Therapeutic effect of aqueous extract from *Ampelocissus africana* (Lour) Merr rhizomes on testosterone-induced benign prostatic hyperplasia in Wistar rats

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**Implication for health policy/practice/research/medical education:**

This article provides scientific evidence of the protective effect of the aqueous extract from *Ampelocissus africana* (AAA) on testosterone-propionate (TP)-induced benign prostatic hyperplasia (BPH) model.


**Abstract**

**Introduction:** *Ampelocissus africana* is a medicinal plant used to treat several diseases, including those affecting the prostate. This study investigated the effects of the aqueous extract of rhizomes from *Ampelocissus africana* (AAA) on a testosterone propionate (TP)-induced benign prostatic hyperplasia (BPH) model.

**Methods:** The effect of the extract at 50, 150, and 300 mg/kg on BPH in male rats was assessed using daily subcutaneous injections of 3 mg/kg TP for four consecutive weeks. After the induction schedule, physical parameters, prostate index (PI), serum biochemical parameters, homogenate antioxidant, enzyme parameters, and prostate histopathological studies were carried out. Triterpenes and sterols were also assessed.

**Results:** Treatment with AAA reduced the PI level in a dose-dependent manner (up to 75.91% at the dose of 300 mg/kg). The inflammatory marker measured in the present study, namely C-reactive protein, increased significantly in the BPH group and improved with the treatments. In addition, AAA reduced oxidative stress by decreasing the malondialdehyde (MDA) level and increasing the catalase (CAT) and superoxide dismutase (SOD) levels in comparison to the positive control group. A histopathological examination corroborated the result of the analysis of the physical and biochemical parameters. The study also showed interesting levels of triterpenes (119.5 ± 4.5 mg UAE/g) and sterols (104.6 ± 3.06 mg CE/g).

**Conclusion:** These results suggest that the aqueous extract of *Ampelocissus africana* could be used as a natural herbal therapy to treat BPH.

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**Keywords:** *Ampelocissus africana* Benign prostatic hyperplasia Antioxidant Anti-inflammation Herbal medicine

**Introduction**

The increasing aging of the world population has recently aroused particular interest in age-related diseases. These diseases have a negative socio-economic impact on the lives of older people (1,2). Benign prostatic hyperplasia (BPH) is arguably one of humanity’s most common male disorders (1,3). Its global prevalence is 26% (4). The etiology of BPH has not been fully elucidated (5,6). However, several authors sustain that various conditions are crucial in developing and progressing...
After obtaining the herbal material, the concentration of uricosuric (URS), anti-inflammatory, and antioxidant activities were determined. The fresh rhizomes of *A. africana* were collected in September 2019 around Dedougou, Burkina Faso. The plant was authenticated and a voucher specimen was deposited at the National Herbarium of Burkina Faso (HNBU) under number 8754. The herbal material was dried at ambient temperature and then crushed into powder with a mechanic grinder.

Thirty grams of the powder was macerated in 300 mL of distilled water, and the extract was frozen and freeze-dried. The extract obtained was stored at 4 °C for various analyses.

### Materials and Methods

#### Chemicals and reagents

Testosterone propionate (TP), hydrogen peroxide (H₂O₂), 2-thiobarbituric acid (TBA), epinephrine, trichloroacetic acid (TCA), ethylenediaminetetraacetic acid (EDTA), Iron dichloride (FeCl₂) were purchased from Sigma-Aldrich (St Louis, USA). Chloroform, and Ethanol were procured from Prolabo (France), Ketamine from CAMEG (Burkina Faso, France), phosphate buffer saline (PBS-tablets) from Medicago (Canada), and Finasteride from Cristers@ (France). Kits used to measure biochemical parameters such as aspartate aminotransferase (AST), blood sugar (BS), alanine aminotransferase (ALT), creatinine (Cr), triglycerides (TAG), total protein (TPr), total cholesterol (TCh), low-density lipoprotein (LDL), high-density lipoprotein (HDL), and C-reactive protein (CRP) were obtained from Cobas 6000, USA.

#### Plant material and extraction

The fresh rhizomes of *A. africana* were collected in September 2019 around Dedougou, Burkina Faso. The plant was authenticated and a voucher specimen was deposited at the National Herbarium of Burkina Faso (HNBU) under number 8754. The herbal material was dried at ambient temperature and then crushed into powder with a mechanic grinder.

Thirty grams of the powder was macerated in 300 mL of distilled water, and the extract was frozen and freeze-dried. The extract obtained was stored at 4 °C for various analyses.

### Dosage of triterpenes and sterols

The total contents of triterpenes and sterols were determined using the vanillin colorimetric assay (21). A spectrophotometer (Agilent 8453, software ChemStation UV-vis) was used to measure the sample at 548 nm against a standard curve of urolic acid (R² = 0.99) and at 640 nm against cholesterol (R² = 0.99), respectively for triterpenes and sterols. Total triterpenes content was expressed in mg urolic acid equivalent per g of the extract (mg UAE/g extract) and total content in mg cholesterol equivalent per g of the extract (mg CE/g extract).

#### Animals care and treatment

Healthy male Wistar rats aged 8 to 12 weeks with an average weight of 350 ± 100 g were procured from the “International Center for Research and Development on Live-stock in the Subhumid Zone” (CIRDES). After 14 days of acclimatization in the Institute of Research in Health Science (IRSS) animal lab at 23-25 °C, with a 12 hours light/dark cycle, the rats were randomly distributed into experimental groups. After obtaining ethics committee approval, the experiment was conducted following international standards as described in the Guide for the Care and Use of Laboratory Animals.

#### Experimental design: Induction of BPH and extract administration

The induction of BPH in the rat model and the experimental procedures were performed according to the modified protocol of Shin et al (5). A total of 42 male rats were divided into six groups (each n = 7) with free access to pellets and water: Group A (normal control group), which received distilled water orally (p.o) and corn oil injected subcutaneously (s.c); Group B (BPH group), which received water (p.o) and TP at a dose of 3 mg/kg body weight (bw) dissolved in corn oil; Group C (positive control group) received finasteride 1 mg/kg (p.o) and TP 3 mg/kg (s.c); Groups D, E, and F (test groups) received the aqueous extract of *A. africana*, respectively, at the doses of 50, 150, 300 mg/kg (p.o) and TP 3 mg/kg (s.c).
All treatments were administered to the animals once daily for four weeks. After the last treatment, the rats were fasted overnight for 18 hours, anesthetized with ketamine 150 mg/kg intraperitoneally, and then euthanized. The blood was drawn by cardiac puncture and then centrifuged at 3000 rpm for ten minutes to separate the serum.

Measurement of physical parameters
The body weight and food and water consumption were recorded weekly throughout the experiment. The prostates and specific vital organs such as the liver, kidneys, and spleen, were removed, immediately weighed, and observed fresh. The relative weight of each organ (RWO) was calculated as the ratio of organ weight to body weight:

\[
\text{The relative weight of each organ (\%)} = \frac{\text{Absolute organ weight (g)}}{\text{Rat weight on the day of sacrifice (g)}} \times 100
\]

PI and percentage of inhibition
The PI and percentage of inhibition of index (IPI) were also determined as follows (7):

\[
\text{The prostate index (mg)} \times \text{Rat weight (g)}
\]

The percentage inhibition of index = 100 – \( \frac{\text{PI}_{\text{TG}} - \text{PI}_{\text{NC}}}{\text{PI}_{\text{BPH}}} \) \times 100

Where PI: Prostate index, TG: treated groups (extract or finasteride), NC: Normal control, \( \text{PI}_{\text{TG}} \): Prostate index of treated group, \( \text{PI}_{\text{NC}} \): Prostate index of normal control group, \( \text{PI}_{\text{BPH}} \): Prostate index of benign prostatic hyperplasia group.

Biochemical parameters analysis
The separated serum served to assay the biochemical parameters namely AST, ALT, Cr, BS, TPr, TAG, TCh, LDL, HDL, and CRP using the commercial kits (Cobas 6000, USA).

Preparation of the homogenates
One of the two prostatic lobes of each rat was rinsed and then ground in a fresh solution of PBS at pH 7.2 at a proportion of 10%. The homogenate obtained was then centrifuged at 12,000 g for 15 minutes at 4 °C (14). The supernatant was recovered and used to carry out the antioxidant activity in vivo: Malondialdehyde (MDA), catalase (CAT), and superoxide dismutase (SOD).

Malondialdehyde assay
The lipid peroxidation was measured by the level of MDA using the thiobarbituric acid reactive substance (TBARS) as previously described (14). 50 µL of FeCl\(_3\) (0.5 mM) and 50 µL of H\(_2\)O\(_2\) (0.5 mM) were added to 1 mL of 10% prostate homogenate. After incubation at 37°C for 30 minutes, 1 mL of TCA (15%) and 1 mL of TBA (0.67%) were added. The mixture was heated in boiling water for 15 minutes, centrifuged at 3000 rpm for 10 minutes after cooling, and then the absorbances were read at 532 nm. The MDA level was expressed in mmol/mg of protein.

Catalase assay
To 10 µL of prostate homogenate was added 190 µL of phosphate buffer (pH 7.0) and 100 µL of substrate 30 mM H\(_2\)O\(_2\) (22). Absorbances were read at a wavelength of 240 nm using a 96-well enzymatic microplate against a blank without substrate. CAT activity was expressed in millimoles (mmol) of H\(_2\)O\(_2\) consumed/mg of protein.

Superoxide dismutase assay
The inhibiting epinephrine-adrenochrome transition method was used to test SOD (23). 500 µL of distilled water was added to 500 µL of prostate homogenate. 250 µL of ice-cold ethanol and 150 µL of chloroform were then successively added to the mixture. After centrifugation, 200 µL of EDTA (0.6 mM), 400 µL of Na\(_2\)CO\(_3\) (0.25 M), 200 µL of epinephrine (3 mM), and 300 µL of water were added. The absorbances were measured using a spectrophotometer (Agilent 8453) at 420 nm. The enzyme activity was expressed in mmol/mg of protein.

Histopathological investigation
The second half of the harvested prostate was fixed with 10% buffered formalin and embedded in paraffin. The standard hematoxylin-eosin staining method allowed histomorphological analysis (24). Sections were visualized under a light microscope (×10) and recorded.

Statistical analysis
Data were expressed as mean ± SEM. Statistical analysis was done by GraphPad Prism version 8.4.3 one-way ANOVA followed by Dunnett’s multiple comparison test. \( P < 0.05 \) was considered significant.

Results
Total triterpenes and sterols contents
Total triterpenes were 119.5 ± 4.5 mg UAE/g with a sterol content of 104.6 ± 3.06 mg CE/g, suggesting that the aqueous extract of A. africana is rich in terpenes.

Protective effect of the extract on BPH
Body weight changes
The evolutions of the average weekly body weight in the rats of control groups and daily treated with finasteride and the aqueous extract of A. africana at doses of 50, 150, and 300 mg/kg for 4 weeks were recorded in. During the experimental period, there was a significant weight gain in all groups (Figure 1).

Water and food consumption
Table 1 presents food and water consumed per group in mg/wk and mL/wk, respectively. The weekly food
consumption of the control and treated rats decreased markedly over the 4 weeks. However, water consumption in all groups increased during the test period.

Despite the decrease in food consumption, the body weight of the animals increased in all the control and treated batches with the extract. The administration of extract at different doses (50 mg/kg, 150 mg/kg, and 300 mg/kg) did not affect the behavior (food and water) of the animals during the test period compared to the NC group.

Evaluation of the prostate enlargement

Induction of BPH increased prostate weight in all groups treated with TP (BPH model) compared to the NC group (Table 2). This suggests that the prostatic hyperplasia was indeed induced. Treatment with finasteride and the extract significantly decreased prostate weight compared to the BPH group. A significant increase in PI was observed in the BPH rats compared to the treated groups. All treatments reduced the PI by more than 50%, as shown in Table 2.

Effect of AAA on the relative organs’ weight

The liver, kidneys, and spleen were removed, observed, and weighed to determine the relative weights of other organs (Table 2). This process was initiated to verify that extract or TP had no toxic effects on vital organs. So, the relative weight of organs did not show any significant difference ($P > 0.05$) among various groups of rats.

Effect of AAA on the biochemical parameters

Figure 2 shows the results of the biochemical tests on the serum of the rats. These results indicate that administering TP and extract on rats for four weeks did not change specific biochemical parameters such as ALT.

Table 1. Weekly food (A) and water (B) consumption of control and treated rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>P</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>FC</td>
<td>1365</td>
<td>1164</td>
<td>1077</td>
<td>942</td>
</tr>
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<td>WC</td>
<td>2335</td>
<td>2850</td>
<td>2740</td>
<td>2880</td>
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<tr>
<td>BPH</td>
<td>FC</td>
<td>1134</td>
<td>1129</td>
<td>1099</td>
<td>938</td>
</tr>
<tr>
<td></td>
<td>WC</td>
<td>1750</td>
<td>2475</td>
<td>2750</td>
<td>2830</td>
</tr>
<tr>
<td>Finasteride</td>
<td>FC</td>
<td>1380</td>
<td>1009</td>
<td>966</td>
<td>893</td>
</tr>
<tr>
<td></td>
<td>WC</td>
<td>2035</td>
<td>2455</td>
<td>2400</td>
<td>2430</td>
</tr>
<tr>
<td>AAA50</td>
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<td>1230</td>
<td>1098</td>
<td>1051</td>
<td>923</td>
</tr>
<tr>
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<td>WC</td>
<td>1935</td>
<td>2385</td>
<td>2385</td>
<td>2700</td>
</tr>
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<td>FC</td>
<td>1247</td>
<td>1201</td>
<td>1016</td>
<td>1171</td>
</tr>
<tr>
<td></td>
<td>WC</td>
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<td>2515</td>
<td>2225</td>
<td>2235</td>
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<tr>
<td>AAA300</td>
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<td>1292</td>
<td>1097</td>
</tr>
<tr>
<td></td>
<td>WC</td>
<td>2050</td>
<td>2760</td>
<td>2570</td>
<td>2415</td>
</tr>
</tbody>
</table>

AAA: *Ampelocissus africana* aqueous extract, AAA50: The extract at 50 mg/kg, AAA150: The extract at 150 mg/kg, AAA300: The extract at 300 mg/kg, BPH: Benign prostatic hyperplasia, P: Parameters, FC: Food consumption (mg/wk), WC: Water consumption (mL/wk).
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Table 2. Effect of *Ampelocissus africana* aqueous extract on relative organs weight (liver, kidney, spleen, and prostate) and prostate enlargement in TP-induced BPH rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Relative weight (RW)</th>
<th>PI</th>
<th>IPI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Kidney</td>
<td>Spleen</td>
</tr>
<tr>
<td>Control</td>
<td>2.43 ± 0.06</td>
<td>0.62 ± 0.01</td>
<td>0.17 ± 0.01</td>
</tr>
<tr>
<td>BPH</td>
<td>2.40 ± 0.09</td>
<td>0.65 ± 0.06</td>
<td>0.16 ± 0.01</td>
</tr>
<tr>
<td>Finasteride</td>
<td>2.39 ± 0.02</td>
<td>0.63 ± 0.05</td>
<td>0.17 ± 0.02</td>
</tr>
<tr>
<td>AAA50</td>
<td>2.47 ± 0.05</td>
<td>0.64 ± 0.01</td>
<td>0.16 ± 0.01</td>
</tr>
<tr>
<td>AAA150</td>
<td>2.43 ± 0.03</td>
<td>0.65 ± 0.01</td>
<td>0.16 ± 0.01</td>
</tr>
<tr>
<td>AAA300</td>
<td>2.44 ± 0.09</td>
<td>0.63 ± 0.03</td>
<td>0.16 ± 0.01</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM (n = 7). ANOWA one-way, Dunnett test: *P < 0.05, ***P < 0.001 against the control group. AAA: *Ampelocissus africana* extract, AAA50: The extract at 50 mg/kg, AAA150: The extract at 150 mg/kg, AAA300: The extract at 300 mg/kg, BPH: Benign prostatic hyperplasia, PI: Prostate index, IPI: Inhibition of prostate index.

Creatinine, fasting BS, TPr, TAG, TCh, and LDL. However, AST decreased in the groups treated with finasteride and *Ampelocissus africana* at 150 mg/kg (Figure 2A). HDL level increased in the group treated with extract at 150 mg/kg (Figure 2B). The inflammatory marker assayed in the present study was CRP. CRP was significantly increased in the BPH group compared to the NC group (Figure 2B).

**Effect of AAA on the lipid peroxidation product and antioxidant enzymes**

As indicated in Figure 3, testosterone caused a significant elevation in the prostate MDA level (*P < 0.001*) compared

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**Figure 2.** Effects of *Ampelocissus africana* aqueous extract on AST, ALT, Cr, BS, TPr (A) and TAG, TCh, LDL, HDL, CRP (B) levels in TP-induced BPH rats. The data represent the mean of seven values. ANOWA one-way, Dunnett test: *P < 0.05, **P < 0.01, ***P < 0.001; ****P < 0.0001 compared with the control group. AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, Cr: Creatinine, BS: Blood sugar, TPr: Total protein, TAG: Triglycerides, TCh: Total cholesterol, LDL: Low-density lipoprotein, HDL: High-density lipoprotein, CRP: C-reactive protein.
to the control group. Interestingly, the administration of extract and finasteride decreased the level of MDA compared to the untreated BPH group. The rats from the BPH model group had low \( P < 0.0001 \) prostatic levels of SOD and CAT compared to the normal control. The administration of extract enhanced the activities of SOD and CAT. As the dose increases, the levels return to normal. Thus, at the dose of 300 mg/kg, there was no significant difference compared to the normal group.

Effect AAA on the prostate tissues by the histopathological examination

The results of the prostate histopathology after hematoxylin-eosin staining are shown in Figure 4. Normal prostate histology is presented in Figure 4A. Rats treated with TP (Figure 4B-F) showed hyperplasia. This hyperplasia was much more marked in the BPH group (Figure 4B).

**Discussion**

Plants are treasures bestowed upon the peoples of the world. Indeed, they produce natural products constituting necessary sources for discovering new molecules that can benefit human health (25). Triterpenes and steroids are compounds found in plants and used in phytotherapy to treat several diseases (26). Previous investigations report that ursolic acid (triterpenoid compound) and β-sitosterol (phytosterol) are effective remedies for managing BPH (5,8). The high content of triterpenes and sterols could be attributed to the extract of *Ampelocissus africana*’s protective properties against BPH. In the present study, the Wistar rats were used for the BPH induction test using TP (13). The enlargement and the increase in prostate weight are vital markers in the BPH model (12,27). Daily injection of TP for 4 weeks substantially increased the relative prostate weight and the levels of PI in BPH rats.

Finasteride, a 5a-reductase inhibitor, is the representative therapeutic agent for BPH; it is known to reduce prostate size (28). AAA and finasteride significantly inhibited PI increase compared to the BPH model.

Some enzymes and proteins, such as ALT, AST, and TPr, can indicate hepatocellular effects. Also, creatinine is considered to be a biomarker of nephron functional lesions (29). AAA at 150 mg/kg treatment showed no significant difference in liver and kidney-specific parameters except a reduction in AST. According to Affy et al, the decrease in AST could exhibit a hepatoprotective action (30). These data confirm the macroscopic observations (relative weight) of the liver and kidneys mentioned in the present study.

Inflammation plays a crucial role in the pathogenesis of BPH (31). CRP increases significantly in response to injury, infection, and inflammation (32). The increase of CRP levels in the BPH rats made it possible to highlight the presence of inflammation. AAA improved CRP levels dose-dependently. The extract has shown anti-inflammatory effects in vivo and in vitro by inhibiting lipoxygenase (19,20).

Oxidative stress is a contributing factor to inflammation (33). It is defined as a cellular condition that occurs when there is an imbalance between ROS production and antioxidants in the body (34). Wu et al claimed that oxidative stress was involved in prostatic hyperplasia. This is often accompanied by an increase in MDA levels and a decrease in antioxidant enzymes (such as SOD and CAT) activities (11). It has been verified with BPH rats in this experiment. AAA successfully reversed changes in antioxidant enzymes and MDA levels. CAT and SOD protect prostate cells, which activate the body’s defense system against the damage caused by increased lipid peroxidation (35,36). These results confirm the antioxidant effect in vivo of the extract elucidated in the previous studies (19).

Previous observations have shown that the prostate's size increases due to a complex interference of mechanisms (37). Among these mechanisms is the change in the histoarchitecture of the prostate. Histologically, BPH is characterized by the proliferation of fibromuscular cells associated with the nodular epithelial hyperplasia of the central paraurethral regions of the prostate (38). Indeed, the external administration of TP produces histological changes in the prostate tissue. This increases prostatic acinar and stromal areas, proving the induction of prostatic hyperplasia in rats (39). The rats treated with testosterone showed prostatic hyperplasia with a flattened endoluminal epithelium compared to normal control animals, characterized by well-defined cells with normal glands showing a scalloped lumen and columnar epithelium. (38) reporting that at low magnification, BPH is by the presence of the cystic glands in the central part of the prostatic epithelial tissue (Figure 4B). Treatments
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with finasteride and AAA improved the development of the cystic areas produced by testosterone, and the glands appeared less cystic with an architecture tending towards normal. These histological data corroborate the biochemical and prostate enlargement results.

\textbf{Conclusion}

This research revealed that the oral administration of the aqueous extract of \textit{A. africana} for 28 days in a BPH rat model significantly reduced prostate hyperplasia. The protective effect of the extract is probably due to the reduction of oxidative stress, the regulation of CRP levels, and the improvement of the histoarchitecture of prostate tissue. This study also indicates that the extract inhibits the activity of phospholipase \textit{A$_2$}. These findings could be helpful in BPH, although further work is needed to elucidate the complete pharmacological mechanisms of action of \textit{A. africana} rhizomes.

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\textbf{Conceptualization}: Wendkouni Leila Marie Esther Belem-Kabré, Noufou Ouédraogo.


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\textbf{Supervision}: Noufou Ouédraogo.

\textbf{Validation}: Ollo Da, Assita Lamien-Sanou, Noufou Ouédraogo.

\textbf{Writing original draft}: Wendkouni Leila Marie Esther Belem-Kabré.

\textbf{Writing, review & editing}: Ollo Da, Lazare Belemnaba, Assita Lamien-Sanou, Noufou Ouédraogo.

\textbf{Conflict of interests}

The authors declare no conflict of interest.

\textbf{Ethical considerations}

The protocol of this study was reviewed and approved by the ethics committee on protecting animals at University Joseph KI-ZERBO under the approval number: CEEA-UJKZ/2020-05.

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The authors declare that they have not received any financial

\textbf{References}


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