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Fucoidan Ameliorates Testosterone-Induced Benign Prostatic Hyperplasia (BPH) in Rats

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Purpose: Benign prostatic hyperplasia (BPH) is a major urological health issue for men globally. Fucoidan, a sulfated polysaccharide, displays diverse bioactivities such as anti-inflammatory, anti-tumor, antioxidant, and immunoregulatory effects. This 28-day study examined the effects of *Undaria pinnatifida* fucoidan on testosterone-induced BPH in rats.

Methods: Forty-eight Sprague Dawley (SD) rats were randomly divided into six groups; G1- vehicle control, G2- testosterone alone BPH control group (3 mg/kg), G3- finasteride (10 mg/kg) + testosterone, G4- fucoidan (40 mg/kg) + testosterone, G5- fucoidan (400 mg/kg) + testosterone, and G6- fucoidan alone (400 mg/kg). The animals were observed for clinical signs, body weight, feed consumption, prostate weight, prostate index, and biochemical markers such as tumor necrosis factor-alpha (TNF-α), interleukin- 1β (IL-1β), prostate-specific antigen (PSA) and messenger ribonucleic acid (mRNA) expression of BCL-2-associated X protein (BAX) and B-cell lymphoma-2 (BCL-2) in serum. Testosterone and dihydrotestosterone (DHT) levels were evaluated in both serum and prostate.

Results: Fucoidan significantly prevented an increase in prostate weight and prostate index induced by testosterone. DHT levels in the prostate of the intervention groups were significantly lower than in the BPH control group ($p < 0.05$); however, no significant difference was observed in serum levels. Similarly, a significant reduction was observed in serum and prostate testosterone levels in the intervention groups compared to the BPH control group ($p < 0.05$). Biochemical analyses showed PSA levels were significantly lower in the fucoidan groups compared to the BPH control group ($p<0.05$). Although not statistically significant, fucoidan groups showed a trend of reducing IL-1β and TNF-α levels. Fucoidan demonstrated pro-apoptotic potential in its ability to decrease BCL-2 and increase BAX. Histopathological evidence revealed fewer microscopic lesions in the fucoidan groups compared to the BPH control group.

Conclusion: The results suggest *Undaria pinnatifida* fucoidan can reduce testosterone-induced BPH symptoms in SD rats.

Keywords: fucoidan, *Undaria pinnatifida*, benign prostatic hyperplasia, brown seaweed, dihydrotestosterone

Introduction

Benign prostatic hyperplasia (BPH) is a non-malignant condition involving progressive hyperplastic changes in stromal and epithelial cells in the prostate.[1] This condition is influenced by dihydrotestosterone (DHT), an active metabolite of testosterone with a high affinity for androgen receptors.[2] The binding of DHT to these receptors leads to hyperplasia of prostatic epithelial and stromal cells, contributing to BPH development.[3] Inflammation, often seen in BPH, can cause prostate tissue damage, which in turn leads to elevated PSA levels and increased cytokines production.[4] Additionally, dysregulation of cell proliferation and/or apoptosis plays an important role in enlargement of the prostate. Specifically, the balance between pro-apoptotic (eg, BAX) and anti-apoptotic (eg, BCL-2) factors are involved.[5] These factors lead to the hyperplastic changes resulting in the formation of discrete nodules, inflammation, and fibrosis in the prostate's transitional zone and alterations in smooth muscle activity. Consequently, these changes result in potential partial or complete urethral obstruction,[6] which along with the heightened bladder muscle tone and subsequent detrusor dysfunction, give rise to lower urinary tract symptoms.[7,8]

Globally, BPH's prevalence ranges from 20 to 62% among men aged 50 and older.[9,10] Multiple studies demonstrated a notable increase in prevalence of BPH with age indicating approximately 10%, 50% and 80% upsurge of hyperplasia in the fourth, sixth and ninth decade of life, respectively.[11,12]

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Management of BPH is a complex endeavor owing to its multifaceted etiology.[13] Approaches for addressing BPH symptoms encompass a range of strategies, including pharmaceutical interventions such as α-blockers, 5α-reductase inhibitors, phosphodiesterase inhibitors and surgical intervention. Nonetheless, these drugs have limitations and can lead to side effects, such as erectile dysfunction, headache, dizziness, hypotension, and asthenia.[14] Consequently, it is important to find ways to manage BPH symptoms through diet, lifestyle changes, or herbal medicines before commencing a drug intervention.

Fucoidan, a natural component of brown seaweed, is a complex sulfated polysaccharide, that has been shown to have a diverse range of biological activities, including anti-inflammatory,[15] anti-oxidative,[16] immunoregulatory and anti-tumor activities.[17,18] Fucoidan is known as a selectin blocker and, therefore, can potentially prevent the intrusion of neutrophils into the tissue and consequently reduce the inflammatory response.[19] In prostate health, fucoidan has been shown to increase the proliferation and activity of human immune cells, such as ex vivo peripheral blood mononuclear cells. It can have a direct inhibitory effect on prostate cancer cells.[17] Furthermore, an extract from a brown seaweed *Sargassum horneri* demonstrated an ability to reduce BPH progression in vitro and in vivo. It was considered that this effect was due to an inhibition of the androgen receptor pathway, which reduces the conversion of testosterone to dihydrotestosterone (DHT).[20] Based on the available evidence, it could be speculated that fucoidan may potentially improve BPH symptoms.

The current study investigated the effect of fucoidan extracted from the brown seaweed species *Undaria pinnatifida* (UPF) on testosterone-induced BPH using an in vivo model. The study aimed to assess the impact of UPF on testosterone and DHT levels in serum and prostate, prostate-specific antigen (PSA) levels in serum, and the mRNA expression of BCL-2-associated X protein (BAX) and B-cell lymphoma-2 (BCL-2). It assessed various biochemical parameters such as Interleukin-1β (IL-1β), tumor necrosis factor-alpha (TNF-α) and included an assessment of histopathological changes.

This investigation also aimed to compare the effects of UPF on BPH with finasteride.[21,22] Finasteride, an FDA-approved drug, is a 5α-reductase inhibitor (5-ARI) commonly used in the treatment of BPH. It works by inhibiting the 5α-reductase enzyme, which is responsible for converting testosterone into dihydrotestosterone (DHT).[23]

Material and Methods

Chemicals, Reagents and Kits

Kits for testosterone enzyme-linked immunosorbent assay (ELISA) (Batch No. RT0423), DHT ELISA (Batch No. RDHT0423), TNF-α ELISA (Batch No. RTNFA323), Interleukin β (IL-1β) ELISA (Batch No. RIL1B0423), PSA ELISA (Batch No. RPSA0423) were purchased from Krishgen Biosystems, India. Testosterone propionate (Batch No. 0000497972) was purchased from Himedia, India; corn oil (Batch No. MKCM3364) was purchased from Sigma-Aldrich, USA; finasteride (Batch No. MN56J003) was purchased from Torrent Pharmaceuticals Ltd, India. SYBR Green PCR master mix (Batch No. SGM2023005) was purchased from Aura Biotechnologies Pvt. Ltd. India; BAX (Batch No. 2303061115-9/14) and Reverse Primer (Batch No. 2303061115-10/14); BCL-2 (Batch No. 2303061115-11/14) and Reverse Primer (Batch No. 2303061115-12/14) were purchased from Barcode Biosciences, India. All the chemicals and reagents used for the trial were of analytical grade.

Preparation

Fucoidan extract from *Undaria pinnatifida* (UPF) (Batch No.: UPF2022532) was provided by Marinova Pty Ltd. (Tasmania, Australia). The material with a quoted fucoidan purity of 89.3% (dry weight), was manufactured using a proprietary aqueous extraction process. Fucoidan purity is calculated as the sum of total carbohydrates, sulfation, acetylation and cations. The carbohydrate profile was determined using a gas chromatography (GC) method for the accurate determination of individual monosaccharide ratios in a sample. Total carbohydrate content was determined spectrophotometrically using the phenol-sulfuric technique,[24] while uronic acid content was determined by spectrophotometric analysis in the presence of 3-phenylphenol.[25] Sulfate content was determined spectrophotometrically using a $BaSO_4$ precipitation technique[26] and cations were determined by Flame Atomic Absorption Spectroscopy. The chemical composition of fucoidan used in this study is described in Table 1 and Table 2.

Fucoidan has been shown to be safe and effective in human use. This has been evidenced by multiple clinical studies, including those using fucoidan extracted by Marinova. For instance, Irhimeh et al published three human clinical studies using a fucoidan dose of 3 g.[27–29] While Cooper et al used a dose of up to 2.2 g in their clinical trial.[30] In a trial by Takahashi et al, 4 g

Table 1 Absolute Mass Percentages of UPF Extract

Fucoidan Extract	Neutral Carbohydrates (%)	Sulfate (%)	Fucoidan (%)	Polyphenols (%)
UPF2022532	46.1	28.3	89.3	<2

Abbreviation: UPF, *Undaria pinnatifida* fucoidan.

Table 2 Carbohydrate Breakdown (Mass %) of Neutral Carbohydrates in UPF Extract

Fucoidan Extract	Fucose (%)	Xylose (%)	Galactose (%)	Arabinose (%)	Rhamnose (%)
UPF2022532	22.5	0.3	19	0.6	0.6

Abbreviation: UPF, *Undaria pinnatifida* fucoidan.

daily of fucoidan were safely and effectively administered to cancer patients.[31] While in a clinical study conducted by Myers et al, participants received 300 mg fucoidan daily.[32] Using the dose translation formula by Reagan Shaw, a human dose of 4000 mg or a 300 mg translates into a 411 mg/kg or 30.8 mg/kg rat dose, respectively.[33] Therefore, the current study selected 400 mg/kg and 40 mg/kg UPF as the two intervention doses. 5α- reductase inhibitors are a choice of drug for BPH management. Therefore, finasteride, a commonly used medication in the treatment of BPH was selected as a positive control for this study.

Finasteride and UPF dosages were prepared prior to each dosing based on the individual body weights of the animals. The dose was calculated using the below formula:

$$\text{Dose Calculation}: \frac{\text{Standard Dose} \times \text{Animal body weight (g)}}{1000\text{g}}$$

Experimental Animals and Welfare

The study was conducted in India following the guidelines specified by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), the Institutional Animal Ethics Committee (IAEC), and as per Vedic Lifesciences Standard Operating Procedures (Approval No. LBPL-IAEC-005-02/2023). These procedures are in accordance with the guidelines for animal care and are accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC), USA.

Adult male Sprague Dawley (SD) rats (~6-8 weeks, mean body weight of ~200 to 248 g) were procured from a CPCSEA-approved vendor in compliance with ethical practices.

All animals were subjected to veterinary examination and allowed to acclimatize to the laboratory environment for five days. The animals were provided access to feed and water *ad libitum* as per experimental conditions (temperature 20.0 to 22.9 °C; relative humidity 32-77%, and 12h alternate light/dark cycle with 12-15 cycles/h of air change and a sound level of <80 dB). Reverse osmosis water and commercial pellet feed were provided *ad libitum* for 4 weeks.

Experimental Design

The study was conducted using 48 adult male SD rats, which were randomly divided into six groups (n=8):

- Group 1 (G1): Vehicle Control (no intervention).
- Group 2 (G2): BPH Control (Testosterone 3 mg/kg).
- Group 3 (G3): Reference Control 10 mg/kg (positive control) finasteride + testosterone (3 mg/kg).
- Group 4 (G4): Fucoidan UPF (40 mg/kg) + testosterone (3 mg/kg).
- Group 5 (G5): Fucoidan UPF (400 mg/kg) + testosterone (3 mg/kg).
- Group 6 (G6): Fucoidan UPF (400 mg/kg).

UPF was dissolved in distilled water and finasteride tablets were crushed into powder and dissolved in 0.5% carboxy methyl cellulose (CMC). The dose formulation was freshly prepared and administered within two hours of preparation.

The prepared dose formulation was continuously stirred using a magnetic stirrer to maintain homogeneity. Finasteride (G3) and UPF (G4, G5 and G6) were orally administered two hours before the administration of testosterone. Testosterone was dissolved in corn oil and administered subcutaneously at a dose of 3 mg/kg body weight.

The dose formulations were administered to the respective intervention groups for 28 consecutive days. On day 29, three mL of blood samples were collected with a capillary tube by retro-orbital plexus puncture under isoflurane anesthesia. The animals were sacrificed using the carbon dioxide asphyxiation method. Gross pathology examinations were performed on all euthanized animals, and the ventral lobe of prostate was collected for histopathological examination. All carcasses were disposed of through Medicare Environmental Management Pvt. Ltd., India.

Oral Administration

The required quantities of the test and reference materials were weighed and mixed with distilled water and 0.5% CMC. The prepared dose formulation was administered to the respective animal groups via oral gavage using a 3 mL disposable syringe attached to a 16/18 oral gavage. The dose volume administered to each animal was 10 mL/kg body weight, calculated based on the most recent body weight.

In-Life Observations

Animals were monitored weekly for body weight gain and feed consumption. Additionally, any clinical signs (changes in skin, fur, eyes, mucous membranes, the occurrence of secretions and excretions and autonomic activity [lacrimation, piloerection, pupil size, and unusual respiratory pattern], changes in gait, posture and response to handling as well as the presence of clonic or tonic movements, stereotypes [eg, excessive grooming, repetitive circling] or bizarre behavior [self-mutilation, walking backwards]) were recorded daily during the treatment period.

Biochemical Parameters

All blood samples were allowed to stand for complete clotting (30-40 minutes) and then centrifuged at 2500 rpm for 15 minutes at 4°C. The collected serum was separated to determine various markers including IL-1β, TNF-α, testosterone and DHT concentration in both serum and prostate, PSA in serum and mRNA expression of BAX and BCL-2. The ELISA method was used as per the Bioassay Technology Laboratory protocol.[34–36] For more details, please refer to Supplementary File.

Organ Weight

All the animals were sacrificed, and prostate organ weight and prostate index were calculated.

$$\text{Prostate Index} = (\text{Prostate Weight}/\text{Body weight}) \times 1000$$

Statistical Analysis

The data were analyzed using One-way and Two-way ANOVA followed by Dunnett's test and Sidak's multiple comparisons test, respectively, to compare all the groups with the BPH control group (G2). All analyses and comparisons were evaluated at the 5% ($p<0.05$) level.

Results

Clinical Signs, Body Weight and Feed Consumption

Throughout the treatment period, no mortality, morbidity, or clinical signs (changes in skin, fur, eyes, mucous membranes, the occurrence of secretions and excretions and autonomic activity like lacrimation, piloerection, pupil size, and unusual respiratory pattern), changes in gait, posture as well as the presence of clonic or tonic movements, stereotypes (eg, excessive grooming, repetitive circling) or bizarre behavior (self-mutilation, walking backwards) were observed in any of the animals across the six groups.

Individual animal body weight (g) was recorded on day 0 before test item administration and weekly thereafter on days 7, 14, 21, and 28 for all groups. Fasting body weight was also measured before the animals were sacrificed on day 29.

An increase in body weight in all the groups was observed; however, a significant increase was observed in groups G1, G3 and G6 at day 28 compared to G2 (Figure 1).

During the treatment period, feed consumption was measured weekly on days 0, 7, 14, 21 and 28. The average cage-wise feed intake (g/rat/day) was calculated, and any feed spillage was weighed and recorded during each feed leftover recording session/during cage change. No significant differences were observed across all groups on day 1-7 and day 8-14. However, during days 15-21, G1, G3 and G6 demonstrated a significant increase in feed consumption (385.31 ±21.43, $p < 0.0001$), (340.48±16.98, $p < 0.0028$), (330.15±44.35, $p < 0.0092$) respectively. Similarly, from days 22 to 28, there was an increase in feed consumption in groups G1 (373.99±39.65, $p < 0.0001$), G3 (319.27±36.08, $p < 0.0425$), and G6 (330.78±38.53, $p < 0.0131$) compared to the G2 group (Figure 2).

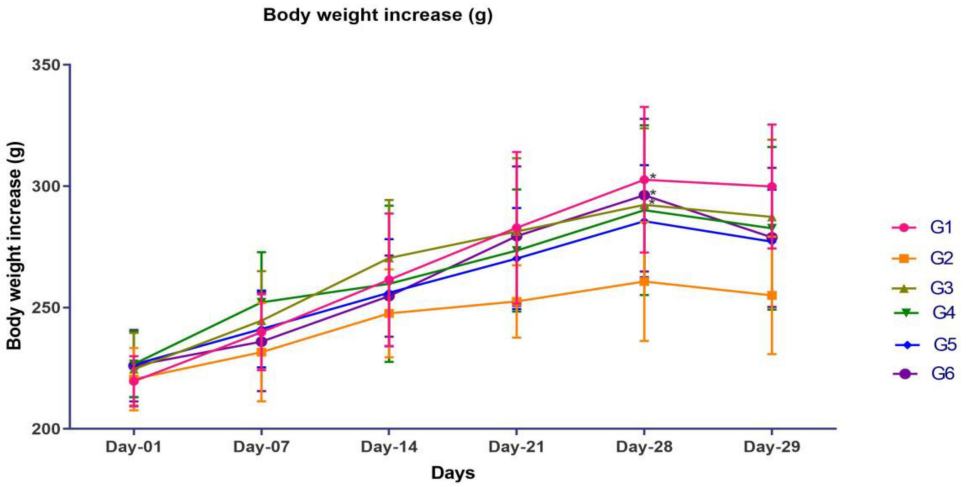


Figure 1 Effect of UPF on body weight. Error bars represent standard deviations.
Notes: Day 1, Day 7, Day 14, Day 21: There was no significant difference in body weight between all groups; Day 28: *G1, G3, and G6, had significantly higher body weights compared to G2 (P<0.0027, 0.0431, 0.0161 respectively).

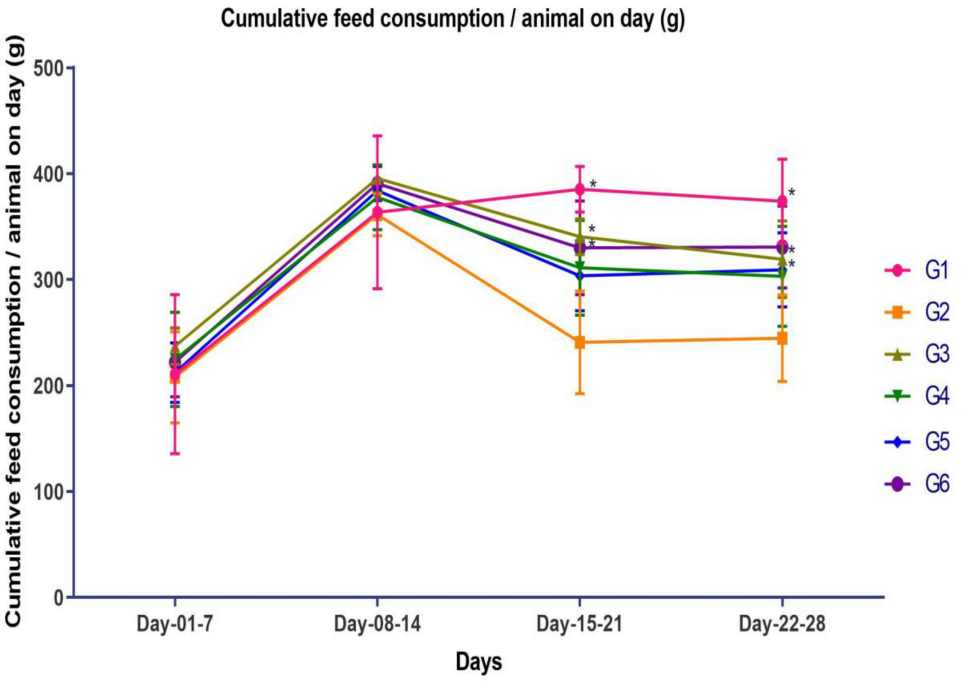


Figure 2 Effect of UPF on weekly feed consumption. Error bars represent standard deviations.
Notes: Days 15-21: *G1, G3, and G6 had significant increase in feed consumption compared to G2 (P <0.0001, 0.0028, 0.0092 respectively); Days 22-28: *G1, G3, and G6 had significant increase in feed consumption compared to G2 (P <0.0001, 0.0425, 0.0131 respectively).

IL-1β Levels

On terminal sacrifice, serum IL-1β (pg/mL) exhibited different trends among the groups. Specifically, an increase in IL-1β levels was observed in the BPH control group only (G2). Conversely, compared to G2 group, a major decrease in IL-1β level was observed in the intervention group G5 while the G3 and G4 groups demonstrated a slight reduction, however, these changes were not statistically significant with p-value > 0.05 (Table 3).

Table 3 Summary of Biochemical Parameters Collected Post Autopsy

Parameters	Group	G1	G2	G3	G4	G5	G6
	No. of Rats	**8**					
Testosterone Serum (pg/mL)	Mean	216.90	358.01	198.69	140.61	114.83	266.71
	±SD	81.48	95.63	66.91	68.43	89.12	139.80
	p-value	0.0184#	–	0.0065#	0.0002#	<0.0001#	0.2026
Testosterone Prostate (pg/mL)	Mean	253.28	411.91	249.73	245.12	292.07	226.28
	±SD	111.01	88.48	101.67	48.60	79.90	130.15
	p-value	0.0429#	–	0.0033#	0.0024#	0.0409#	0.0007#
DHT Serum (pg/mL)	Mean	7.25	7.46	6.70	7.06	4.97	6.27
	±SD	1.35	1.37	0.76	1.77	0.77	3.24
	p-value	0.9997	–	0.8606	0.9886	0.0321#	0.7656
DHT Prostate (pg/mL)	Mean	10.83	16.42	9.63	9.84	10.99	12.62
	±SD	2.62	5.04	3.42	2.68	4.02	2.96
	p-value	0.0133#	–	0.0020#	0.0029#	0.0171#	0.1423
PSA (pg/mL)	Mean	490.25	998.00	191.75	313.63	294.25	449.00
	±SD	214.42	748.34	142.82	125.36	123.74	223.97
	p-value	0.0268#	–	0.0002#	0.0017#	0.0004#	0.0147#
TNF-α (pg/mL)	Mean	23.60	74.94	17.41	14.79	10.17	12.66
	±SD	25.63	158.70	17.46	12.06	6.77	9.22
	p-value	0.4030	–	0.2994	0.2613	0.2032	0.2332
IL-1β (pg/mL)	Mean	90.00	131.85	115.36	118.81	108.51	99.53
	±SD	43.54	24.76	22.31	23.70	31.13	55.57
	p-value	0.0913	–	0.8211	0.9193	0.5598	0.2613
BAX	Mean	26.43	19.63	21.59	23.40	25.53	23.60
	±SD	7.08	2.85	6.47	7.83	12.35	6.90
	p-value	0.1994	–	0.9812	0.7907	0.4171	0.7576
BCL-2	Mean	19.41	30.26	19.15	21.88	20.00	24.10
	±SD	3.85	4.58	7.94	8.05	3.55	9.18
	p-value	0.0084#	–	0.0067#	0.0309#	0.0135#	0.2308

Note: #Statistically significant decrease as compared to G2.

TNF-α Levels

On autopsy, serum TNF-α (pg/mL) levels were higher in the BPH control group (G2). In contrast, the greatest reductions in TNF-α (pg/mL) levels were observed in the G5 and G6 groups, followed by the G4 and G3, when compared to the G2 group. The differences were not statistically significant with p-value > 0.05 (Table 3).

Serum Levels of Testosterone and DHT

The highest testosterone levels were observed in the BPH control group (G2) which were significantly higher when compared with G3 (p <0.0065), G4 (p <0.0002) and G5 (p <0.0001), as evident in Table 3. No significant difference in serum testosterone levels was observed between the groups G1 and G6.

DHT levels (pg/mL) were lower in G1, G3, G4, G5 and G6 groups compared to G2; however, only G5 results were statistically different to G2 (p < 0.0321) (Table 3).

Prostate Levels of Testosterone and DHT

There were significantly lower prostate testosterone levels in the intervention groups G3 (p <0.0033), G4 (p <0.0024), and G5 (p <0.0409), when compared to G2 (BPH control group). In the UPF alone (G6) group, a significantly lower prostate testosterone level was observed in comparison to the BPH control group (G2) (p-value <0.0007), but no significant difference was observed between G1 and G6 groups (Table 3).

The intervention groups, G3, G4 and G5 had significantly lower DHT levels (p-values <0.0020, <0.0029 and <0.0171, respectively) when compared to G2. There was no significant difference between the BPH control group (G2) and UPF alone group (G6) with p-value of 0.1423 (Table 3).

PSA Levels

There were significantly lower serum PSA levels in the intervention groups G3 (p <0.0002), G4 (p <0.0017), and G5 (p <0.0004), when compared to G2 (BPH control group). Additionally, the UPF alone (G6) group, also demonstrated significantly lower PSA levels in comparison to the BPH control group (G2) with a p-value <0.0147 (Table 3 and Figure 3).

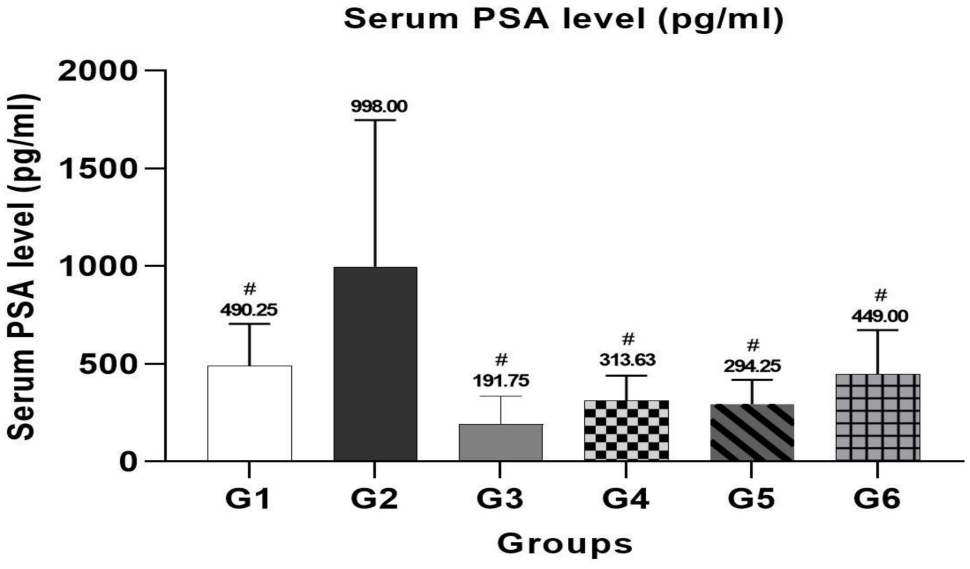


Figure 3 Serum PSA levels.
Note: #Significantly lower concentration compared to G2.

Prostate Weight, Prostate Index and Histopathology

In the BPH control group (G2), prostate weight and prostate index were significantly higher compared to the vehicle control (G1). The UPF alone (G6) group demonstrated significantly lower prostate weight and index followed by G4 and G5 group which were similar to the reference control (G3) group (Figures 4 and 5).

Upon microscopic examination of the prostate, the vehicle control group (G1) and UPF alone (G6) showed no lesions of pathological significance. BPH control group (G2) showed multifocal hyperplasia in the acinar epithelial lining of the prostate. This finding was characterized based on the irregular shapes of villous projection in the lumen of the gland, which is a possible trait of adenomatous hyperplasia in comparison with a control group (G1). These changes were also present in groups G3, G4 and G5 but reduced in severity and incidence (ie a reduction in hyperplasia of lining epithelium, alveoli folding, and height of the secretory epithelium) (Table 4 and Figure 6).

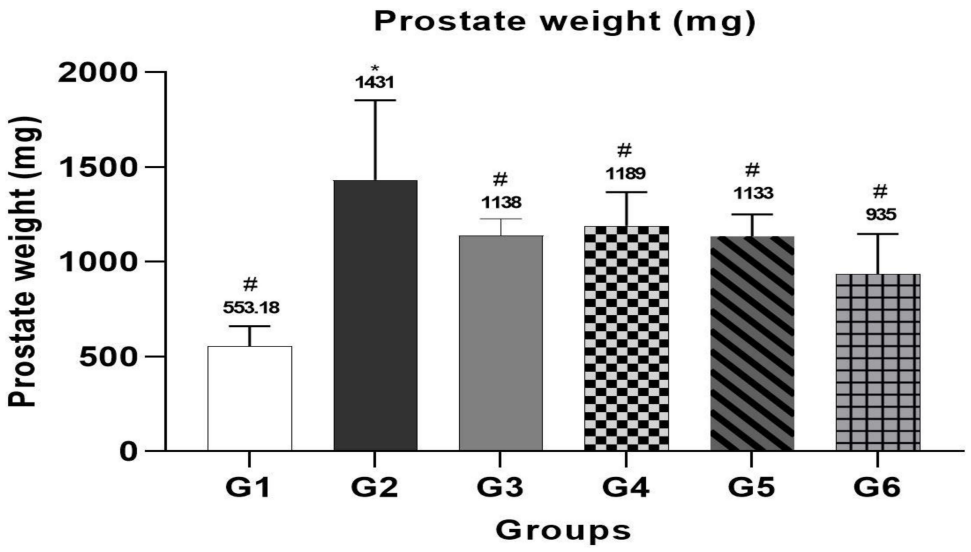


Figure 4 Effect of UPF on prostate weight.
Notes: *Significantly higher prostate weight as compared to G1. #Significantly lower prostate weight as compared to G2.

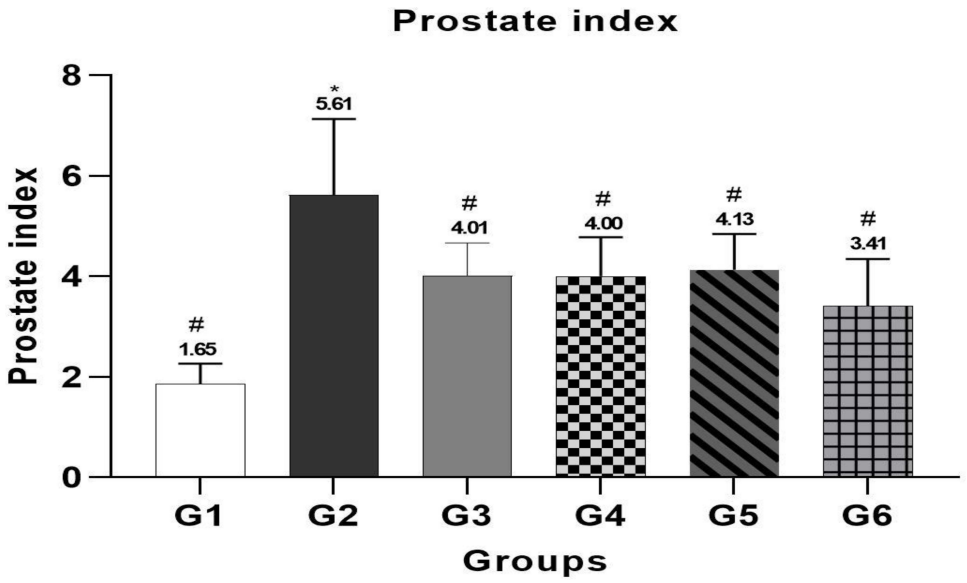


Figure 5 Effect of UPF on prostate index.
Notes: *Significantly higher prostate index as compared to G1. #Significantly lower prostate index as compared to G2.

Table 4 Summary of Histopathology Findings

Dose Group	G1	G2	G3	G4	G5	G6
Number of Animals per Group (n)	8					
Prostate						
No Abnormality detected (n)	8	1	0	0	2	8
Hyperplasia, lining epithelium, acinar, multifocal Minimal	0	3	5	5	5	0
Hyperplasia, lining epithelium, acinar, multifocal Mild	0	4	3	3	1	0

Notes: minimal: very small amount of change <10%; mild: lesion is easily identified with limited severity 11–25%; focal: lesion located at a single area of the tissue section; multifocal: lesion located at one or more foci of the tissue section.

Expression of BAX and BCL-2

Testosterone supplementation decreased the mRNA expression of the pro-apoptotic protein BAX in the BPH control group (G2). On the other hand, finasteride (G3) as well as UPF (G4, G5, and G6) groups increased BAX expression compared to G2. Moreover, G5 was the most efficient treatment in increasing BAX expression (Figure 7).

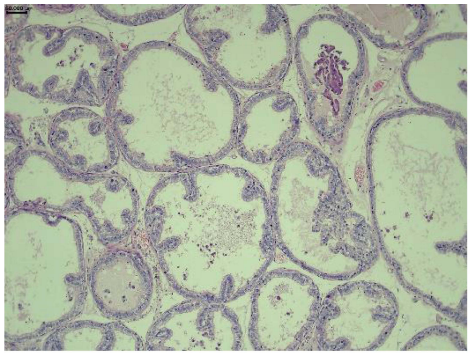
The G2 group enhanced BCL-2 mRNA expression compared to all other groups (G3, G4, G5 and G6). The G1 group showed a significant decrease in BCL-2 mRNA expression compared to G2 group with a p-value of <0.0084. Significant differences were also noted in the intervention groups G3 ($p<0.0067$), G4 ($p<0.0309$) and G5 ($p<0.0135$) when compared to G2 (Table 3 and Figure 8).

Discussion

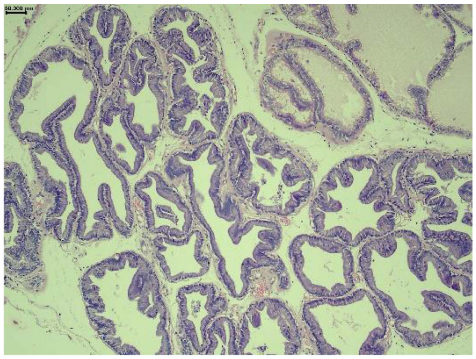
This is the first study to examine the activity of UPF on benign prostate hyperplasia in vivo. The most important finding was that UPF treatment significantly inhibited the progression of testosterone-induced BPH in rats. This was confirmed by the reduction in testosterone and DHT levels in both the prostate and serum, along with a decrease in PSA levels. Compared to the BPH control group, noticeable reductions in prostate weight, prostate index, and histopathological changes were observed following treatment with UPF.

In previous research conducted using rat models, change in prostate weight has been assessed as one of the primary objectives for evaluating the efficacy of a test item on the progression and management of BPH.[37] In BPH, the enlargement of the prostate results in the narrowing of the urethral canal, creating partial or complete blockages in urinary flow. This obstruction often contributes to an increase in prostate weight.[38] Due to these reasons, prostate weight was measured to assess the inhibitory effect of fucoidan on the development of BPH. In the present study, BPH control group rats showed an increase in prostate weight as well as prostate index. In contrast, finasteride and UPF groups (G3, G4, G5 and G6) elicited lower prostate weight and index. In addition, a high dose of UPF (G5) demonstrated lower prostate weight than the finasteride group (G3). In line with previous research, finasteride, a standard BPH treatment, showed substantially lower prostate volume and improvement in BPH symptoms.[35]

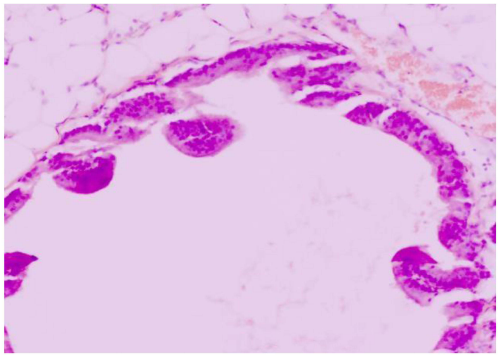
The condition of prostate gland enlargement is primarily identified through histological diagnosis, marked by the proliferation of prostate cellular elements, encompassing both stromal and epithelial components.[39] Histopathology of the BPH control group (G2) revealed mild and multifocal hyperplasia in the acinar lining epithelium characterized by increased alveoli folding and height of the secretory epithelium; while control group (G1) showed no lesions of pathological significance. Comparatively, finasteride (G3) as well as UPF along with testosterone treated groups recorded reduced or minimal hyperplasia in the lining epithelium of the acinar marked by decreased alveoli folding and height of the secretory epithelium in comparison to the BPH control group (G2). Additionally, the high dose fucoidan alone group (G6) showed normal lining of epithelial cells and did not show any lesions compared to the BPH control group (G2). In this study, the BPH control group (G2) was treated with 3 mg/kg of testosterone for 28 days. Other studies investigating the effects of nutraceuticals on testosterone-induced BPH in rats used different concentrations of testosterone such as 10 mg/kg and therefore severity of histopathological results may vary.[40]



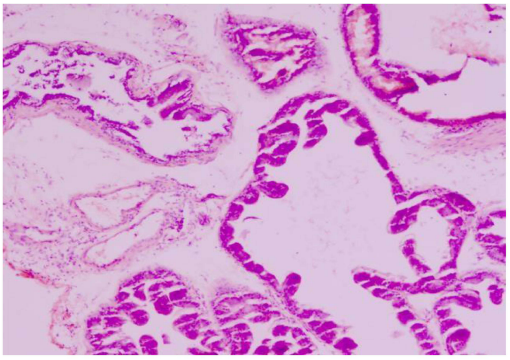
G1-Normal acini of prostate glands



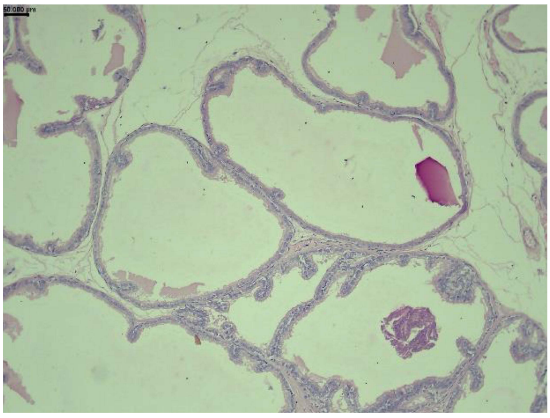
G1-Normal acini of prostate glands



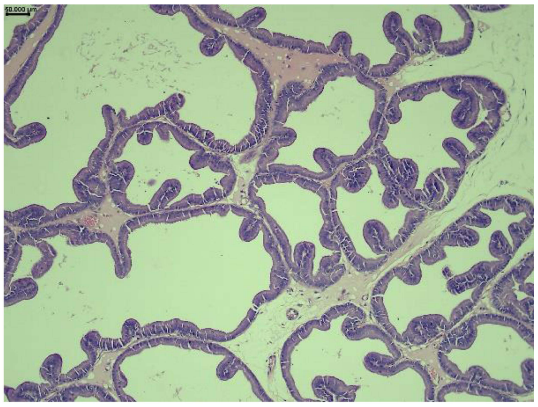
G2- Prostate; Hyperplasia, lining epithelium, acinar, multifocal, Mild.



G2- Prostate; Hyperplasia, lining epithelium, acinar, multifocal, Mild.

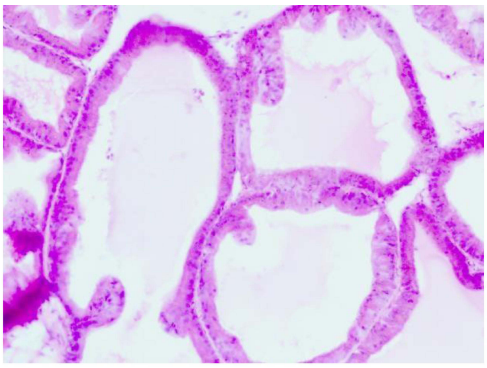


G3-Prostate; Reduced Hyperplasia, lining epithelium, acinar, multifocal, Minimal.

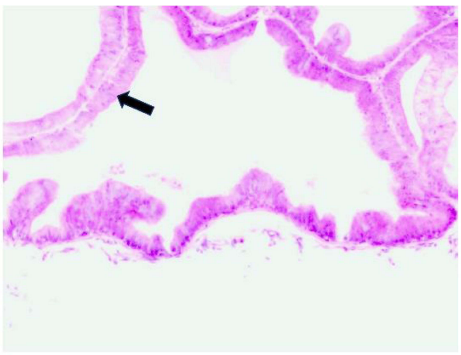


G3- Prostate; Reduced Hyperplasia, lining epithelium, acinar, multifocal, Minimal.

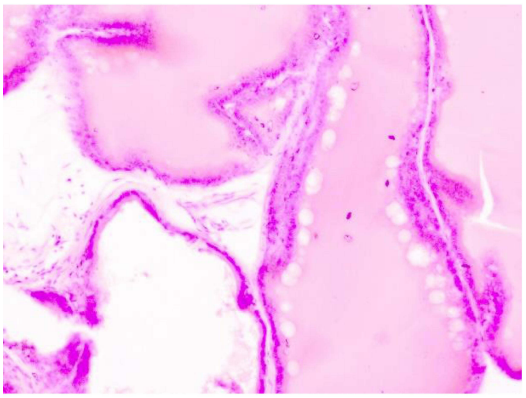
Figure 6 Continued.



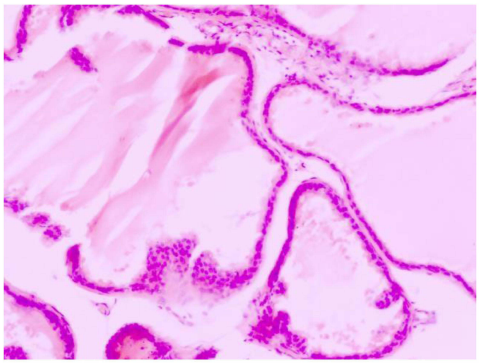
G4-Prostate; Reduced Hyperplasia, lining epithelium, acinar, multifocal, Minimal.



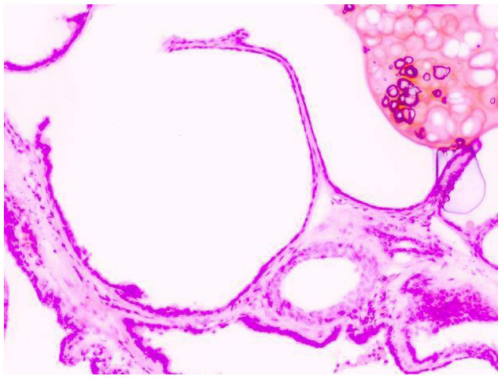
G4-Prostate; Reduced Hyperplasia, lining epithelium, acinar, multifocal, Minimal.



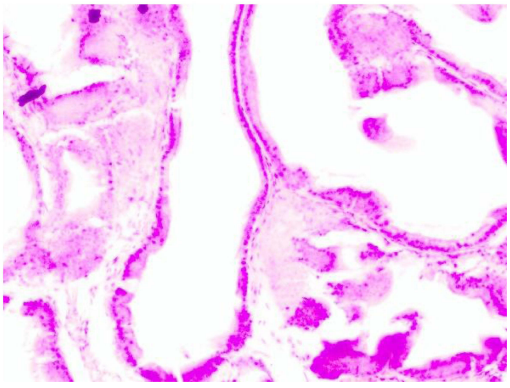
G5- Prostate; Reduced Hyperplasia, lining epithelium, acinar, multifocal, Minimal.



G5- Prostate; Reduced Hyperplasia, lining epithelium, acinar, multifocal, Minimal.



G6-normal acini of prostate glands.



G6- normal acini of prostate glands.

Figure 6 Histopathological images.

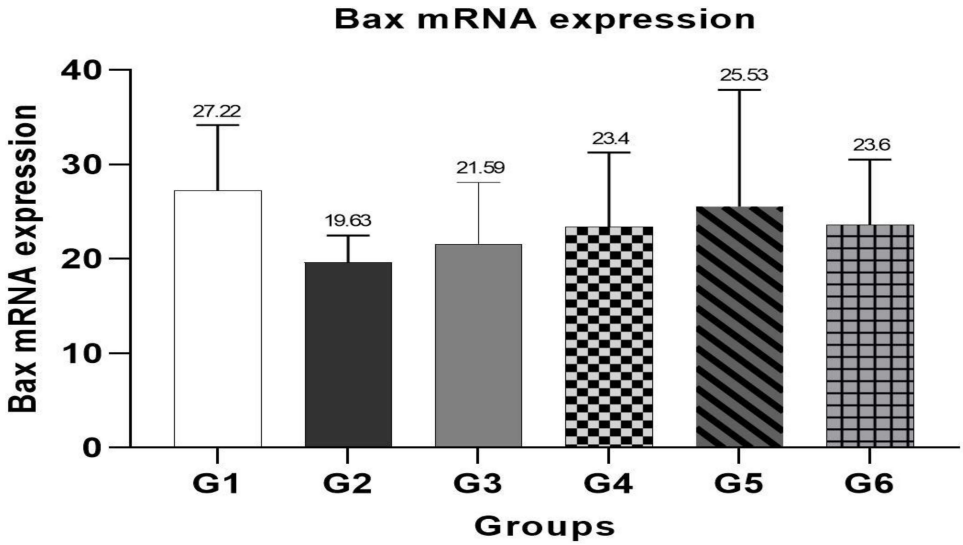


Figure 7 BAX mRNA expression.
Note: No significant increase in BAX mRNA expression when compared to G2.

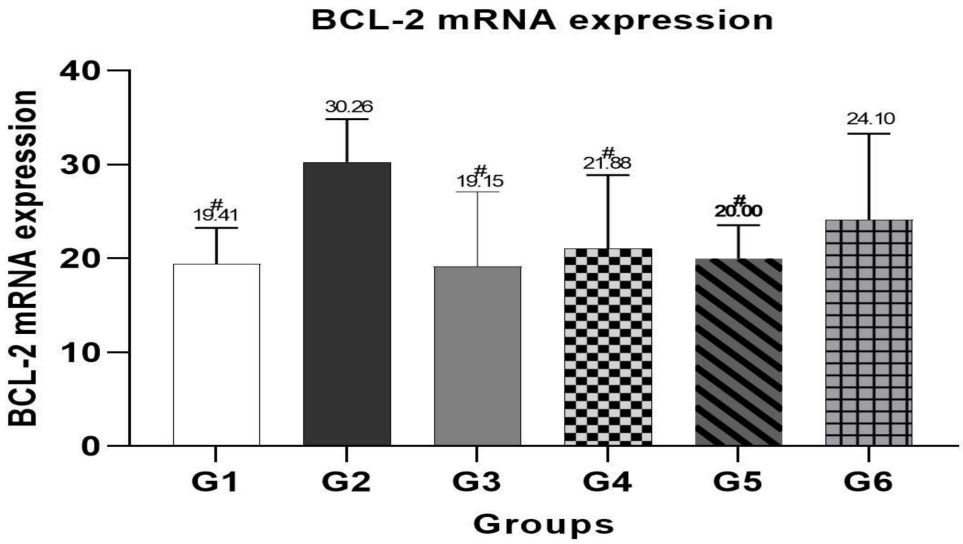


Figure 8 BCL-2 mRNA expression.
Note: #Significantly lower BCL-2 mRNA expression when compared with G2.

Dihydrotestosterone (DHT) is an active metabolite of testosterone produced by the enzyme 5α-reductase enzyme which plays a crucial role in the development of BPH.[41] Therefore, a reduction in DHT levels can improve BPH-related symptoms. This is supportive of the findings in the current study, where some significant reductions in both testosterone and DHT levels in the serum and prostate were found. For future studies, it may be interesting to observe the effect of UPF on androgen receptor antagonism. Testosterone levels in both serum and prostate were decreased in the finasteride and UPF groups (G3, G4, G5) when compared to the BPH control group (G2). Prostate DHT levels were significantly lower in the intervention groups (G3, G4 and G5) compared to G2. Additionally, the high dose UPF group (G5) not only showed a marked reduction in serum DHT levels compared to G2, but also had significantly lower serum levels of both testosterone and DHT compared to the low dose UPF group (G4).

Prostate-specific antigen (PSA), a glycoprotein produced by the prostate glandular tissue, is usually elevated in BPH. Epithelial cell damage or disruption of the blood-epithelial barrier can lead to a significant increase in serum PSA levels

owing to its entry into the bloodstream.[42] In the present study, we observed decreased PSA levels in finasteride as well as UPF groups (G3, G4, G5 and G6) compared to G2. This could be a consequence of decreased hyperplasia, possibly due to inhibition of 5α-reductase, which could lead to improved urinary flow and a reduction in urinary obstruction. This finding was consistent with a previous study using a *Sargassum horneri* extract in which PSA levels were found to be significantly decreased in testosterone-induced BPH in vitro and in vivo.[20]

Inflammation is frequently observed in BPH, potentially leading to tissue damage. As a response to this damage, the inflammatory cells produce cytokines and elicit a self-protective response.[43] Increased expressions of proinflammatory cytokines, like IL-1β and TNF-α, are reported as potent growth factors for prostatic epithelial and stromal cells, as indicated in previous studies of BPH models.[44,45] In addition to that, IL-1β being a member of IL-1 family, has a wide range of target cells and acts to promote antigen specific immune responses, inflammation and tissue repair.[46] In the present study, in the intervention groups G3, G4, and G5, lower serum levels of TNF-α and IL-1β were observed when compared to the BPH control group (G2). The differences were not statistically significant but indicate that finasteride and UPF may possess anti-inflammatory activities which potentially aid in BPH management.

A growing body of evidence suggests that inhibition of cell apoptosis is linked to the development of BPH.[47,48] When apoptosis is inhibited, it leads to an increase in the total number of stromal and epithelial cells in the prostate, eventually resulting in a larger prostate size. Numerous proteins are known to have significant roles in the regulation of apoptosis, with the BCL-2 family proteins being the most extensively studied. BCL-2, a member of the anti-apoptosis group within the BCL-2 family, promotes apoptosis inhibiting effect, while BAX, a pro-apoptosis member, promotes cell death.[49] In the present study, we observed that testosterone administration alone increased anti-apoptotic protein BCL-2, leading to a decrease in cell death and a subsequent increase in prostate size. However, comparatively, in the intervention groups, the expression of this BCL-2 was significantly decreased in the rat prostates compared to the BPH control group ($p < 0.05$). Furthermore, the expression of pro-apoptotic protein BAX increased in the intervention groups, although the changes were not statistically significant. The findings suggest pro-apoptotic potential of fucoidan in the hypertrophic prostate of rats, and this mechanism may be one explanation on how fucoidan may counteract the effects of BPH.

The study also reported that there was no major impact of UPF on body weight and feed consumption during the experimental trial compared to the BPH control group. No clinical signs, mortality and morbidity were observed during the trial.

Conclusion

This study demonstrated that fucoidan extracted from *Undaria pinnatifida* effectively inhibits the progression of testosterone-induced BPH in rats, as evidenced by reductions in testosterone, DHT, PSA levels, IL-1β, TNF-α, and prostate weight, and preserved the morphology of prostate tissue. The findings suggest that UPF may exert its effects through mechanisms such as reducing DHT levels, decreasing hyperplasia, and enhancing pro-apoptotic activity. Overall, these results indicate that fucoidan extracted from *Undaria pinnatifida* may provide benefit in supporting healthy prostate function.

Abbreviations

BAX, BCL-2-associated X protein; BCL-2, B-cell lymphoma-2; DHT, dihydrotestosterone; IL-1β, Interleukin-1β; PSA, prostate-specific antigen; TNF-α, tumor necrosis factor-alpha; G1, Vehicle control; G2, BPH control; G3, Reference control; G4, Fucoidan UPF (40 mg/kg) + testosterone (3 mg/kg); G5, Fucoidan UPF (400 mg/kg) + testosterone (3 mg/kg); G6, Fucoidan UPF (400 mg/kg).

Data Sharing Statement

The data presented in this study are available on request from the corresponding author with due permission from the sponsor.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

Dr Corinna Dwan and Dr Barbara Wimmer are employees of Marinova Pty Ltd. The authors declare no other conflict of interest.

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