL-1β, IL-2, and TNF-α, along with other growth factors [19, 20]. In the current study, prostatic tissue of the BPH group exhibited significant elevations in inflammatory markers IL-6, IL-1β, and TNF-α compared to the normal control group. However, treatment with probenecid, eugenol, or their combination significantly reduced the prostatic content of these markers. This highlights the potential of probenecid and eugenol in mitigating

BPH by alleviating high UA-induced inflammation. Prostatic inflammation is an essential factor in the pathogenesis of BPH [56, 57]. BPH patients with increased prostate tissue inflammation exhibit an association with more severe symptoms and larger prostate volumes [58]. Consistently, experimental investigations have established that inflammation is a pathway by which testosterone can induce benign BPH, and protective agents have exhibited significant anti-inflammatory properties against it [59, 60].

The IL-6 receptor, a tyrosine kinase-linked receptor, triggers the activation of NFκB, PI3-K, and consequently Akt. Akt activation, in turn, stimulates mTOR, prompting its translocation into the nucleus and upregulation of pro-survival genes. These genes serve to inhibit apoptosis and promote the growth of prostatic tissue [21–24]. The current results show elevated prostatic levels of PI3-K, Akt, mTOR (Porta et al., 2014; Rittler et al., 2019) as well as elevated gene expression of NFκB in BPH group compared to the normal control group, while treatment with either probenecid, eugenol, or their combination significantly mitigated these elevations, indicating the potential anti-hyperproliferative effect of these drugs via amelioration of the presented pro-survival pathway (Roy et al., 2023).

According to the current findings, the combination treatment group showed remarkable efficacy in attenuating testosterone-induced BPH across multiple parameters compared to either probenecid or eugenol alone. Specifically, the combination treatment led to a more pronounced reduction in prostate index, serum uric acid levels, oxidative stress markers, inflammatory cytokines, and expression levels of cell survival markers such as cyclin D1. Furthermore, histopathological examination revealed that the combination treatment resulted in a greater mitigation of histopathological alterations compared to monotherapy with either probenecid or eugenol.

5 | Conclusion

In conclusion, this study provides insights into the pathophysiological processes that contribute to the development of BPH induced by testosterone and assesses the potential therapeutic effects of probenecid and eugenol in slowing the progression of BPH. The results of this study indicate that the administration of testosterone induces prostatic hyperplasia, increased uric acid levels, oxidative stress, inflammation, and the activation of pro-survival pathways. These results underscore the complex and multifaceted process by which BPH develops. Significantly, the application of probenecid, eugenol, or their combination reduces these pathological alterations with efficacy, indicating their potential as therapeutic agents in the management of BPH. The findings presented in this study enhance the comprehension of the pathogenesis of BPH and offer significant contributions to the development of potential therapeutic approaches for this prevalent urological disorder. Future studies are warranted to explore the therapeutic potential of these agents in established BPH using post-induction treatment models. Although no animal model fully replicates human BPH, this study offers mechanistic insights into uric acid-mediated prostatic hyperplasia and supports novel drug repurposing strategies. These findings highlight the importance of identifying safer, mechanism-based alternatives or adjuncts to standard BPH therapies, particularly those that target inflammation and metabolic dysregulation.

Author Contributions

All authors contributed to the study conception and design. Nibrass Taher Abdali and Hassan Afify contributed to the material preparation, data collection, and analysis. The first draft of the manuscript was written by Nibrass Taher Abdali and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Acknowledgments

The authors acknowledge Dr. Mohamed A Khattab, Department of Cytology and Histology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt, for his contribution to the histopathological and immunohistochemical examinations. The authors received no specific funding for this work.

Ethics Statement

The experimental protocol was approved by the Ethics Committee for Animal Experimentation at the Faculty of Pharmacy, Cairo University, and complied with the guidelines outlined in the Guide for Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 2011). The protocol approval code is (PT 1390).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data will be made available on request.

References

1. B. Chughtai, J. C. Forde, D. D. Thomas, et al., "Benign Prostatic Hyperplasia," *Nature Reviews Disease Primers* 2, no. 1 (2016): 16031, https://doi.org/10.1038/nrdp.2016.31.

2. J. Maliakal, E. E. Mousa, and V. Menon, "Giant Prostatic Hyperplasia: Fourth Largest Prostate Reported in Medical Literature," *Sultan Qaboos University Medical Journal* 14, no. 2 (2014): 253–256.

3. S. Vesely, T. Knutson, J. E. Damber, M. Dicuio, and C. Dahlstrand, "Relationship Between Age, Prostate Volume, Prostate-Specific Antigen, Symptom Score and Uroflowmetry in Men With Lower Urinary Tract Symptoms," *Scandinavian Journal of Urology and Nephrology* 37, no. 4 (2003): 322–328, https://doi.org/10.1080/00365590310014760.

4. G. D. Grossfeld and F. V. Coakley, "Benign Prostatic Hyperplasia: Clinical Overview and Value of Diagnostic Imaging," *Radiologic Clinics of North America* 38, no. 1 (2000): 31–47, https://doi.org/10.1016/S0033-8389(05)70148-2.

5. D. Elterman, B. Gao, S. Lu, N. Bhojani, K. C. Zorn, and B. Chughtai, "New Technologies for Treatment of Benign Prostatic Hyperplasia," *Urologic Clinics of North America* 49, no. 1 (2022): 11–22, https://doi.org/10.1016/j.ucl.2021.07.007.

6. A. Koudonas, A. Anastasiadis, S. Tsiakaras, et al., "Overview of Current Pharmacotherapeutic Options in Benign Prostatic Hyperplasia," *Expert Opinion on Pharmacotherapy* 24, no. 14 (2023): 1609–1622.

7. B. Chislett, D. Chen, M. L. Perera, E. Chung, D. Bolton, and L. G. Qu, "5-alpha Reductase Inhibitors Use in Prostatic Disease and Beyond," *Translational Andrology and Urology* 12, no. 3 (2023): 487–496, https://doi.org/10.21037/tau-22-690.

8. H. Afify, A. M. Abo-Youssef, H. M. Abdel-Rahman, S. Allam, and A. A. Azouz, "The Modulatory Effects of Cinnamaldehyde on Uric Acid Level and IL-6/JAK1/STAT3 Signaling as a Promising Therapeutic Strategy Against Benign Prostatic Hyperplasia," *Toxicology and Applied Pharmacology* 402 (2020): 115122, https://doi.org/10.1016/j.taap.2020.115122.

9. S. Madersbacher, N. Sampson, and Z. Culig, "Pathophysiology of Benign Prostatic Hyperplasia and Benign Prostatic Enlargement: A Mini-Review," *Gerontology* 65, no. 5 (2019): 458–464.

10. T. Jarvis, B. Chughtai, and S. Kaplan, "Testosterone and Benign Prostatic Hyperplasia," *Asian Journal of Andrology* 17, no. 2 (2015): 212–216, https://doi.org/10.4103/1008-682x.140966.

11. X.-H. Tang, Z.-Y. Liu, J.-W. Ren, et al., "Comprehensive RNA-seq Analysis of Benign Prostatic Hyperplasia (BPH) in Rats Exposed to Testosterone and Estradiol," *Scientific Reports* 15, no. 1 (2025): 2750, https://doi.org/10.1038/s41598-025-87205-2.

12. N.-H. Kim, J. Jegal, Y. N. Kim, et al., "The Effects of *Aronia melanocarpa* Extract on Testosterone-Induced Benign Prostatic Hyperplasia in Rats, and Quantitative Analysis of Major Constituents Depending on Extract Conditions," *Nutrients* 12, no. 6 (2020): 1575.

13. L.-Y. Li, J. Han, L. Wu, et al., "Alterations of Gut Microbiota Diversity, Composition and Metabonomics in Testosterone-Induced Benign Prostatic Hyperplasia Rats," *Military Medical Research* 9, no. 1 (2022): 12, https://doi.org/10.1186/s40779-022-00373-4.

14. R. S. Swerdloff, R. E. Dudley, S. T. Page, C. Wang, and W. A. Salameh, "Dihydrotestosterone: Biochemistry, Physiology, and Clinical Implications of Elevated Blood Levels," *Endocrine Reviews* 38, no. 3 (2017): 220–254.

15. R. O. Alvim, J. H. Siqueira, D. Zaniqueli, D. M. Dutra, P. R. Oliosa, and J. G. Mill, "Influence of Muscle Mass on the Serum Uric Acid Levels in Children and Adolescents," *Nutrition, Metabolism, and Cardiovascular Diseases* 30, no. 2 (2020): 300–305, https://doi.org/10.1016/j.numecd.2019.08.019.

16. E. Russo, D. Verzola, F. Cappadona, et al., "The Role of Uric Acid in Renal Damage-A History of Inflammatory Pathways and Vascular Remodeling," *Vessel Plus* 5 (2021): 15.

17. Y. Deng, F. Liu, X. Yang, and Y. Xia, "The Key Role of Uric Acid in Oxidative Stress, Inflammation, Fibrosis, Apoptosis, and Immunity in the Pathogenesis of Atrial Fibrillation," *Frontiers in Cardiovascular Medicine* 8 (2021): 641136.

18. Y. Zhou, M. Zhao, Z. Pu, G. Xu, and X. Li, "Relationship Between Oxidative Stress and Inflammation in Hyperuricemia: Analysis Based on Asymptomatic Young Patients With Primary Hyperuricemia," *Medicine* 97, no. 49 (2018): e13108, https://doi.org/10.1097/md.0000000000013108.

19. F. Sangkop, G. Singh, E. Rodrigues, E. Gold, and A. Bahn, "Uric Acid: A Modulator of Prostate Cells and Activin Sensitivity," *Molecular and Cellular Biochemistry* 414, no. 1–2 (2016): 187–199, https://doi.org/10.1007/s11010-016-2671-8.

20. O. Dienz and M. Rincon, "The Effects of IL-6 on CD4 T Cell Responses," *Clinical Immunology* 130, no. 1 (2009): 27–33, https://doi.org/10.1016/j.clim.2008.08.018.

21. P. N. Kelly and A. Strasser, "The Role of Bcl-2 and Its Pro-Survival Relatives in Tumourigenesis and Cancer Therapy," *Cell Death & Differentiation* 18, no. 9 (2011): 1414–1424, https://doi.org/10.1038/cdd.2011.17.

22. H. J. Choi, J. H. Lee, S. Y. Park, J. H. Cho, and J. S. Han, "STAT3 Is Involved in Phosphatidic Acid-Induced Bcl-2 Expression in Hela Cells," *Experimental and Molecular Medicine* 41, no. 2 (2009): 94–101, https://doi.org/10.3858/emm.2009.41.2.012.

23. H. C. Dan, M. J. Cooper, P. C. Cogswell, J. A. Duncan, J. P. Y. Ting, and A. S. Baldwin, "Akt-Dependent Regulation of NF-κB Is Controlled by mTOR and Raptor in Association With IKK," *Genes & Development* 22, no. 11 (2008): 1490–1500, https://doi.org/10.1101/gad.1662308.

24. S. S. Sokar, M. A. Alkabbani, E. S. Akool, and S. E. Abu-Risha, "Hepatoprotective Effects of Carvedilol and Crocin Against Leflunomide-Induced Liver Injury," *International Immunopharmacology* 113, no. Pt A (2022): 109297, https://doi.org/10.1016/j.intimp.2022.109297.

25. Z. Onódi, S. Koch, J. Rubinstein, P. Ferdinandy, and Z. V. Varga, "Drug Repurposing for Cardiovascular Diseases: New Targets and Indications for Probenecid," *British Journal of Pharmacology* 180, no. 6 (2023): 685–700, https://doi.org/10.1111/bph.16001.

26. M. M. Abdel-Fattah, M. E. Abo-El Fetoh, H. Afify, L. A. A. Ramadan, and W. R. Mohamed, "Probenecid Ameliorates Testosterone-Induced Benign Prostatic Hyperplasia: Implications of PGE-2 on ADAM-17/EGFR/ERK1/2 Signaling Cascade," *Journal of Biochemical and Molecular Toxicology* 37 (2023): e23450.

27. R. Mahfouz, S. He-r, A. Ig, E. Sheta, M. Aa, and S. S. A. Ibrahim, "L-Carnitine Augments Probenecid Anti-Inflammatory Effect in Monoiodoacetate-Induced Knee Osteoarthritis in Rats: Involvement of miRNA-373/P2X7/NLRP3/NF-κB Milieu," *Inflammopharmacology* 32 (2023): 715–731, https://doi.org/10.1007/s10787-023-01376-w.

28. J. Huttunen, J. Tampio, J. Järvinen, A. B. Montaser, M. Markowicz-Piasecka, and K. M. Huttunen, "Amino Acid Derivative of Probenecid Potentiates Apoptosis-Inducing Effects of Vinblastine by Increasing Oxidative Stress in a Cancer Cell-Specific Manner," *Chemico-Biological Interactions* 388 (2024): 110833, https://doi.org/10.1016/j.cbi.2023.110833.

29. M. H. Cheng and S. J. Kim, "Inhibitory Effect of Probenecid on Osteoclast Formation via JNK, ROS and COX-2," *Biomolecules & Therapeutics* 28, no. 1 (2020): 104–109, https://doi.org/10.4062/biomolther.2019.047.

30. J. Kerosenewala, P. Vaidya, V. Ozarkar, Y. Shirapure, and A. P. More, "Eugenol: Extraction, Properties and Its Applications on Incorporation With Polymers and Resins—A Review," *Polymer Bulletin* 80, no. 7 (2023): 7047–7099.

31. A. Sharma, G. Bhardwaj, H. S. Sohal, and A. Gohain, "Chapter 9 - Eugenol," in *Nutraceuticals and Health Care*, eds. Kour J. and Nayik G. A. (Academic Press, 2022), 177–198.

32. B. Yogalakshmi, P. Viswanathan, and C. V. Anuradha, "Investigation of Antioxidant, Anti-Inflammatory and Dna-Protective Properties of Eugenol in Thioacetamide-Induced Liver Injury in Rats," *Toxicology* 268, no. 3 (2010): 204–212.

33. A. B. Tiku, S. K. Abraham, and R. K. Kale, "Eugenol as an In Vivo Radioprotective Agent," *Journal of Radiation Research* 45, no. 3 (2004): 435–440.

34. S. K. Abraham, "Anti-Genotoxicity of Trans-Anethole and Eugenol in Mice," *Food and Chemical Toxicology* 39, no. 5 (2001): 493–498.

35. D. O. Saleh, S. M. Baraka, G. A. A. Jaleel, A. Hassan, and O. A. Ahmed-Farid, "Eugenol Alleviates Acrylamide-Induced Rat Testicular Toxicity by Modulating AMPK/p-AKT/mTOR Signaling Pathway and Blood–Testis Barrier Remodeling," *Scientific Reports* 14, no. 1 (2024): 1910, https://doi.org/10.1038/s41598-024-52259-1.

36. M. L. Abdullah, O. Al-Shabanah, Z. K. Hassan, and M. M. Hafez, "Eugenol-Induced Autophagy and Apoptosis in Breast Cancer Cells via PI3K/AKT/FOXO3a Pathway Inhibition," *International Journal of Molecular Sciences* 22, no. 17 (2021): 9243, https://doi.org/10.3390/ijms22179243.

37. A. M. Abo-Youssef, H. Afify, A. A. Azouz, H. M. Abdel-Rahman, A. B. Abdel-Naim, and S. Allam, "Febuxostat Attenuates Testosterone-Induced Benign Prostatic Hyperplasia in Rats via Inhibiting JAK/STAT Axis," *Life Sciences* 260 (2020): 118414, https://doi.org/10.1016/j.lfs.2020.118414.

38. K. J. Livak and T. D. Schmittgen, "Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2−ΔΔCT

Method," *Methods* 25, no. 4 (2001): 402–408, https://doi.org/10.1006/meth.2001.1262.

39. C. F. A. Culling, *Handbook of Histopathological and Histochemical Techniques: Including Museum Techniques*. (Butterworth-Heinemann, 2013).

40. L. O. Mendes, A. C. S. Castilho, C. F. Pinho, et al., "Modulation of Inflammatory and Hormonal Parameters in Response to Testosterone Therapy: Effects on the Ventral Prostate of Adult Rats," *Cell Biology International* 42, no. 9 (2018): 1200–1211.

41. S. Słuczanowska-Głąbowska, M. Laszczyńska, M. Wylot, W. Głąbowski, M. Piasecka, and D. Gącarzewicz, "Morphological and Immunohistochemical Comparison of Three Rat Prostate Lobes (Lateral, Dorsal and Ventral) in Experimental Hyperprolactinemia," *Folia Histochemica et Cytobiologica* 48, no. 3 (2010): 447–454.

42. M. Thiyagarajan, C. L. Kaul, and P. Ramarao, "Enhancement of α-adrenoceptor-mediated Responses in Prostate of Testosterone-Treated Rat," *European Journal of Pharmacology* 453, no. 2 (2002): 335–344, https://doi.org/10.1016/S0014-2999(02)02452-4.

43. H. H. Mosli, A. Esmat, R. T. Atawia, S. M. Shoieb, H. A. Mosli, and A. B. Abdel-Naim, "Metformin Attenuates Testosterone-Induced Prostatic Hyperplasia in Rats: A Pharmacological Perspective," *Scientific Reports* 5 (2015): 15639, https://doi.org/10.1038/srep15639.

44. Y. J. Jang, H. Y. Jung, J. Y. Myeong, et al., "Effects of Alginate Oligosaccharide on Testosterone-Induced Benign Prostatic Hyperplasia in Orchiectomized Rats," *Nutrients* 15, no. 3 (2023): 682, https://doi.org/10.3390/nu15030682.

45. P.-H. Cheng, P.-C. Chiu, J.-C. Chang, et al., "Inhibition of Testosterone-Mediated Benign Prostatic Enlargement of Orchiectomized Sprague-Dawley Rats by Diets Supplemented With Bio-Elicited Peanut Sprout Powder (BPSP) and Three New BPSP-Extracted Natural Compounds Identified," *Journal of Functional Foods* 79 (2021): 104383, https://doi.org/10.1016/j.jff.2021.104383.

46. W. Zhong, J. Peng, H. He, et al., "Ki-67 and PCNA Expression in Prostate Cancer and Benign Prostatic Hyperplasia," *Clinical and Investigative Medicine* 31, no. 1 (2008): E8–E15, https://doi.org/10.25011/cim.v31i1.3136.

47. J. Lin, J. Zhou, W. Xu, X. Zhong, Z. Hong, and J. Peng, "Qianliening Capsule Treats Benign Prostatic Hyperplasia via Suppression of the EGF/STAT3 Signaling Pathway," *Experimental and Therapeutic Medicine* 5, no. 5 (2013): 1293–1300, https://doi.org/10.3892/etm.2013.1008.

48. E. S. Ahmed, L. S. Elnour, R. Hassan, et al., "Immunohistochemical Expression of Cyclin D1 Among Sudanese Patients Diagnosed With Benign and Malignant Prostatic Lesions," *BMC Research Notes* 13, no. 1 (2020): 295, https://doi.org/10.1186/s13104-020-05138-7.

49. M. Pisano, G. Pagnan, M. Loi, et al., "Antiproliferative and Pro-Apoptotic Activity of Eugenol-Related Biphenyls on Malignant Melanoma Cells," *Molecular Cancer* 6, no. 1 (2007): 8, https://doi.org/10.1186/1476-4598-6-8.

50. A. T. Zari, T. A. Zari, and K. R. Hakeem, "Anticancer Properties of Eugenol: A Review," *Molecules* 26, no. 23 (2021): 7407, https://doi.org/10.3390/molecules26237407.

51. J. Nomura, N. Busso, A. Ives, et al., "Xanthine Oxidase Inhibition by Febuxostat Attenuates Experimental Atherosclerosis in Mice," *Scientific Reports* 4 (2014): 4554, https://doi.org/10.1038/srep04554.

52. H. Choobineh, M. A. Sadighi Gilani, P. Pasalar, I. Jahanzad, R. Ghorbani, and G. Hassanzadeh, "The Effects of Testosterone on Oxidative Stress Markers in Mice With Spinal Cord Injuries," *International Journal of Fertility & Sterility* 10, no. 1 (2016): 87–93, https://doi.org/10.22074/ijfs.2016.4773.

53. S. Holmes, M. Singh, C. Su, and R. L. Cunningham, "Effects of Oxidative Stress and Testosterone on Pro-Inflammatory Signaling in a Female Rat Dopaminergic Neuronal Cell Line," *Endocrinology* 157, no. 7 (2016): 2824–2835, https://doi.org/10.1210/en.2015-1738.

54. E. G. Gulec Peker and K. Kaltalioglu, "Cinnamaldehyde and Eugenol Protect Against LPS-Stimulated Oxidative Stress and Inflammation in Raw 264.7 Cells," *Journal of Food Biochemistry* 45, no. 12 (2021): e13980.

55. L. Trumper, L. A. Monasterolo, and M. M. Elías, "Probenecid Protects Against in Vivo Acetaminophen-Induced Nephrotoxicity in Male Wistar Rats," *Journal of Pharmacology and Experimental Therapeutics* 284, no. 2 (1998): 606–610.

56. B. Chughtai, R. Lee, A. Te, and S. Kaplan, "Role of Inflammation in Benign Prostatic Hyperplasia," *Reviews in Urology* 13, no. 3 (2011): 147–150.

57. A. B. Abdel-Naim, T. Neamatallah, B. G. Eid, et al., "2-Methoxyestradiol Attenuates Testosterone-Induced Benign Prostate Hyperplasia in Rats Through Inhibition of HIF-1α/TGF-β/Smad2 Axis," *Oxidative Medicine and Cellular Longevity* 2018 (2018): 4389484, https://doi.org/10.1155/2018/4389484.

58. G. Robert, A. Descazeaud, N. Nicolaïew, et al., "Inflammation in Benign Prostatic Hyperplasia: A 282 Patients' Immunohistochemical Analysis," *Prostate* 69, no. 16 (2009): 1774–1780, https://doi.org/10.1002/pros.21027.

59. H. Cai, G. Zhang, Z. Yan, and X. Shang, "The Effect of Xialiqi Capsule on Testosterone-Induced Benign Prostatic Hyperplasia in Rats," *Evidence-Based Complementary and Alternative Medicine* 2018 (2018): 5367814, https://doi.org/10.1155/2018/5367814.

60. Y. Jung, J. Park, H. L. Kim, et al., "Vanillic Acid Attenuates Testosterone-Induced Benign Prostatic Hyperplasia in Rats and Inhibits Proliferation of Prostatic Epithelial Cells," *Oncotarget* 8, no. 50 (2017): 87194–87208, https://doi.org/10.18632/oncotarget.19909.