

Biochemical Characterization of the Pharmacological Effects of *Yagari* in Experimentally Induced Benign Prostatic Hyperplasia



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Key words

Nauclea latifolia, *Erythrophleum suaveolens*, Rubiaceae, Fabaceae, *Yagari*, prostatic hyperplasia, prostatitis

received 18.04.2021

revised 30.11.2021

accepted 22.12.2021

Bibliography

Planta Med Int Open 2022; 9: e90–e107

DOI 10.1055/a-1729-3499

ISSN 2509-9264

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ABSTRACT

Benign prostatic hyperplasia is a progressive pathologic condition common in ageing men, constituting a health burden. Benign prostatic hyperplasia is characterized by the proliferation of prostatic tissues, prostate enlargement, and lower urinary tract symptoms. The use of herbal medicine in the management and treatment of benign prostatic hyperplasia has shown some promise. The efficacy and potency of some polyherbal extracts have been scientifically proven in the management and treatment of diseases, while many others are yet to be investigated. The aim of this study was to determine the effect of the herbal mixture *Yagari* on experimentally induced benign prostatic hyperplasia in rats and to identify its pharmacologically active agents. The effect of the herbal mixture on experimentally induced benign prostatic hyperplasia in rats was determined using 36 male Wistar rats grouped randomly into 6 groups of 6 rats each. The test rats were treated subcutaneously using a combination of dihydrotestosterone and estradiol valerate in a 10:1 ratio for 28 days according to the standard method. The test rats were thereafter treated with the herbal mixture for 21 days. Prostate-specific antigen, inflammatory cytokines, assay of prostate function hormonal and prostate function enzyme activities, and kidney function test were evaluated in the blood samples collected by ocular puncture applying standard methods. Prostates were harvested and examined for histopathological changes. Biological activity-guided fractionation of *Yagari* in a silica gel column was carried out and using phospholipase A₂ activity as a biomarker. The identity of the bioactive compounds was determined using mass spectroscopy and nuclear magnetic resonance. The herbal mixture showed a positive effect on prostatic hyperplasia by decreasing urinary obstruction through the inhibition of 5-alpha reductase, anti-inflammatory activity, and decreased level of sex hormones. Characterization by spectral studies revealed apigenin (4',5,7-trihydroxyflavone, molecular weight).

Introduction

Benign prostatic hyperplasia (BPH) involves the stromal and epithelial elements of the prostate arising in the periurethral and transition

zones of the gland. It occurs when the cells of the prostate gland begin to multiply [1, 2]. BPH is characterized as a slow, progressive enlargement of the prostate gland, which eventually causes obstruction and

subsequent problems with urination. However, BPH is believed to be neither a premalignant lesion nor a precursor of prostate cancer [3]. The incidence of BPH increases dramatically with age from about 50 % at 50 years of age to 90 % by the 9th decade of life [4]. BPH progression is characterized by hyperplasia of both the stromal and epithelial compartments. BPH is a nonmalignant adenomatous overgrowth of the periurethral prostate gland [5]. In adult men, there is normally a balance between prostate cell growth (proliferation) and cell death (apoptosis). BPH develops when this balance becomes tipped in favor of increased cell proliferation, resulting in prostatic enlargement [6]. In BPH, prostatic enlargement causes narrowing of the urethra where it passes through the prostate, leading, in turn, to bladder outlet obstruction and lower urinary tract symptoms [7]. The etiology is unknown but probably involves hormonal changes associated with ageing [8]. According to Wang et al. [9], BPH almost exclusively affects men aged over 40 because prostate size increases slowly.

Herbal medicine is commonly used as an alternative source of treatment for BPH [10]. A lot of plants with multiparmacological effects in the management of BPH have been scientifically proven for efficacy [11]. However, others are yet to be investigated. One such phytotherapeutic treatment that is yet to be scientifically investigated for efficacy is a local- *Yagari*, popularly used to treat BPH in the eastern part of Nigeria. Many patients who had reached the catheter/surgery stage have smiled (relieved not to have surgery) after treatment with the herbal mixture [12]. *Yagari* is a polyherbal formulation consisting of 62.4 % *Nauclea latifolia* and 37.6 % *Erythrophleum suaveolens*. To ascertain the efficacy of this herbal mixture and to identify the active component present, experimental screening was necessary. The aforementioned statement justified and warranted the study. The active ingredient could be developed as a new drug in the future.

Results

On preliminary phytochemical screening, *Yagari* divulged the presence of phenols, tannins, alkaloids, steroids, saponins, flavonoids, terpenoids, glycosides, and reducing sugars. Their relative abundance was alkaloids (2333 ± 0.006 mg/g), flavonoids (883.949 ± 0.020 mg/g), phenols (1740.645 ± 0.004 mg/g), tannins (788.420 ± 0.001 mg/g), terpenoids (2177.030 ± 0.006 mg/g), and saponins (63.230 ± 0.002 mg/g), as shown in ► **Table 12**. The acute toxicity test of *Yagari* showed that the herbal mixture does not cause death with doses up to 5000 mg/kg body weight. ► **Table 13** shows the observations in phases I and II of the acute toxicity test (LD_{50}) of *Yagari*. These observations indicated that no mortality or uncoordinated movement was recorded in the mice treated with different doses of the extract in both phases of the study, which is an indication that *Yagari* was well tolerated by the mice.

The results (► **Table 1**) show that the mean body weight of the rats induced with BPH only and rats induced with BPH and treated with 400 mg/kg body weight of the *Yagari* extract (197.46 ± 3.46 g and 184.68 ± 3.60 g, respectively) were significantly higher ($p < 0.05$) when compared to that of the normal control group (170.89 ± 2.61 g) after 21 days of treatment. Animals treated with a lower concentration of the extract (100 and 200 mg/kg) and the standard drug (finasteride) had a nonsignificant ($p > 0.05$) increase in mean body weight of 170.68 ± 1.54 g, 175.58 ± 1.54 g, and 176.45 ± 2.15 g, respective-

ly. There was a significant decrease in mean body weight of all animals when compared to those of Group 2. ► **Table 1** also shows the mean prostate weight of the animals. There was a significant ($p < 0.05$) increase in the mean prostate weight of groups induced with BPH (Groups 2–6) when compared with that of Group 1 (normal control). The result of the mean prostate:body weight (P:BW) ratio (relative prostate weight) in ► **Table 1** shows that there was a significant ($p < 0.05$) increase in relative prostate weight of rats induced with BPH (Groups 2–6) compared to that of normal control (Group 1). The mean P:BW ratio of the treated groups (Groups 3–6) revealed a significant ($p < 0.05$) decrease when compared with that of Group 2.

Results in ► **Table 2** show the increase in relative prostate weight (P:BW), percentage increase in relative prostate weight, and percentage recovery in relative prostate weight. They reveal that the increases in the P:BW ratio in Groups 2–6 were calculated to be 7.24, 1.58, 4.38, 2.89, and 1.74, respectively, thereby demonstrating that the increases in the P:BW ratio of the induced groups (Groups 2–6) were greater than that of the normal control (Group 1). They also indicate that the increases in P:BW of the induced and treated groups (Groups 3–6) were less than that of Group 2. The percentage increase in the P:BW ratio was calculated based on the increase in the P:BW ratio of Group 2, BPH induced and untreated, compared to that of the BPH-induced groups and treated (Groups 3–6). The percentage increases in the P:BW ratio of Groups 2–6 were calculated to be 100, 21.82, 60.5, 39.92, and 24.03 %, respectively. These indicate that there were reductions in the percentage increase in the P:BW ratio of the treated groups (3–6) compared to the untreated group (Group 2). The percentage recovery in the P:BW ratio of the BPH-treated groups (Groups 3–6) were calculated to be 78.18, 39.5, 60.08, and 75.97 %, respectively. The results demonstrated that Groups 3 and 6 had the highest percentage recovery in the P:BW ratio with 78.18 and 75.97 %, respectively.

The mean urine output was measured to denote the clinical implications of the study, as urine flow is seriously obstructed in cases of hyperplasia of the prostate. In the case of the vehicle-treated control group (Group 1), there was practically no change in urine output. As hyperplasia progressed with dihydrotestosterone-estradiol valerate treatment, urine output was reduced, and a drastic reduction was observed after 21 days of treatment in the negative control group (Group 2) animals. When the *Yagari* extract were administered to Groups 3–5, a significant improvement in urine output over the negative control group was observed. The % obstruction in urine output for the extract-treated groups in doses of 100, 200, and 400 mg/kg was 12.97 ± 4.40 , 5.71 ± 1.19 , and 2.45 ± 0.59 %, respectively, compared to 77.38 ± 0.21 % in the negative control group. In the finasteride-treated group (Group 6), the % obstruction recorded was 1.96 ± 0.98 % (20 mg/kg) per oral administration. The relative efficacy of the extract in reducing the obstruction caused by dihydrotestosterone-estradiol valerate can be stated in the following order: finasteride > 400 mg/kg extract > 200 mg/kg extract > 100 mg/kg extract. The results are depicted in ► **Table 3**.

As revealed in ► **Table 4**, the concentration of creatinine in rats in the negative control group that received dihydrotestosterone-estradiol valerate and remained untreated was 1.121 ± 0.041 mg/dL, which differed significantly ($p < 0.05$) from 0.983 ± 0.061 mg/dL of those in the normal control group. Per oral treatment with the standard drug, finasteride (Group 3) reduced the creatinine concentration

► **Table 1** Effect of *Yagari* extract on the body weight, prostate weight, and prostate:body weight (P:BW) ratio of experimentally induced BPH in rats.

Groups	Day 0 (g)	Day 21 (g)	Prostate weight (mg)	P:BW
1	160.78 ± 3.52 ^B	170.89 ± 5.84 ^B	233.00 ± 8.59 ^B	1.36 ± 0.03 ^B
2	177.24 ± 8.55 ^A	197.46 ± 7.73 ^A	1695.52 ± 8.35 ^A	8.60 ± 0.30 ^A
3	171.45 ± 2.62	176.45 ± 4.81 ^B	518.70 ± 518.70 ^{A, B}	2.94 ± 0.05 ^{A, B}
4	159.44 ± 2.58 ^B	170.68 ± 3.30 ^B	979.53 ± 11.94 ^{A, B}	5.74 ± 0.07 ^{A, B}
5	169.93 ± 4.16 ^{A, B}	175.58 ± 3.45 ^B	745.05 ± 8.32 ^{A, B}	4.25 ± 0.04 ^{A, B}
6	175.91 ± 6.63 ^A	184.68 ± 8.05 ^{A, B}	572.83 ± 3.96 ^{A, B}	3.10 ± 0.11 ^{A, B}

Data are presented as the mean ± SD (n = 6). ^Ap < 0.05 compared to normal control; ^Bp < 0.05 compared to negative control. Group 1 – normal control (vehicle only), Group 2 – BPH-induced and untreated (negative control), Group 3 – BPH-induced and treated with 20 mg/kg body weight of the standard drug (finasteride), Group 4 – BPH-induced and treated with 100 mg/kg body weight of *Yagari* extract, Group 5 – BPH-induced and treated with 200 mg/kg body weight of *Yagari* extract, Group 6 – BPH-induced and treated with 400 mg/kg body weight of *Yagari* extract.

► **Table 2** Effect of *Yagari* extract on the increase of relative prostate weight, percentage increase, and percentage recovery of experimentally induced BPH in rats.

Groups	Increase in P:BW	% Increase in P:BW	% Recovery in P:BW
1	0	–	–
2	7.24	100	0
3	1.58	21.82	78.18
4	4.38	60.50	39.50
5	2.89	39.92	60.08
6	1.74	24.03	75.97

Data are presented as the mean ± SD (n = 6). Group 1 – normal control (vehicle only), Group 2 – BPH-induced and untreated (negative control), Group 3 – BPH-induced and treated with 20 mg/kg body weight of the standard drug (finasteride), Group 4 – BPH-induced and treated with 100 mg/kg body weight of *Yagari* extract, Group 5 – BPH-induced and treated with 200 mg/kg body weight of *Yagari* extract, Group 6 – BPH-induced and treated with 400 mg/kg body weight of *Yagari* extract.

significantly ($p < 0.05$) by 0.065 ± 0.017 mg/dL with respect to Group 2, the BPH-untreated group. Groups (4–6) treated with different concentrations (100, 200, and 400 mg/kg) of the *Yagari* extract demonstrated a significant ($p < 0.05$) decrease in the mean concentration of creatinine. ► **Table 4** also shows that the concentration of urea of all groups induced with BPH (Groups 2–6) increased significantly ($p < 0.05$) compared to that of the normal control group. Groups treated with different doses of the extract (100, 200, and 400 mg/kg body weight) had significant reductions ($p < 0.05$) in their serum urea concentrations (23.766 ± 0.050 , 23.084 ± 0.160 , and 21.954 ± 0.081 mg/dL, respectively) when compared to Group 2 (BPH untreated 37.234 ± 0.272 mg/dL). The finasteride-treated group showed a significant decrease ($p < 0.05$) in urea concentration (24.799 ± 0.298 mg/dL) compared to Group 2.

Data in ► **Table 5** show the effect of the *Yagari* extract on the prostate function enzyme activities, prostatic acid phosphatase (PAP), and 5-alpha reductase. The table reveals that the PAP activity of animals in Groups 2–6 increases significantly ($p < 0.05$) compared to that of the normal control (Group 1). Treatment of animals with finasteride, the standard drug for prostate hyperplasia, significantly decreased ($p < 0.05$) PAP activity (2.045 ± 0.037 IU/L) with respect to

Group 2 (induced with BPH and untreated, 4.291 ± 0.016 IU/L). Groups 4–6 treated with different doses of the extract (100, 200, and 400 mg/kg body weight, respectively) showed a significant reduction ($p < 0.05$) in PAP activity at 2.288 ± 0.012 , 2.232 ± 0.084 , and 2.084 ± 0.104 IU/L, respectively, when compared to Group 2.

Steroid 5-alpha reductase activity of rats induced with BPH (Group 2, negative control) increased significantly ($p < 0.05$) by 0.330 ± 0.009 IU/mL with respect to the normal control group (1.514 ± 0.015 IU/mL). Treatment of animals with 100, 200, and 400 mg/kg body weight of the extract significantly decreased ($p < 0.05$) 5-alpha reductase activity (1.611 ± 0.218 , 1.608 ± 0.028 , and 1.606 ± 0.220 IU/mL, respectively) compared to Group 2 (1.844 ± 0.006 IU/mL).

The data in ► **Table 6** show that the serum testosterone concentration of Group 2, rats treated with dihydrotestosterone-estradiol valerate only (3.642 ± 0.072 ng/mL), decreased significantly ($p < 0.05$) when compared to that of Group 1, administered with the vehicle, 2% Tween 80 solution. Groups (4–6) administered with different concentration of the *Yagari* extract (100, 200, and 400 mg/kg body weight, respectively) after BPH induction had a significant increase ($p < 0.05$) in their serum testosterone concentrations. The effect was dose-dependent with 5.568 ± 0.086 , 5.685 ± 0.144 , and 6.349 ± 0.134 , respectively, when compared to that of Group 2. The animals in Group 3 treated with 20 mg/kg body weight of finasteride had a significant increase of serum testosterone concentration by 2.977 ± 0.016 ng/mL ($p < 0.05$) with respect to Group 2. ► **Table 6** also shows that the serum dihydrotestosterone (DHT) concentration of Group 2, treated with dihydrotestosterone-estradiol valerate only (901.330 ± 14.639 pg/mL), increased significantly ($p < 0.05$) by 482.874 ± 9.171 pg/mL when compared to that of Group 1 (418.456 ± 5.468 pg/mL). The administration of different concentrations (100, 200, and 400 mg/kg) of the *Yagari* extract after BPH induction with dihydrotestosterone-estradiol valerate in groups 4, 5, and 6, respectively, caused a significant ($p < 0.05$) decrease in the serum DHT concentrations. The serum DHT concentration of groups treated with 100, 200, and 400 mg/kg of the extract were significantly decreased ($p < 0.05$) by 211.682 ± 13.339 , 245.252 ± 1.455 , and 283.274 ± 20.981 , respectively, when compared to that of Group 2. There was also a significant decrease ($p < 0.05$) in the serum DHT concentration in Group 3 treated with finasteride by 315.174 ± 2.620 pg/mL when compared to Group 2.

► **Table 3** Effect of *Yagari* extract on urine output (mL) of experimentally induced BPH in rats.

Groups	Day 0	Day 7	Day 14	Day 21	% Obstruction
1	1.05 ± 0.03	1.07 ± 0.02 ^B	1.08 ± 0.03 ^B	1.05 ± 0.02 ^B	0 ^B
2	1.02 ± 0.04	0.88 ± 0.03 ^A	0.41 ± 0.04 ^A	0.23 ± 0.01 ^A	77.38 ± 0.21 ^A
3	1.02 ± 0.01	1.03 ± 0.07 ^B	0.74 ± 0.24 ^{A, B}	1.00 ± 0.01 ^{A, B}	1.96 ± 0.98 ^B
4	1.07 ± 0.06	0.98 ± 0.02 ^{A, B}	0.64 ± 0.04 ^{A, B}	0.93 ± 0.0 ^{A, B}	12.97 ± 4.40 ^{A, B}
5	0.99 ± 0.03	0.98 ± 0.03 ^{A, B}	0.66 ± 0.04 ^{A, B}	0.94 ± 0.03 ^{A, B}	5.71 ± 1.19 ^{A, B}
6	0.96 ± 0.04 ^A	0.95 ± 0.03 ^{A, B}	0.44 ± 0.04 ^A	0.93 ± 0.04 ^{A, B}	2.45 ± 0.59 ^{A, B}

Data are presented as the mean ± SD (n = 6). ^AP < 0.05 compared to normal control; ^Bp < 0.05 compared to negative control. Group 1 – normal control (vehicle only), Group 2 – BPH-induced and untreated (negative control), Group 3 – BPH-induced and treated with 20 mg/kg body weight of the standard drug (finasteride), Group 4 – BPH-induced and treated with 100 mg/kg body weight of *Yagari* extract, Group 5 – BPH-induced and treated with 200 mg/kg body weight of *Yagari* extract, Group 6 – BPH-induced and treated with 400 mg/kg body weight of *Yagari* extract.

► **Table 4** Effect of *Yagari* extract on kidney function biomarkers of experimentally induced BPH in rats.

Groups	Creatinine (mg/dL)	Urea (mg/dL)
1	0.983 ± 0.061 ^B	16.731 ± 0.069 ^B
2	1.121 ± 0.041 ^A	37.234 ± 0.272 ^A
3	1.056 ± 0.024 ^{A, B}	24.799 ± 0.298 ^{A, B}
4	1.006 ± 0.018 ^B	23.766 ± 0.050 ^{A, B}
5	0.982 ± 0.031 ^B	23.084 ± 0.160 ^{A, B}
6	0.915 ± 0.020 ^{A, B}	21.954 ± 0.081 ^{A, B}

Data are presented as the mean ± SD (n = 6). ^AP < 0.05 compared to normal control; ^Bp < 0.05 compared to negative control. Group 1 – normal control (vehicle only), Group 2 – BPH-induced and untreated (negative control), Group 3 – BPH-induced and treated with 20 mg/kg body weight of the standard drug (finasteride), Group 4 – BPH-induced and treated with 100 mg/kg body weight of *Yagari* extract, Group 5 – BPH-induced and treated with 200 mg/kg body weight of *Yagari* extract, Group 6 – BPH-induced and treated with 400 mg/kg body weight of *Yagari* extract.

► **Table 6** Effect of *Yagari* extract on the prostate function hormonal concentration of experimentally induced BPH in rats.

Groups	Testosterone (ng/mL)	DHT (pg/mL)
1	6.569 ± 0.072 ^B	418.456 ± 5.468 ^B
2	3.642 ± 0.072 ^A	901.330 ± 14.639 ^A
3	6.619 ± 0.056 ^B	586.156 ± 17.259 ^{A, B}
4	5.568 ± 0.086 ^{A, B}	689.648 ± 27.978 ^{A, B}
5	5.685 ± 0.144 ^{A, B}	656.078 ± 16.094 ^{A, B}
6	6.349 ± 0.134 ^B	618.056 ± 35.620 ^{A, B}

Data are presented as the mean ± SD (n = 6). ^AP < 0.05 compared to normal control; ^Bp < 0.05 compared to negative control. Group 1 – normal control (vehicle only), Group 2 – BPH-induced and untreated (negative control), Group 3 – BPH-induced and treated with 20 mg/kg body weight of the standard drug (finasteride), Group 4 – BPH-induced and treated with 100 mg/kg body weight of *Yagari* extract, Group 5 – BPH-induced and treated with 200 mg/kg body weight of *Yagari* extract, Group 6 – BPH-induced and treated with 400 mg/kg body weight of *Yagari* extract.

► **Table 5** Effect of *Yagari* extract on prostate function enzyme activity of experimentally induced BPH in rats.

Groups	Prostatic acid phosphatase (IU/L)	Steroid 5-alpha reductase (IU/mL)
1	2.311 ± 0.081 ^B	1.514 ± 0.015 ^B
2	4.291 ± 0.016 ^A	1.844 ± 0.006 ^A
3	2.045 ± 0.037 ^{A, B}	1.564 ± 0.009 ^B
4	2.288 ± 0.012 ^{A, B}	1.611 ± 0.218 ^B
5	2.232 ± 0.084 ^{A, B}	1.608 ± 0.028 ^B
6	2.084 ± 0.104 ^{A, B}	1.606 ± 0.220 ^B

Data are presented as the mean ± SD (n = 6). ^AP < 0.05 compared to normal control; ^Bp < 0.05 compared to negative control. Group 1 – normal control (vehicle only), Group 2 – BPH-induced and untreated (negative control), Group 3 – BPH-induced and treated with 20 mg/kg body weight of the standard drug (finasteride), Group 4 – BPH-induced and treated with 100 mg/kg body weight of *Yagari* extract, Group 5 – BPH-induced and treated with 200 mg/kg body weight of *Yagari* extract, Group 6 – BPH-induced and treated with 400 mg/kg body weight of *Yagari* extract.

Results in ► **Table 7** show that the serum TNF-α concentration of Group 2, rats treated with dihydrotestosterone-estradiol valerate only, increased significantly (p < 0.05) by 34.331 ± 2.496 pg/mL when compared to that of Group 1, which received vehicle only, 2 % Tween 80 solution. There were decreases by 26.928 ± 1.147, 30.026 ± 1.666, and 32.037 ± 2.319 pg/mL (p < 0.05) in TNF-α concentration in Groups 4, 5, and 6, respectively, treated with 100, 200, and 400 mg/kg body weight of the *Yagari* extract correspondingly with respect to Group 2 (58.019 ± 2.980 pg/mL). Similarly, there was a significant (p < 0.05) decrease by 34.351 ± 0.901 in Group 3 rats treated with 20 mg/kg of finasteride relative to Group 2. ► **Table 7** also shows that the serum interferon-gamma (INF-γ) concentrations of rats treated with dihydrotestosterone-estradiol valerate only increased significantly (p < 0.05) by 151.516 ± 4.883 pg/mL when compared to Group 1 (28.189 ± 1.962). There was a significant decrease in the serum INF-γ level (p < 0.05) in groups (4–6) administered with different doses of the *Yagari* extract (100, 200, and 400 mg/kg body weight) by 49.302 ± 1.893, 64.364 ± 1.676, and 68.458 ± 3.049 pg/mL, respectively, when compared to Group 2 (179.705 ± 6.845). Also, Group 3 animals administered with 20 mg/kg of finasteride had a

► **Table 7** Effect of *Yagari* extract on the concentration of inflammatory cytokines of experimentally induced BPH in rats.

Groups	TNF- α (pg/mL)	INF- γ (pg/mL)
1	23.688 \pm 0.484 ^B	28.189 \pm 1.962 ^B
2	58.019 \pm 2.980 ^A	179.705 \pm 6.845 ^A
3	23.668 \pm 2.079 ^B	110.890 \pm 5.586 ^{A, B}
4	31.760 \pm 1.833 ^{A, B}	130.403 \pm 4.952 ^{A, B}
5	27.993 \pm 1.314 ^{A, B}	115.341 \pm 5.169 ^{A, B}
6	25.982 \pm 0.661 ^B	111.247 \pm 3.796 ^{A, B}

Data are presented as the mean \pm SD (n = 6). ^Ap < 0.05 compared to normal control; ^Bp < 0.05 compared to negative control. Group 1 – normal control (vehicle only), Group 2 – BPH-induced and untreated (negative control), Group 3 – BPH-induced and treated with 20 mg/kg body weight of the standard drug (finasteride), Group 4 – BPH-induced and treated with 100 mg/kg body weight of *Yagari* extract, Group 5 – BPH-induced and treated with 200 mg/kg body weight of *Yagari* extract, Group 6 – BPH-induced and treated with 400 mg/kg body weight of *Yagari* extract.

► **Table 8** Effect of *Yagari* extract on the prostate-specific antigen (PSA) and zinc concentrations of experimentally induced BPH in rats.

Groups	PSA (ng/mL)	Zinc (ug/dL)
1	1.492 \pm 0.072 ^B	22.990 \pm 0.868 ^B
2	3.478 \pm 0.149 ^A	27.612 \pm 0.746 ^A
3	1.593 \pm 0.062 ^B	23.476 \pm 0.494 ^B
4	1.872 \pm 0.113 ^{A, B}	24.552 \pm 0.347 ^{A, B}
5	1.686 \pm 0.047 ^{A, B}	24.144 \pm 0.722 ^{A, B}
6	1.617 \pm 0.028 ^{A, B}	23.198 \pm 0.635 ^B

Data are presented as the mean \pm SD (n = 6). ^Ap < 0.05 compared to normal control; ^Bp < 0.05 compared to negative control. Group 1 – normal control (vehicle only), Group 2 – BPH-induced and untreated (negative control), Group 3 – BPH-induced and treated with 20 mg/kg body weight of the standard drug (finasteride), Group 4 – BPH-induced and treated with 100 mg/kg body weight of *Yagari* extract, Group 5 – BPH-induced and treated with 200 mg/kg body weight of *Yagari* extract, Group 6 – BPH-induced and treated with 400 mg/kg body weight of *Yagari* extract.

► **Table 9** Effect of *Yagari* extract fractions on phospholipase A₂ activities.

	n-Hexane	Ethyl acetate	Ethanol	Aqueous
Control	0.787 \pm 0.020	0.787 \pm 0.020	0.787 \pm 0.020	0.787 \pm 0.020
0.5 mg/mL	0.561 \pm 0.002 (28.70) [*]	0.554 \pm 0.019 (29.60) [*]	0.482 \pm 0.042 (38.89) [*]	0.535 \pm 0.002 (32.40) [*]
1.0 mg/mL	0.535 \pm 0.002 (32.03) [*]	0.509 \pm 0.001 (35.30) [*]	0.180 \pm 0.004 (77.10) [*]	0.289 \pm 0.012 (63.30) [*]
2.0 mg/mL	0.508 \pm 0.002 (35.50) [*]	0.508 \pm 0.002 (35.60) [*]	0.153 \pm 0.007 (80.60) [*]	0.227 \pm 0.002 (71.20) [*]

Values of absorbance shown are the mean \pm SD of triplicate determination. Values in parentheses are the percentage of inhibition of phospholipase A₂ calculated relative to the control. ^{*}p < 0.05 compared to the control

significant decrease (p < 0.05) in the serum INF- γ level by 68.815 \pm 1.259 pg/mL when compared to Group 2.

The data in ► **Table 8** show that the serum prostate-specific antigen (PSA) concentrations of Group 2 rats treated with dihydrotestosterone-estradiol valerate only (3.478 \pm 0.149 ng/mL) increased significantly (p < 0.05) when compared to Group 1, administered with 2 % Tween 80 solution. Groups (4–6) administered with different doses of *Yagari* extract (100, 200, and 400 mg/kg body weight) had significant decreases (p < 0.05) in their serum PSA levels. The effect was concentration-dependent with 1.872 \pm 0.113, 1.686 \pm 0.047, and 1.617 \pm 0.028 ng/mL, respectively, when compared to that of Group 2. The animals in Group 3, treated with 20 mg/kg of finasteride, had a significant decrease by 1.885 \pm 0.087 ng/mL (p < 0.05) in their serum PSA level when compared to that of Group 2. ► **Table 8** shows that the zinc concentration of Group 2, treated with dihydrotestosterone-estradiol valerate only (27.612 \pm 0.746 μ g/dL), increased significantly (p < 0.05) when compared to Group 1, the normal control (22.990 \pm 0.868 μ g/dL). Groups (4–6) administered different concentrations of the *Yagari* extract (100, 200, and 400 mg/kg) had significant reductions (p < 0.05) in their serum zinc concentration by values of 3.060 \pm 0.399, 3.468 \pm 0.024, and 4.414 \pm 0.111 μ g/dL, respectively, when compared to that of Group 2. Relatedly, Group 3 rats treated with 20 mg/kg body weight of finasteride had a significant reduction by 4.136 \pm 0.252 μ g/dL (p < 0.05) in its serum zinc concentration with respect to Group 2 (27.612 \pm 0.746 μ g/dL).

The prostate epithelial cells of the animals in the control group showed normal histological (Fig. 1) features characterized by regular size and cuboidal shape. The prostate tissues of animals in the BPH-treated group showed abnormal histological features seen as an enlarged gland characterized by hyperplastic epithelial cells (Fig. 2). The histological characteristics of prostate tissues of animals in the standard drug-treated and treated with the extract (100, 200, and 400 mg/kg body weight) groups showed reduced hyperplasia (Figs 3–6). The BPH group showed an enlarged gland with hyperplastic cells characterized by papillary epithelial cells with multi-vacuolated cytoplasm projecting into the glandular lumen and a decreased glandular luminal area. The standard drug group showed reduced hyperplasia (Fig. 6). The effects of *Yagari* extract on the prostate histology are shown in Figs 3–5. The rats administered with 100 and 200 mg/kg of the extract showed reduced hyperplasia, while those administered with 400 mg/kg of the extract showed almost normal prostate cell features when compared to the normal control group.

Data in ► **Table 9** show biological activity-guided fractionation of the *Yagari* extract with solvents of increasing polarity such as *n*-hexane, ethyl acetate, ethanol, and water. The ethanol fraction yielded the best activity against phospholipase A₂, inhibiting the enzyme activity by 80.60 %, while *n*-hexane, ethyl-acetate, and the aqueous fraction showed 35.50, 35.60, and 71.20 % inhibition of enzyme activity, respectively. The inhibitory effect of all fractions

was concentration dependent. Further fractionation of the ethanol fraction, as shown in ► **Table 10**, yielded the most active fraction of *Yagari* extract, hereafter referred to as Active Fraction.

► **Fig. 3** shows the HPLC chromatogram of the Active Fraction with a single sharp peak at 10.37 min to yield a pure yellow crystalline isolate called YAF5. ESI-MS spectra of YAF5 in ► **Fig. 4** provide the molecular weight of the main compound or the most abundant compound. The molecular ion $[M - H]^-$ peak was displayed at m/z 269. Fourier-transform infrared spectra of YAF5 in ► **Fig. 5** revealed the presence of different functional groups as follows: OH stretching band (3338.68 cm^{-1}), CH stretching band (2941.91 and 2831.51 cm^{-1}), C = O in chromone carbonyl of the keto group (1635.80 cm^{-1}), CH bending band in the aromatic carbonyl of the phenyl group (1448.23 cm^{-1}), OH bending band (1115.84 cm^{-1}), C-O single bond stretching vibration (1021.52 cm^{-1}), and CH bend in the para-distributed aromatic compound.

The ^1H NMR spectrum in ► **Fig. 6** shows the presence of two meta-coupled aromatic doublets at δ 6.470 and 6.178 corresponding to protons H-6 and H-8, a pair of doublets at δ 6.920 and 7.926 for H-3' / H-5' and H-2' / H-6' protons in the aromatic region, and a singlet at δ 6.777 corresponding to H-3. The ^1H NMR spectrum also showed the presence of three singlets at δ 12.958, 10.854, and 10.374 corresponding to three hydroxyl groups. The ^{13}C NMR spectra, as shown in ► **Fig. 7**, revealed the presence of twelve aromatic carbons: seven quaternary carbons, five methine carbons, and an unsaturated carbonyl carbon. The ^1H and ^{13}C NMR values for all the carbons were assigned on the basis of HSQC and HMBC correlations (► **Figs. 9** and ► **10**), which were further supported by the COSY and HMBC correlations, as shown in ► **Figs. 8** and ► **10**. The HSQC spectrum allowed for the assignment of all protonated carbons, as shown in ► **Fig. 10**, which suggested there were three hydroxyls, including one phenolic hydroxyl proton at 10.374 ppm, in the ^1H -NMR spectrum. Further investigation of HMBC supported the assignment of all the quaternary carbons. The structure was elucidated by ^1H - ^1H COSY and HMBC correlations using two benzene rings as the starting points. In the HMBC spectrum, C-2 correlated with H-3, H-6', and H-2', C-10 correlated with H-6 and H-8, C-7 correlated with H-6 and H-8, C-4' correlated with H-3', H-5', and H-6', C-9 correlated with H-8, C-2' correlated with H-3', and C-1' correlated H-2'. From COSY, four ^1H peaks at 7.926, 10.374, 10.854, and 12.958 were correlated with each other. The distortionless enhancement by polarization transfer (DEPT) experiment gave three singlets and two doublets. The most downfield shifted peak was 187.27 ppm, which was assigned a ketone group (C-4). The next downfield shifted peak, 163.93, was C-2.

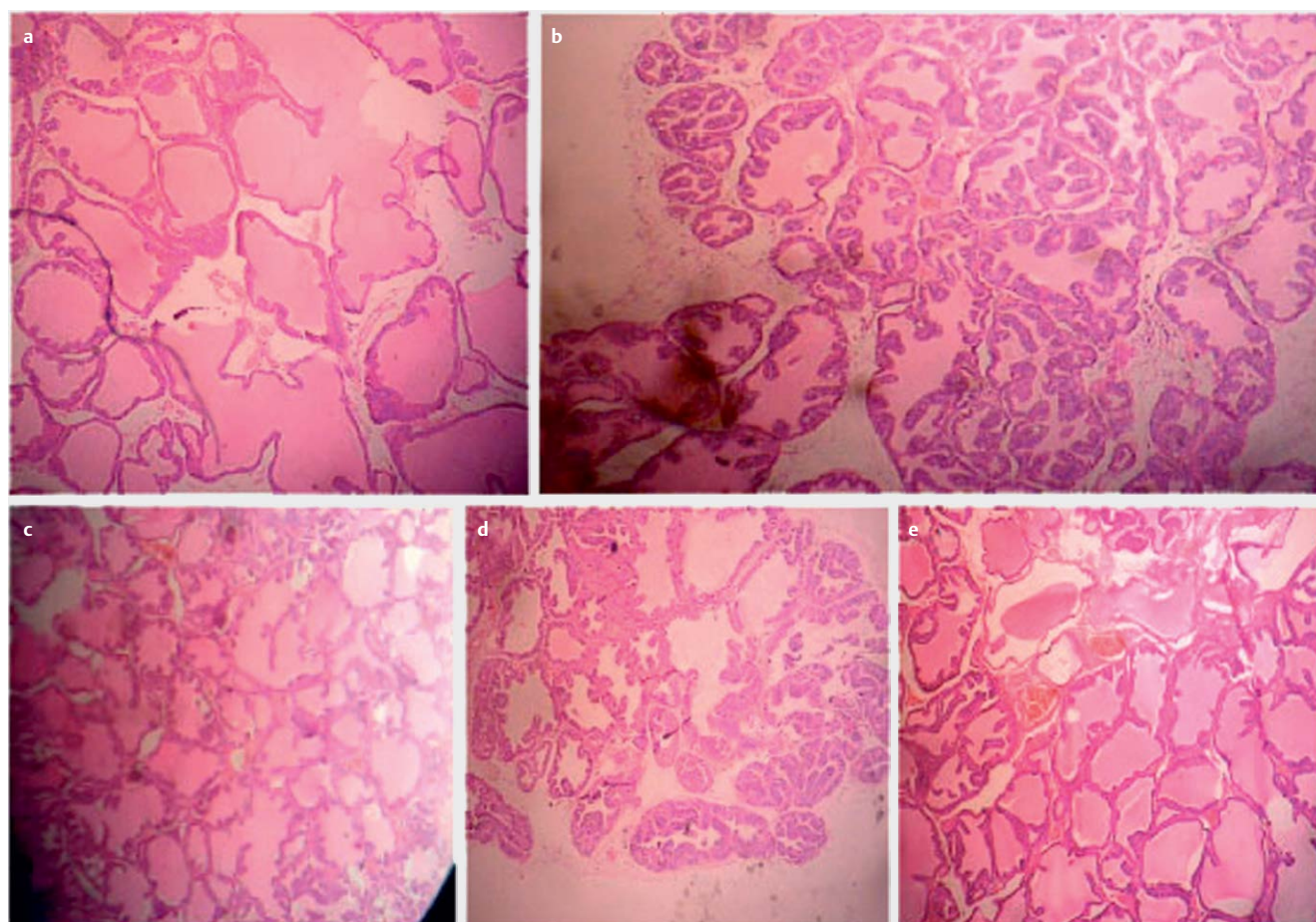
UV-Vis spectrophotometric spectra (► **Fig. 11**) showed absorption peaks at 268, 344 nm. UV-Vis spectra of the flavones possessed characteristic absorption peak bands at 300–380 and 240–280 nm due to their chemical structure.

A comparison of the spectra obtained from ^1H NMR, ^{13}C NMR, and ^2D NMR involving DEPT, COSY, HSQC, and HMBC of YAF5 with the published library of compounds established the identity of the isolate YAF5 as 5,7-dihydroxy-2-(4'-hydroxyphenyl)-4H-chromen-4-one, or apigenin. A search in the literature confirmed the spectral data was consistent with 4',5,7-trihydroxyflavone, also known as apigenin.

► **Table 10** Effect of solvent gradient fractions on phospholipase A_2 activity.

	Fraction 1	Fraction 2	Fraction 3	Mean OD ₄₁₈	Fraction 4	Fraction 5	Fraction 6	Fraction 7	Fraction 8
Control	0.871 ± 0.027	0.871 ± 0.027	0.871 ± 0.027	0.871 ± 0.027	0.871 ± 0.027	0.871 ± 0.027	0.871 ± 0.027	0.871 ± 0.027	0.871 ± 0.027
1.0 mg/mL	0.749 ± 0.005 (14.00)*	0.543 ± 0.002 (37.70)*	0.345 ± 0.012 (60.40)*	0.352 ± 0.006 (59.60)*	0.133 ± 0.002 (84.70)*	0.247 ± 0.001 (71.60)*	0.235 ± 0.003 (73.02)*	0.235 ± 0.003 (73.02)*	0.351 ± 0.005 (59.70)*
2.0 mg/mL	0.744 ± 0.001 (14.60)*	0.438 ± 0.007 (49.70)*	0.332 ± 0.006 (61.90)*	0.330 ± 0.007 (62.20)*	0.109 ± 0.004 (87.50)*	0.241 ± 0.004 (72.33)*	0.235 ± 0.003 (73.02)*	0.235 ± 0.003 (73.02)*	0.334 ± 0.002 (61.65)*

Values of absorbance shown are the mean ± SD of triplicate determination. Values in parentheses are the percentage of inhibition of phospholipase A_2 calculated relative to the control. * $P < 0.05$ compared to the control.



► **Fig. 1** Photomicrograph of sections of a prostate from a 1a: Prostate of normal control rat. 1b: Prostate induced with BPH, Untreated. 1c: Prostate induced with BPH, Treated with 20 mg/kg b.w of finasteride. 1d: Prostate induced with BPH, Treated with 100 mg/kg b.w of herbal mixture. 1e: Prostate induced with BPH, Treated with 400 mg/kg b.w of herbal mixture. H&E $\times 100$, L = lumen, S = stromal.

Data in ► **Table 11** show R_f values of YAF5 and standard apigenin. When the developed plates were sprayed with 5 % ethanol ferric chloride solution and placed in iodine chamber, they showed a spot that had a brownish color. The R_f value (0.74) of YAF5 corresponded with the R_f value of standard apigenin.

Structure of apigenin Figure. 12

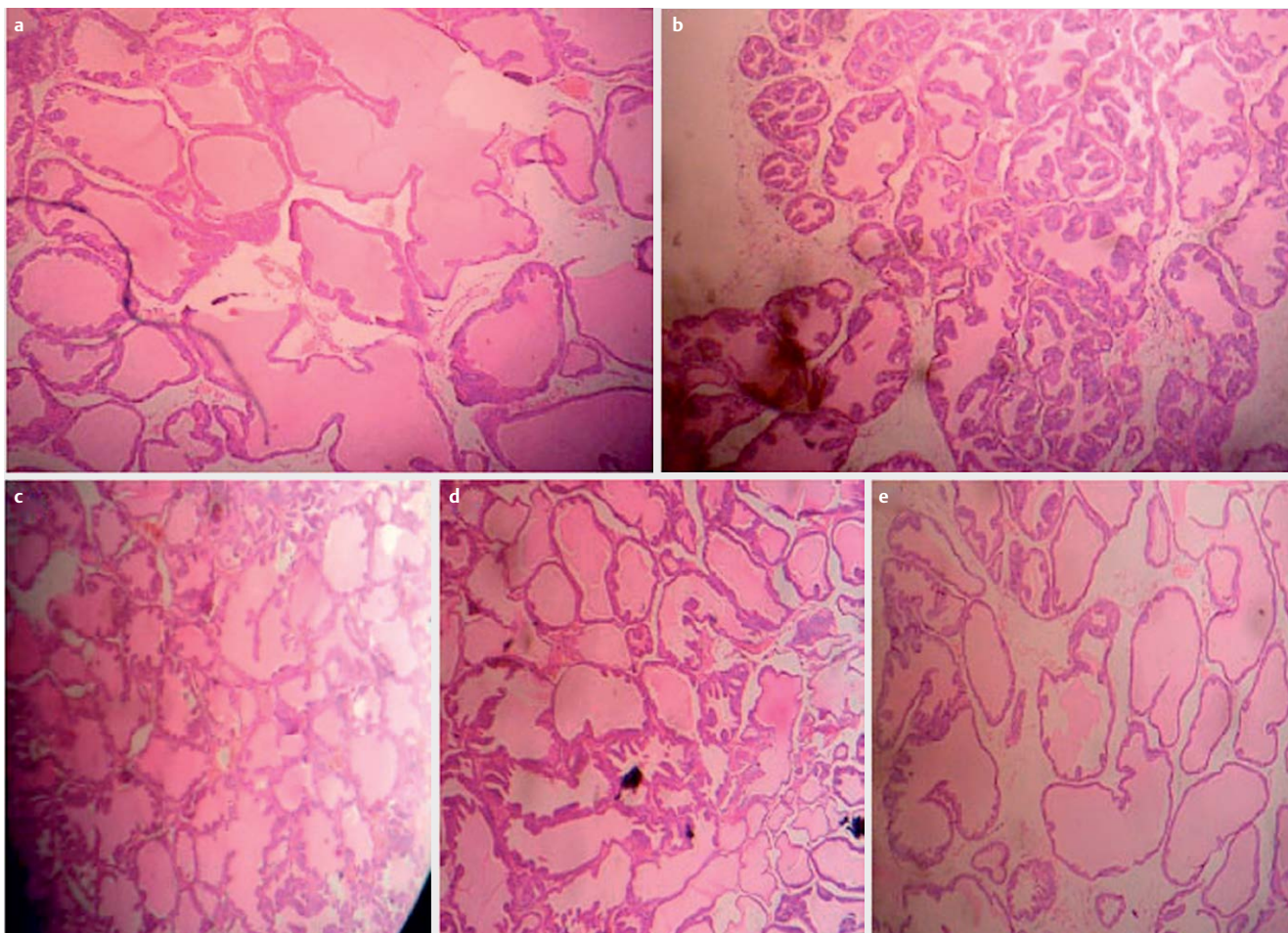
Discussion

The elevated incidence of BPH is a cause of increasing public health concern. BPH is the most frequent urological problem of ageing men, which is manifested as a severe obstruction in urinary flow with discomfort and pain. The principal hypothesis for the hypertrophic reaction of prostate tissue is steroid-mediated cellular proliferation and inflammatory response to local infection. The use of herbal medicine in the management and treatment of BPH has shown some promise [16]. This study was designed to assess the effect of a locally used herbal mixture called *Yagari* on markers of experimentally induced BPH in rats and to identify its pharmacologically active principles.

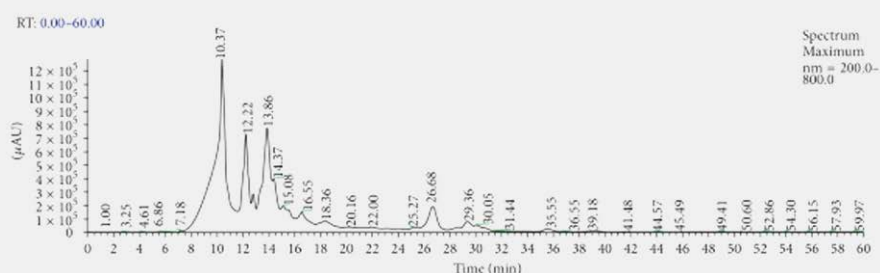
BPH experimentally induced by treatment of animals with dihydrotestosterone-estradiol valerate causes a marked increase in

mean body weight, prostate weight, and P:BW ratio. Increased body weight could be attributed to an increase in appetite stimulated by the herbal mixture. This finding is in agreement with that of Ramani et al. [27] who reported an increase in mean body weight in BPH-induced animals, but in contrast with that of Kim et al. [28] who observed a significant reduction in body weight gain in a BPH model.

A prostatic enlargement as an increase in prostate weight has been used as a vital marker of BPH progression [29]. In this study, there was a significant increase in the prostate weight of animals induced with BPH. The treatment of the animals with the *Yagari* extract decreased the dihydrotestosterone-estradiol valerate-induced increase in prostate weight. This result suggests that the herbal mixture possesses antiproliferative effects. This could be attributed to the apigenin, which has been demonstrated in this investigation to be present in the extract through NMR spectra studies. Apigenin has been reported to inhibit prostate cell proliferation [30]. The relative prostate weight in the present study was the absolute prostate weight to body weight ratio. In this study, BPH induced by dihydrotestosterone-estradiol valerate markedly increased the prostate volume, which is consistent with previous studies [31, 32]. The present study demonstrated that the extract significantly de-



► **Fig. 2** Photomicrograph of sections of a 2a: Prostate of normal control rat, 2b: Prostate induced with BPH, Untreated, 2c: Prostate induced with BPH, Treated with 20 mg/kg b.w of finasteride. 2d: Prostate induced with BPH, Treated with 200 mg/kg b.w of herbal mixture. 2e: Prostate induced with BPH, Treated with 400 mg/kg b.w of herbal mixture. H&E $\times 100$, L = lumen, S = stromal.



► **Fig. 3** HPLC Chromatogram.

creased the relative prostate weight, suggesting that the herbal mixture exerts an attenuating effect on the aberrant growth of the prostate. Histological examination of prostate tissues paralleled the results of prostate weight measurement. The mathematical expression resulting in a remarkable variance in the increase in relative prostate weight, the percentage increase in the relative prostate weight, and the recovery in the prostate weight also infers that the herbal mixture has an antiproliferative effect that is compara-

ble to finasteride. These results are in tandem with those obtained in the studies by Yang et al. [33].

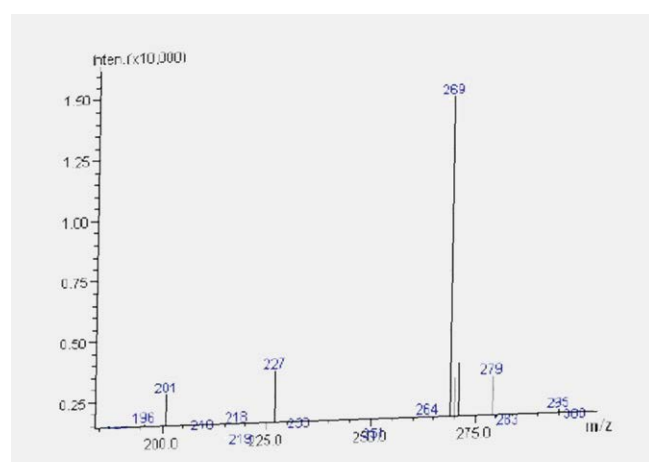
The effect of the *Yagari* extract on urine output of experimentally induced BPH in rats was studied. It was found that urine output was decreased drastically in the DHT-estradiol valerate-treated group due to the enlargement of the prostate gland. In the extract- and finasteride-treated groups, significant decreases in prostatic weights were noted with a increase in urinary output. In most of

the cases, urine output returned to near normal values as on day zero of the study. Obstruction in urine output shown by the DHT-estradiol valerate-treated group was significantly greater than in the extract- and finasteride-treated groups. The group treated with the herbal mixture as well as finasteride exhibited a significant improvement in urine output as compared to the DHT-estradiol valerate-treated group. Obstruction of urinary discharge, painful micturition, reduced urine flow, urinary urgency, etc., are major pathological problems of clinical significance in patients with BPH [34]. The results of the study indicate possible usefulness of the herbal mixture in the stated conditions.

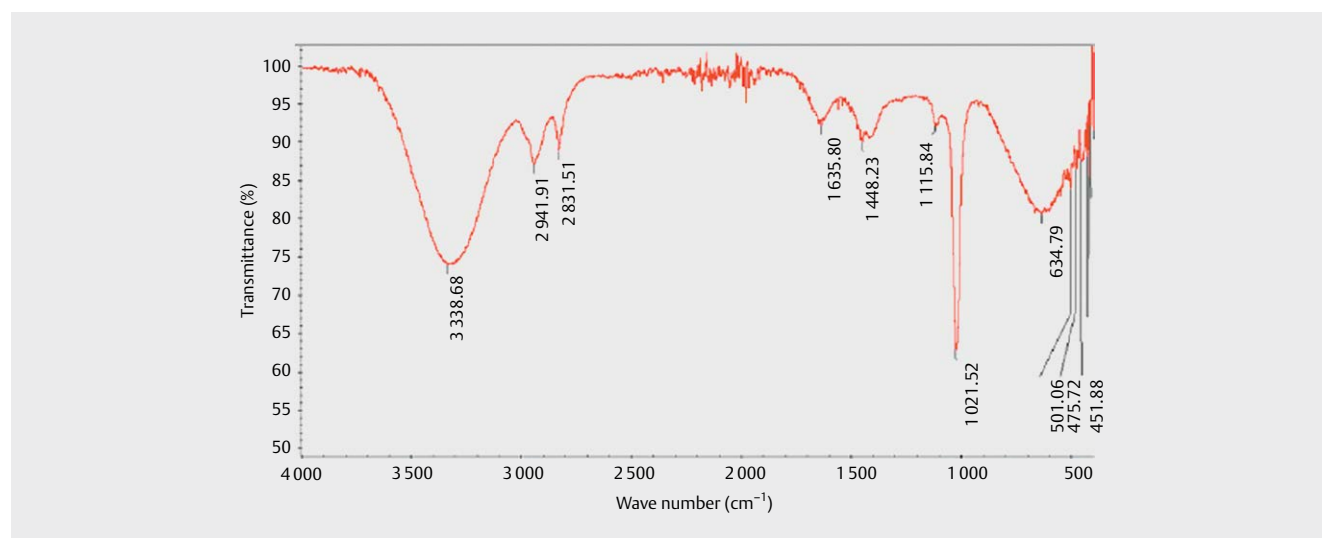
BPH is a frequent cause of obstructive uropathy, which occurs when urine cannot drain through a ureter due to functional hindrance of normal urine flow [35]. The urine backs up into the kidney and causes it to become swollen (hydronephrosis) [35]. In this

study, the effect of the herbal mixture (*Yagari*) extract on kidney function of rats with experimentally induced BPH revealed a significantly high concentration of creatinine and urea relative to the normal control. This increase could be as a result of the proliferation of the prostate tissue, leading to prostatic enlargement that impaired and led to compression and thinning of the renal cortex with an obvious impact on renal functions. Upper urinary tract dilation or hydronephrosis is consistent with chronic renal failure due to obstructive uropathy. This result agrees with that of Homma et al. [36] who reported an increase in kidney status markers in BPH. Reynolds et al. [37] also reported increased serum creatinine concentrations in BPH patients. The significant reduction observed in serum urea and creatinine concentrations of the treated groups compared to the negative control group (untreated group) reveals the ability of the herbal mixture to increase kidney functionality, playing an active role in clearing urea and creatinine off the blood.

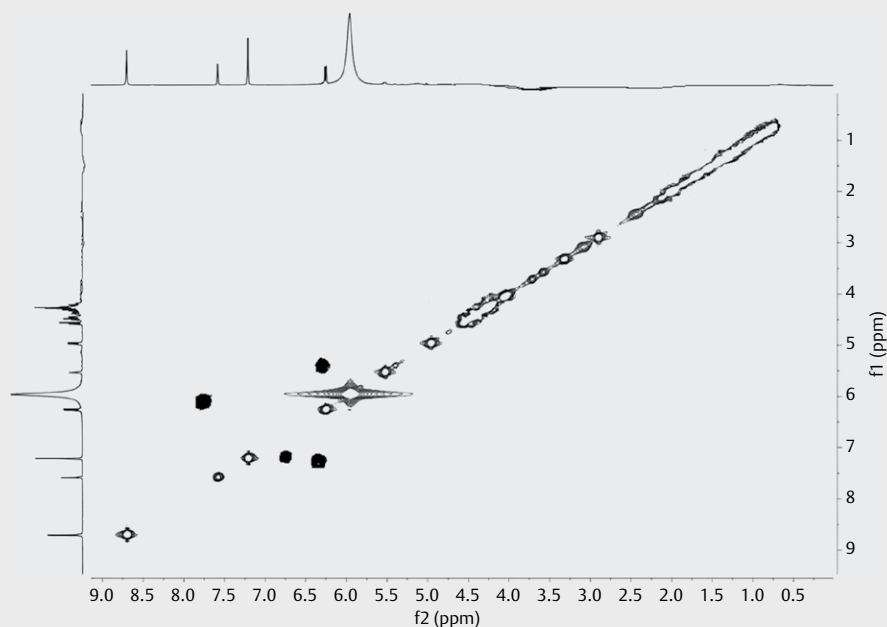
PAP activity was significantly higher ($p < 0.05$) in BPH subjects when compared to the normal control group. PAP is one of the prostatic secretions used as a marker of prostatic disease and as an indicator of treatment progress. Elevations in PAP levels have been reported in animals treated with androgen and estrogen and may be due to increased lysosomal activity [14]. The higher values of PAP activity in BPH-induced groups indicate the increase in secretory activity (due to hyperplasia). Recent advances reveal that cellular PAP functions as a protein tyrosine phosphatase, which causes prostatic cell proliferation under an androgen-reduced condition [38]. Being a prostate epithelial cell differentiation enzyme, serum PAP activity is significantly elevated with BPH. The observations of this study are in agreement with the findings of Ejike and Ezeanyika [13] showing increased PAP activity in BPH-induced rats. The reduction in mean serum PAP activities of the herbal mixture-treated groups and finasteride-treated group when compared to the mean serum PAP activity of the negative control group suggests a positive effect of the herbal mixture on serum PAP activity, which is essential for normal functioning of the prostate gland. This



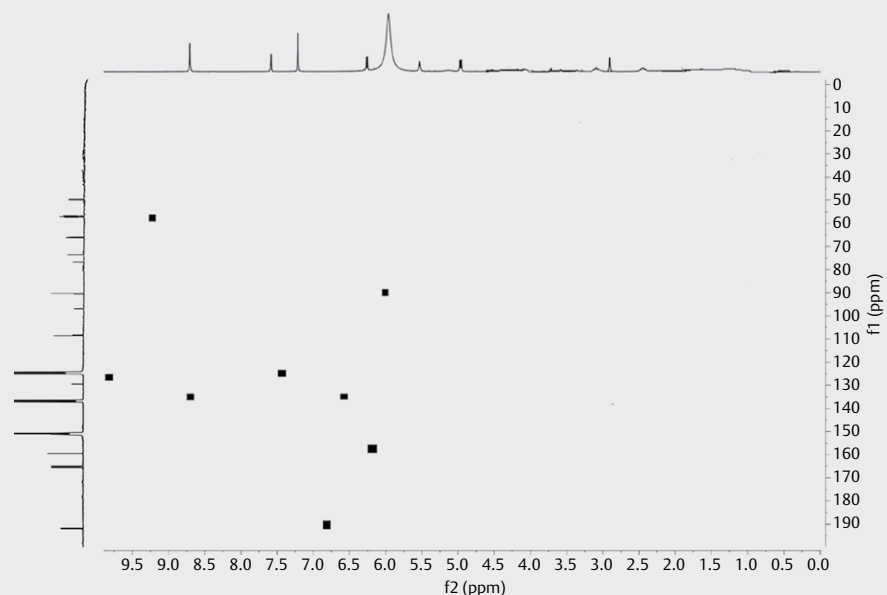
► Fig. 4 ESI-MS Spectra.



► Fig. 5 FT-IR Spectra.



► Fig. 8 COSY Spectra.

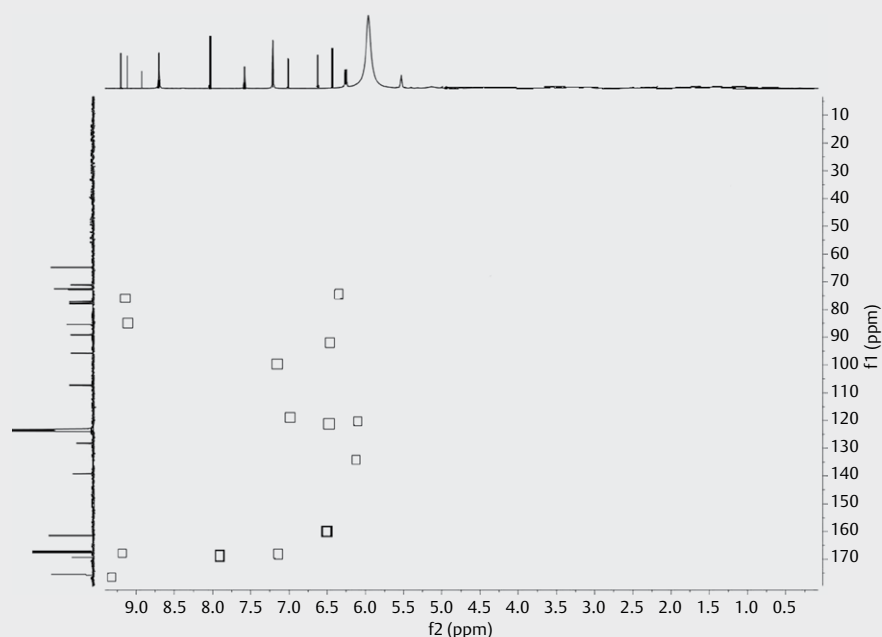


► Fig. 9 HSQC NMR Spectra.

effect also buttresses its effectiveness in forestalling aberrant growth of the prostate and is in tandem with the report of Ejike and Ezeanyika [13].

Steroid 5- α reductase metabolizes testosterone to DHT, which is responsible for BPH and PCa Prostate cancer in older men [39]. In this study, the activity of 5- α reductase was effectively inhibited by treatment with the *Yagari* extract, as shown by the sig-

nificant decrease in the enzyme's activity in animals induced with BPH and treated with different concentrations of the extract. This reduction in steroid 5- α reductase activity in the extract-treated groups, which was also observed in that of the finasteride-treated group, may be attributed to the inhibitory effect of the herbal mixture on DHT-estradiol valerate-induced BPH since there was no significant difference between them.



► **Fig. 10** HMBC NMR Spectra.

► **Table 11** R_f values of the isolated compounds and standard.

Sample	Solvent system (25:5:3 v/v/v)	R_f values
Standard apigenin	<i>n</i> -butanol-water-acetic acid	0.73
Isolated compound (YAF5)	<i>n</i> -butanol-water-acetic acid	0.74
Isolated compound (YAF5)	<i>n</i> -butanol-water-acetic acid	0.74

► **Table 12** Phytochemical composition of *Yagari*.

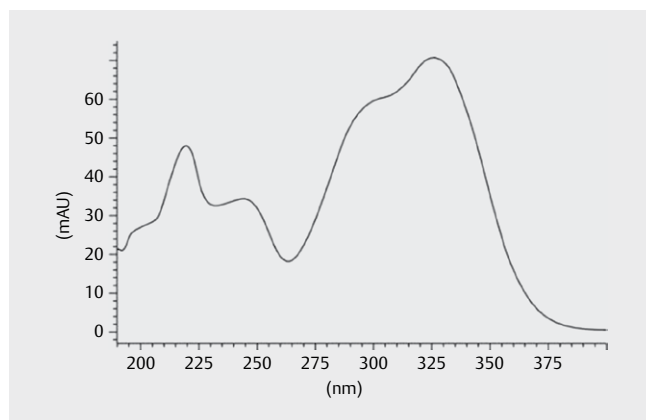
Phytochemical	Relative abundance (mg/g)
Reducing Sugars	72.870 ± 0.003
Tannins	788.420 ± 0.001
Flavonoids	883.949 ± 0.020
Phenols	1740.645 ± 0.004
Steroids	41.580 ± 0.005
Terpenoids	2177.030 ± 0.006
Saponins	63.200 ± 0.002
Glycosides	38.157 ± 0.005
Alkaloids	2333.339 ± 0.006
Results are expressed in means ± SD (n = 3)	

Hormonal alterations have been proposed to be involved in BPH pathogenesis and progression [40]. Indeed, the development of BPH requires the presence of testicular androgens [41]. Testosterone is an important agent in BPH because of its involvement in prostate cell proliferation [42]. The hormonal assays showed that

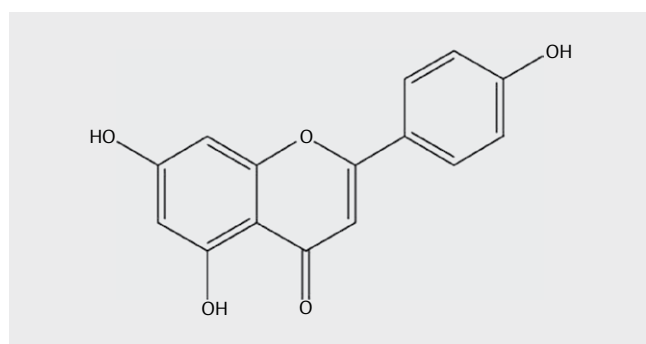
► **Table 13** Acute toxicity study of *Yagari* in mice.

Groups	Dosage (mg/kg body weight)	Mortality (Phase I)
Group 1	10	0/3
Group 2	100	0/3
Group 3	1000	0/3
Groups	Dosage (mg/kg body weight)	Mortality (Phase II)
Group 1	1600	0/3
Group 2	2900	0/3
Group 3	5000	0/3

testosterone concentrations were lower in BPH subjects when compared to the normal control. This finding agrees with that of Morote et al. [43], who reported decreased serum total testosterone concentrations in BPH subjects but found no correlation between prostate-specific antigen (PSA) and testosterone. This significant decrease in the testosterone concentration may be due to conversion of the testosterone to DHT. Treatment of animals with the herbal mixture resulted in an elevation of testosterone concentrations, suggesting inhibitory effects of the herbal mixture on 5- α reductase activity. Steroid 5- α reductase converts testosterone to DHT, an active form of androgen in the prostate; high levels of DHT result in the development of prostatic hyperplasia [29]. DHT has a 10 times higher affinity for the androgen receptor than testosterone and easily binds to the androgen receptor, which stimulates the transcription of growth factors that are mitogenic for the epithelial and stromal cells of the prostate [11]. In this study, the effect of the *Yagari* extract on the DHT concentration in experimentally induced BPH in rats showed a significant ($p < 0.05$) elevation in



► Fig. 11 UV VIS Spectra



► Fig. 11 Apigenin

DHT concentration in groups induced with BPH. Treatment of animals with the extract reduced the concentration significantly ($p < 0.05$) relative to the untreated negative control group (Group 2). This demonstrated the inhibitory potential of the herbal mixture on 5- α reductase activity comparably to the finasteride-treated group. This report is in agreement with the study by Lee et al. [44], who reported a significant decrease in DHT concentration in BPH-induced animals treated with *Melandrium firmum* methanol extract.

In the last few years, the role of prostatic inflammation as a crucial part of BPH pathogenesis and progression has emerged. Interestingly, it has been hypothesized that an inflammatory infiltrate leads to tissue damage and to a chronic process of wound healing that might subsequently determinate prostatic enlargement [45, 46]. The effect of the *Yagari* extract on inflammatory cytokines was considered. The extract was highly effective in reducing the elevated concentrations of inflammatory cytokines (TNF- α and IFN- γ) in BPH-induced groups. TNF- α is a cell-signaling protein involved in systemic inflammation and is one of the cytokines that make up the acute phase reaction. Inflammation is an important feature, since it appears to be involved in the pathogenesis, symptomatology, and progression of BPH [47].

The chronic inflammatory condition may contribute to tissue injury, activate cytokine release, and increase the concentration of growth factors, thus creating a local vicious cycle. In this context, the upregulation of proinflammatory cytokines has been widely re-

ported in prostatic tissues of patients with BPH [48]. TNF- α affects cellular function via activation of inflammatory mediators. The primary role of TNF- α is in the regulation of immune cells. Inflammation-induced damage of the prostatic tissue represents a chronic process of wound healing that activates hyperproliferative processes, resulting in the reactivation of prostatic inflammation. The extract's ability to reduce the concentration of TNF- α demonstrates its therapeutic benefit observed in the treatment of the BPH-induced groups. This could be attributed to the herbal mixture's anti-inflammatory activity due to the presence of flavonoids.

IFN- γ is a potent multifunctional cytokine that is secreted primarily by activated natural killer cells and T cells, originally characterized based on its ability to inhibit viral replication directly and, most importantly, from its immunostimulatory and immunomodulatory effects [49]. It has been shown that IFN- γ exerts antiproliferative, immunoregulatory, and proinflammatory activities [22]. The upregulation of IFN- γ has been reported in BPH tissues [50].

In this study, the concentration of IFN- γ was elevated in the BPH-induced groups probably due to the proliferation of prostate tissue. The IFN- γ concentration increases in response to proliferation. The treatment of groups with the *Yagari* extract significantly reduced the concentration of IFN- γ . By implication, the release of inflammatory cytokines was inhibited, thereby accounting for a decrease in the concentration of IFN- γ . This result is in agreement with that Comalada et al. [51], who reported a decrease in IFN- γ concentrations of BPH-treated rats relative to the negative control, which is animals induced with BPH and untreated.

The PSA is the main tool for early detection, risk stratification and monitoring of BPH [52]. Variations in serum concentration of PSA indicate the severity of prostatic lesion and are used to monitor treatment outcome or response to therapy [53]. In this study, the serum concentrations of PSA in rats treated with dihydrotestosterone-estradiol valerate significantly ($p < 0.05$) increased, indicating prostate hyperplasia. Treatment with the extract decreased the induced elevated concentration of PSA, indicating that the herbal mixture had a protective effect against the development of BPH. This could be due to the presence of apigenin, which was demonstrated in this investigation to be present in the extract through NMR spectra studies (► Fig. 10). Apigenin has been reported to inhibit prostate hyperplasia [54]. This result is in agreement with the reports of Mbaka et al. [55], which showed an increase in serum PSA concentration in induced BPH subjects and a decrease in PSA concentration of treated subjects.

The effect of the *Yagari* extract on the zinc concentration of BPH-induced rats in serum was studied. Zinc is an important regulator of prostate function and plays a unique role in prostate health by helping the prostate cells resist malignant transformation [56]. In this study, the zinc concentration was observed to increase in BPH-induced subjects when compared to normal control subjects. The elevated zinc concentration could be attributed to an increased accumulation of zinc as a result of increased cell production, which occurs in BPH [57]. However, treatment with the extract significantly ($p < 0.05$) reduced the serum concentration of zinc in a dose-dependent manner.

The effect of *Yagari* extract on BPH, as investigated in this study, revealed that the herbal mixture has an upbeat result on prostate enlargement by shrinking urinary obstruction through inhibition

of 5- α reductase, anti-inflammatory activity, and decreased concentration of sex hormones. The fractionation, purification, and characterization of *Yagari* were studied to identify its pharmacologically active principle. The result of the fractionation using column chromatography indicated that the ethanol fraction exerted the highest enzyme inhibitory activity (by 80.60%) on phospholipase A₂ when compared to the aqueous, ethyl acetate, and *n*-hexane fractions. The greater activity of the ethanol fraction could be attributed to the functional chemical composition of the active principle. Ethanol extracts more compounds with polar properties [58]. It could therefore be inferred that the active principle is a polar compound. Previous studies revealed that ethanol can dissolve polar compounds, such as sugars, amino acids, glycoside compounds, and phenolic compounds, with low and medium molecular weights and medium polarity [59]. Ethanol also dissolves aglycon flavonoids and flavones [60]. The extractive capability of phenolic components from herb material is considerably dependent on the type of solvent. The polarity of a molecule is a characteristic value known as the electric moment. Polar substances dissolve in polar substances because they are thermodynamically favorable for the solvent/solute forces [61]. The ethanol fraction was further fractionated with a gradient mixture of ethanol and water, yielding eight pooled fractions. Activity tests on the fractions revealed fraction 5 had the greatest activity on the inflammatory index. Characterization of fraction 5, YAF5 after purification with HPLC, showed 4',5,7-trihydroxyl flavone, trivially known as apigenin.

Apigenin (4',5,7-trihydroxyflavone), which is found in many plants, is a natural product belonging to the flavone class, which is the aglycone of several naturally occurring glycosides. Apigenin has been described for its ability to induce growth arrest on cell proliferation and cell cycle progression [54]. The anti-inflammatory effect of apigenin that is inhibiting the expression of inflammatory mediators and AP-1 factors involved in the inflammation and its importance in the treatment of inflammatory diseases has been demonstrated [62]. Like many other flavonoids, apigenin has been reported to exert anti-inflammatory effects such as lowering oxidative stress and preventing the expression of several inflammatory factors [63]. The protective effects of apigenin in biological systems are ascribed to their capacity to transfer hydrogen or electron free radicals, activate antioxidant enzymes, chelate metal catalysts, reduce α -tocopherol radicals, and inhibit oxidases [64]. The presence of the hydroxyl groups in ring A and a 4'-hydroxyl group in ring B seems to enhance the antioxidant activity in radical assays. The hydroxyl substituents on radical scavengers are considered to be the source of hydrogen atoms that neutralize radical species [64]. The presence of a C-2-C-3 double bond and C-4 ketonic group are two essential structural features in the bioactivity of apigenin. Studies suggest that the protective mechanism of apigenin may be attributed partly to the decreased production of proinflammatory cytokines through the inhibition of COX-2 and NF- κ B activation [65]. Apigenin modified the expression levels of cell cycle regulatory proteins leading to a dose-dependent decrease of cyclin D1 and an increase of p21/WAF1 expression, as determined by immunoblot analysis. As apigenin leads to a decrease of the phosphorylation status of ERK1 and 2, it is possible that the mechanism of apigenin action is mediated through the mitogen activated protein kinases (MAPK) pathway. Studies have demonstrated that apigenin

is an effective inhibitor of aromatase and 17 β -hydroxysteroid dehydrogenase activities and also suppresses prostate cell growth through estrogen receptor β 1 [66]. In response to apigenin treatment, prostatic stromal cells showed a dose-dependent inhibition of cell proliferation, which may be due to cell cycle growth arrest and seems to occur via the MAPK pathway [67]. These results might be indicative of a possible beneficial effect of apigenin in the prevention and/or treatment of BPH.

The results indicate that the herbal mixture could be useful in the management of BPH by decreasing urinary obstruction through inhibition of 5- α reductase, anti-inflammatory activity, and decreasing the levels of sex hormones. Biological activity-guided fractionation of the herbal mixture identified the ethanol fraction as the most active, with 80.60% inhibition activity against phospholipase A₂ activity, while *n*-hexane, ethyl-acetate, and aqueous fractions gave 35.50, 35.60, and 71.20% inhibition activities, respectively. Further fractionation of the ethanol fraction revealed the presence of a biologically active compound, which, on characterization by spectral studies, was found to be apigenin (4',5,7-trihydroxyflavone, molecular weight: 270.24 g/mol and chemical formula: C₁₅H₁₀O₅) with potent beneficial effects in the prevention and/or treatment of BPH.

Material and Methods

The herbal mixture *Yagari* was obtained from a traditional herbal shop in Nsukka, Enugu State, Nigeria, and authenticated by Mr. Alfred Ozioko, an ethnobotanist at the herbarium unit of the Biore-sources Development and Conservation Programme (BDGP) Research Centre, Nsukka, Enugu, with voucher number 900324. The herbal mixture *Yagari* was filtered with muslin cloth via Whatman No. 4 filter paper. The filtrate was concentrated in a rotary evaporator and stored in a refrigerator for further analysis.

Thirty-six male Wistar albino rats, weighing 120–200 g, were procured from the animal house facility of the Department of Pharmacology, University of Nigeria, Nsukka, and handled according to the guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health, USA. The rats were maintained at 25.0 \pm 2 °C on a 12-h light/dark cycle with access to standard animal feed and water *ad libitum* for 7 days before the commencement of the experiment.

All the chemicals and reagents were of analytical grade, and were used as obtained from Crystal Chem, Elabscience, and other chemical suppliers. Finasteride 100 mg (purity > 98%) was purchased from Sigma-Aldrich.

Methods

Studies on the effect of the *Yagari* extract on BPH in rats were carried out. BPH was induced by using a modification [13] of the method of Jeyaraj et al. [14]. The animals (36 male Wistar albino rats, weighing 120–200 g) were acclimatized to laboratory conditions for 7 days and randomly divided into 6 groups of 6 animals each. They were maintained under optimal atmospheric and hygienic conditions and allowed access to both feed and water *ad libitum*. The route of administration was subcutaneous. The groups and doses administered are summarized below.

Group 1 – normal control (vehicle only)

Group 2 – BPH-induced and untreated (negative control)

Group 3 – BPH-induced and treated with 20 mg/kg body weight of the standard drug, finasteride

Group 4 – BPH-induced and treated with 100 mg/kg body weight of *Yagari* extract

Group 5 – BPH-induced and treated with 200 mg/kg body weight of *Yagari* extract

Group 6 – BPH-induced and treated with 400 mg/kg body weight of *Yagari* extract

The animals in Groups 2–6 were administered a 0.2-mL subcutaneous injection of hormones containing 9 mg/kg body weight DHT and 0.9 mg/kg body weight estradiol valerate dissolved in olive oil every other day for 28 days. Animals in Group 1 received a subcutaneous injection of olive oil in place of the hormones. At the end of day 28, the animals (Group 3–6) were treated once per day with the *Yagari* extract and standard drug for 21 days while animals in Groups 1 and 2 received 2% v/v Tween 80 solution, which was used in dissolving the extract and the drug. The route of administration of treatment was oral. At the end of the induction/treatment period, all animals were fasted for 18 h, then blood samples were collected into centrifuge tubes (non-heparinized sample bottles) through the plexus in the eye. Each blood sample was allowed to clot, and the serum was obtained by centrifugation at 3000 rpm for 10 min to enable a complete separation of the serum from the clot. The clear serum obtained as the supernatant was then carefully aspirated with a syringe and used for the analyses below.

Body and prostate weights of benign prostate hyperplasia-induced rats

The body weight of the animals was recorded prior to the commencement of treatment, that is day 28 of induction or day 0 of treatment and at the end of treatment (day 21) as outlined by Nandecha et al. [15]. At the end of treatment, each rat was anesthetized, and the body was promptly dissected. The prostates were carefully excised, freed of external fasciae, washed in cold normal saline, blotted with filtered paper, and weighed on a sensitive balance to determine prostate weight. The mean body weight and the mean P:BW ratio (relative prostatic weight) were calculated for each group of animals as outlined by Ejike and Ezeanyika [16]. Meanwhile, the increase in relative prostatic weight, percentage increase in prostatic weight, and percentage recovery of relative prostatic weight after treatment were calculated using the method described by Nandecha et al. [15]. Relative prostatic weight was calculated as the ratio of the prostate weight to the body weight.

Determination of serum urea and creatinine concentration

The determination of urea in serum was carried out using the Urease Berthelot method according to Fawcett and Scott [17]. Creatinine concentration determination was based on Jaffe's alkaline picrate reaction method using direct endpoint according to Henry [18].

Determination of serum testosterone and dihydrotestosterone concentration

Serum testosterone concentration was determined spectrophotometrically with a Diametra ELISA kit according to the method of Ismail et al. [19]. Serum DHT concentration was determined spectrophotometrically by the method of Bassett [20] with an Alpco ELISA kit.

Assay of prostatic acid phosphatase and steroid 5-alpha reductase activity

The assay of acid phosphatase activity was carried out using the method of Gutman and Gutman [21]. The activity of steroid 5-alpha reductase was assayed according to the method of Gupta et al. [22] using an ELISA kit.

Determination of serum tumor necrosis factor-alpha and interferon-gamma concentration

The concentration of serum TNF- α was determined according to the method of Bonavida [23] using an ELISA kit. The concentration of serum IFN- γ was determined according to the method of Gupta et al. [22] using ELISA kit.

Determination of prostate-specific antigen and zinc concentration

The serum concentration of total PSA was estimated using the ELISA method of Stowell et al. [24]. Serum zinc concentration was determined spectrophotometrically using commercial kits according to the method of Johnsen and Eliasson [25].

Biological activity-guided fractionation of the *Yagari* extract

Fractionation of the *Yagari* extract was carried out with column chromatography (3.2 mm internal diameter and 30 cm length column) and investigating fractions' anti-inflammatory properties through its effect on phospholipase A₂. A concentrated amount (40 g) of the extract was subjected to solvent-guided fractionation in a 2 \times silica gel (120 mesh size) column successively eluted with 2 L of 100% *n*-hexane, ethyl acetate, ethanol, and water. The solvent fractions were collected in large volumes (250 mL) and concentrated in a rotary evaporator under vacuum to yield *n*-hexane, ethyl acetate, ethanol, and aqueous fractions. The fractions were screened for anti-inflammatory properties through their effect on phospholipase A₂ activity. Based on the magnitude of inhibition of phospholipase A₂ activity, the ethanol fraction was the most active. Consequently, the ethanol fraction was subjected to further separation in a silica gel (120 mesh size) column eluted with graded mixtures of ethanol and water (100:0, 80:20, 70:30, 60:40, 50:50, 40:60). The fractions were collected in 25 mL volumes, and based on the TLC profiles, the fractions were pooled and finally eight fractions obtained. Among the eight fractions, fraction 5 showed better anti-inflammatory properties against phospholipase A₂ activity and hence, it was regarded as the Active Fraction, which upon concentration with a rotary evaporator, yielded a yellow crystalline solid compound. The Active Fraction was further purified to represent the entire fractions by an HPLC instrument to yield a subfraction isolate with a single sharp peak in its chromatogram. Collection of the subfraction from the HPLC was guided by

the appearance of the chromatogram on a computer screen. This was collected in a vial and labelled as YAF5. YAF5 was characterized and the structure elucidated using electrospray ionization mass spectrometry (ESI-MS), Fourier transform-infrared (FT-IR), NMR, and UV spectrometry and TLC. Analyses were carried out using an Agilent 1200 HPLC system interfaced with an Agilent 6520 hybrid quadrupole time-of-flight mass spectrometer (Agilent Technologies). The NMR spectra (^1H NMR 400 MHz, ^{13}C NMR 100 MHz) were recorded using Agilent Technologies NMR Avance II 400 spectrometer at a temperature of 298 K. The NMR spectra of ^1H NMR, ^{13}C NMR, COSY, heteronuclear single quantum correlation (HSQC), HMBC, and DEPT were collected in DMSO- d_6 . Mass spectrometry was performed with a Q Exactive Orbitrap MS (Thermo Fisher) using heated an ESI source for the ionization of the target compounds in the negative ion mode.

Histopathological examination

The histopathological examination of the prostate tissue section was carried out according to the method of Culling [26].

Phytochemical analyses of Yagari

The qualitative phytochemical analyses of *Yagari* were carried out according to the methods of Harborne [68], methods as described by AOAC [69], and Trease and Evans [70].

Acute toxicity study of Yagari

The acute toxicity study of the herbal mixture (*Yagari*) was carried out by the method of Lorke [71] to define the range of lethal dose and safe dose for the crude extract. Eighteen albino mice were utilized in the study. They were starved of food for 18 h but allowed access to water prior to the study. The study involved two stages. In stage one, the animals were grouped into three groups of three mice each and were administered 10, 100, and 1000 mg/kg body weight of the herbal mixture, after which they were observed for 24 h for signs of toxicity and/or mortality. Based on the results of the first phase, nine mice were again divided into three groups of three mice each and were also treated with the herbal mixture at doses of 1600, 2900, and 5000 mg/kg body weight, respectively, in the second phase. The animals were observed for 24 h for nervousness, dullness, in-coordination, and/or death. The extract was dispersed in normal saline and administered orally. The median lethal dose (LD_{50}) was estimated as the geometric mean of the maximum dose that caused 0% death and the maximum dose that caused 100% death.

Mathematically, LD_{50} =

D_0 = Highest dose that gave no mortality

D_{100} = Lowest dose that produced mortality

Data and statistical analysis

All data are expressed as the mean \pm SEM and statistical differences between means were determined by one-way ANOVA followed by Duncan's post hoc test for multiple comparison tests using Statistical Package and Service Solution. Values were considered significant at $p \leq 0.05$.

Ethical approval

All experimental protocols, including the use of animal models, were approved and followed the guidelines of the Faculty of Biological Sciences Ethical Committee of the University of Nigeria, Nsukka, Nigeria. The ethical approval number is UNN/FBS/2016-0183.

Conflict of Interest

The authors declare that they have no conflict of interest.

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