

# Pharmacological Effects of the Lipidosterolic Extract from *Kigelia africana* Fruits in Experimental Benign Prostatic Hyperplasia Induced by Testosterone in Sprague Dawley Rats

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**Background:** The use of phytotherapies is very frequent in men with prostatic diseases, sexual disorders and infertility, and many associations are commercially available. Various vegetable products used as drugs or nutraceuticals are attributed to possess the capacity to exert benefic effects on the reproductive system, and most of these drugs have a rich and varied lipidosterolic fraction, primarily responsible for the effects related to the male genital sphere. *Kigelia africana* (Lam.) Benth. (Bignoniaceae) is a plant used in African folk medicine as a vegetal remedy for various diseases, including some disorders of the male reproductive system; however, its potential activities have not yet been fully explored. The aim of the present study was to investigate whether the lipidosterolic hexane extract (LHE) from *K. africana* fruits, analyzed by comprehensive two-dimensional gas chromatography-mass spectrometry/flame ionization detection (GC×GC-MS/FID), can prevent or reverse benign prostatic hyperplasia (BPH) in rats.

**Methods:** BPH was induced in experimental groups by daily subcutaneous injections of testosterone propionate (TP) for four weeks.  $\beta$ -sitosterol ( $\beta$ -s) was used as positive control. On day 28, the animals were sacrificed by cervical dislocation after anesthesia. Prostates were excised, weighed, and used for macroscopic and histological studies. Testosterone and dihydrotestosterone (DHT) levels in prostate were measured.

**Results:** The results showed that LHE significantly reduced the prostatic weight, prostatic index, prostatic levels of testosterone and DHT, and the histopathological alterations (including the epithelial thickness, stromal proliferation, and lumen area) induced by testosterone. These effects were superior to those demonstrated by  $\beta$ -s and appear to be due to a partial antiandrogenic activity of LHE.

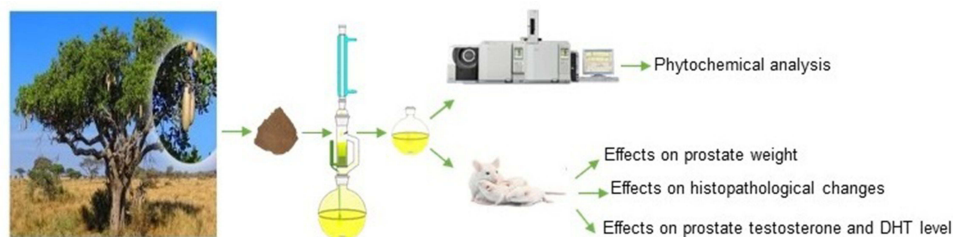
**Conclusion:** The results obtained showed that the LHE can prevent, and reverse testosterone induced prostatic hyperplasia, and support the traditional use of *Kigelia africana* in some disorders of the reproductive system.

**Keywords:** *Kigelia africana*, hexane extract, benign prostatic hyperplasia, GC×GC-MS/FID, histological studies

## Introduction

Benign prostatic hyperplasia (BPH) is a frequent condition seen in senior men, characterized by a non-cancerous prostatic swelling associated with urinary tract disorders including difficulty in starting urination, urinary urgency and frequency, incomplete bladder emptying, urinary retention and histomorphological changes, mainly due to the proliferation of luminal epithelial cells and fibromuscular tissue.<sup>1</sup> Androgens have a facilitating role in the development and growth of the prostate. Dihydrotestosterone (DHT) is an important intermediary of prostate growth, which is produced in the prostate from hematic testosterone by 5 $\alpha$ -reductase. DHT production increases with aging and leads to hyperplasia and increased growth of the prostate.<sup>2</sup> 5 $\alpha$ -reductase inhibitor drugs (such as finasteride) significantly reduce prostate size and the DHT content in the prostate and have been shown to be effective drugs for the treatment of BPH.<sup>3</sup>

## Graphical Abstract



The use of commercial natural products is very frequent in men with prostatic diseases and sexual disorders. Various vegetable products used as drugs or nutraceuticals are attributed to possess the capacity to exert benefic effects on the reproductive system, and most of these drugs have a rich and varied lipidosterolic fraction, primarily responsible for the effects related to the male genital sphere. The use of phytosterols as treatment for urinary tract symptoms due to a prostatic disease has constantly increased.<sup>4</sup> *Kigelia africana* (Lam.) Benth. (Bignoniaceae) is a plant used in African folk medicine as a vegetal remedy for various diseases (malaria, diarrhea, psoriasis), including some disorders of the male reproductive system (treatment of erectile dysfunction, sexual asthenia and infertility), but its potential activities have not yet been fully explored. The main phytochemical constituents of *K. africana* fruits are iridoids, naphthoquinones, lignan, flavonoids, as well as phytosterols and fatty acids.<sup>5,6</sup>

The aim of the present study was to investigate whether the lipidosterolic hexane extract (LHE) from *K. africana* fruits, identified and quantified (one-point calibration) by comprehensive two-dimensional gas chromatography-mass spectrometry and comprehensive two-dimensional gas chromatography-mass spectrometry/flame ionization detection (GC×GC-MS and GC×GC/FID), can prevent or reverse benign prostatic hyperplasia induced by testosterone in rats.

## Materials and Methods

### Plant Material

Collection of plant material was carried out in accordance with guidelines provided by the University of Messina and West African guidelines. Mature fruits of *K. africana* were collected, in Gallè village (Mali) in June 2016 and were identified at the Department of Traditional Medicine (DMT), University of Bamako (Mali). The fruits were air-dried, pulverized, and stored in airtight containers under vacuum until the preparation of the extract. A voucher specimen (nr. 03128) was deposited in the Department of Chemical, Biological, Pharmaceutical and Environmental Sciences of the University of Messina (Italy).

### Preparation of the Extract

60 g of fruits were exhaustively extracted in a Soxhlet extractor with hexane (500 mL) for 8 hours. The crude extract obtained was then filtered and concentrated to dryness under vacuum with a rotating evaporator (Buchi R-205). The lipidosterolic extractive residue obtained was 1.65g (2.75% of dry drug). The residue was stored in sealed bottles under vacuum at a low temperature. The residue preserved in such a manner remains stable and its chemical composition does not vary over time, as demonstrated by the phytochemical analysis performed at different time intervals. In order to prepare the test solutions, the residues were suspended in Tween 80 at the desired concentrations, immediately before use.

### Phytochemical Analysis

The lipidosterolic residue was saponified with a KOH/ethanol solution, and with heating at 80°C under reflux. The unsaponifiable fraction was extracted with diethyl ether. The extraction was repeated twice. The organic solution was

dried with anhydrous  $\text{Na}_2\text{SO}_4$ , filtered then concentrated to dryness with a rotating evaporator. The unsaponifiable dry extract was 0.82g (49.7% of the dry lipid extract). The fatty acid (FA) composition in the saponifiable fraction was investigated by using gas chromatography-quadrupole mass spectrometry, with 11 FAs subjected to identification (as methyl esters).

The unsaponifiable fraction, dissolved in 500  $\mu\text{L}$  of chloroform, was treated with 200  $\mu\text{L}$  of BSTFA [N, O-bis(trimethylsilyl) trifluoroacetamide] (1% TMCS - trimethylchlorosilane) and 200  $\mu\text{L}$  of pyridine, and then heated at 70°C for 20 min. The derivatized sample was then ready for GC injection. The BSTFA + 1% TMCS kit was kindly supplied by Sigma–Aldrich (Milan, Italy).

### Qualitative Analysis

Analysis of the unsaponifiable fraction of *K. africana* lipids was carried out by comprehensive two-dimensional gas chromatography-mass spectrometry (GC×GC-MS), using a Shimadzu GC×GC system, consisting of two independent GC2010 gas chromatographs and a QP2010 Ultra quadrupole mass spectrometer (Shimadzu, Kyoto, Japan). The primary GC system was equipped with an AOC-20i auto-injector, a split-split-less injector (350°C), and a cable extension for the MS connection (due to the presence of the second GC oven).

Data were acquired by using the GCMS solution software ver.4.41 (Shimadzu). Bidimensional chromatograms were generated by using the Chrome-Square software ver. 2.3 (Shimadzu Europe, Duisburg, Germany). Mass spectral identification was performed through the use of a sterols database (in-lab constructed) together with the support of the linear retention indices (LRIs) obtained injecting a C7-C40 alkanes (Sigma–Aldrich) standard solution.

### Quantitative Analysis

The flow-modulation GC×GC-FID applications were carried out on a GC2030 Nexis gas chromatograph. Semi-quantification was performed using an external standard solution of stigmaterol trimethylsilyl ether (purity 95%), at a concentration of 50 ppm (v/v), in chloroform. Hence, single-point calibration was performed. The procedure used to obtain the silanization of the standard compound has been reported previously.<sup>7</sup> Absolute quantities of the sterols and triterpenic alcohols contained in the unsaponifiable fraction of *K. africana* were determined.

## Animals and Experimental Design

Male Sprague-Dawley rats, weighing 210–240 g, were used (Envigo RMS of Udine, Italy). The animals were kept under standard laboratory conditions (12 h light: 12 h dark and at  $23 \pm 2^\circ\text{C}$  with a relative humidity of 40–60%). They were allowed unrestricted access to water and standard laboratory diet (rat pellets). Animal care and experimental procedures were approved by the animal welfare Committee of the University of Messina (ID 16/2016) and the Italian Ministry of Health (authorization number 814/2016 PR). The animals were handled throughout the experiment according to international laboratory animal use and care guidelines.<sup>8</sup>

BPH was induced in experimental groups by daily subcutaneous injections of testosterone propionate (TP, Sigma Chemical Co., St. Louis, MO), for four weeks.  $\beta$ -sitosterol ( $\beta$ -s) was used as positive control. A first series of experiment was conducted to investigate the ability of LHE to prevent BPH, using five groups of 5 rats for group: testosterone group (5 mg/kg b.w., solubilized in soy oil, for 4 weeks); LHE groups (administered daily orally at a dose of 10 or 100 mg/kg b. w. suspended in Tween 80, along with the TP injections, for 4 weeks);  $\beta$ -s group (administered daily orally at a dose of 1 mg/kg b.w. together with the TP injections, for 4 weeks); negative control group (no treatment); vehicle control group (vehicle, Tween 80, by oral gavage and soy oil by subcutaneous injection). The dosage of 10 and 100 mg/kg of LHE was chosen based on the positive results obtained from our preliminary research. The second series of experiments was carried out, in two groups of 5 animals, to investigate the ability of LHE to reverse BPH. The rats were treated daily with testosterone. After 2 weeks of testosterone-treatment, the rats were orally treated with LHE (100 mg/kg b.w) or  $\beta$ -s (1 mg/kg b.w) together with the TP injections for other 2 weeks. The control animals were treated with only vehicle (Tween 80, by oral gavage and soy oil by subcutaneous injection). All rats were weighed prior to the start of the experimentation and subsequently were weighed weekly during the experiment. The administered dose/animal was calculated based on the most recent recorded body weight of individual animals. Twenty-four hours after the last

administration, the animals were weighted and sacrificed by cervical dislocation after anesthesia. Whole prostates were immediately removed, and weighted. Relative organ weights (prostate index) were calculated as the ratio of prostate weight (g) to body weight (g) x 100. Ventral prostate lobe was fixed with 10% neutral buffered formalin and embedded in paraffin for histological studies. The remainder of each prostate was stored at  $-20^{\circ}\text{C}$  and used to evaluate testosterone and DHT levels.

## Macroscopic Examination

After excision, entire prostates were examined for any macromorphological alterations, and the prostate sizes were compared in all the experimental groups.

## Histological Examination

After 24 hours of fixation in formalin, prostates were embedded in paraffin and sectioned (5- $\mu\text{m}$  thick sections were cut) using a Rotary Microtome RM2125RT (Leica Instruments Nussloch Germany). The sections were stained with haematoxylin and eosin for microscopic observation of structural changes in the prostates, using a Nikon Ci-L microscope (Nikon Instruments Tokyo Japan). Each section was examined, and photomicrographs were taken. All low magnification images were obtained with a 10X objective, while the high magnification micrographs with a 40X objective.

## Determination of Testosterone and DHT Concentrations in Prostate

Testosterone and DHT concentrations in the prostate were determined using enzyme-linked-immunosorbent assay (ELISA) and values were expressed for mg of total protein in prostate. In summary, prostates were homogenized (1/10 w/v) with a tissue lysis/extraction reagent that contained a protease inhibitor mixture (Sigma). Homogenates were centrifuged at 12,000 rpm, g-force 11,270, for 30 min at  $4^{\circ}\text{C}$ , and the protein concentration in the supernatant fractions were determined using Bradford reagent (Sigma).

## Acute Toxicity

The acute toxicity was assessed by daily clinical observation of the behavior and of physiological functions of all animals during the treatment period using the Irwin test.<sup>9</sup>

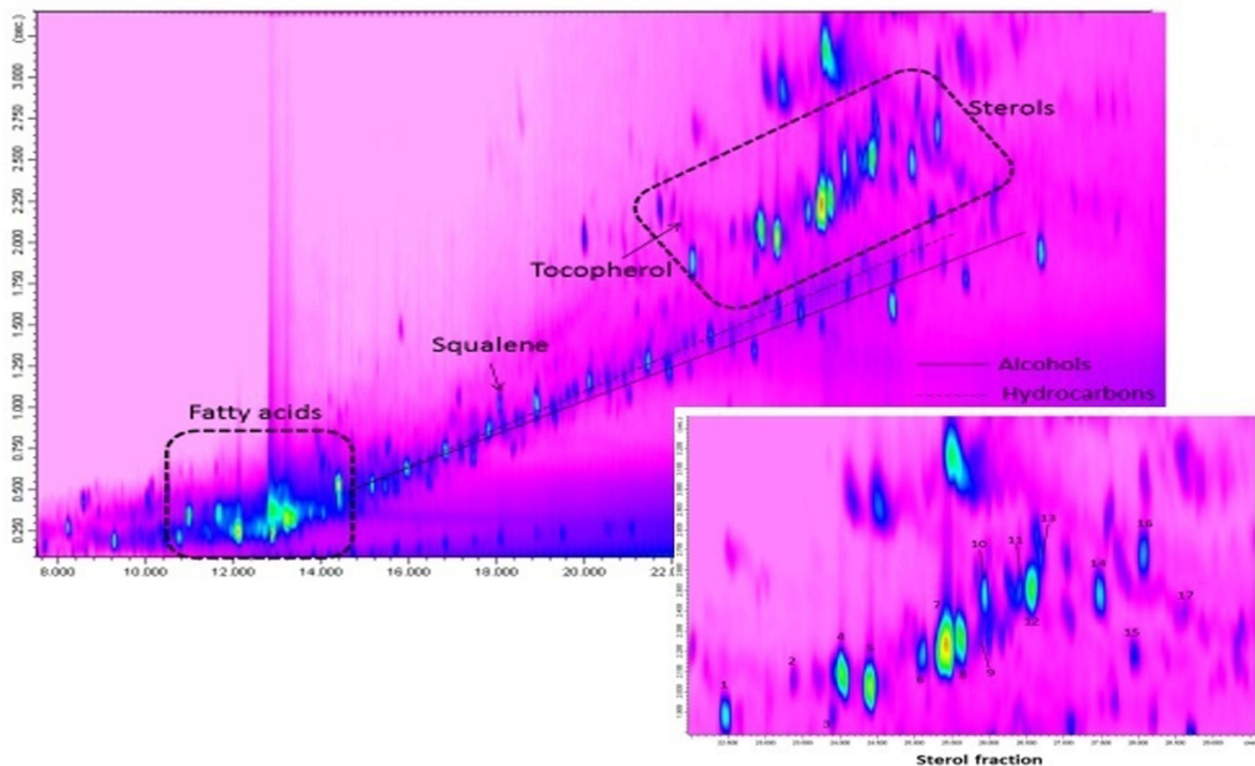
## Statistical Analysis

All data are expressed as mean  $\pm$  SD. Statistical analysis was performed using both one-way variance analysis (ANOVA) and Dunnett's *t*-test. Values of  $p < 0.05$  were considered as statistically significant.

## Results

### Phytochemical Examination

The phytochemical analyses showed that the lipidosterolic extract from *K. africana* fruits, contained numerous sterols. In particular, 17 sterols were identified (Figure 1):  $\beta$ -sitosterol, stigmasterol, campesterol, cholesterol, lathosterol, campestanol, clerosterol,  $\Delta^{-5}$ -avenasterol,  $\Delta^{-7}$ -stigmasterol (4- $\alpha$ -methylsterols), citrostadienol (4-des-methylsterols),  $\beta$ -amyirin,  $\alpha$ -amyirin, cycloartanol, 24-Me-cycloartanol, parkeol (4,4-dimethylsterols), erythrodiol and uvaol (triterpenic dialcohols). The absolute quantities and the relative percentage abundances of the sterols and triterpenic alcohols contained in the unsaponifiable fraction of *K. africana* are reported in Table 1. In the lipidosterolic extract, the overall content of phytosterols was found to be 24 mg (1.46%), with an individual content of  $\beta$ -sitosterol of 13.6 mg (0.83%). The results of the phytochemical analysis also showed that the lipid extract from *K. africana* fruits contains numerous fatty acids such as long chain and unsaturated fatty acids. The most abundant FAs, in a decreasing order, were linolenic, linoleic and oleic acids. Saturated FAs were present in low quantities and were represented mainly by palmitic and stearic acid (Table 2).



**Figure 1** Comprehensive 2D GCxGC-MS chromatographic expansion highlighting the sterols contained in the lipid extract of the fruits of *K. africana*.

## Effects of LHE on Prostate Weight

Testosterone-induced BPH group showed a significant increase in relative prostate weight ( $P < 0.01$ ) compared with the vehicle control group (Table 3). The co-treatment (for 4 or 2 weeks) with plant extract at a concentration of 10 mg/kg reduced prostatic index in an insignificant manner whereas at 100 mg/kg (which contains an almost equivalent amount of  $\beta$ -s) the prostatic index was significantly reduced ( $p < 0.05$ ) as compared to the testosterone group. In addition, also PI values for the ( $\beta$ -s) groups were all significantly lower than those of the testosterone group ( $p < 0.05$ ).

**Table 1** Absolute Quantities and Percentual Relative Abundances of the Sterols and Triterpenic Alcohols Contained in the Unsaponifiable Fraction of *K. africana* Fruits

N°	Compound	$\mu\text{g}/\text{mg}$	%*
1	Cholesterol	0.30	1.01
2	Lathosterol	0.02	0.05
3	Campestanol	0.04	0.14
4	Campesterol	1.42	4.86
5	Stigmasterol	3.43	11.71
6	Clerosterol	0.29	0.98
7	$\beta$ -Sitosterol	16.60	56.57
8	$\Delta^3$ -Avenasterol	0.83	2.83
9	Parkeol	0.16	0.53
10	$\beta$ -Amyrin	0.67	2.30

(Continued)



**Table 1** (Continued).

N°	Compound	µg/mg	%*
11	Δ <sup>7</sup> -Stigmastenol	0.16	0.56
12	Cycloartanol	3.68	12.54
13	α-Amyrin	0.13	0.43
14	24-Me-Cycloartanol	0.78	2.66
15	Erythrodiol	0.20	0.69
16	Citrostadienol	0.29	0.98
17	Uvaol	0.34	1.17

Note: \*Percentage related to the compounds listed in the table.

**Table 2** Qualitative Composition of Fatty Acids Contained in the Hexane Extract of *K. africana* Fruits. The Similarity Values of the MS Database, the Experimental Linear Retention Index Values and Database are Reported

Compound	Match	LRI <sub>exp</sub> [FAMES]	LRI <sub>lib</sub> [FAMES]	ΔLRI	LRI <sub>exp</sub> [FAMES]	LRI <sub>lib</sub> [FAMES]	ΔLRI
1. Me. C14:0; Myristate	*	1400	1400	0	1363	1362	1
2. Me. C16:0; Palmitate	95	1600	1600	0	1565	1563	2
3. Me. C17:0; Heptadecanoate	80	1702	1700	2	1666	1664	2
4. Me. C18:0; Stearate	95	1800	1800	0	1766	1765	1
5. Me. C18:1n9; Oleate	94	1820	1819	1	1786	1784	2
6. Me. C18:2n6; Linolelaidate	91	1870	1874	-4	1834	1838	-4
7. Me. C18:3n3; Linolenate <alpha-, methyl->	93	1934	1928	6	1901	1893	8
8. Me. C20:0; Arachidate	87	2000	2000	0	1968	1967	1
9. Me. C20:1n9; Eicos-(11Z)-enoate	*	2021	2022	-1	1988	1989	-1
10. Me. C22:0; Behenate	*	2201	2200	1	2168	2167	1
11. Me. C24:0; Lignocerate	*	2401	2400	1	2370	2369	1

Note: \*Database match lower than 80%.

Abbreviations: Match, MS database; LRI exp FAMES, experimental linear retention index values; LRI lib FAMES, linear retention index values and database.

**Table 3** Effects of Lipidosterolic Hexane Extract from *K. africana* Fruits and of β-Sitosterol on Body and Prostate Weights in Testosterone Treated Rats for Subcutaneously Route

Treatment	Dose (mg/Kg/Oral)	Body Weights (g)		Prostate Weights	
		Initial	Final	Absolute Weight (g)	Relative Weight (%)
Negative control	–	228.0 ± 4.0	275.5 ± 6.30	0.455 ± 0.08	0.17 ± 0.07
Vehicle control	–	224.6 ± 3.5	280.4 ± 7.20	0.448 ± 0.04	0.16 ± 0.02
Testosterone	5 (s.c.)	221.6 ± 2.3	288.4 ± 8.73	0.942 ± 0.09*	0.33 ± 0.03*
β-s + TP (co-treatment for 4 weeks)	1+5	222.8 ± 7.3	292.2 ± 7.20	0.857 ± 0.1**	0.29 ± 0.02**
LHE + TP (co-treatment for 4 weeks)	100+5	225.0 ± 6.24	299.2 ± 9.75	0.780 ± 0.1**	0.26 ± 0.02**
β-s + TP (co-treatment for 2 weeks)	1+5	224.2 ± 8.40	297.4 ± 11.00	0.789 ± 0.06**	0.27 ± 0.01**
LHE + TP (co-treatment for 2 weeks)	100+5	223.0 ± 5.17	299.8 ± 6.47	0.732 ± 0.08**	0.25 ± 0.01**

Notes: \*p<0.01 Vs vehicle control group; \*\*<0.05 Vs testosterone group (n=5).

Abbreviations: LHE, lipidosterolic hexane extract; β-s, β-sitosterol; TP, testosterone propionate; S.C., subcutaneously route.

## Macroscopic Examination

Macroscopic examination of the prostate revealed that rats treated with only testosterone showed increases in prostate size compared visually with the control group. The prostate was enlarged, especially in the transition area, with fibroadenomatous nodules, while the fibromuscular part was peripherally displaced without tissue infiltration. On the

other hand, treated rats with LHE or  $\beta$ -s displayed a marked reduction in prostate sizes and did not show other morphological alterations, compared visually with the testosterone-group (Figure 2). Considering that the weight gain of the prostate is a valid parameter for the evaluation of its size, these results are in agreement with the statistical comparison of the various groups relating to the weight of the prostate shown in Table 3, which, in fact, shows that the weight of the prostate was significantly increased in the TP group compared to the control group, while it was significantly reduced by the administration of LHE and  $\beta$ -s.

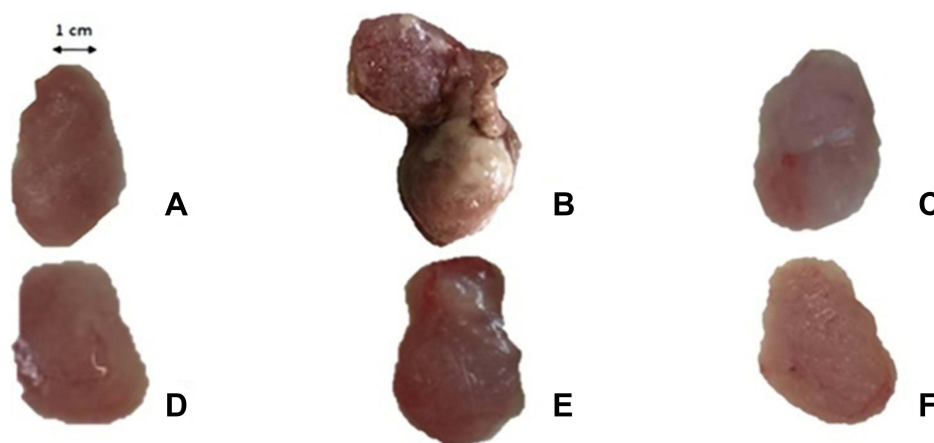
## Effects of LHE on Histopathological Changes in Hyperplastic Prostates

In the control group, histological examination of the prostate showed tubular glands containing secreted material (corpora amylacea), of variable diameter and irregular lumen with secretory luminal cells lined with a single layer of low columnar epithelium. The interglandular fibromuscular matrix rich in vessels contained a normal strip of smooth muscle fibers.

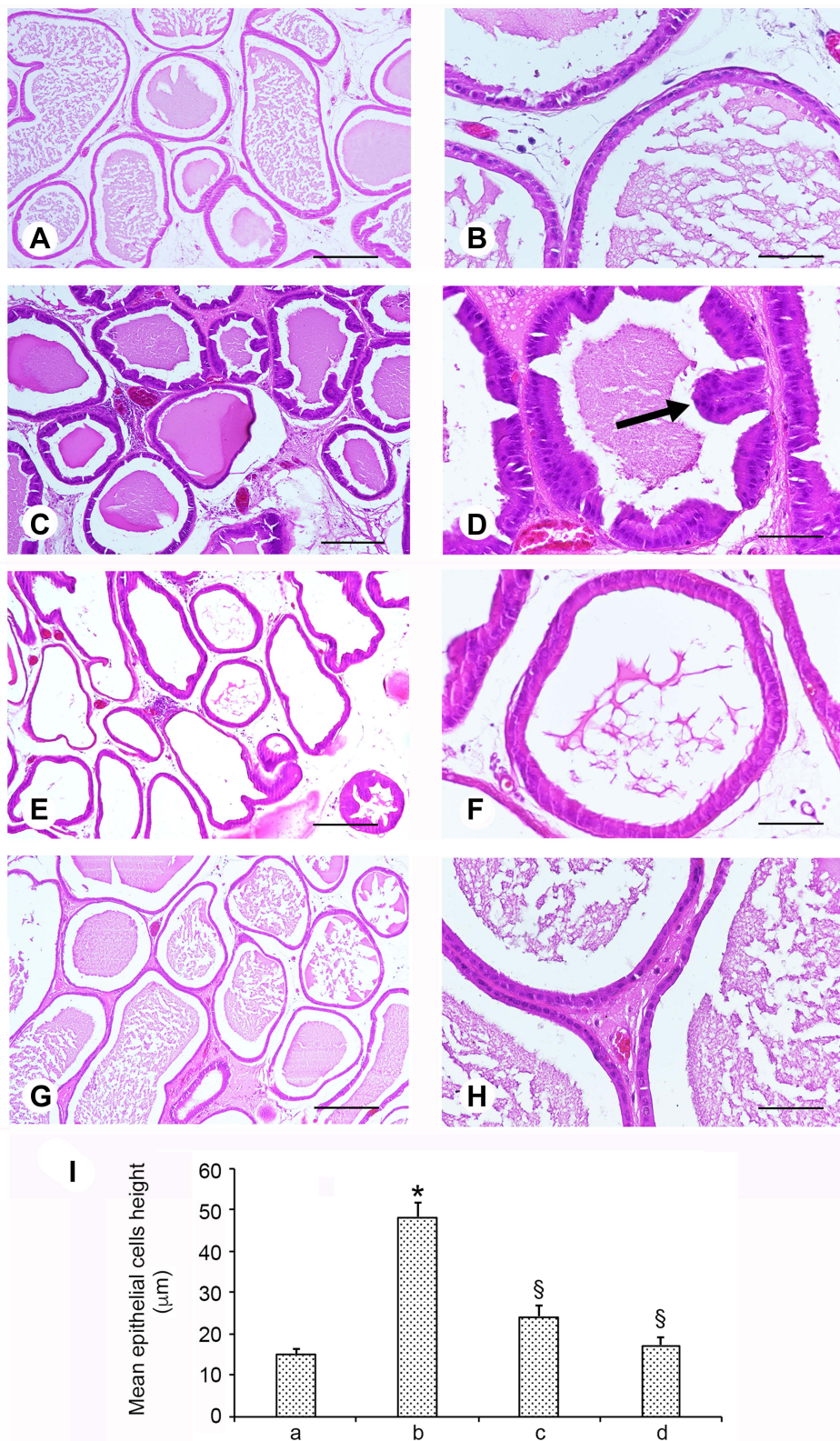
In the testosterone group (Figure 3), the density of interglandular fibromuscular stromal was significantly increased, the gland walls were thickened, the intraglandular epithelial cells proliferated to significantly higher levels and the prostatic glandular cavities were also markedly enlarged than in the vehicle control group. In the plant extract groups (for 4 or 2 weeks) and in the ( $\beta$ -s) groups fibromuscular stromal and both epithelial cell proliferation and glandular cavity enlargement were markedly reduced. The morphological data were confirmed by a statistical evaluation of the mean epithelial height: a significant increase in the testosterone alone group and a significant decrease in both treated groups, more evident in the extract + testosterone group (Figure 3I) were demonstrated.

## Effects of LHE on Prostate Testosterone and DHT Concentrations

The results obtained showed that in the prostate, the levels of testosterone ( $4.75 \pm 0.30$  ng/mL,  $P < 0.01$ ) and DHT ( $420.35 \pm 25.42$  pg/mL,  $P < 0.01$ ) were increased in the testosterone group as compared to the vehicle control group, in the  $\beta$ -s co-treated group (for 4 weeks at 1 mg/kg) a decrease in testosterone ( $2.88 \pm 0.22$  ng/mL,  $P < 0.01$ ) and DHT ( $315.39 \pm 34.20$  pg/mL,  $P < 0.05$ ) was observed. Similar to the results of  $\beta$ -s treated group, but with a greater potency, in the LHE-co-treated groups (for 4 weeks or 2 weeks) at 100 mg/kg, the level of testosterone ( $2.03 \pm 0.6$  ng/mL,  $P < 0.01$ ) and DHT ( $245.24 \pm 42.67$  pg/mL,  $P < 0.01$ ) were significantly reduced compared with those of the BPH group.



**Figure 2** Macroscopic comparison of the prostate tissues. Representative photographs: (A) normal prostate in vehicle control; (B) prostatic hyperplasia induced by testosterone in T-treated group; (C) prostate in  $\beta$ -s + TP co-treated group for 4 weeks; (D) prostate in LHE + TP co-treated group for 4 weeks. (E) prostate in  $\beta$ -s treated group for 2 weeks after induction of prostatic hyperplasia; (F) prostate in LHE-treated group for 2 weeks after induction of prostatic hyperplasia.



**Figure 3** Histological observations of the effects of  $\beta$ -sitosterol and *Kigelia* extract on testosterone induced hyperplasia in the rat prostate. Sections of prostate in control group (**A** and **B**) show acini of variable size lined by a low columnar epithelium; acini are filled with pale eosinophilic material. In testosterone-treated group (**C** and **D**), the epithelial cells show increased number and height, with proliferation of the fibromuscular stroma. The walls of acini are thicker with many infoldings projecting into the lumen (arrow). In  $\beta$ -sitost+Testost (**E** and **F**) and extract+Testost groups (**G** and **H**), a reduction of epithelial cell height and of stromal proliferation is evident. Statistical evaluation of the mean epithelial height in the different groups (a = control; b = testosterone treated; c =  $\beta$ -sitosterol + testosterone treated; d = extract + testosterone treated). \* $p < 0.05$  versus vehicle control; § $p < 0.05$  versus testosterone treated) (**I**) (Scale bar: A, C, E, G = 250  $\mu$ m; B, D, F, H = 50  $\mu$ m).



## Acute Toxicity

Signs of acute toxicity effects like difficult or labored breathing, lacrimation, hair erection, convulsion, agitation, or dizziness were not observed during the whole observation period and all animals survived the experimentation period. Similarly, no macroscopic lesions of the autopsy organs were observed in all the groups treated up to highest dose of lipidosterolic hexane extract (100 mg/kg body weight) or  $\beta$ -sitosterol (1 mg/kg).

## Discussion

Benign prostatic hyperplasia is one of the most common conditions in senior men.  $5\alpha$ -reductase inhibitors (such as finasteride) are frequently used for the treatment of BPH because they reduce the level of DHT.  $\alpha$ -blockers drugs are also used for BPH, as they relax the smooth muscles of the prostate. However, both classes of drugs show different types of side effects including erectile and ejaculatory dysfunctions decreased libido, hypotension, and nasal congestion, which limit their therapeutic use. Therefore, phytochemicals are considered as a valid alternative to use, because of reduced side effects. Several plant extracts, including *Serenoa repens*, *Pygeum africanum*, and *Secale cereal*, have been evaluated in clinical trials for the treatment of BPH. The lipidosterolic extract of *Serenoa repens* (Permixon<sup>®</sup>), is frequently used for the treatment of BPH. This extract contains phytosterols (including  $\beta$ -sitosterol, stigmasterol, campesterol), aliphatic alcohols and a mixture of fatty acids including linoleic, oleic, palmitic and lauric acids, to which the pharmacological action is attributed, mainly due to the inhibition of  $5\alpha$ -reductase.

The results of the phytochemical analysis showed that the extract from *K. africana* fruits has a very similar chemical composition to that of the extract of *Serenoa repens* in fact, it contains numerous sterols as  $\beta$ -sitosterol, stigmasterol, campesterol, and unsaturated fatty acids as linolenic, linoleic, and oleic. Phytosterols are regarded as the most important contributors to the pharmacological activity of various vegetable products used as phytotherapies concerning prostatic hyperplasia. Positive effects of these phytochemicals on malign prostate hyperplasia and pro-apoptotic effects on prostate cancer cells have also been reported.<sup>10</sup> Amongst the diverse group of phytosterols,  $\beta$ -sitosterol has widely been described as exceptionally active in several clinical studies on BPH,<sup>11</sup> proving to be capable of preventing and reversing this condition. This clinical activity has been attributed due to a partial  $5\alpha$ -reductase inhibition, by this individual phytosterol,<sup>12</sup> which has not been reported in the same manner from other associates of this compound group. In addition, also unsaturated fatty acids, like oleic, linolenic, and linoleic acids can play an important role in the inhibition of the  $5\alpha$ -reductase enzyme, thus, suggesting a competitive mechanism of inhibition with the sterols.<sup>13</sup>

The increase of relative prostate weight (prostate index) is considered as significant index of BPH.

The results obtained in relation to the prostate index showed that the LHE can prevent, and reverse testosterone induced prostatic hyperplasia. As can be seen from Table 3, the prostate index values are lower in the 2-week treatment (reversal) groups than in the 4-week (prevention) respective treatment groups and this indicates that LHE is more effective in reversing than preventing BPH.

Prostatic growth is used as one of important marker of BPH. Testosterone and DHT have a fundamental role in the growth of male reproductive organs. In the prostate, testosterone is turned by the  $5\alpha$ -reductase enzyme, into the active form dihydrotestosterone, that is commonly associated with BPH. Our results, in particular the reduction of DHT content in rats with BPH, indicating the capacity of the LHE to inhibit the  $5\alpha$ -reductase activity in the prostate.

In summary, our results demonstrate that the LHE can prevent, and reverse testosterone induced prostatic hyperplasia by a significant reduction of the prostatic weight, prostatic index, prostatic levels of testosterone and DHT, and by a marked reduction of the histopathological alterations (including the epithelial thickness, stromal proliferation, and lumen area) induced by testosterone. These effects appear to be due to a partial antiandrogenic activity of LHE.

In addition, the results obtained show also that the effects of LHE were superior to those demonstrated by  $\beta$ -s; therefore, it can be speculated that although phytosterols seem to contribute more significantly to the effects described above, synergism with other constituents of the lipidic extract such as fatty acids and antioxidants as  $\alpha$ -tocopherol (a compound with established antioxidant properties) probably plays an important additional role. Therefore, the lipid extract of the fruits of *K. africana* could potentially be considered as a typical phytotherapeutic product, the activity of which is due to the synergistic interaction of numerous constituents of the extract.

## Conclusion

In conclusion, we evaluated the effects of the lipidsterolic hexane extract from *K. africana* fruits, on prostate size and DHT and testosterone levels in prostate tissue in a testosterone-induced prostatic hyperplasia rat model. The results obtained show that the LHE can prevent, and reverse testosterone induced prostatic hyperplasia, and support the traditional use of *Kigelia africana* in some disorders of the reproductive system.

## Abbreviations

BPH, Benign prostatic hyperplasia; GC×GC-MS/FID, gas chromatography-mass spectrometry/flame ionization detection. LHE, lipidsterolic hexane extract;  $\beta$ -s,  $\beta$ -sitosterol; TP, testosterone propionate; DHT, dihydrotestosterone.

## Acknowledgments

We declare that part of the abstract of this manuscript was presented at the following conference: [https://pinpick.it/wpcontent/uploads/documenti/BIBLIOGRAFIA/programma\\_ed\\_abstract.pdf](https://pinpick.it/wpcontent/uploads/documenti/BIBLIOGRAFIA/programma_ed_abstract.pdf).

## Disclosure

The authors report no conflicts of interest in this work.

## References

1. Pais P. Potency of a novel saw palmetto extract, SPET-085, for inhibition of 5 $\alpha$ -reductase II. *Adv Ther*. 2010;27:555–563. doi:10.1007/s12325-010-0041-6
2. Carson C, Rittmaster R. The role of dihydrotestosterone in benign prostatic hyperplasia. *Urology*. 2003;61:2–7. doi:10.1016/S0090-4295(03)00045-1
3. Paba S, Frau R, Godar SC, Devot P, Marrosu F, Bortolato M. Steroid 5 $\alpha$  reductase as a novel therapeutic target for schizophrenia and other neuropsychiatric disorders. *Curr Pharm Des*. 2011;17:151–167. doi:10.2174/138161211795049589
4. Buck AC. Is there a scientific basis for the therapeutic effects of *Serenoa repens* in benign prostatic hyperplasia? Mechanisms of action. *J Urol*. 2004;172:1792–1799. doi:10.1097/01.ju.0000140503.11467.8e
5. Kokwaro JO. *Medicinal Plants of East Africa*. 3rd ed. Nairobi, Kenya: University of Nairobi Press; 2009.
6. Bello I, Shehu MW, Musa M, Zaini Asmawi M, Mahmus R. *Kigelia africana* (Lam) Benth. (Sausage tree): phytochemistry and pharmacological review of a quintessential African medicinal plant. *J Ethnopharmacol*. 2016;189:253–276. doi:10.1016/j.jep.2016.05.049
7. Tranchida PQ, Salivo S, Bonaccorsi I, Rotondo A, Dugo P, Mondello L. Analysis of the unsaponifiable fraction of lipids belonging to various milk-types by using comprehensive two-dimensional gas chromatography with dual mass spectrometry/flame ionization detection and with the support of high-resolution time-of-flight mass spectrometry for structural elucidation. *J Chromatogr*. 2013;1313:194–201.
8. National Research Council. *Guide for the Care and Use of Laboratory Animals*. National Academies Press; 2010.
9. Irwin S. Comprehensive observational assessment: a systematic, quantitative procedure for assessing the behavioral and physiologic state of the mouse. *Psychopharmacologia*. 1968;13:222–257. doi:10.1007/BF00401402
10. Ifere GO, Barr E, Equan A, et al. Differential effects of cholesterol and phytosterols on cell proliferation, apoptosis and expression of a prostate specific gene in prostate cancer cell lines. *Cancer Detect Prev*. 2009;32:319–328. doi:10.1016/j.cdp.2008.12.002
11. Prager N, Bickett K, French N, Marcovici G. A randomized, double-blind, placebo-controlled trial to determine the effectiveness of botanically derived inhibitors of 5- $\alpha$ -reductase in the treatment of androgenetic alopecia. *J Altern Complement Med*. 2002;8:143–152. doi:10.1089/10755302317371433
12. Gutendorf B, Westendorf J. Comparison of an array of in vitro assays for the assessment of the estrogenic potential of natural and synthetic estrogens, phytoestrogens and xenoestrogens. *Toxicology*. 2001;166:79–89. doi:10.1016/S0300-483X(01)00437-1
13. Liang T, Liao S. Inhibition of steroid 5  $\alpha$ -reductase by specific aliphatic unsaturated fatty acids. *Biochem J*. 1992;285:257–262. doi:10.1042/bj2850557

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