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 aging [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 multipharmacological 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 justifies 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 1. 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 1 and 3 of the acute toxicity test (LD50) 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 (170.68 ± 1.54 g, 175.58 ± 1.54 g, and 176.45 ± 2.15 g, respectively). 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...
Thieme

<table>
<thead>
<tr>
<th>Groups</th>
<th>Day 0 (g)</th>
<th>Day 21 (g)</th>
<th>Prostate weight (mg)</th>
<th>P:BW</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>160.78 ± 3.52&lt;sup&gt;a&lt;/sup&gt;</td>
<td>170.89 ± 5.84&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>233.00 ± 8.59&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.36 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>177.24 ± 8.55&lt;sup&gt;a&lt;/sup&gt;</td>
<td>197.46 ± 7.73&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>169.52 ± 8.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.60 ± 0.30&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>171.45 ± 2.62</td>
<td>176.45 ± 4.81&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>518.70 ± 518.70&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.94 ± 0.05&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>159.44 ± 2.58&lt;sup&gt;b&lt;/sup&gt;</td>
<td>170.68 ± 3.30&lt;sup&gt;b&lt;/sup&gt;</td>
<td>979.53 ± 119.44&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>5.74 ± 0.07&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>169.93 ± 4.16&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>175.58 ± 3.45&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>745.05 ± 8.32&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>4.25 ± 0.04&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>6</td>
<td>175.91 ± 6.63&lt;sup&gt;a&lt;/sup&gt;</td>
<td>184.68 ± 8.05&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>572.83 ± 3.96&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.10 ± 0.11&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data are presented as the mean ± SD (n = 6).<sup>a</sup>p<0.05 compared to normal control;<sup>b</sup>p<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.

Data in ▶ Table 1 show the effect of the Yagari extract on the body weight, prostate weight, and prostate:body weight (P:BW) ratio of experimentally induced BPH in rats.

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>Increase in P:BW</th>
<th>% Increase in P:BW</th>
<th>% Recovery in P:BW</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>7.24</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>1.58</td>
<td>21.82</td>
<td>78.18</td>
</tr>
<tr>
<td>4</td>
<td>4.38</td>
<td>60.50</td>
<td>39.50</td>
</tr>
<tr>
<td>5</td>
<td>2.89</td>
<td>39.92</td>
<td>60.08</td>
</tr>
<tr>
<td>6</td>
<td>1.74</td>
<td>24.03</td>
<td>75.97</td>
</tr>
</tbody>
</table>

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.

Data in ▶ Table 2 show the effect of the Yagari extract on the increase of relative prostate weight, percentage increase, and percentage recovery of experimentally induced BPH in rats.

The table reveals that the PAP activity of animals in 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 concentrations 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.
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 32.026 ± 2.319 pg/mL (p<0.05) in TNF-α concentration in Groups 4, 5, and 6, respectively, when compared to Group 2 (38.019 ± 2.980 pg/mL). Similarly, there was a significant (p<0.05) decrease by 34.351 ± 0.901 in Group 3 rats treated with 200 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 (58.019 ± 2.980 pg/mL). 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
significant decrease (p < 0.05) in the serum INF-γ level by 68.815 ± 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 ± 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 ± 0.113, 1.686 ± 0.047, and 1.617 ± 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 ± 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 ± 0.746 μg/dL), increased significantly (p < 0.05) when compared to Group 1, the normal control (22.990 ± 0.868 μg/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 ± 0.399, 3.468 ± 0.024, and 4.414 ± 0.111 μg/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 ± 0.252 μg/dL (p < 0.05) in its serum zinc concentration with respect to Group 2 (27.612 ± 0.746 μg/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 multivacuolated 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

<table>
<thead>
<tr>
<th>Groups</th>
<th>PSA (ng/mL)</th>
<th>Zinc (μg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.492 ± 0.072 A</td>
<td>22.990 ± 0.868 A</td>
</tr>
<tr>
<td>2</td>
<td>3.478 ± 0.149 A</td>
<td>27.612 ± 0.746 A</td>
</tr>
<tr>
<td>3</td>
<td>1.593 ± 0.062 B</td>
<td>23.476 ± 0.494 B</td>
</tr>
<tr>
<td>4</td>
<td>1.872 ± 0.113 A, B</td>
<td>24.552 ± 0.347 A, B</td>
</tr>
<tr>
<td>5</td>
<td>1.686 ± 0.047 A, B</td>
<td>24.144 ± 0.722 A, B</td>
</tr>
<tr>
<td>6</td>
<td>1.617 ± 0.028 A, B</td>
<td>23.198 ± 0.635 A</td>
</tr>
</tbody>
</table>

Data are presented as the mean ± SD (n = 6). *P < 0.05 compared to normal control; †P < 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 7** Effect of Yagari extract on the concentration of inflammatory cytokines of experimentally induced BPH in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>TNF-α (pg/mL)</th>
<th>INF-γ (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>23.688 ± 0.484 A</td>
<td>28.189 ± 1.962 A</td>
</tr>
<tr>
<td>2</td>
<td>58.019 ± 2.980 A</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>23.668 ± 2.079 A</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>31.760 ± 1.833 A, B</td>
<td>130.403 ± 4.952 A, B</td>
</tr>
<tr>
<td>5</td>
<td>27.993 ± 1.314 A, B</td>
<td>115.341 ± 5.169 A, B</td>
</tr>
<tr>
<td>6</td>
<td>25.982 ± 0.661 B</td>
<td>111.247 ± 3.796 A, B</td>
</tr>
</tbody>
</table>

Data are presented as the mean ± SD (n = 6). †P < 0.05 compared to normal control; †P < 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.

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>n-Hexane</th>
<th>Ethanol</th>
<th>Aqueous</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSA (ng/mL)</td>
<td>Mean OD418</td>
<td>Ethanol</td>
<td>Aqueous</td>
</tr>
<tr>
<td>0.5 mg/mL</td>
<td>0.561 ± 0.002 (28.70)*</td>
<td>0.554 ± 0.019 (29.60)*</td>
<td>0.482 ± 0.042 (38.89)*</td>
</tr>
<tr>
<td>1.0 mg/mL</td>
<td>0.535 ± 0.002 (32.03)*</td>
<td>0.509 ± 0.001 (35.30)*</td>
<td>0.180 ± 0.004 (77.10)*</td>
</tr>
<tr>
<td>2.0 mg/mL</td>
<td>0.508 ± 0.002 (35.50)*</td>
<td>0.508 ± 0.002 (35.60)*</td>
<td>0.153 ± 0.007 (80.60)*</td>
</tr>
</tbody>
</table>

Values of absorbance shown are the mean ± 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.
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), 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 in the para-distributed aromatic compound.

The ¹H NMR spectrum in Fig. 6 shows the presence of two meta-coupled aromatic doublets at δ 6.6470 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 ¹³C 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 ¹³C 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 with 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 downshifted peak was 187.27 ppm, which was assigned a ketone group (C-4). The next downshifted peak was 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, ¹³C 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 A₂ activity.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Mean OD₄₁₈ (Mean ± SD)</th>
<th>1.0 mg/mL</th>
<th>0.5 mg/mL</th>
<th>0.2 mg/mL</th>
<th>0.1 mg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.871 ± 0.027</td>
<td>0.871 ± 0.027</td>
<td>0.871 ± 0.027</td>
<td>0.871 ± 0.027</td>
<td>0.871 ± 0.027</td>
</tr>
<tr>
<td>1.0 mg/mL</td>
<td>0.794 ± 0.005 (14.400)</td>
<td>0.543 ± 0.002 (37.70)</td>
<td>0.354 ± 0.012 (66.40)</td>
<td>0.332 ± 0.006 (61.90)</td>
<td>0.332 ± 0.006 (61.90)</td>
</tr>
<tr>
<td>0.5 mg/mL</td>
<td>0.744 ± 0.001 (14.60)</td>
<td>0.438 ± 0.007 (49.70)</td>
<td>0.332 ± 0.007 (62.20)</td>
<td>0.109 ± 0.004 (87.50)</td>
<td>0.109 ± 0.004 (87.50)</td>
</tr>
<tr>
<td>0.2 mg/mL</td>
<td>0.714 ± 0.001 (14.40)</td>
<td>0.383 ± 0.007 (49.70)</td>
<td>0.332 ± 0.007 (62.20)</td>
<td>0.133 ± 0.006 (61.90)</td>
<td>0.133 ± 0.006 (61.90)</td>
</tr>
<tr>
<td>0.1 mg/mL</td>
<td>0.714 ± 0.001 (14.40)</td>
<td>0.383 ± 0.007 (49.70)</td>
<td>0.332 ± 0.007 (62.20)</td>
<td>0.133 ± 0.006 (61.90)</td>
<td>0.133 ± 0.006 (61.90)</td>
</tr>
</tbody>
</table>

Values of absorbance shown are the mean ± 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.
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-
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 comparable 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 an 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. 6 \textsuperscript{1}H NMR Spectra.

Fig. 7 \textsuperscript{13}C 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-alpha 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-alpha reductase was effectively inhibited by treatment with the Yagari extract, as shown by the significant decrease in the enzyme’s activity in animals induced with BPH and treated with different concentrations of the extract. This reduction in steroid 5-alpha 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.
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 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 (PCA) 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-alpha reductase activity. Steroid 5-alpha 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

---

**Table 11** R<sub>p</sub> values of the isolated compounds and standard.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Solvent system (25:5:3 v/v/v)</th>
<th>R&lt;sub&gt;p&lt;/sub&gt; values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard apigenin</td>
<td>n-butanol-water-acetic acid</td>
<td>0.73</td>
</tr>
<tr>
<td>Isolated compound (YAF5)</td>
<td>n-butanol-water-acetic acid</td>
<td>0.74</td>
</tr>
<tr>
<td>Isolated compound (YAF5)</td>
<td>n-butanol-water-acetic acid</td>
<td>0.74</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Phytochemical</th>
<th>Relative abundance (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reducing Sugars</td>
<td>72.870 ± 0.003</td>
</tr>
<tr>
<td>Tannins</td>
<td>788.420 ± 0.001</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>883.949 ± 0.020</td>
</tr>
<tr>
<td>Phenols</td>
<td>1740.645 ± 0.004</td>
</tr>
<tr>
<td>Steroids</td>
<td>41.580 ± 0.005</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>2177.030 ± 0.006</td>
</tr>
<tr>
<td>Saponins</td>
<td>63.200 ± 0.002</td>
</tr>
<tr>
<td>Glycosides</td>
<td>38.157 ± 0.005</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>2333.339 ± 0.006</td>
</tr>
</tbody>
</table>

Results are expressed in means ± SD (n = 3)

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dosage (mg/kg body weight)</th>
<th>Mortality (Phase I)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>10</td>
<td>0/3</td>
</tr>
<tr>
<td>Group 2</td>
<td>100</td>
<td>0/3</td>
</tr>
<tr>
<td>Group 3</td>
<td>1000</td>
<td>0/3</td>
</tr>
<tr>
<td>Group 1</td>
<td>1600</td>
<td>0/3</td>
</tr>
<tr>
<td>Group 2</td>
<td>2900</td>
<td>0/3</td>
</tr>
<tr>
<td>Group 3</td>
<td>5000</td>
<td>0/3</td>
</tr>
</tbody>
</table>

---

**Fig. 10** HMBC NMR Spectra.
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-alpha 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 determine 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 reported 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-alpha 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-trihydroxy 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, 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-alpha 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 (BDCP) 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 ± 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 purchase 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 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. 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 prostate weight) were calculated for each group of animals as outlined by Ejike and Ezeanyika [16]. Meanwhile, the increase in relative prostate weight, percentage increase in prostate weight, and percentage recovery of relative prostate weight after treatment were calculated using the method described by Nandecha et al. [15]. Relative prostate 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 x 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, 13C 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, 13C NMR, COSY, heteronuclear single quantum correlation (HSQC), HMBC, and DEPT were collected in DMSO-d6. 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 (LD50) was estimated as the geometric mean of the maximum dose that caused 0% death and the maximum dose that caused 100% death.

Mathematically, LD50 =

\[ D_0 = \text{Highest dose that gave no mortality} \]

\[ D_{100} = \text{Lowest dose that produced mortality} \]

Data and statistical analysis
All data are expressed as the mean ± 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 ≤ 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.

References


[12] Nwodo OFC, Omeje KO. Personal communication on herbal mixtures for BPH treatment at Nsukka, Enugu State, Nigeria; 2016


Agatemor Uzuazokaro MM, Nwodo Okwesili FC. Biochemical Characterization of the... Planta Med Int Open 2022; 9: e90–e107 | © 2022, The Author(s).


Mishra VC, Allen DJ, Nicolau C, Shafir H, Hudd C, Karim OM. Does intraprostatic inflammation have a role in the pathogenesis and progression of benign prostatic hyperplasia? BJU Int 2007; 100: 327–331


Schoenborn JR, Wilson CB. Regulation of interferon-gamma during the pathogenesis of benign prostatic hyperplasia. Lab Invest 2003; 83: 1131–1146


