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RESEARCH

Ameliorative effect of *Hibiscus sabdariffa* leaf flavonoid extract on the PSA/CEA/AKT/MMP-9 signaling pathway in benign prostatic hyperplasia-induced rats

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Abstract
Benign prostatic hypertrophy (BPH) is a prevalent condition affecting the prostate gland of elderly men. This study investigated the effects of *H. sabdariffa* leaf flavonoid extracts on BPH induction in rats. Thirty-six male Wistar rats were split into six groups: normal controls, BPH-induced rats (3 mg/kg testosterone), and rats treated with BPH-induced with flavonoid extract (300 mg/kg and 150 mg/kg) or a standard drug (1 mg/kg finasteride). After 28 days, biochemical, histological, and molecular parameters were assessed. The extract rich in flavonoids from *H. sabdariffa* markedly ($p < 0.05$) decreased the amount of MDA and enhanced the activities of antioxidant enzymes in rats administered with BPH. The extracts substantially attenuated inflammatory markers such as IL-6, IL-1β, and TNFα levels. Histological examination revealed improved prostate architecture in the treated groups in contrast to those in BPH-induced rats. Additionally, substantial ($p < 0.05$) downregulation of CEA, AKT, MMP9, and PSA in the extract-treated groups was observed. HPLC characterization identified prominent flavonoids, with quercetin being the most abundant bioactive compound. The outcome of this study proffers the therapeutic activities of *H. sabdariffa* flavonoid extract on BPH rat model.

Keywords Benign · Flavonoid · Gene expression · Hyperplasia · Testosterone

Introduction

An enlarged prostate, also known as benign prostatic hyperplasia (BPH), is not malignant, which may induce recurrent urination, a weak urine stream, and difficulty urinating. In severe situations, it might result in full blockage, infections, bladder stones, and even kidney damage (Sershon, et al., 2012). The causes of BPH are unclear. However, certain factors increase the risk, such as family history, obesity, diabetes, lack of exercise, and erectile dysfunction. Some medications, such as anticholinergics, calcium channel blockers, and pseudoephedrine, can also worsen BPH symptoms. This happens because the enlarged prostate squeezes the urinary canal, and the urinary tract, making it harder to urinate (Kim et al. 2016a). After ruling out other potential reasons, the diagnosis is typically based on indications and investigation. This condition is very common, affecting an estimated 105 million men worldwide (Vos et al. 2015).

BPH is one of the most common illnesses affecting elderly men. By their 40 s, approximately 30–40% of men have some signs of BPH, and this frequency rises steadily to 70–80% in older adults. However, it is crucial to differentiate lower urinary tract symptoms (LUTS), which typically necessitate medical consideration, and benign prostatic enlargement (BPE), defined as an inflamed prostate (Guilbert 2003). In addition to BPH, other conditions that worsen with age include BPE, LUTS, and aberrant urodynamic patterns. Moderate to severe LUTS affects approximately 20% of men at the attainment of age 50, at age 60, 30% and 40% at the age 80. Out of the total populace of 8.7 million, an estimated 350,000 Austrian men over 40 years are either having acute or chronic LUTS, according to a prevalence study based on 2096 men living in the country. This number will significantly increase to almost 500,000 in the next 20 years due to changes in the population, underscoring its

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socioeconomic significance (Madersbacher et al. 2016). Although BPH is quite common and has a significant economic impact, the disease mechanism is still not really unknown. A series of traditional medications are currently used for BPH treatment; nevertheless, these medications have other complications aside been expensive and inaccessible in rural areas. *Hibiscus sabdariffa* is among the plants that are commonly used locally with no or minimal side effects. *Hibiscus sabdariffa* is a flowering plant of the *Hibiscus* species, native to Africa, mostly West Africa. It is an annual or perennial woody-based subshrub plant, growing to a height 2–2.5-m (7–8 ft) tall.

This plant has different names depending on the Nigeria region. The Yoruba people call it Ìsápá, while the Hausa people in the north know it Yakuwa. Interestingly, the Hausa have specific names for different Roselle parts: Gurguzu for the seeds and Zoborodo or Zobo for the flower calyx. This versatile plant is well known for its health benefits. Roselle leaf and its derivatives are recognized as a good source of flavonoid components, kaempferol, and quercetin. The leaves are endowed with antioxidants constituents such as-neochlorogenic, chlorogenic, and crytochlorogenic acids, while the florae contain protocatechuic and anthocyanins acid. Flavonoids, including gossypetin, hibiscetine, and sabdaretine, are present in dried calyces (Odigie et al. 2003; Olatunji et al. 2005). Little is known on the flavonoid extracts of *Hibiscus sabdariffa* in the treatment of this disease. Hence, this study aimed to investigate the activities of flavonoid extract of *Hibiscus sabdariffa* leaf on PSA, CEA, AKT, and MMP-9 in benign prostatic hyperplasia rats.

Materials and methods

Collection and identification of plant

The purchase of *H. sabdariffa* leaves was in Oja-Oba market in Ado-Ekiti, Ekiti State, Nigeria. Leaf identification and authentication were performed at Forestry Research Institute of Nigeria (FRIN) Ibadan with voucher number *FHI 113742*. The drying of the *H. sabdariffa* leaves took 2 weeks at room temperature. The dried leave was grinded into powder with blender.

Extraction of flavonoid-rich extract

A known gram of the powdered sample was defatted by soaking in 80% methanol for 72 h. Rotary evaporator was utilized to concentrate the extract. A known gram of the filtrate was then dissolved in 200 mL of 10% H_2SO_4 and heated in a water bath to 100 °C for 30 min to initiate hydrolysis. To precipitate flavonoid aglycones, the mixture was placed on ice. The flavonoid aglycone was dissolved in 50 mL of warm 95% ethanol. This was followed by filtering the mixture into a 100-mL volumetric flask and adjusting the volume to the top with 95% ethanol. The solution was concentrated using a rotary evaporator and concentrated ammonium hydroxide was employed to precipitate the filtrate. The whole mixture was left to settle for the extraction of the flavonoid extract. The sediment was collected and washed with diluted ammonium hydroxide.

Experimental animals

Thirty-six male albino rats (Wistar strain) weighing 150 to 250 g were obtained from the Animal House, Show Gold at Idofin, Oye; Ekiti, Ekiti State, Nigeria. The animals were acclimatized for 2 weeks. The rats were given water and rat chow ad libitum and were kept under standard temperature conditions with a 12-h dark/light cycle. All animal experiments were conducted according to the guidelines of the National Institute of Health (NIH publication 85–23, 2010) for laboratory animal care and use. All procedures were performed in line with the Federal University Oye-Ekiti, Faculty of Science Ethical Research Committee.

Induction of BPH and animal grouping

The animals in groups B to E were treated with BPH according to Shabani et al. (Shabani et al. 2021) through subcutaneous (SQ) inoculation of 3 mg/kg body weight testosterone dissolved in 1 mL/kg of olive oil for 28 days. This was followed by treatment in groups C to E and the grouping of the animals into six groups ($n = 6$):

Group A: Olive oil for 28 days (NC)
Group B: Testosterone (3 mg/kg SQ) for 28 days (BPH)
Group C: 3 mg/kg SQ testosterone + 1 mg/kg finasteride, standard drug for 28 days (BPH + STD drug)
Group D: Testosterone (3 mg/kg SQ) + 150 mg/kg flavonoid-rich extract of *H. Sabdariffa* (BPH + 150 HS)
Group E: Testosterone (3 mg/kg SQ) + 300 mg/kg flavonoid-rich extract of *H. Sabdariffa* (BPH + 300 HS)
Group F: Uninduced rats + 300 mg/kg flavonoid-rich extract of *H. sabdariffa* only (300 HS)

Sample collection and preparation

The experimental rats were fasted overnight at the end of the experiment, weighed, and sacrificed under sodium pentobarbital euthanasia. Blood from each rat was obtained via cardiac puncturing, which was allowed to clot and then centrifugation at 1500 × g for 5 min. The prostrate sample was collected from each rat testis. The obtained prostrate was homogenized using ice-cold phosphate buffer solution

at pH 6.2 and the supernatant was centrifuged at 5000 × g for 10 min.

Biochemical parameters studied

The protocol described by Misra and Fridovich (1972) was employed to determine the superoxide dismutase activity. The activity of catalase (CAT) was assessed through the previously defined procedure (Beers and Sizer 1952). The GPx activity was evaluated based on the described procedure by Paglia and Valentine (1967). The Ellman (Ellman 1959) procedure was employed in the assessment of the GSH concentration. The concentration of malondialdehyde (MDA) was determined by Valenzuela (1991) method. Miranda et al. (Miranda et al. 2001) reported procedure was employed to assess nitric oxide (NO) levels. ELISA kits were used to determine cyclooxygenase-2, interleukins (1β, IL-6, IL-10), together with TNFα, testosterone, and dihydrotestosterone.

Histopathological studies

Histology was performed using the method given by Blume et al. 1997 with hematoxylin and eosin (H&E) staining.

Absolute gene expression of AKT, CEA, MMP-9, and PSA

Quick-RNA MiniPrep™ was used to isolate total RNA from the prostate. This was RNA was converted to cDNA. Finally, the method of Elekofehinti et al. (2020) was used for agarose gel electrophoresis and PCR amplification. The primers used are shown in Table 1.

HPLC characterization of Hibiscus sabdariffa flavonoid extract

An Agilent 1100 HPLC (serial #: DE03011634), as described previously (Banwart et al. 1985) with some modifications.

Table 1 Primer sequences

Gene	Sequence
PSA	Forward: CAGCACCAGGCCAGATAAG Reversed: CACAGGTTGAGAAGCAGACA
AKT	Forward: TCACCTCTGAGACCGACACC Reversed: ACTGGCTGAGTAGGAGAACTGG
MMP 9	Forward: CCCAACCTTTACCAGCTACTC Reversed: GTCAGAACCGACCCTACAAAG
CEA	Forward: CTGGGTTCTACACCCTACGC Reversed: TGAGGAAAGGGAGGTGTCCA

Statistical examination

All the data were represented as the means ± standard deviations of six separate treatments. Significant differences were assessed between the control and treated groups using ANOVA. The obtained result was subjected to GraphPad statistical software version 6.0; $p < 0.05$ to designate statistical significance.

Results

Prostate redox indicators of flavonoid-rich H. sabdariffa extract in rats induced with benign prostatic hyperplasia

Malondialdehyde (MDA) levels were substantially lower ($p < 0.05$) in the BPH group compared to the control. However, all the treated groups exhibited no significant ($p > 0.05$) differences when compared with the normal control group. Additionally, reduced GSH, GST, catalase, and SOD, activities were substantially ($p < 0.05$) higher in the BPH-treated rat's prostate tissues compared to that of BPH group (Fig. 1).

Prostate inflammatory biomarkers of Hibiscus sabdariffa flavonoid-rich extracts in induced prostatic hyperplasia benign rats

Induction with BHP increased IL-β, IL-6, IL-10, and TNFα levels (Fig. 2) in the rat's prostate ($p < 0.05$) when compared with both the treated and control groups. However, the administration of flavonoid-rich extracts from *Hibiscus sabdariffa* substantially at ($p < 0.05$) decreased IL-1β and IL-6 levels in comparison with those in BPH group. However, rats administered *Hibiscus sabdariffa* flavonoid-rich extracts at 300 mg/kg body weight showed noticeable substantial ($p < 0.05$) differences against those in the normal, BPH, and treated groups.

Prostate histological examination of Hibiscus sabdariffa flavonoid-rich extracts in benign prostatic hyperplasia-induced rats

The normal control (NC) rats showed usual intraglandular epithelial convolution (V) into the lumen, while the untreated BPH rats demonstrated severe disruption of the epithelial lining (EP), clear lumen, and vascularized stroma. Additionally, treatment with BPH + STD indicated mild disruption of the epithelial lining (EP), clear lumen, and vascularized stroma; however, treatment with BPH + 150 HS resulted in a normal epithelial lining (EP), stroma (S), and blood vessels. As shown in Fig. 3, rats treated with BPH + 300 HS exhibited a normal epithelial

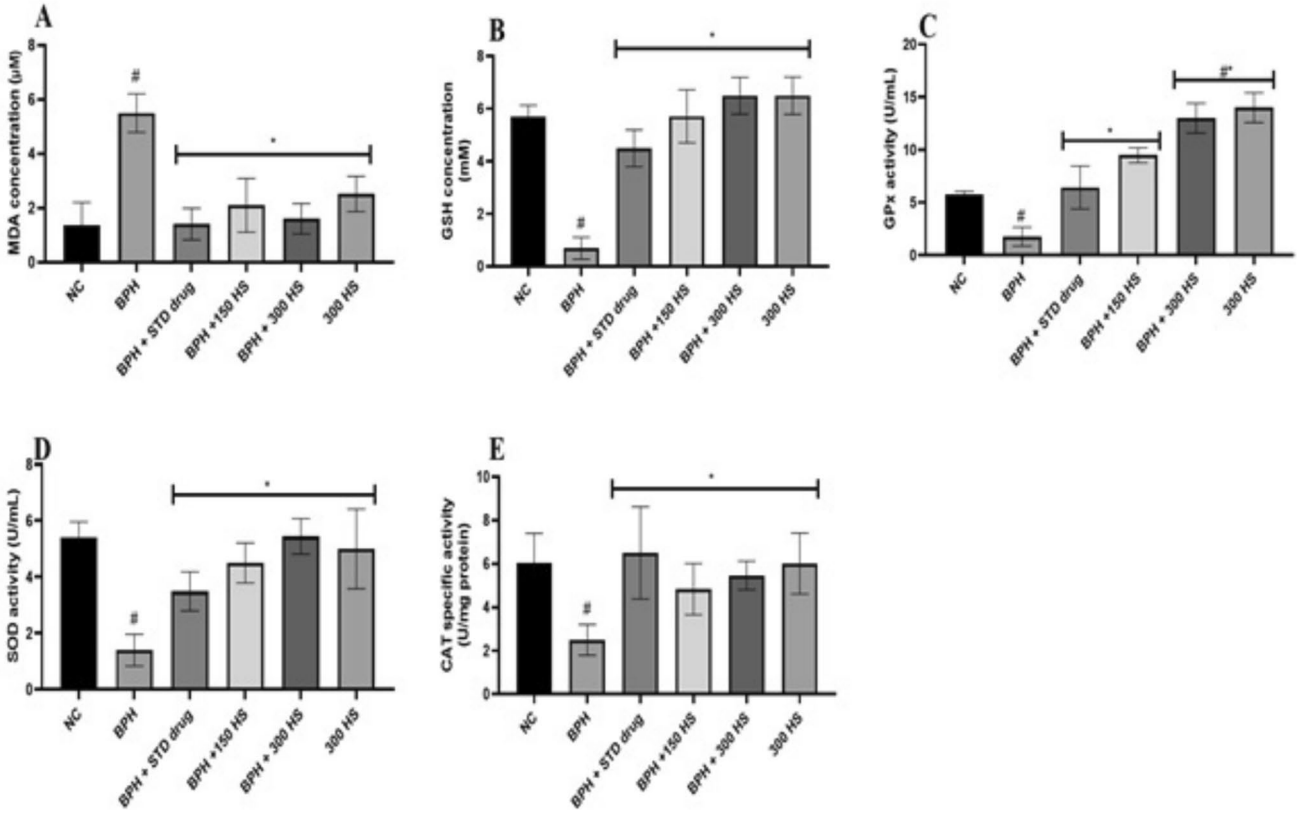

Fig. 1 Prostate redox biomarkers of flavonoid-rich extracts from *Hibiscus sabdariffa* leaf in benign prostatic hyperplasia-induced rats. Each value is the mean of eight determinations ± SD. #$p < 0.05$ vs. NC, *$p < 0.05$ vs. DC. Legend: NC, normal control; BPH, benign prostatic hyperplasia-induced rats; BPH + STD drug, benign prostatic hyperplasia-induced rats administered 1 mg/kg finasteride; BPH + 150 HS, benign prostatic hyperplasia-induced rats administered a low dose (150 mg/kg body weight) of a flavonoid-rich extract of *Hibiscus sabdariffa*; BPH + 300 HS, benign prostatic hyperplasia-induced rats administered a high dose (300 mg/kg body weight) of a flavonoid-rich extract of *Hibiscus sabdariffa*; 300 HS, rats administered a high dose (300 mg/kg body weight) of a flavonoid-rich extract of *Hibiscus sabdariffa*

lining (EP), normal prostatic concretions at the lumen, and stroma (S). Finally, rats administered only 300 HSs exhibited a normal epithelial lining (EP), with prostatic concretions at the lumen and stroma (S).

Dihydrotestosterone and testosterone levels of Hibiscus sabdariffa flavonoid-rich extracts in hyperplasia-induced benign prostatic animal

The testosterone and dihydrotestosterone levels in the prostate were substantially ($p < 0.05$) higher in the BPH group than in the control rats. BPH rats administered either 150 or 300 mg/kg body weight *Hibiscus sabdariffa* showed a significant ($p < 0.05$) reduction compared with the BPH group, whereas no significant difference was observed in the normal control group (Fig. 4).

Comparative expression of genes AKT, MMP9, CEA, and PSA in Hibiscus sabdariffa flavonoid-rich extracts in induced benign prostatic hyperplasia animals

A substantial ($p < 0.05$) rise in the prostate expression of AKT in the BPH group was observed when compared to the other groups. Moreover, BPHs induced with 150 mg/kg *Hibiscus sabdariffa* flavonoid-rich extracts exhibited noticeable differences between normal and BPH groups. Nevertheless, no difference was seen in BPH treated with 300 mg/kg *Hibiscus sabdariffa* flavonoid-rich extract, BPH treated with drugs, and normal rats (Fig. 5). The MMP-9 comparative gene expression in the BPH group is lower than that of the control group. However, BPH rats treated with 150 or 300 mg/kg *Hibiscus sabdariffa*

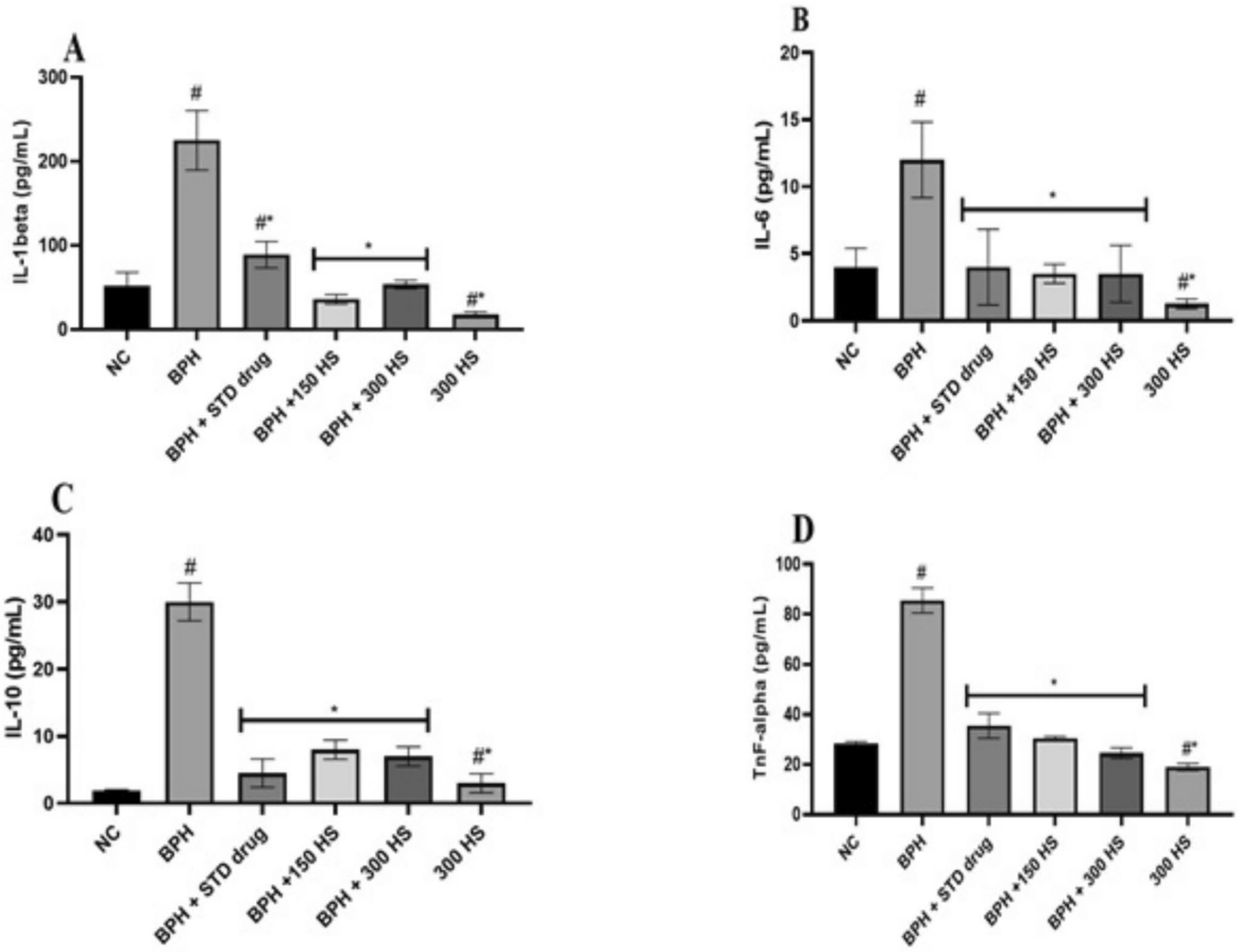

Fig. 2 Prostate inflammatory biomarkers of flavonoid-rich extracts from *Hibiscus sabdariffa* leaf in benign prostatic hyperplasia-induced rats. Each value is the mean of eight determinations ± SD. #$p < 0.05$ vs. NC, *$p < 0.05$ vs. DC. Legends: NC, normal control; BPH, benign prostatic hyperplasia-induced rats; BPH + STD drug, benign prostatic hyperplasia-induced rats administered 1 mg/kg finasteride; BPH + 150 HS, benign prostatic hyperplasia-induced rats administered a low dose (150 mg/kg body weight) of the flavonoid-rich extract of *Hibiscus sabdariffa*; BPH + 300 HS, benign prostatic hyperplasia-induced rats administered a high dose (300 mg/kg body weight) of the flavonoid-rich extract of *Hibiscus sabdariffa*; 300 HS, rats administered a high dose (300 mg/kg body weight) of the flavonoid-rich extract of *Hibiscus sabdariffa*

flavonoid-rich extract exhibited noticeable differences compared with both the normal and BPH groups.

As indicated in Fig. 5, CEA and PSA relative gene expression levels were substantially ($p < 0.05$) lower in BPH-treated rats with 150 and 300 mg/kg *Hibiscus sabdariffa* flavonoid-rich extracts than in the BPH group, but the differences were not significant compared to those in normal rats. However, if compared with both normal and treated groups, only 300 mg/kg *Hibiscus sabdariffa* flavonoid-rich extract markedly decreased the comparative gene expression of AKT, CEA, MMP9, and PSA.

HPLC characterization of Hibiscus sabdariffa's flavonoid-rich extract

Based on the HPLC result, the flavonoid-rich extract of *Hibiscus sabdariffa* revealed 14 peaks, as shown in Fig. 6. However, the identities of six out of 14 patients were unknown because we do not have a standard for them. As indicated in Table 2, quercetin was the most abundant phytochemical present in the extract. This was followed by ellagic acid, while the compound was isoquercitrin.

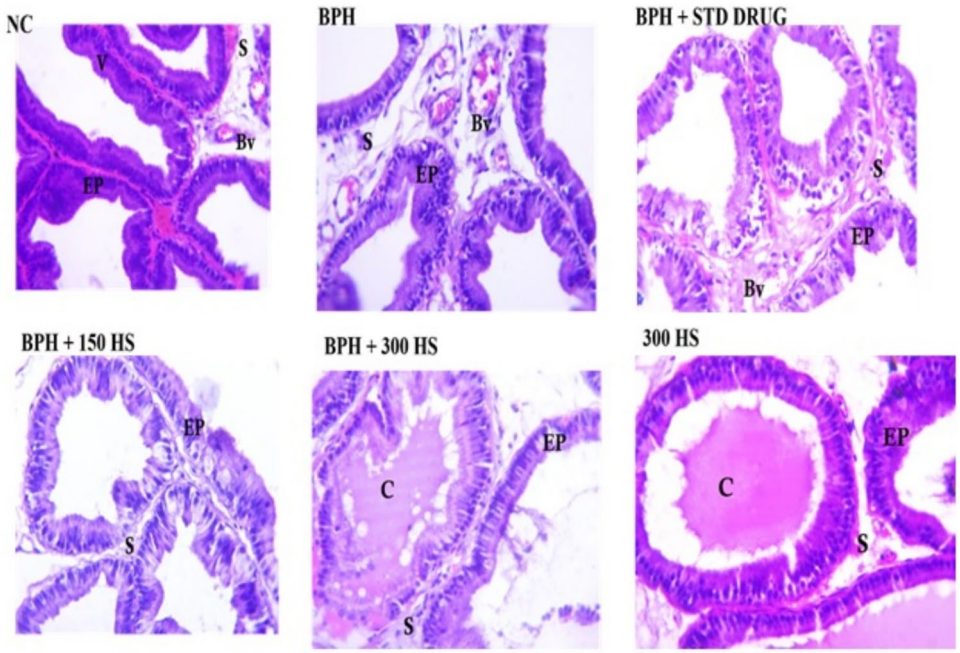

Fig. 3 Prostate histological examination of flavonoid-rich extracts from *Hibiscus sabdariffa* leaf in benign prostatic hyperplasia-induced rats. H&E staining scale bar, 50 µm; magnification, 800×. Legend: NC, normal control; BPH, benign prostatic hyperplasia-induced rats; BPH + STD drug, benign prostatic hyperplasia-induced rats administered 1 mg/kg finasteride; BPH + 150 HS, benign prostatic hyperplasia-induced rats administered a low dose (150 mg/kg body weight) of a flavonoid-rich extract of *Hibiscus sabdariffa*; BPH + 300 HS, benign prostatic hyperplasia-induced rats administered a high dose (300 mg/kg body weight) of a flavonoid-rich extract of *Hibiscus sabdariffa*; 300 HS, rats administered a high dose (300 mg/kg body weight) of a flavonoid-rich extract of *Hibiscus sabdariffa*; EP, epithelial lining; Bv, blood vessels; S, stroma

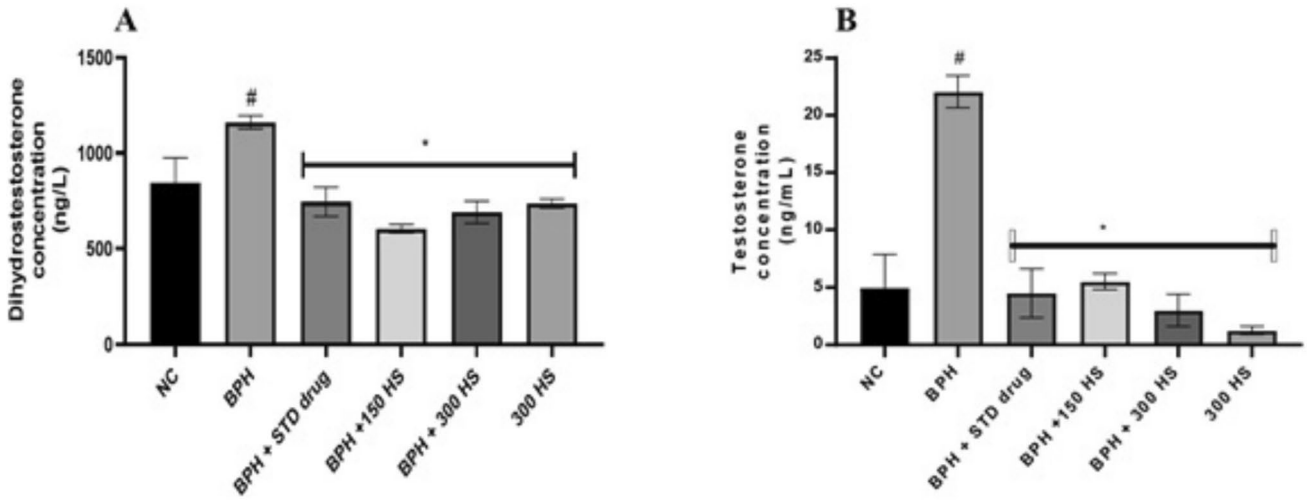

Fig. 4 Dihydrotestosterone and testosterone levels of flavonoid-rich extracts from *Hibiscus sabdariffa* leaf in benign prostatic hyperplasia-induced rats. Each value is the mean of eight determinations ± SD. #$p < 0.05$ vs. NC, *$p < 0.05$ vs. DC. Legend: NC, normal control; BPH, benign prostatic hyperplasia-induced rats; BPH + STD drug, benign prostatic hyperplasia-induced rats administered 1 mg/kg finasteride; BPH + 150 HS, benign prostatic hyperplasia-induced rats administered a low dose (150 mg/kg body weight) of a flavonoid-rich extract of *Hibiscus sabdariffa*; BPH + 300 HS, benign prostatic hyperplasia-induced rats administered a high dose (300 mg/kg body weight) of a flavonoid-rich extract of *Hibiscus sabdariffa*; 300 HS, rats administered a high dose (300 mg/kg body weight) of a flavonoid-rich extract of *Hibiscus sabdariffa*

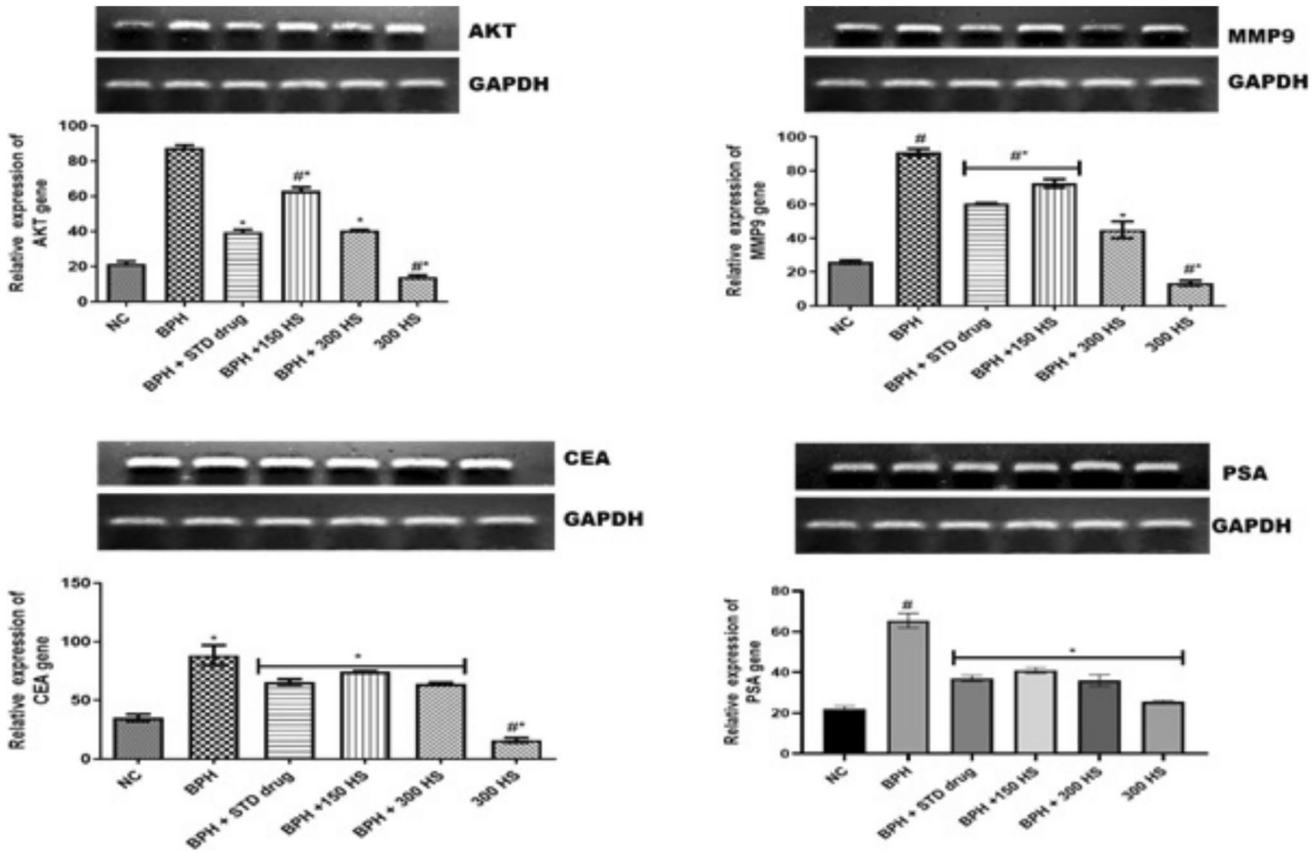

Fig. 5 Relative gene expression levels of AKT, MMP9, CEA, and PSA in *Hibiscus sabdariffa* leaf flavonoid-rich extracts from benign prostatic hyperplasia-induced rats. Each value is the mean of eight determinations ± SD. #$p < 0.05$ vs. NC, *$p < 0.05$ vs. DC. Legend: NC, normal control; BPH, benign prostatic hyperplasia-induced rats; BPH + STD drug, benign prostatic hyperplasia-induced rats administered 1 mg/kg finasteride; BPH + 150 HS, benign prostatic hyperplasia-induced rats administered a low dose (150 mg/kg body weight) of a flavonoid-rich extract of *Hibiscus sabdariffa*; BPH + 300 HS, benign prostatic hyperplasia-induced rats administered a high dose (300 mg/kg body weight) of a flavonoid-rich extract of *Hibiscus sabdariffa*; 300 HS, rats administered a high dose (300 mg/kg body weight) of a flavonoid-rich extract of *Hibiscus sabdariffa*

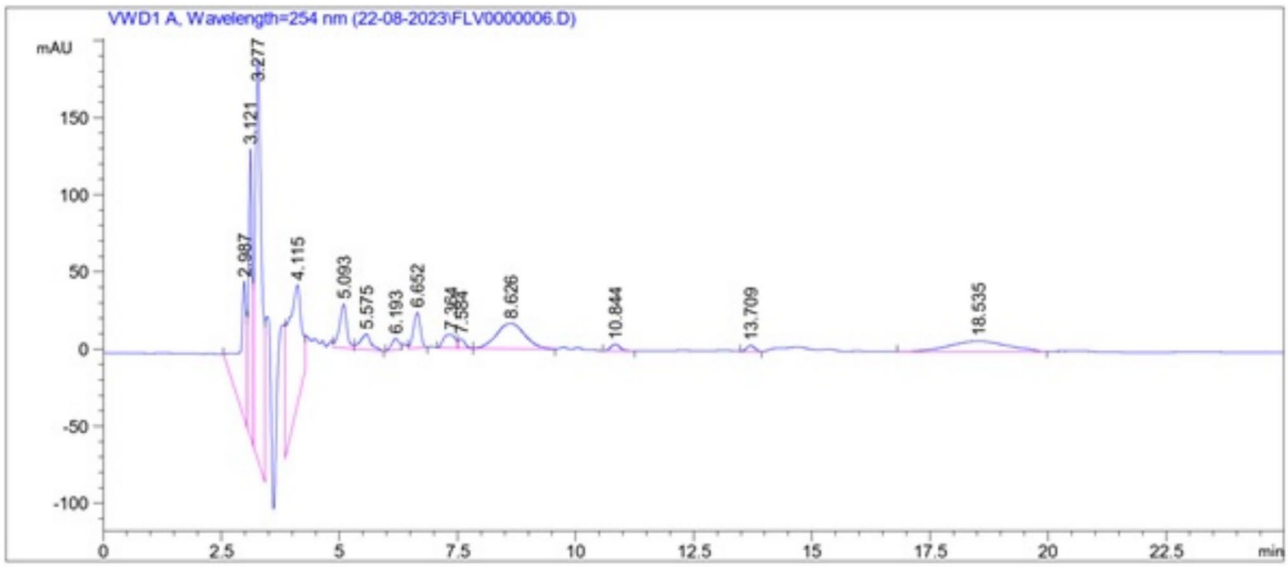

Fig. 6 HPLC chromatogram of the *Hibiscus sabdariffa* flavonoid-rich extract

Table 2 HPLC quantification of *Hibiscus sabdariffa* flavonoid-rich extracts

S/N	Quantity (µg/g)	Compounds	Molecular formula
1	0.648 ± 0.002	Isoquercitrin	$C_{21}H_{20}O_{12}$
2	1.344 ± 0.02	Unknown	N/A
3	**7.620 ± 0.713**	**Quercetin**	$C_{15}H_{10}O_7$
4	1.254 ± 0.075	Kaempferol	$C_{15}H_{10}O_6$
5	1.527 ± 0.105	Caffeic acid	$C_9H_8O_4$
6	1.103 ± 0.008	Unknown	N/A
7	1.133 ± 0.041	Unknown	N/A
8	1.254 ± 0.018	Unknown	N/A
9	1.164 ± 0.011	Unknown	N/A
10	1.184 ± 0.011	Unknown	N/A
11	2.781 ± 0.18	Ellagic acid	$C_{14}H_6O_8$
12	0.806 ± 0.003	Gallic acid	$C_7H_6O_5$
13	0.888 ± 0.008	Chlorogenic acid	$C_{16}H_{18}O_9$
14	0.900 ± 0.001	Tannin	$C_{76}H_{52}O_{46}$

Discussion

The ameliorative potential of flavonoid-rich extracts from *Hibiscus sabdariffa* leaves on specific signaling pathways associated with BPH in an albino rat model was investigated in this study. The present study assessed the impact of *Hibiscus sabdariffa* leaf flavonoid extract on redox biomarkers in BPH-treated rats' prostate tissue. Going by a recent report (Dearakhshandeh et al. 2019), the induction of testosterone at $p < 0.05$ substantially altered the levels/activities of redox modulatory biomarkers such as CAT, MDA, GPx, GSH, and SOD. Redox imbalance due to disparity in the formation and neutralization of ROS significantly influences various pathological conditions. In BPH-induced rats, this stress triggers intricate molecular and cellular mechanisms, intensifying the progression of the condition (Ebenyi et al. 2022). Redox biomarkers, including MDA and antioxidant defenses, exhibit significant alterations in the BPH-induced prostate tissue rats, indicating a compromised antioxidant system and consequential oxidative damage (Li et al. 2019). After treatment with *Hibiscus sabdariffa* leaf flavonoid extract, a substantial ($p < 0.05$) reduction in the MDA content indicated a reduction in lipid peroxidation, which is indicative of the antioxidant properties of the extract. Additionally, the potential of flavonoid-rich extracts to enhance the antioxidant defense system is seen by a significant rise in the activities of antioxidant enzymes such as SOD, CAT, GPx, and GSH. These findings suggest that the extract may mitigate oxidative stress associated with BPH, which is crucial in the pathogenesis of this condition (Akanni et al. 2022).

BPH progression depends mainly on prostate inflammation. As shown in Fig. 2, the administration of BPH increased at $p < 0.05$ significantly on the levels of proinflammatory cytokines such as IL-1β, IL-6, IL-10, and TNFα in prostate tissues. Our findings are in agreement with the previously published report by Rho et al. (2020). The role of inflammatory biomarker levels in BPH is crucial for understanding its pathogenesis. Higher levels of inflammatory markers, including IL-1β, IL-6, IL-10, and TNFα, indicate a proinflammatory microenvironment in the prostate. This inflammation is closely linked to redox imbalance, exacerbating the progression and severity of BPH (Kim et al. 2016b). The increased expression of these biomarkers reflects immune system activation, potentially contributing to tissue damage and cellular alterations in the prostate. Interestingly, the levels of IL-1β, IL-6, and TNFα were considerably ($p < 0.05$) lowered after treatment with the flavonoid-rich extract of *Hibiscus sabdariffa* leaves, indicating an anti-inflammatory activity (Umeoguaju et al. 2021). This anti-inflammatory property may contribute to the overall protective effects of the extract against BPH-induced damage.

Histological examination revealed valuable evidence of structural variability in the prostate gland, as depicted in Fig. 3. The untreated BPH rats showed severe disruption of the epithelial lining, clogged lumen, and abnormally vascularized stroma, which was not inconsistent with previous literature (Sasidharan et al., 2022). Severe disruption of the epithelial lining suggests structural damage to the cells covering the prostate surface. This disruption can compromise the usual prostate gland functioning, thereby affecting its ability to produce and secrete fluids essential for reproductive processes (Rasheed et al. 2023). The obstruction of the lumen, the central channel through which urine passes, can lead to difficulties in urination. In BPH, prostate enlargement compresses the urethral canal, partially or completely obstructing the flow of urine. The clogged lumen contributes to the characteristic urinary symptoms associated with BPH, such as hesitancy, a weak stream, and incomplete bladder emptying (Nnaemeka et al. 2018)). Changes in the vascularity of the stroma indicate alterations in the blood supply to the prostate tissue. Abnormal vascularization may lead to inadequate nutrient and oxygen delivery to prostate cells, further compromising their function and contributing to the overall pathology of BPH (Raafat et al. 2022). However, the *Hibiscus sabdariffa* flavonoid-rich extract-treated groups showed improvements in these histological features similar to what was reported in Adeyemi and Adewole (Adeyemi and Adewole 2019), with the high-dose extract group demonstrating a normal epithelial lining, clear lumen, prostatic concretions, and normally vascularized stroma. These histopathological findings align with the observed biochemical changes, indicating the potential of the extract to preserve the structural integrity of the prostate gland.

As shown in Fig. 4, this study investigated the levels of dihydrotestosterone (DHT) and testosterone, two hormones implicated in the progression and development of BPH.

The results indicate a significant ($p < 0.05$) rise of these hormones in BPH-induced rats, which is consistent with prior reported works (Kim et al. 2015) as well as the hormonal changes observed in clinical BPH cases. Understanding the role of DHT and testosterone levels is crucial for understanding the pathology of BPH, with their modulation being a central focus in therapeutic strategies for managing this condition. Elevated levels of DHT, which is a potent testosterone metabolite, stimulate prostate cell growth by binding to androgen receptors, thus fostering cellular proliferation and hypertrophy. This process is fundamental to the development and progression of BPH (Yu-Rong et al. 2017; Choi et al. 2022). Treatment with *Hibiscus sabdariffa* flavonoid-rich extract caused a drastic and significant decrease at $p < 0.05$ in DHT and testosterone levels in the BPH + 300HS-treated rats. This hormonal modulation may contribute to the extract's protective effects against BPH, possibly by influencing hormone-sensitive pathways involved in prostate growth.

The relative gene expression analysis provides molecular information on the regulatory pathways involved in BPH and the potential impact of the *Hibiscus sabdariffa* flavonoid-rich extract. This study focused on genes associated with BPH, such as prostate-specific antigen (PSA), carcinoembryonic antigen (CEA), matrix metalloproteinase-9 (MMP-9), and protein kinase B (AKT). The treatment of testosterone substantially at $p < 0.05$ upregulated the expression of these genes (PSA/CEA/AKT/MMP-9) in non-BPH-treated rats, consistent with previous research (Park et al. 2019; Liu et al. 2021). The prostate gland produces the enzyme PSA which is traditionally associated with the evaluation of prostate health. It liquefies semen, allowing sperm to swim freely. PSA is commonly used as a marker for monitoring the response to BPH treatments. A reduction in PSA levels may indicate a positive therapeutic outcome (Akanni et al. 2017; Ibukun et al. 2023). CEA, a glycoprotein crucial for cell adhesion and tissue structure, becomes aberrant in colorectal cancer, driving invasive behavior. Elevated CEA, a tumor marker, indicates the presence of cancer, but its non-specificity limits its diagnostic accuracy. Despite its elevation in nonmalignant conditions such as BPH, CEA aids in diagnosing, prognosticating, and monitoring cancer and BPH treatments, guiding therapeutic decisions (Onyegeme-Okerenta et al. 2022; Desai and Guddati 2023). AKT is a serine/threonine kinase involved in several biological processes, plus cell survival and proliferation. AKT is implicated in the development and survival of prostate cells. Its overactivation can promote the progression and development of BPH. Inhibiting AKT activity is a latent therapeutic approach to control the abnormal growth of prostate cells in BPH (Omar and Tolba 2018; Binmahfouz et al. 2022). MMP-9 is an enzyme involved in the extracellular matrix protein degradation. MMP-9 is associated with tissue remodeling and may contribute to the structural changes observed in the prostate during BPH (Baspinar et al. 2017; Gilardoni et al. 2017). According to the reported anticancer effect of *H. sabdariffa* leaf from in vitro studies (Chiu et al. 2015; Worawattananutai et al. 2014), the anti-invasive activity of *H. sabdariffa* leaf in cancer cells was enumerated. The report shows that *H. sabdariffa* leaf extract inhibited the secretion of MMP-9 in LNCaP cells. In contrast to our study on the induction of BPH in animal models, the flavonoid-rich extract of *H. sabdariffa* downregulated the mRNA expression of MMP-9, showing that our study agrees with the report of Chiu et al. (2015). Treatment with flavonoid-rich extract of *Hibiscus sabdariffa* significantly at $p < 0.05$ downregulated the expression of these genes (PSA/CEA/AKT/MMP-9), suggesting a regulatory effect on key signaling pathways implicated in BPH, similar to what was alluded to by Ke et al. (2019). Notably, the extract had a dose-dependent effect, with the high-dose group exhibiting more pronounced changes in gene expression.

HPLC analysis showed various flavonoids present in the *Hibiscus sabdariffa* leaf extract, in line with a previous report (Oboh et al. 2018), with quercetin identified as the predominant compound. Flavonoids are identified for their anti-inflammatory (Ajiboye et al. 2018; Maleki et al. 2019) and antioxidant (Agbo et al. 2015; Ajuwon et al. 2015; Akinmoladun et al. 2018) properties, and the observed biological activities in this study align with the flavonoid composition. The identification of specific flavonoids provides a basis for understanding the possible bioactive substances responsible for the extract's therapeutic effects.

Conclusions

This study demonstrates the ability of the flavonoid extract of *Hibiscus sabdariffa* leaf on BPH in a testosterone-treated rat model by ameliorating redox status, inflammatory markers, prostate histological examination, dihydrotestosterone, and testosterone. The extract also ameliorated the molecular gene expression of AKT, MMP9, CEA, and PSA. These observations may be attributed to the phytochemicals present in this extract, such as quercetin, ellagic acid, and isoquercitrin.

The findings also support the traditional use of *Hibiscus sabdariffa* in managing prostate-related disorders.

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Data Availability The data that support the findings of this study are available from the authors.

Declarations

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