Iranian Journal of Pharmaceutical Sciences
2025: 21 (1): 165-176
https://journals.sbmu.ac.ir/IJPS

Research Article

OPEN ACCESS

Protective Effects of Quercetin-Loaded Pumpkin Seed Oil Nano-Emulsion on Testosterone-Induced Benign Prostatic Hyperplasia in Rats

Mohammad Shirmohammadi Yazdi[a], Reza Heidari[b,c], Negar Panahi[a], Mohsen Chamanara[d,*] , Abbas Nourmohammadi [e,*]

[a] *Student Research Committee, AJA University of Medical Sciences, Tehran, Iran.*
[b] *Cancer Epidemiology Research Center, AJA University of Medical Sciences, Tehran, Iran.*
[c] *Medical Biotechnology Research Center, AJA University of Medical Sciences, Tehran, Iran.*
[d] *Toxicology Research Center, AJA University of Medical Sciences, Tehran, Iran.*
[e] *Faculty of Aerospace and Diving Medicine, AJA University of Medical Sciences, Tehran, Iran.*

Received: September 3, 2024 **Last Revision:** January 27, 2025 **Accepted:** March 8, 2025 **Available online:** May 07, 2025.

Abstract

Oxidative stress and inflammation play a significant role in the development of testosterone-induced benign prostatic hyperplasia (BPH) and prostate cancer. Quercetin, a polyphenolic compound with antioxidant and anti-inflammatory properties, has limited gastrointestinal absorption. Pumpkin seed oil, rich in β-sitosterol, provides anti-inflammatory and antioxidant benefits. This study evaluated the protective effects of a quercetin-loaded pumpkin seed oil nano-emulsion on BPH in rats. Thirty-six adult male Wistar rats were divided into six groups (n=6 each): control, healthy, quercetin-loaded pumpkin seed oil nano-emulsion, pumpkin seed oil nano-emulsion, quercetin, and finasteride. Except for the healthy group, all groups received intramuscular testosterone enanthate (100 mg/mL) for five consecutive days each week over four weeks. Blood and pathological assessments were conducted to evaluate the effects of the treatments. Statistical analyses were performed using One-Way ANOVA, Paired T-Test, Kruskal-Wallis, and IBM SPSS version 25, with a significance level of $p < 0.05$. Significant improvements in inflammatory and antioxidant markers were observed in the quercetin-loaded pumpkin seed oil nano-emulsion group and the finasteride group compared to the quercetin, pumpkin seed oil nano-emulsion, and control groups ($P<0.05$). Hyperplastic and hypertrophic lesions were markedly reduced in the quercetin-loaded pumpkin seed oil nano-emulsion group compared to the control group, demonstrating superior efficacy relative to finasteride ($P<0.05$). The quercetin-loaded pumpkin seed oil nano-emulsion showed better outcomes in treating or preventing BPH than finasteride, with fewer side effects. This nano-emulsion could be a promising alternative or adjunct therapy for individuals at risk of BPH, such as athletes and the elderly.

Keywords: Quercetin; Pumpkin Seed Oil; Nano-emulsion; Benign Prostatic Hyperplasia; Testosterone; Antioxidant.

1. Introduction

Oxidative stress and inflammation play a crucial role in prostate cancer and testosterone-induced prostatic hypertrophy in athletes. Several reports highlight the link between oxidative stress and prostatic hypertrophy. This excessive production of free radicals and reactive oxygen

*** Corresponding Authors**:
Mohsen Chamanara, Toxicology Research Center, AJA University of Medical Sciences, Tehran, Iran. E-mail: chamanaramohsen@gmail.com.
Abbas Nourmohammadi, Faculty of Aerospace and Diving Medicine, AJA University of Medical Sciences, Tehran, Iran. E-mail: dr.nourmohamadi@yahoo.com

Cite this article as: Shirmohammadi Yazdi M., Heidari R., Panahi N., Chamanara M., Nourmohammadi A. Protective Effects of Quercetin-Loaded Pumpkin Seed Oil Nano-Emulsion on Testosterone-Induced Benign Prostatic Hyperplasia in Rats. Iran. J. Pharm. Sci., 2025, 21 (1): 165- 176.

DOI: https://doi.org/10.22037/ijps.v21i1.46132

species (ROS) is suggested to be related to androgen exposure, leading to the development of adenomas and prostate cancer. Benign prostatic hyperplasia (BPH) is a pathological change causing lower urinary tract symptoms and is one of the most common urological diseases in middle-aged men [1]. BPH treatment involves reducing or eliminating androgen stimulation of the prostate. A significant reduction in serum dihydrotestosterone (DHT) concentration has been observed within days to weeks after surgery [2].

Multiple studies have considered treatments such as 5-alpha reductase inhibitors (finasteride) [3], androgen receptor inhibitors (androgen antagonists) [4], aromatase inhibitors, and other anti-estrogen drugs and gonadotropin-releasing hormone (GnRH) agonists [5]. In men, lower urinary tract symptoms are common complications of BPH, and an effective treatment involves using alpha-1 adrenergic receptor antagonists to relax smooth muscles in the lower urinary tract. However, due to the absence of smooth muscle in the prostate, this treatment is not used for prostate enlargement itself. Finasteride blocks the activity of 5α-reductase, an enzyme that converts testosterone to DHT. DHT is a biologically active hormone essential for activating prostatic hyperplasia in individuals. Finasteride reduces the conversion of testosterone to DHT, maintaining testosterone concentration while eliminating DHT production [6, 7].

Alpha-1 adrenergic receptor antagonists and 5-alpha reductase enzyme inhibitors, which block the conversion of testosterone to DHT, are the first-line drugs in BPH treatment. A correlation exists between the accumulation of DHT in the prostate and aging, resulting in cell growth and hyperplasia. Due to side effects such as decreased libido, erectile dysfunction, and nasal congestion, the use of these drugs has limitations. Therefore, identifying better and safer therapeutic compounds becomes significant. Recent studies indicate that using several medicinal plants effectively treats and inhibits the progression of benign prostatic hyperplasia [8]. The development of adenomas and prostate cancer is linked to high ROS production. Most inflammatory cells have low antioxidant capacity, causing oxidative stress and ROS. High levels of ROS, especially hydrogen peroxide and superoxide radicals, have been found in prostate cancer [9, 10].

Polyphenolic compounds like quercetin, abundant in various vegetables and fruits, exhibit significant pharmacological effects, such as antioxidant, anti-inflammatory, and anti-atherogenic activities. Unfortunately, the absorption of this antioxidant from the digestive system is very low (about 5%) [11]. Pumpkin seed oil is also known as a suitable source of beta-sitosterol. Beta-sitosterols benefit from treating prostatic hypertrophy due to their anti-inflammatory, antioxidant, and anti-cancer properties [12]. Recently, the nano-emulsion drug delivery system (NEDDS), an emulsification method, has gained more attention as it can enhance the oral bioavailability of hydrophobic drugs and bioactive food components and has various applications in drug delivery [13].

This study evaluates the protective effects of a novel quercetin-loaded pumpkin seed oil nano-emulsion on testosterone-induced benign prostatic hyperplasia (BPH) in rats. The increasing prevalence of BPH, particularly in aging male populations, underscores the necessity of finding effective and safe treatments that minimize side effects. While current pharmacological treatments, such as 5-alpha reductase inhibitors like finasteride, have proven efficacy, they are often associated with significant adverse effects, including decreased libido and erectile dysfunction. This research aims to explore whether combining the antioxidant and anti-inflammatory properties of quercetin with the bioavailability-enhancing capabilities of a nano-emulsion delivery system can offer a superior alternative. The nano-emulsion is designed to enhance quercetin's therapeutic effects and maximize its absorption, potentially providing a more potent and safer treatment option for individuals with BPH. Investigating biochemical markers and histopathological changes in this animal model will contribute to a deeper understanding of how natural compounds can be harnessed to address the complex pathology of BPH, paving the way for novel therapeutic strategies.

2. Materials and Methods

2.1. Preparation of Nano-Emulsion

The nano-emulsion was formulated based on initial solubility studies. A mixture consisting of Tween 80 (50%), polyethylene glycol (PEG) 200 (20%), and pumpkin seed oil (30%) was used. A total of 5 mg of

quercetin was added to the mixture and vortexed for five minutes to prepare the nano-emulsion. This formulation was then subjected to ultrasonication for 50 seconds.

2.2. Nano-Emulsion Characterization

2.2.1. Scanning Electron Microscopy (SEM)

SEM was utilized to analyze the quercetin-loaded pumpkin seed oil nano-emulsion and nano-emulsion's surface morphology. The samples were mounted on aluminum stubs, coated with a thin gold layer, and examined at various magnifications. This allowed for detailed observation of particle shape and surface features.

2.2.2. Dynamic Light Scattering (DLS)

The nano-emulsions' particle size distribution and polydispersity index (PDI) were determined using DLS, following the method described in a previous report [14]. The samples were appropriately diluted with distilled water and analyzed at 25°C. This technique provided information on the average particle size and the distribution uniformity of the nano-emulsions.

2.2.3. Zeta Potential Analysis

The zeta potential of the quercetin-loaded pumpkin seed oil nano-emulsion and pumpkin seed oil nano-emulsion was measured to evaluate their surface charge and stability. After diluting the samples with distilled water, measurements were taken at 25°C, following the method described in a previous report [15].

2.3. Animal Model

Thirty-six adults male Wistar rats, each weighing approximately 220 grams, were obtained from the Pasteur Institute. Rats were selected due to their physiological and anatomical similarities to humans, small size, ease of maintenance, and strong immune system. They were housed in cages (15×42×26.5 cm) with access to food and water. The rats were fed a specialized diet from the Pasteur Institute and tap water from Tehran through water bottles. They were kept in a controlled environment with a temperature of 25±5°C, a 12-hour light/dark cycle, and 40-60% relative humidity. After a one-week acclimation period, the rats were divided into six groups (n=6 each) and treated as follows:

1. Negative Control Group: Received 10 mg/kg testosterone enanthate subcutaneously daily for 4 weeks.
2. Finasteride (Fin) Group: Received 10 mg/kg testosterone enanthate subcutaneously and 1 mg/kg finasteride orally via gavage daily for 4 weeks.
3. Quercetin (Q) Group: Received 10 mg/kg testosterone enanthate subcutaneously and 25 mg/kg quercetin orally via gavage daily for 4 weeks.
4. Quercetin-Loaded Pumpkin Seed Oil Nano-Emulsion (NPOSQ) Group: Received 10 mg/kg testosterone enanthate subcutaneously and 0.5 mL of quercetin-loaded pumpkin seed oil nano-emulsion orally via gavage daily for 4 weeks.
5. Pumpkin Seed Oil Nano-Emulsion (NPOS) Group: Received 10 mg/kg testosterone enanthate subcutaneously and 0.5 mL of pumpkin seed oil nano-emulsion orally via gavage daily for 4 weeks.
6. Positive Control (Healthy) Group: Maintained without testosterone or drug treatment under similar conditions for 4 weeks.

All experimental procedures followed the guidelines for the Care and Use of Laboratory Animals by the National Institutes of Health (USA) and approved by the Internal Research Ethics Committee (Approval ID: IR.AJAUMS.REC.1402.029).

2.4. Euthanasia and Sample Collection

After 4 weeks, the rats were euthanized under deep anesthesia using ketamine (35 mg/kg) and xylazine (10 mg/kg). Weekly body weights were recorded. At the end of the 28-day treatment period, after a 48-hour fasting period, blood samples were collected from the heart ventricle using a 5 mL syringe under anesthesia. The blood samples were centrifuged at 3,000 g for 15 minutes to separate the serum, which was then stored at -80°C for further analysis of antioxidant and anti-inflammatory parameters and dihydrotestosterone levels. Prostate tissues (anterior, posterior, and lateral lobes) were harvested, measured, and weighed. The remaining tissue was stored at -70°C for further inflammatory and antioxidant analysis.

2.5. Biochemical Assays

2.5.1. Dihydrotestosterone (DHT) Measurement

Blood samples were collected from the rats for serum separation and DHT evaluation. Serum samples were stored at -80°C. DHT levels were measured using Biocompare kits according to the manufacturer's instructions.

2.5.2. Measurement of Oxidative Stress and Inflammatory Markers

Portions of the prostate tissue stored at -80°C were homogenized in cold normal saline and centrifuged at 13,634 g for 20 minutes at 4°C. The homogenized and centrifuged tissues were then used to measure malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPx), total antioxidant capacity (TAC), and tumor necrosis factor-alpha (TNF-α) levels with ZellBio (GmbH) kits, following the manufacturer's instructions.

2.6. Histopathological Analysis

Prostate tissue samples, including all three lobes, were fixed in formalin. Light microscopy assessed These samples for qualitative pathological evaluation, epithelial cell size, and thickness. Hematoxylin-eosin staining was performed according to standard protocols.

2.7. Statistical Analysis

All experimental data were subjected to statistical analysis using IBM SPSS software version 25.0. The results were expressed as mean ± standard deviation (SD) for each group. The normality of the data distribution was assessed using the Shapiro-Wilk test. A one-way analysis of variance (ANOVA) was performed for comparisons between groups, followed by Tukey's post hoc test to determine the significance of differences among multiple groups. In cases where the data did not meet the assumptions for ANOVA, the non-parametric Kruskal-Wallis test was employed, followed by Dunn's post hoc test. The paired t-test was used to compare the pre-treatment and post-treatment data within the same group. A p-value of less than 0.05 was considered statistically significant for all analyses, indicating that the observed differences were unlikely to have occurred by chance. All statistical analyses were designed to rigorously evaluate the efficacy of the treatments in reducing BPH-related parameters, ensuring robust and reliable results.

3. Results

3.1. Characterization of Nano-Emulsions

The synthesized nano-emulsions were characterized using scanning electron microscopy (SEM), Dynamic Light Scattering (DLS), and zeta potential analysis. The SEM images revealed that the nano-emulsions exhibited a uniform spherical shape with a smooth surface, indicating well-dispersed particles. The average particle diameter observed in SEM was consistent with the size range anticipated for optimal nano-emulsion performance (**Figure 1**).

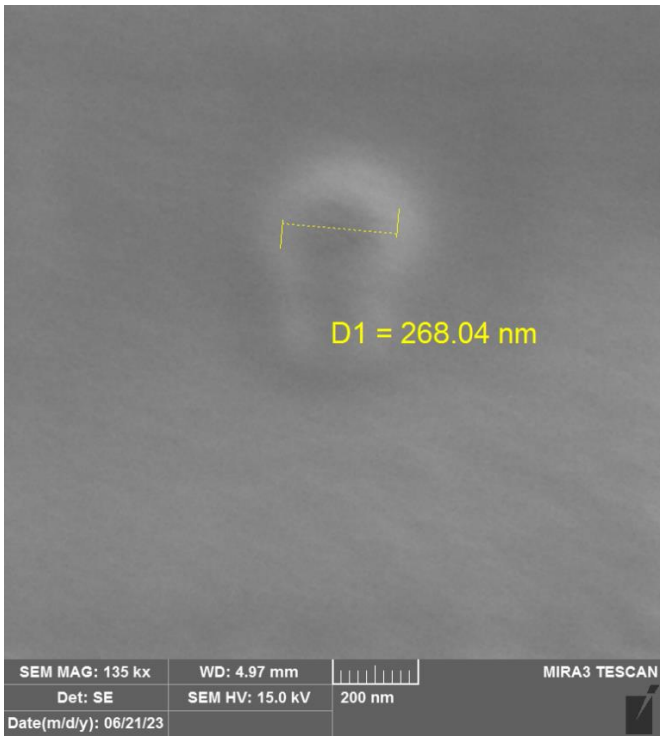

Figure 1. Scanning Electron Microscopy (SEM) Image of the Quercetin-Loaded Pumpkin Seed Oil Nano-Emulsion

The DLS analysis indicated an average particle size of approximately 275.3 ± 123.0 nm, with a PDI value below 0.3, signifying a relatively narrow size distribution and homogeneity among the particles. These characteristics are crucial for ensuring the stability and uniformity of the nano-emulsions in biological systems (**Figure 2**).

The mean zeta potential value was determined to be −19 mV, suggesting moderate emulsion stability due to sufficient electrostatic repulsion between particles. This negative surface charge is essential in preventing particle aggregation, thereby enhancing the shelf-life and bioavailability of the nano-emulsion (**Figure 2**).

Figure 2 depicts the size and zeta potential distribution of nano-emulsions. DLS analysis of nanoparticles showed a peak with an average diameter of 275.3 ± 123.0 nm. The mean zeta potential of nano-emulsions was determined to be −19 mV, indicating an overall acceptable stability.

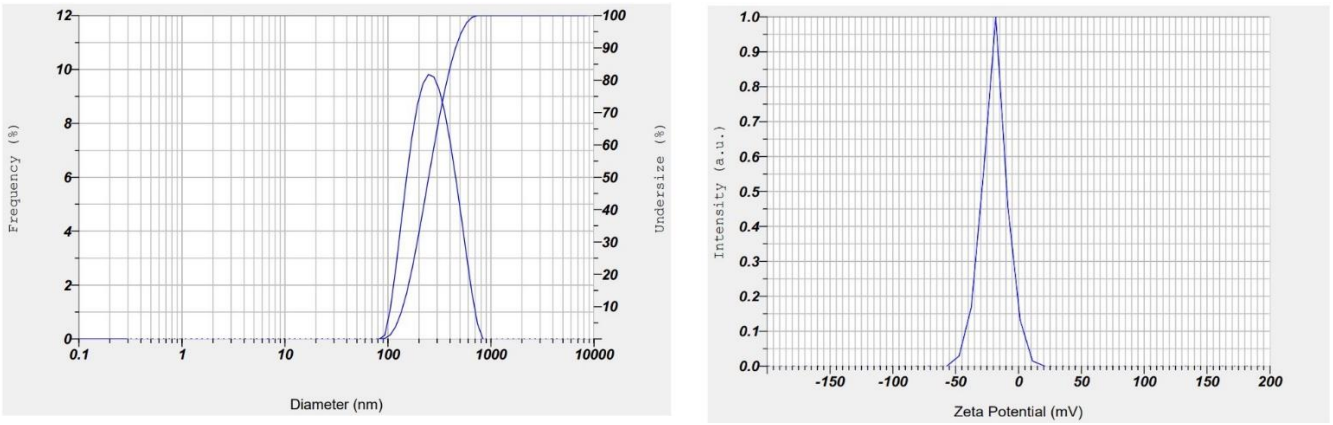

Figure 2. Particle Size Distribution and Zeta Potential of Quercetin-Loaded Pumpkin Seed Oil Nano-Emulsion**. (A)** Particle Size Distribution: The histogram illustrates the size distribution of the nano-emulsion particles as determined by Dynamic Light Scattering (DLS). The average particle diameter is approximately 275.3 ± 123.0 nm, with a relatively narrow distribution as indicated by the polydispersity index (PDI) of less than 0.3. **(B)** Zeta Potential Distribution: The graph shows the zeta potential of the nano-emulsion, with a mean value of −19 mV.

3.2. DHT Levels in Serum

Figure 3 shows the serum levels of DHT in different groups. The untreated control animals showed substantially higher DHT levels than healthy animals. Treatment with Quercetin (Q) resulted in a significant reduction of serum DHT compared to the control group ($P < 0.01$). Additionally, treatment with NPOS, NPOSQ, and Finasteride (Fin) was accompanied by significant reductions in the level of DHT in each group compared to that of the control untreated animals ($P < 0.0001$).

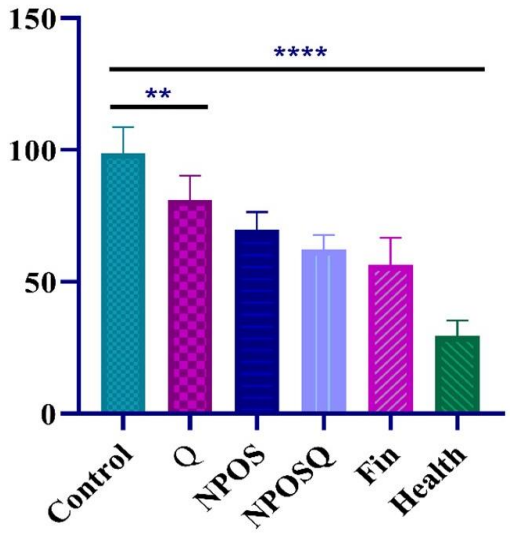

Figure 3. Serum Dihydrotestosterone (DHT) Levels in Different Experimental Groups. The bar graph displays the serum DHT levels in rats from various treatment groups, including control, quercetin, pumpkin seed oil nano-emulsion, quercetin-loaded pumpkin seed oil nano-emulsion, and finasteride. Data are presented as mean ± SD. Asterisks indicate statistically significant differences from the control group, with **P < 0.01 and ****P < 0.0001.

3.3. Levels of MDA, SOD, and TAC in Serum and Prostate Tissue

MDA, a product of fatty acid peroxidation, is used as a marker of oxidative stress. **Figure 4** shows the serum and prostate tissue MDA levels in different groups of animals. The levels of MDA were significantly higher in the serum and prostate tissue of untreated control animals compared to those of healthy animals ($P < 0.0001$). Treatment with Q alone significantly decreased the level of MDA in the prostate tissue; however, it was not associated with a meaningful change in the serum level of MDA. Similarly, NPOS was not found to impact the serum level of MDA significantly. Conversely, treatment with NPOS, NPOSQ, and Fin was accompanied by markedly diminished levels of MDA both in serum and prostate tissue, with Fin showing the highest degree of effect, followed by NPOSQ and NPOS ($P < 0.0001$).

Superoxide dismutase (SOD) is a marker of antioxidative activity, as it facilitates degradation of reactive oxygen species (ROS). As anticipated, the untreated control animals demonstrated the lowest serum and tissue levels of SOD, which were significantly lower than their healthy counterparts (**Figure 5**). All therapeutic interventions, including Q, Fin, NPOS, and NPOSQ, were found to significantly upregulate SOD both in the serum and prostate tissue of animals, compared to the control group ($P < 0.0001$). In terms of comparative efficacy, the effects of Fin, NPOS, and NPOSQ on serum SOD were not significantly different. However, the tissue level of SOD was found to be markedly affected by Fin, compared to NPOS and NPOSQ, in a positive manner ($P < 0.01$).

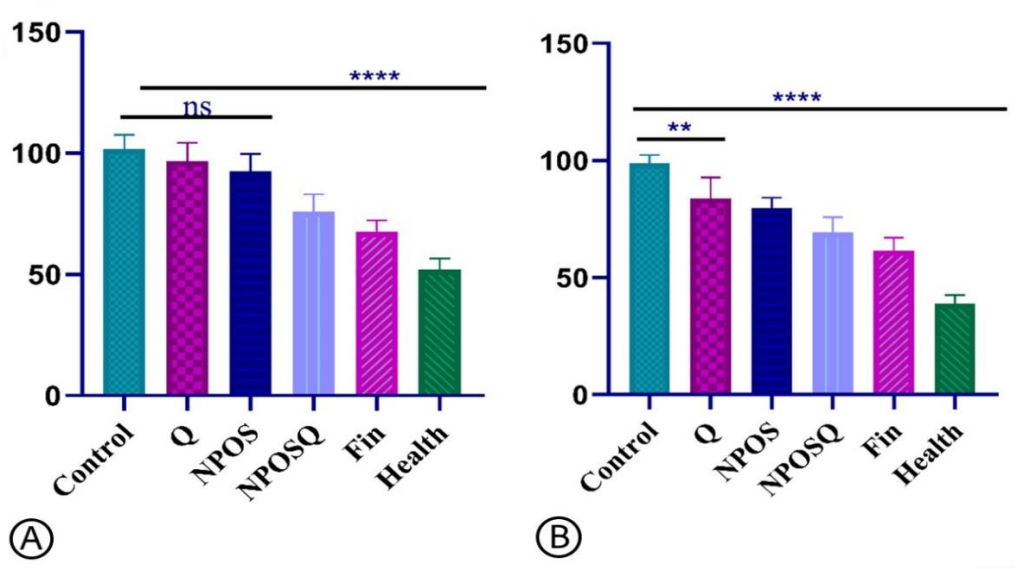

Figure 4. Malondialdehyde (MDA) Levels in Serum and Prostate Tissue. **(A)** Serum MDA Levels: The bar graph shows MDA levels in serum from different experimental groups. The untreated control group has significantly higher MDA levels compared to all treated groups. **Significance levels: *P < 0.01, **P < 0.001, ****P < 0.0001. **(B)** Prostate Tissue MDA Levels: The bar graph illustrates MDA levels in prostate tissue. Significant reductions in MDA levels are observed in the quercetin-loaded pumpkin seed oil nano-emulsion, pumpkin seed oil nano-emulsion, and finasteride groups compared to the control. **Significance levels: *P < 0.01, ****P < 0.0001.

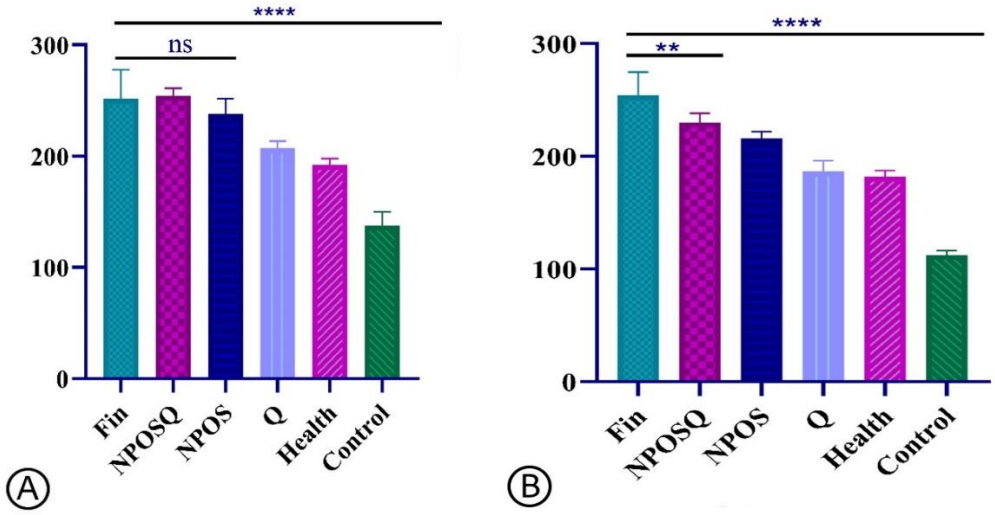

Figure 5. Superoxide Dismutase (SOD) Levels in Serum and Prostate Tissue. (A) Serum SOD Levels: The bar graph shows SOD levels in serum across different treatment groups. All treatment groups demonstrate significantly higher SOD levels compared to the control. **Significance levels: **P < 0.01, ******P < 0.0001. (B) Prostate Tissue SOD Levels:** The bar graph displays SOD levels in prostate tissue. The finasteride group shows the highest SOD levels, followed by the quercetin-loaded pumpkin seed oil nano-emulsion and pumpkin seed oil nano-emulsion groups. **Significance levels: **P < 0.01**, ****P < 0.0001.**

Glutathione peroxidase (GPx) is a cytosolic antioxidant enzyme that facilitates the degradation of hydrogen peroxide and, hence, is a marker of antioxidative activity. As depicted in **Figure 6**, the untreated control animals had the lowest serum and tissue levels of GPx. Compared to the control group, all therapeutic groups showed markedly increased serum and tissue levels of GPx ($P < 0.01$). The highest level of GPx was observed in the Fin group, followed by NPOSQ, NPOS, and Q group. Fin and NPOSQ were equally effective at elevating GPx in prostate tissue, as the difference was deemed insignificant. Collectively, NPOSQ and NPOS were found to be significantly more effective than Quercetin alone.

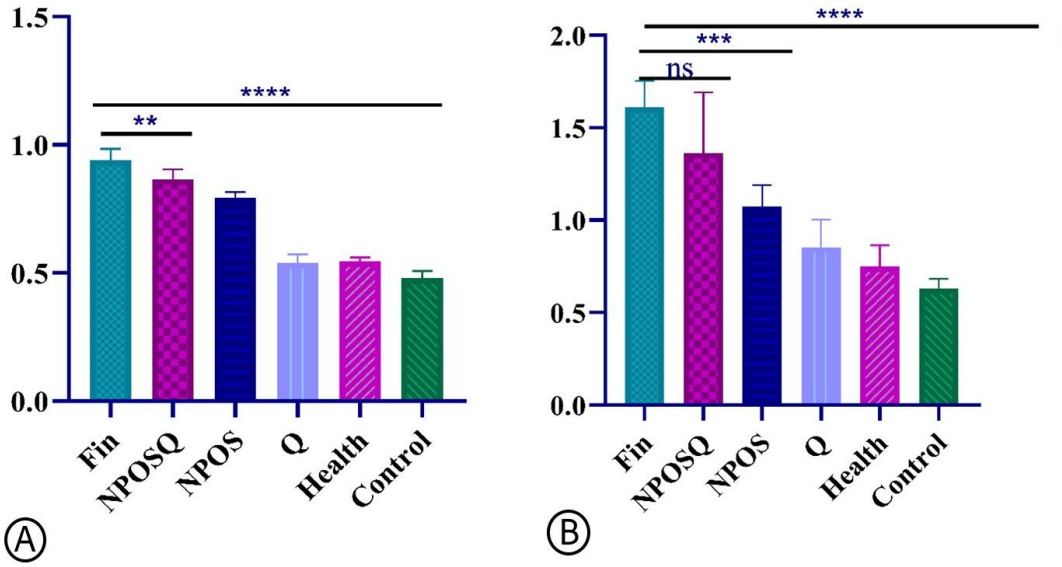

Figure 6. Glutathione Peroxidase (GPx) Levels in Serum and Prostate Tissue. (A) Serum GPx Levels: The bar graph presents GPx levels in serum from various experimental groups. All treatment groups show significantly increased GPx levels compared to the control. **Significance levels: **P < 0.01, ***P < 0.001, ******P < 0.0001. (B) Prostate Tissue GPx Levels:** The bar graph illustrates GPx levels in prostate tissue. The finasteride and quercetin-loaded pumpkin seed oil nano-emulsion groups exhibit the highest GPx levels. **Significance levels: **P < 0.01, ***P < 0.001, ******P < 0.0001.**

The total antioxidant capacity (TAC) was measured and illustrated in **Figure 7** to determine the additive effects of antioxidants. TAC's baseline serum and tissue levels were higher in healthy animals than their untreated control counterparts. Treatment with Q, NPOS, NPOSQ, and Fin led to a significant increase in the serum and prostate tissue levels of TAC compared to the control group. While the effect of NPOS was comparatively lesser than Fin, NPOSQ was found to be as effective as Fin since the difference between NPOSQ and Fin, in both serum and tissue levels of TAC, was determined to be insignificant, indicating the superior efficacy of NPOSQ compared to Q alone.

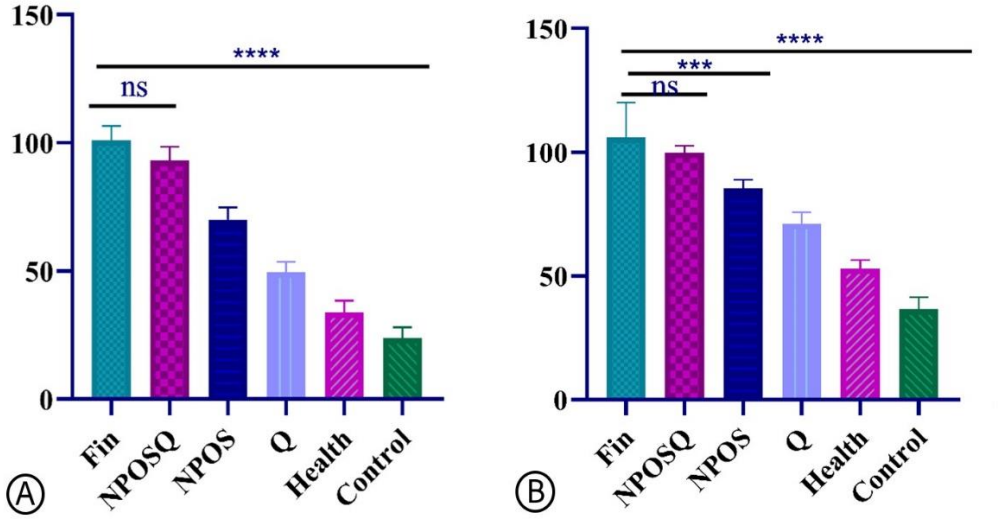

Figure 7. Total Antioxidant Capacity (TAC) in Serum and Prostate Tissue. (A) Serum TAC Levels: The bar graph shows TAC levels in serum across different experimental groups. All treatment groups exhibit significantly higher TAC levels compared to the control. **Significance levels: **P < 0.001, ******P < 0.0001. (B) Prostate Tissue TAC Levels:** The bar graph illustrates TAC levels in prostate tissue. The quercetin-loaded pumpkin seed oil nano-emulsion group shows similar effectiveness to the finasteride group in enhancing TAC levels. **Significance levels: **P < 0.001, ******P < 0.0001.**

3.4. TNF-α Levels in Serum and Prostate Tissue

TNF-α is a pro-inflammatory cytokine whose upregulation is known in the context of prostate cancer and BPH. As anticipated, healthy animals had the lowest serum and tissue levels of TNF-α, while untreated control animals demonstrated the highest levels (**Figure 8**). Treatment with Q, NPOS, NPOSQ, and Fin resulted in significantly reduced levels of TNF-α both in serum and prostate tissue specimens, compared to the controls ($P < 0.01$). The inhibitory effect associated with NPOSQ was significantly higher than Q alone. The insignificant difference between NPOSQ and Fin suggested that NPOSQ could be as effective as Fin in regulating TNF-α.

3.5. Histopathological Findings

Histopathological examination of prostate tissue sections collected from the six groups indicated significant differences between the therapeutic and control groups. As shown in **Figure 9**, the untreated control groups demonstrated significantly more clusters of papillary hyperplastic growths with narrowing of the lumens compared to the healthy group. Administration of Q, NPOS, NPOSQ, and Fin was found to alleviate these histopathological changes associated with BPH. The greatest extent of change was observed in the NPOSQ group, whose histological appearance was reminiscent of normal prostate tissue, as shown in Figure X. Moreover, Fin, NPOS, and Q treatments were also found to significantly reverse the neoplastic changes associated with BPH.

3.7. Body and Prostate Weights

Figure 10 shows differences in body and prostate weights among the test groups. As anticipated, healthy animals exhibited the lowest prostate weight, while untreated controls showed the highest. Treatment with Q and Fin alone did not result in significant decreases in prostate weight, while treatment with NPOS led to a marginal increase in prostate weight compared to controls. However, administration of NPOSQ was associated with a significantly reduced prostate weight compared to all test groups ($P < 0.05$). Similar changes were observed with total body weight. Collectively, we found that NPOSQ was substantially more effective than NPOS, Fin, and Q ($P < 0.05$).

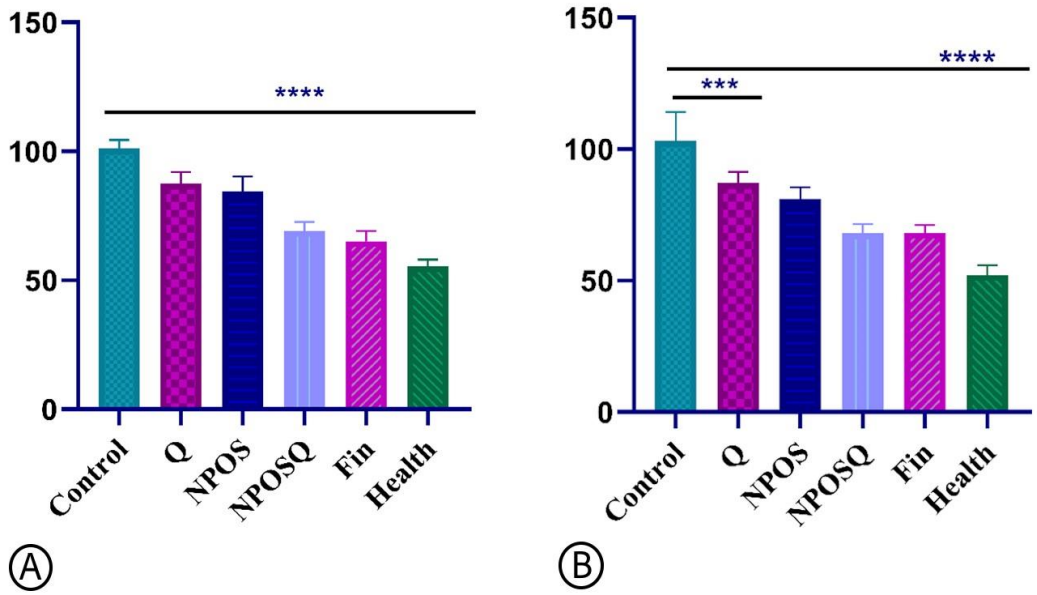

Figure 8. Tumor Necrosis Factor Alpha (TNF-α) Levels in Serum and Prostate Tissue. (A) Serum TNF-α Levels: The bar graph shows TNF-α levels in serum from different experimental groups. All treatment groups have significantly reduced TNF-α levels compared to the control group. **Significance levels: ***P < 0.001, ******P < 0.0001. (B) Prostate Tissue TNF-α Levels:** The bar graph depicts TNF-α levels in prostate tissue. The quercetin-loaded pumpkin seed oil nano-emulsion group demonstrates a significant reduction in TNF-α levels, comparable to the finasteride group. **Significance levels: ***P < 0.001, ******P < 0.0001.**

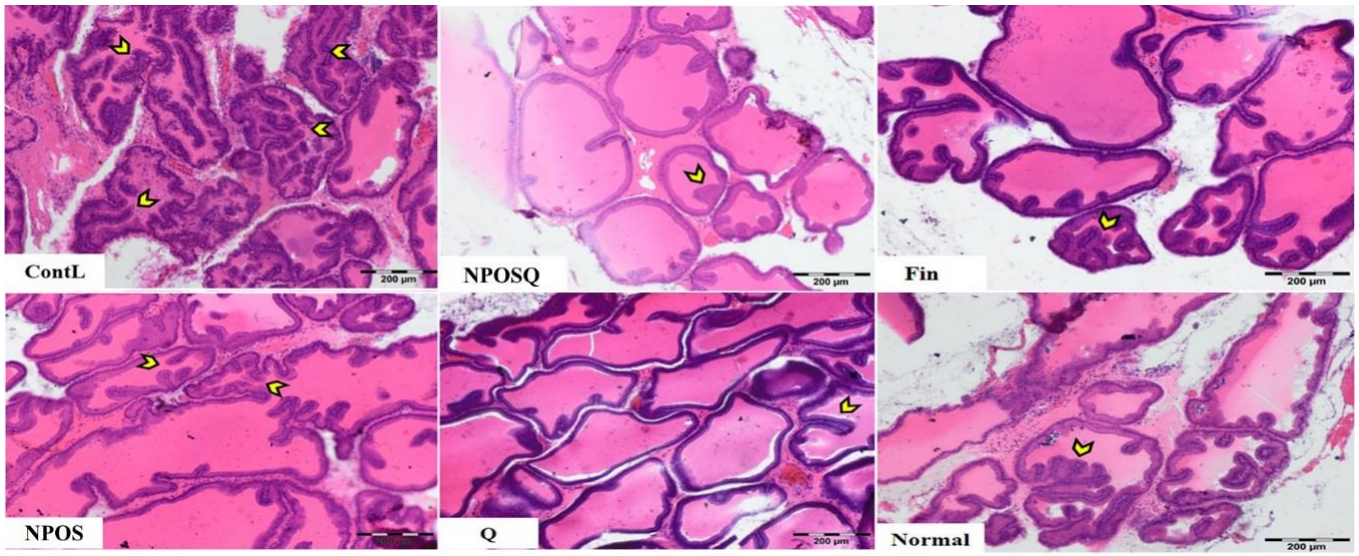

Figure 9. Histopathological Examination of Prostate Tissue. The images show histopathological changes in prostate tissue sections from different experimental groups. The untreated control group displays marked papillary hyperplastic growths with narrowed lumens. The quercetin-loaded pumpkin seed oil nano-emulsion, pumpkin seed oil nano-emulsion, and finasteride groups notably alleviate hyperplastic and hypertrophic lesions.

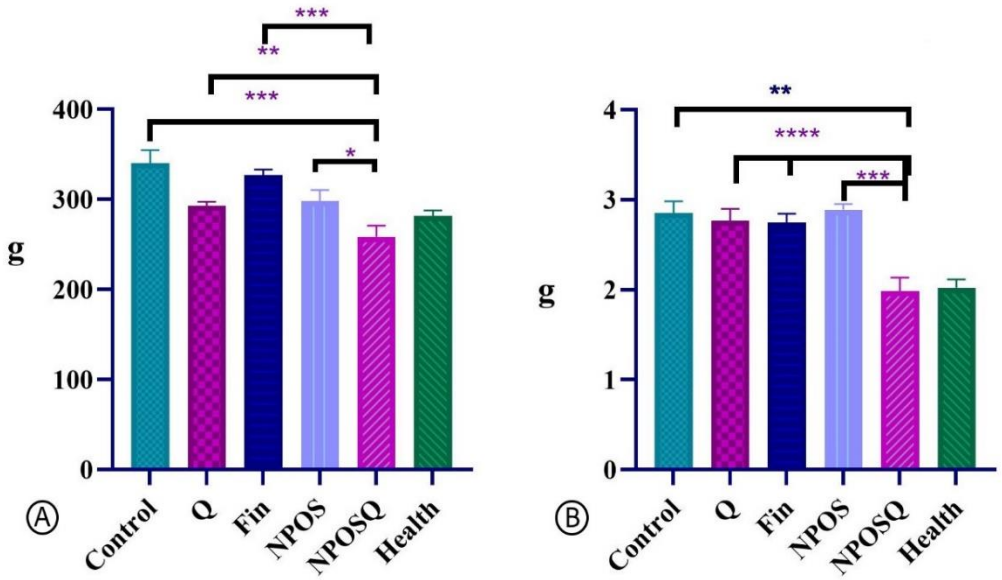

Figure 10. **Body and Prostate Weights in Different Experimental Groups. (A) Body Weight:** The bar graph displays the body weights of rats from various treatment groups. No significant differences in body weight were observed among the treatment groups compared to the control. **(B) Prostate Weight:** The bar graph shows prostate weights for different experimental groups. The quercetin-loaded pumpkin seed oil nano-emulsion group exhibits a significantly reduced prostate weight compared to all other groups. *Significance levels: *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.001.

3.8. Discussion

Our study demonstrates that the quercetin-loaded pumpkin seed oil nano-emulsion significantly reduces inflammation and oxidative stress markers in testosterone-induced benign prostatic hyperplasia (BPH) rats. The nano-emulsion notably decreases dihydrotestosterone (DHT) levels and improves antioxidant enzyme activities in both serum and prostate tissues. Furthermore, histopathological analysis shows a substantial reduction in hyperplastic and hypertrophic lesions, suggesting superior efficacy compared to finasteride. These findings indicate that quercetin-loaded pumpkin seed oil nano-emulsion may be a promising alternative or adjunctive therapy for BPH.

Recent studies corroborate our findings regarding the efficacy of natural compounds in treating benign prostatic hyperplasia (BPH). Achi et al. (2024) demonstrated that *Syzygium malaccense* methanol leaf extract (SMLE) significantly reduced inflammation and oxidative stress in a testosterone-estradiol valerate-induced BPH model in rats [1]. Like our quercetin-loaded pumpkin seed oil nano-emulsion, SMLE-modulated prostatic and serum biomarkers show promising therapeutic potential. Similarly, Lee et al. (2023) showed that finasteride inhibited BPH progression by regulating oxidative stress, inflammation, and ER stress responses [16], mechanisms also targeted by our nano-emulsion treatment.

Comparatively, studies by Binmahfouz et al. (2022) and Elsherbini et al. (2022) highlighted the antiproliferative, antioxidant, and anti-inflammatory properties of 6-Paradol [17] and marjoram extract [18], respectively, in testosterone-induced BPH models. These findings align with our results, where the quercetin-loaded nano-emulsion effectively reduced hyperplastic lesions and improved antioxidant enzyme activities. Additionally, Uroko et al. (2022) and Baek et al. (2022) reported similar therapeutic effects using combined extracts of *Spermacoce radiata* and *Hypselodelphys poggeana* [19] and *Eriochloa villosa* [20], respectively, further supporting the role of natural compounds in managing BPH through oxidative stress and inflammation modulation.

Peng et al. (2021) investigated the anti-BPH effects of a water extract from Prinsepia utilis Royle leaves, identifying ten key flavonoids in the active fraction (Fr. B) and demonstrating significant reductions in DHT, VEGF, and MDA levels in murine models [21]. Similar to our findings, their study highlighted the importance of antioxidant mechanisms, as Fr. B increased SOD, GSH-Px, and CAT levels. In comparison, our research also identified key active constituents that modulate oxidative stress and inflammatory markers, underscoring the therapeutic potential of natural extracts in BPH management [21].

Wang et al. (2021) explored the effects of curcuma oil (CO) on BPH, showing its ability to induce apoptosis and reduce prostate weight and hyperplasia by suppressing 5α-reductase and inhibiting the NF-κB pathway [22]. These results align with our findings on reducing DHT levels and anti-inflammatory effects. Similarly, studies by Ekeyi et al. (2021) on ethanol extract of *Cassia sieberiana* leaves [23] and Farshid et al. (2021) on black mulberry extract [24] demonstrated reductions in prostate weight and improvements in antioxidant parameters, reinforcing the significance of anti-inflammatory and antioxidant pathways in BPH treatment. Comparatively, Eid et al. (2020) demonstrated the efficacy of a piceatannol nanoformulation in modulating oxidative stress and inflammatory responses [25], similar to our findings on bioactive natural compounds improving BPH symptoms.

Abdel-Aziz et al. (2020) showed that febuxostat significantly ameliorated testosterone-induced BPH in rats by reducing oxidative stress, inflammation, and prostatic weight, comparable to finasteride [26]. Similar to our findings, their study highlighted the reduction of pro-inflammatory cytokines and inhibiting key pathways such as iNOS/COX-2 and VEGF/TGF-β. Anosike et al. (2020) reported that methanol leaf extract of *Duranta erecta* (MLEDE) reduced prostate weight, testosterone, DHT, and oxidative stress markers in BPH rats [27]. These outcomes agree with our observations on the importance of antioxidants in managing BPH, demonstrating MLEDE's ability to enhance the antioxidant defense system and improve prostate histoarchitecture.

Elbaz et al. (2020) found that diallyl sulfide (DAS) from garlic reduced prostate weight, testosterone, DHT, and inflammatory markers through the suppression of the ERK pathway [28]. These results are consistent with our findings on natural compounds reducing oxidative stress and inflammation. Similarly, Acheampong et al. (2019) demonstrated the efficacy of lime essential oil (LEO) in lowering prostate weight and PSA levels, enhancing antioxidant capacity, and improving histomorphological characteristics [29], which fall in line with our study's focus on antioxidant and anti-inflammatory mechanisms. Cai et al. (2018) reported that the Xialiqi (XLQ) capsule reduced prostate weight and inflammatory markers while increasing SOD activity and caspase-3 expression [30], corroborating our findings on the multi-faceted therapeutic effects of traditional herbal formulas on BPH. Lastly, Soni et al. (2017) indicated that finasteride, combined with DA-9401, mitigated oxidative stress and apoptosis in testicular tissue [31], reinforcing our results on the role of antioxidant therapies in BPH treatment.

In summary, our study introduces and validates the novel formulation of quercetin-loaded pumpkin seed oil nano-emulsion, demonstrating significant therapeutic potential in mitigating BPH through mechanisms involving oxidative stress reduction, anti-inflammatory effects, and hormonal regulation. Unlike previous studies, this is the first to explore the unique combination of quercetin with pumpkin seed oil in a nano-emulsion delivery system. Our formulation effectively decreased prostate weight, improved histological architecture, and modulated key biochemical markers, highlighting its multifaceted benefits and establishing it as a promising alternative or adjunct to conventional BPH treatments.

4. Conclusion

In this study, the quercetin-loaded pumpkin seed oil nano-emulsion demonstrated significant therapeutic potential in managing testosterone-induced benign prostatic hyperplasia (BPH). The nano-emulsion effectively reduced inflammatory and oxidative stress markers, as evidenced by decreased levels of malondialdehyde (MDA) and increased antioxidant enzyme activities (SOD, GPx, and TAC) in both serum and prostate tissues. Histopathological analysis further revealed a substantial reduction in hyperplastic and hypertrophic lesions in the prostate, indicating the nano-emulsion's superior efficacy compared to finasteride.

The quercetin-loaded nano-emulsion showed comparable or superior results to finasteride, with fewer side effects. This formulation enhances quercetin's bioavailability and efficacy through the nano-emulsion delivery system, addressing the limitations of its poor gastrointestinal absorption. The study's findings support the potential of this nano-emulsion as a promising alternative or adjunctive therapy for individuals at risk of BPH, such as elderly individuals and athletes. Further clinical investigations are warranted to validate these results and explore the therapeutic benefits in human subjects.

Acknowledgment

The authors would like to express their gratitude to the Central Research Laboratory, Aja University of Medical Sciences, for providing the necessary resources and support for this research.

Conflict of interest

The authors declare no competing interests.

Data Availability Statement

All datasets generated in this study will be made available upon reasonable request.

Athors Contributions

The authors confirm their contribution to the paper as follows: Study conception and design: AN and MC; Data collection: MSY, NP and RH; Analysis and interpretation of results: MSY, RH, and NP; Draft manuscript preparation: RH, MSY, NP, MC and AN. All authors reviewed the results and approved the final version of the manuscript.

Athors Orcid numbers:

Mohammad Shirmohammadi Yazdi: 0009-0006-4175-2868
Reza Heidari: 0000-0003-2985-8396
Negar Panahi: 0000-0003-2261-3388
Mohsen Chamanara: 0000-0002-6201-2918
Abbas Nourmohammadi: 0000-0003-1621-767X

Funding

This study was funded by AJA University of Medical Sciences (Grant NO.: IR.AJAUMS.REC.1402.029).

Using artificial intelligence chatbots

There was no use of artificial intelligence in the making of this article.

References

1. Achi, N.K., et al., *Syzygium malaccense leaves methanol extract modulate some biochemical and inflammatory markers and prostate histology of testosterone-estradiol valerate induced benign prostatic hyperplasia in rats.* Avicenna Journal of Phytomedicine, 2024. **14**(3): p. 305-324.
2. Welén, K. and J.E. Damber, *Androgens, aging, and prostate health.* Reviews in Endocrine and Metabolic Disorders, 2022. **23**(6): p. 1221-1231.
3. Li, Y., et al., *The efficacy and safety of dutasteride and finasteride in patients with benign prostatic hyperplasia: a systematic review and meta-analysis.* Translational Andrology and Urology, 2022. **11**(3): p. 313-324.
4. Vickman, R.E., et al., *The role of the androgen receptor in prostate development and benign prostatic hyperplasia: A review.* Asian Journal of Urology, 2020. **7**(3): p. 191-202.
5. Akça, K.T., M.A. Demirel, and I. Süntar, *The Role of Aromatase Enzyme in Hormone Related Diseases and Plant- Based Aromatase Inhibitors as Therapeutic Regimens.* Current Topics in Medicinal Chemistry, 2021. **22**(3): p. 229-246.

6. Foo, K.T., *Pathophysiology of clinical benign prostatic hyperplasia.* Asian Journal of Urology, 2017. **4**(3): p. 152-157.
7. Madersbacher, S., N. Sampson, and Z. Culig, *Pathophysiology of Benign Prostatic Hyperplasia and Benign Prostatic Enlargement: A Mini-Review.* Gerontology, 2019. **65**(5): p. 458-464.
8. Kim, E.H., J.A. Brockman, and G.L. Andriole, *The use of 5-alpha reductase inhibitors in the treatment of benign prostatic hyperplasia.* Asian Journal of Urology, 2018. **5**(1): p. 28-32.
9. Udensi, U.K. and P.B. Tchounwou, *Oxidative stress in prostate hyperplasia and carcinogenesis.* Journal of Experimental and Clinical Cancer Research, 2016. **35**(1): p. 139-139.
10. Vital, P., P. Castro, and M. Ittmann, *Oxidative stress promotes benign prostatic hyperplasia.* Prostate, 2016. **76**(1): p. 58-67.
11. Aghababaei, F. and M. Hadidi, *Recent Advances in Potential Health Benefits of Quercetin.* Pharmaceuticals, 2023. **16**(7): p. 1020-1020.
12. Shaban, A. and R.P. Sahu, *Pumpkin Seed Oil: An Alternative Medicine.* International Journal of Pharmacognosy and Phytochemical Research, 2017. **9**(2): p. 11-11.
13. Preeti, et al., *Nanoemulsion: An Emerging Novel Technology for Improving the Bioavailability of Drugs.* Scientifica, 2023. **2023**: p. 6640103-6640103.
14. Ansari, F.J., et al., *Comparison of the efficiency of ultrafiltration, precipitation, and ultracentrifugation methods for exosome isolation.* Biochemistry and Biophysics Reports, 2024. **38**: p. 101668.
15. Rostami, N., et al., *Synthesis and Characterization of Folic Acid-Functionalized DPLA-co-PEG Nanomicelles for the Targeted Delivery of Letrozole.* ACS Applied Bio Materials, 2023. **6**(5): p. 1806-1815.
16. Lee, G.-H., et al., *The Role of Reactive Oxygen Species, Inflammation, and Endoplasmic Reticulum Stress Response in the Finasteride Protective Effect against Benign Prostate Hyperplasia.* The World Journal of Men's Health, 2024. **42**(3): p. 600-600.
17. Binmahfouz, L.S., et al., *6-Paradol Alleviates Testosterone-Induced Benign Prostatic Hyperplasia in Rats by Inhibiting AKT/mTOR Axis.* Plants, 2022. **11**(19): p. 2602-2602.
18. Elsherbini, D.M.A., et al., *Origanum majorana L. Extract Attenuated Benign Prostatic Hyperplasia in Rat Model: Effect on Oxidative Stress, Apoptosis, and Proliferation.* Antioxidants, 2022. **11**(6): p. 1149-1149.
19. Uroko, R.I., et al., *Combined Spermacoce Radiata and Hypselodelphys Poggeana Extract (CESH) Protect Against Oxidative Stress and Enhances Haematological Parameters in Benign Prostatic Hyperplasia-induced Rats.* Majalah Obat Tradisional, 2022. **27**(3): p. 181-191.
20. Baek, E.B., et al., *Eriochloa villosa Alleviates Progression of Benign Prostatic Hyperplasia in vitro and in vivo.* Research and Reports in Urology, 2022. **14**: p. 313-326.
21. Peng, Y., et al., *Chemical profiles of the active fraction from Prinsepia utilis Royle leaves and its anti-benign prostatic hyperplasia evaluation in animal models.* BMC Complementary Medicine and Therapies, 2021. **21**(1): p. 272-272.
22. Wang, S., et al., *Curcuma oil ameliorates benign prostatic hyperplasia through suppression of the nuclear factor-kappa B signaling pathway in rats.* Journal of Ethnopharmacology, 2021. **279**: p. 113703-113703.
23. Ekeyi, Y., et al., *Ethanol extract of Cassia sieberiana leaves ameliorates deviances associated with benign prostatic hyperplasia in rats.* All Life, 2021. **14**(1): p. 473-483.
24. Farshid, M.A., et al., *Protective effect of black mulberry (Morus nigra L.) fruit hydroalcoholic extract against testosterone-induced benign prostatic hyperplasia in rats.* Revista Internacional de Andrologia, 2021. **19**(1): p. 53-61.
25. Eid, B.G. and A.B. Abdel-Naim, *Piceatannol Attenuates Testosterone-Induced Benign Prostatic Hyperplasia in Rats by Modulation of Nrf2/HO-1/NFκB Axis.* Frontiers in Pharmacology, 2020. **11**: p. 614897-614897.
26. Abdel-Aziz, A.M., et al., *Amelioration of testosterone-induced benign prostatic hyperplasia using febuxostat in rats: The role of VEGF/TGFβ and iNOS/COX-2.* European Journal of Pharmacology, 2020. **889**: p. 173631-173631.
27. Anosike, C.A., et al., *Effect of methanol leaf extract of Duranta erecta in rats induced with benign prostatic hyperplasia.* Tropical Journal of Natural Product Research, 2020. **4**(10): p. 790-795.
28. Elbaz, E.M., et al., *Immunomodulatory effect of diallyl sulfide on experimentally-induced benign prostate hyperplasia via the suppression of CD4+T/IL-17 and TGF-β1/ERK pathways.* Inflammopharmacology, 2020. **28**(5): p. 1407-1420.
29. Acheampong, D.O., et al., *Histoprotective Effect of Essential Oil from Citrus aurantifolia in Testosterone-Induced Benign Prostatic Hyperplasia Rat.* Advances in Urology, 2019. **2019**: p. 3031609-3031609.
30. Cai, H., et al., *The Effect of Xialiqi Capsule on Testosterone-Induced Benign Prostatic Hyperplasia in Rats.* Evidence-Based Complementary and Alternative Medicine, 2018. **2018**: p. 5367814-5367814.
31. Soni, K.K., et al., *Protective effect of DA-9401 in finasteride-induced apoptosis in rat testis: Inositol requiring kinase 1 and c-Jun N-terminal kinase pathway.* Drug Design, Development and Therapy, 2017. **11**: p. 2969-2979.