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In Vitro Effect of D-004, a Lipid Extract of the Fruit of the Cuban Royal Palm (*Roystonea regia*), on Prostate Steroid 5α-Reductase Activity

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ABSTRACT

Background: D-004, a lipid extract of the fruit of the Cuban royal palm (*Roystonea regia*), has been found to reduce prostatic hyperplasia (PH) induced with testosterone (T), but not PH induced with dihydrotestosterone (DHT), in rodents, suggesting the inhibition of prostate 5α-reductase activity.

Objectives: The aims of this study were to assess whether D-004 inhibits prostate 5α-reductase activity in vitro and to examine the effects of D-004 on enzyme kinetics.

Methods: This experimental study was conducted at the Pharmacology Department, Center of Natural Products, National Center for Scientific Research, Havana, Cuba. Soluble rat prostate preparations were used as the source of 5α-reductase, and (^{3}H)-DHT production was measured to determine prostate 5α-reductase activity. Cell-free rat prostate homogenates were pre-incubated with carboxymethyl cellulose 2% alone (control tubes) or D-004 (0.24–125 μg/mL) suspended in the vehicle (treated tubes) for 10 minutes prior to adding the labeled substrate (^{3}H)-T. Once the reaction was stopped, sterols were extracted with chloroform and aliquots were applied on silica gel plates developed in benzene-acetone (4:1, v/v). Areas containing DHT were scraped and radioactivity was counted. The median inhibitory concentration (IC_{50}) was determined by measuring the conversion of T to DHT. The apparent Michaelis-Menten constant (K_m) and V_{max} values before and after adding D-004 were determined in kinetic studies using labeled T (0.5–25 μmol/L).

Results: Compared with controls, D-004 significantly and dose-dependently inhibited the enzymatic reaction at doses of 1.95 to 125.0 μg/mL (all, $P < 0.05$). The IC_{50} of D-004 required to inhibit 5α-reductase activity was 2.25 μg/mL. Enzyme inhibition was noncompetitive, since D-004 lowered the V_{max} from 15.3 to 10.0 nmol DHT/min · mg^{-1} protein, while the K_m (4.54 μmol/L) was almost unaffected.

Conclusions: D-004 dose-dependently and noncompetitively inhibited in vitro 5α-reductase activity in soluble fractions of rat prostate. Although the

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extent of the maximal inhibition was high and the value of IC_{50} was low, the relevance of such inhibition requires further study in vivo. (*Curr Ther Res Clin Exp.* 2006;67:396–405)

Key words: D-004, free fatty acids, prostate hyperplasia, prostate 5α-reductase inhibitors.

INTRODUCTION

Benign prostatic hyperplasia (BPH), a term that describes the histologic basis of a diagnosis of prostate enlargement, is the uncontrolled growth of granular and stromal elements of the prostate gland, which may lead to bladder outflow obstruction and difficulty urinating.[1] BPH is common in older men, with a frequency that increases with age.[1–5]

While the etiology of BPH is not completely understood, it is known to involve hormonal changes that occur with aging. The development and growth of the prostate gland depend on androgen stimulation, mainly by dihydrotestosterone (DHT).[1–7] The 5α-reductase enzyme, present as 2 isoforms (types 1 and 2), converts testosterone (T) to its more potent active metabolite DHT. The type 2 isoform is more abundant than the type 1 isoform within the prostate.[6] Accumulation of DHT in the prostate may encourage cell growth and cause hyperplasia, supporting the static component of BPH.[7,8] BPH also involves a dynamic component, which is associated with an increase in smooth muscle tone mediated through the α_1-adrenoceptors under sympathetic innervation in the bladder neck, urethra, and prostate.[9]

Thus, prostate 5α-reductase inhibitors[10,11] and α_1-adrenoreceptor blockers[12] are commonly used to treat BPH. While prostate 5α-reductase inhibitors are effective in reducing prostate enlargement and complications,[10,11] α_1-adrenoreceptor blockers are effective in relaxing smooth muscle and improving BPH symptoms.[12] Finasteride, the most prescribed α-reductase inhibitor, competitively and specifically inhibits type 2 5α-reductase, reducing prostate volume by 20% to 30% and obstructive symptom scores, while increasing peak urinary flow rate by 25%.[11]

Herbal medicines, mainly the lipidosterolic extracts (LEs) of saw palmetto berries (*Serenoa repens*), are commonly used to treat BPH.[13–15] Many clinical studies have supported the effectiveness of saw palmetto berries, even compared with finasteride and tamsulosin, to treat BPH,[14–18] although recent placebo-controlled trials have found that saw palmetto berries did not improve symptoms or objective measures of BPH more than placebo.[19,20]

Although the mechanisms supporting the efficacy of the LEs of saw palmetto berries in BPH are multiple,[14] it has been found to be an effective dual inhibitor of 5α-reductase isozyme activity in the prostate[21–26] that, unlike other 5α-reductase inhibitors, does not interfere with the cellular capacity to secrete prostate-specific antigen.[25] Free fatty acids that are major components of saw palmetto berry LEs (eg, oleic, lauric, palmitric, myristic, acids) have been found to inhibit

prostate 5α-reductase activity, with differential effects on the type 1 and 2 isoforms.[26] However, one study found that LEs of saw palmetto berries did not inhibit prostate 5α-reductase activity.[27]

D-004, a lipid extract of the fruit of the Cuban royal palm (*Roystonea regia*), contains free fatty acids, with oleic, lauric, palmitic, and myristic acids being the most abundant components.[28,29] As mentioned previously, some of them (ie, oleic, lauric, palmitic, and myristic acids) have been found to inhibit prostate 5α-reductase activity.[26]

Oral treatment with D-004 reduced prostatic hyperplasia (PH) induced with T,[28–31] but not PH induced with DHT,[29] in rodents, suggesting an inhibitory effect on prostate 5α-reductase activity.

In light of these issues, this study assessed whether D-004 could inhibit in vitro 5α-reductase activity in soluble rat prostate fractions. We also investigated the effects of D-004 on enzyme kinetics.

MATERIALS AND METHODS

This experimental study was conducted at the Pharmacology Department, Center of Natural Products, National Center for Scientific Research, Havana, Cuba. Animal handling was conducted in accordance with the Cuban regulations for the use of laboratory animals and ethical principles for animal management. The study protocol was reviewed and approved by an independent institutional review board.

Animals

Male Wistar rats (150–200 g) were acquired from the Center for Laboratory Animal Production (CENPALAB, Havana, Cuba) and maintained under standard laboratory conditions (temperature 25°C ± 2°C, relative humidity 65% ± 5%, and 12-hour light/darkness cycles) with access to food (rodent chow) and water ad libitum.

Treatment

D-004 was obtained from the Chemistry Department of the Center of Natural Products (Havana, Cuba), where its composition and purity were assessed using a validated gas chromatography method. The free fatty acid composition of the tested batch was as follows: caprylic acid ($C_{8:0}$) 0.4%, capric acid ($C_{10:0}$) 0.5%, lauric acid ($C_{12:0}$) 27.0%, myristic acid ($C_{14:0}$) 8.5%, palmitic acid ($C_{16:0}$) 11.1%, palmitoleic acid ($C_{16:1}$) 0.4%, stearic acid ($C_{18:0}$) 2.0%, oleic acid ($C_{18:1}$) 28.5%, linoleic acid ($C_{18:2}$) 9.5%, and linoleic acid ($C_{18:3}$) 2.0%.

Preparation of the Soluble Prostate Fraction

On the day of the assay, the rats were euthanized under ether anesthesia. The ventral prostate glands were immediately removed and blotted prior to their use. Unless specified otherwise, the following procedures were conducted at 4°C.

The soluble rat prostate fraction was prepared as per the method of George et al.[32] In brief, rat prostate tissues were placed in crushed ice and were homogenized in 9 volumes of ice-cold buffer containing sucrose 0.25 mol/L, Tris hydrochloride (HCl) 50 mmol/L, and magnesium chloride ($MgCl_2$) 50 mmol/L using a polytetrafluoroethylene glass tissue homogenizer (Ultra-Turrax T25, Janke and Kunkel GmbH, IKA-Labortechnik, Staufen, West Germany). Homogenates were centrifuged at 800*g* for 20 minutes at 4°C, and cell-free homogenates with a known protein concentration of 7 mg/mL were then obtained.

Effects of D-004 on 5α-Reductase Activity

The in vitro effects of D-004 on enzyme activity were investigated by incubating different concentrations of the tissue preparation and determining the dose-effect relationship.

Prostate 5α-reductase activity was measured using the method of Kitahara et al,[33] with slight modifications. In brief, for 10 minutes prior to adding the labeled substrate, the homogenates were preincubated with carboxymethyl cellulose 2% alone (control tubes) or with different concentrations of D-004 suspended in this vehicle (treated tubes). Thus control and D-004 tubes were all prepared in the same manner, with the only difference being the use of vehicle alone or D-004 and vehicle together, respectively. Protein was estimated using a modification of the Lowry method.[34]

To calculate the median inhibitory concentration (IC_{50}) values required to inhibit prostate 5α-reductase activity, tubes containing increasing doses of D-004 (0.24–125 μg/mL) and those with vehicle alone were preincubated with tissue preparations in the presence of a 25-mol/L sucrose solution (1 mL) containing Tris HCl 50 mmol/L (pH 7.4), nicotinamide adenine dinucleotide phosphate 0.3 mmol/L, and $MgCl_2$ 50 mmol/L.

After 10 minutes, 2 μmol/L of the labeled substrate [1,2,6,7-^{3}H]T (Amersham Biosciences, Piscataway, New Jersey) 3.52 Bq/mmol · L^{-1} containing 20 × 10^4 dpm was added, while stirring constantly. The reaction was carried out in triplicate at 37°C for 1 hour, and 3 mL of cold chloroform was added to stop the reaction. Cold DHT (50 μg) was added to each tube as a carrier and visualization marker.

Sterols were extracted with chloroform 3 times. Combined extracts were dried under nitrogen at 40°C, dissolved again in chloroform (1 mL), and 200-μL aliquots were applied to silica gel plates that were developed in benzene-acetone (4:1, v/v). After drying the plates, DHT was visualized with a 50% sulfuric acid solution by heating the plates, and areas containing DHT were carefully scraped and transferred to scintillation vials. Radioactivity was counted using a scintillation counter (model RACKBETA 1219, LKB Wallac, Turku, Finland). The 5α-reductase activity was expressed as nanomoles of DHT formed/min · mg^{-1} protein.

Twenty rats were used to prepare the soluble prostate homogenates. Differences between control and D-004 tubes were estimated from triplicate experiments at each dose. The linearity of enzyme activity versus the time and protein concentration was established in initial studies (results not shown).

The IC_{50} values were determined graphically from the curve by plotting the percentage of inhibition of enzyme activity versus the different concentrations of D-004. Kinetic studies were done under similar conditions, but the activity of the enzyme was measured in the presence of various concentrations of labeled T (0.5–25 μmol/L). A Lineweaver-Burk plot was generated and the values of the Michaelis-Menten constant (K_m) and V_{max} were calculated. Experiments were performed in triplicate samples.

Statistical Analysis

Data are presented as mean (SD). Statistical comparisons of treated and control samples were performed using the 2-tailed Mann-Whitney *U* test.

Based on the information obtained with LEs of saw palmetto berries, the study was designed to detect a 50% inhibition of enzyme activity with D-004 at 5.0 μg/mL versus control samples, with a power of 80% at an α level of 0.05. Based on this assumption, we estimated a priori that 2 series of triplicate tubes for each dose would be sufficient.

$P < 0.05$ was considered statistically significant. Statistical analyses were performed using Statistics for Windows version 4.2 (StatSoft, Inc., Tulsa, Oklahoma).

RESULTS

The **table** and **Figure 1** show the effects of D-004 on DHT formation in the incubation medium. Compared with controls, D-004 significantly inhibited in a dose-dependent manner the enzymatic reaction at doses of 1.95 to 125.0 μg/mL (all, $P < 0.05$). The inhibition was almost complete (~95%) at concentrations >15.62 μg/mL. The IC_{50} of D-004 required to inhibit 5α-reductase activity was 2.25 μg/mL.

Figure 2 illustrates the effect of D-004 on the kinetics of 5α-reductase activity. The apparent K_m and V_{max} of the enzyme activity were 4.54 μmol/L and 15.3 nmol DHT/min · mg^{-1} protein, respectively. In control conditions, the enzyme appeared to be saturated at concentrations >8 μmol/L. Preincubation with D-004 lowered V_{max} from 15.3 to 10.0 nmol DHT/min · mg^{-1} protein, while K_m remained almost unaffected, indicating that the inhibition was noncompetitive.

DISCUSSION

The steroid 5α-reductase is a key enzyme in converting several 3-keto steroids into their respective 5α-reduced derivatives, governing the conversion of T to DHT.[6,8] An increase in prostate 5α-reductase activity can result in excessively high DHT concentrations in the prostate, leading to BPH.[3,4,6,8] The inhibition of 5α-reductase activity is a first-line pharmacologic treatment strategy for BPH.[1–5,10,11]

In this study, we found that D-004, a lipid extract of the fruit of *R regia*,[28–31] appeared to inhibit 5α-reductase activity of soluble fractions of rat prostate in vitro in a dose-dependent manner. This result is consistent with the fact that oral treatment with D-004 prevented PH induced with T, but not with DHT, in

Table. Effects of D-004 on dihydrotestosterone (DHT) generation in soluble fractions of rat prostate.

Treatment	Enzyme Activity, Mean (SD), nmol DHT/min · mg^{-1} Protein*	Inhibition, %
Control	4.15 (0.352)	–
D-004 dose		
0.24 µg/mL	4.12 (0.213)	0.7
0.48 µg/mL	4.08 (0.162)	1.7
0.97 µg/mL	3.90 (0.095)	6.0
1.95 µg/mL	2.99 (0.195)†	27.9
3.90 µg/mL	1.09 (0.051)†	73.8
15.62 µg/mL	0.56 (0.030)†	86.5
62.5 µg/mL	0.18 (0.011)†	95.7
125.0 µg/mL	0.19 (0.003)†	95.4

*Values are based on 3 replications of each experiment.
†$P < 0.05$ versus control (Mann-Whitney U test).

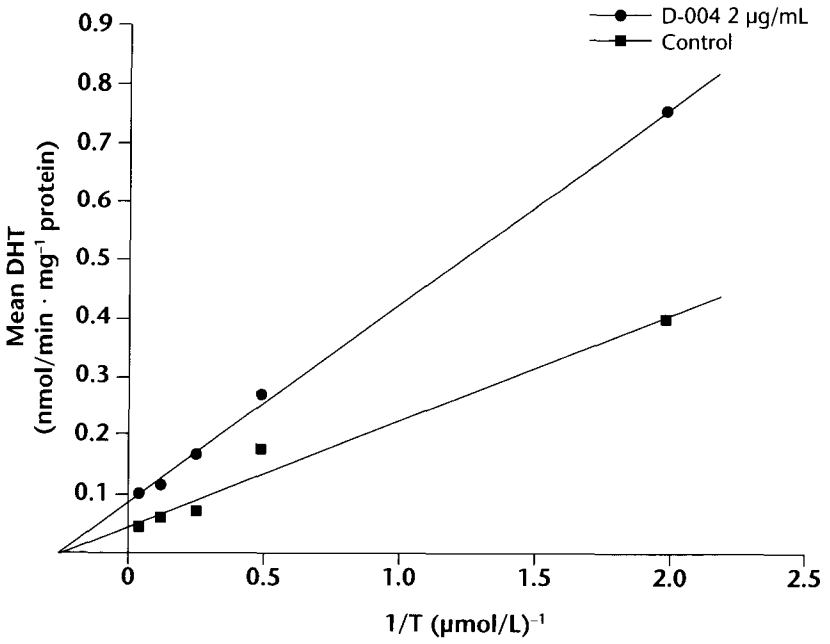

Figure 1. Dose-related relationship of the in vitro effect of D-004 on prostate 5α-reductase activity. DHT = dihydrotestosterone; T = testosterone.

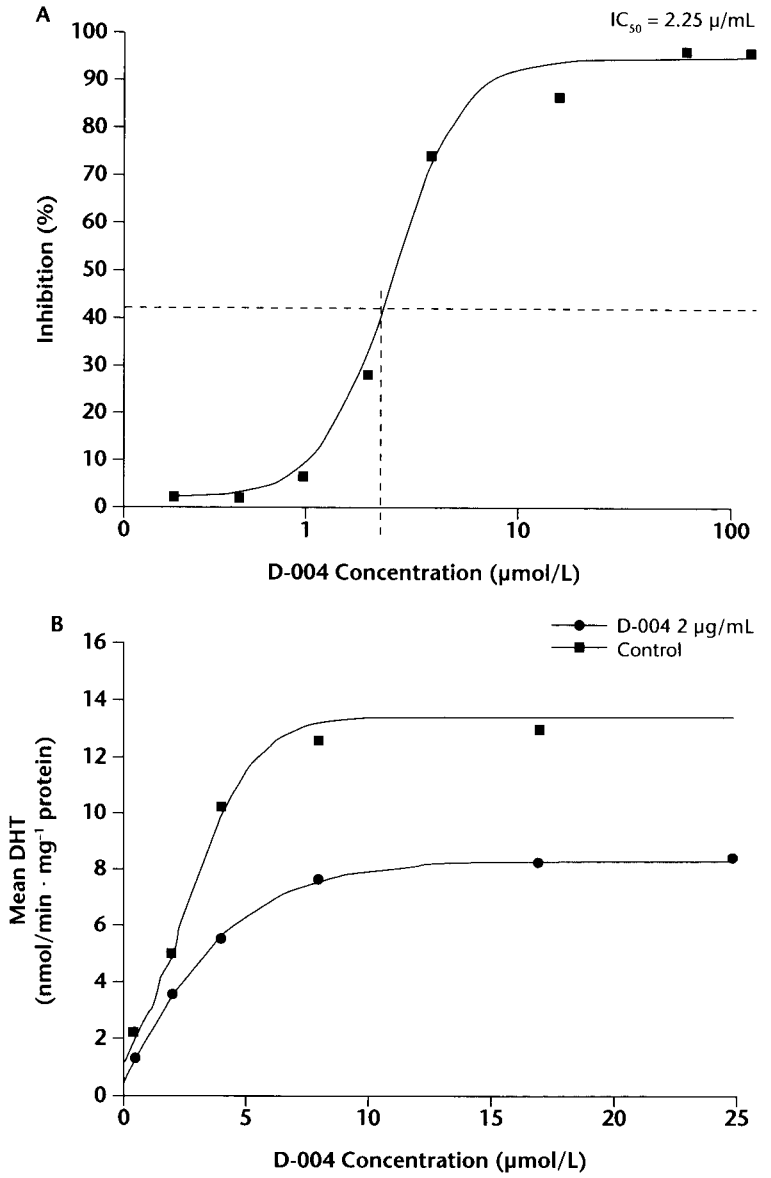

Figure 2. (A) Saturation curve (constructed with mean values of a study performed in triplicate samples, at various concentrations of testosterone in the absence [control] and presence of D-004 added at inhibitory concentrations) and (B) Lineweaver-Burk plot for the inhibition of D-004 on prostate 5α-reductase activity. The apparent Michaelis-Menten constant was 4.54 µmol/L and V_{max} values were 15.3 and 10.0 nmol dihydrotestosterone (DHT)/min · mg^{-1} protein for the control and treated sample, respectively. IC_{50} = median inhibitory concentration.

rats.[29] The most abundant components of D-004 are free fatty acids (≥85%) (eg, oleic, lauric, palmitic, myristic acids), all of which have been found to inhibit prostate 5α-reductase in vitro.[26,35] Hence, our findings are logical.

In addition, several studies have found saw palmetto berries to inhibit prostate 5α-reductase activity, thereby preventing prostate growth,[14,15] although some results have been discrepant.[27] Since D-004 and saw palmetto berries contain similar LEs,[36,37] their effects on PH induced with T in rodents are similar, and their botanic origins are comparable, our findings were expected. Also, the almost complete inhibition of enzyme activity (~95%) achieved with D-004 and the low IC_{50} value (2.25 μg/mL) were similar to the inhibition of enzyme activity and the IC_{50} value (5 μg/mL) reported for the LEs of saw palmetto berries.[22,25]

D-004 inhibited 5α-reductase in a noncompetitive manner because it decreased the V_{max} of the enzyme reaction without changing the K_m, which is different from the action of competitive inhibitors. This suggests that the inhibition induced with D-004, as occurs with the LEs of saw palmetto berries,[22] does not involve the binding of the active component(s) of the extract to the site of enzyme activity.

CONCLUSIONS

The results of this study suggest that D-004 dose-dependently and noncompetatively inhibits in vitro 5α-reductase activity in soluble fractions of rat prostate through a noncompetitive mechanism. Although the maximal inhibition (95%) and the low IC_{50} (2.25 μg/mL) suggest that it could be relevant, the fact that this study investigated only the inhibition of D-004 on 5α-reductase activity in vitro limits its applicability. Therefore, in vivo studies are needed to determine whether the inhibitory effects of D-004 are biologically meaningful.

REFERENCES

1. Clifford GM, Farmer RD. Medical therapy for benign prostatic hyperplasia: A review of the literature. *Eur Urol.* 2000;38:2–19.
2. Simpson RJ. Benign prostatic hyperplasia. *Br J Gen Pract.* 1997;47:235–240.
3. Barry MJ, Roehrborn CG. Benign prostatic hyperplasia [published correction appears in *BMJ.* 2002;324:775]. *BMJ.* 2001;323:1042–1046.
4. Thorpe A, Neal D. Benign prostatic hyperplasia [published correction appears in *Lancet.* 2003;362:496]. *Lancet.* 2003;361:1359–1367.
5. Bhargava S, Canda AE, Chapple CR. A rational approach to benign prostatic hyperplasia evaluation: Recent advances. *Curr Opin Urol.* 2004;14:1–6.
6. Bartsch G, Rittmaster RS, Klocker H. Dihydrotestosterone and the concept of 5alpha-reductase inhibition in human benign prostatic hyperplasia. *Eur Urol.* 2000;37:367–380.
7. Carson C III, Rittmaster R. The role of dihydrotestosterone in benign prostatic hyperplasia. *Urology.* 2003;61(Suppl 1):2–7.
8. Bartsch G, Rittmaster RS, Klocker H. Dihydrotestosterone and the role of 5 alpha-reductase inhibitors in benign prostatic hyperplasia. *Urologe A.* 2002;41:412–424.

9. Michel MC, Taguchi K, Schafers RS, et al. α1-Adrenoceptor subtypes in the human cardiovascular and urogenital systems. *Adv Pharmacol.* 1998;42:394–398.
10. Sandhu JS, Te AE. The role of 5-alpha-reductase inhibition as monotherapy in view of the MTOPS data. *Curr Urol Rep.* 2004;5:274–279.
11. Lam JS, Romas NA, Lowe FC. Long-term treatment with finasteride in men with symptomatic benign prostatic hyperplasia. 10-Year follow-up. *Urology.* 2003;61:354–358.
12. Oelke M, Hofner K, Berges RR, Jonas U. Drug therapy of benign prostatic hyperplasia syndrome with alpha 1-receptor blockers. Basic principles and clinical results. *Urologe A.* 2002;41:425–441.
13. Lowe FC, Dreikorn K, Borkowski A, et al. Review of recent placebo-controlled trials utilizing phytotherapeutic agents for treatment of BPH. *Prostate.* 1998;37:187–193.
14. Plosker GL, Brogden RN. *Serenoa repens* (permixon). A review of its pharmacology and therapeutic efficacy in benign prostate hyperplasia. *Drugs Aging.* 1996;9:379–395.
15. Wilt T, Ishani A, Mac Donald R. *Serenoa repens* for benign prostatic hyperplasia. *Cochrane Database Syst Rev.* 2002;(5):CD001423.
16. Carraro JC, Raynaud JP, Koch G, et al. Comparison of phytotherapy (permixon) with finasteride in the treatment of benign prostate hyperplasia: A randomized international study of 1,098 patients. *Prostate.* 1996;29:231–240.
17. Aliaev IG, Vinarov AZ, Lokshin KL, Spivak LG. Five-year experience in treating with prostatic hyperplasia patients with permixon (*Serenoa repens* Pierre Fabre Medicament). *Urologia.* 2002;1:23–25.
18. Debruyne F, Koch G, Boyle P, et al, for the Groupe d´etude PERMAL. Comparison of a phytotherapeutic agent (permixon) with an alpha-blocker (tamsulosin) in the treatment of benign prostatic hyperplasia: 1-Year randomized international study. *Prog Urol.* 2002;2:384–392.
19. Willetts KE, Clements MS, Champion S, et al. *Serenoa repens* extract for benign prostate hyperplasia: A randomized controlled trial. *BJU Int.* 2003;92:267–270.
20. Bent S, Kane C, Shinohara K, et al. Saw palmetto for benign prostatic hyperplasia. *N Engl J Med.* 2006;354:557–566.
21. Sultan C, Terraza A, Deviller C, et al. Inhibition of androgen metabolism and binding by a liposterolic extract of "Serenoa repens B" in human foreskin fibroblasts. *J Steroid Biochem.* 1984;20:515–519.
22. Weisser H, Tunn S, Behnke B, Krieg M. Effects of the *Sabal serrulata* extract IDS 89 and its subfractions on 5alpha-reductase activity in human prostatic hyperplasia. *Prostate.* 1996;28:300–306.
23. Iehlé CV, Délos S, Guirou O, et al. Human prostatic steroid 5 alpha-reductase isoforms—a comparative study of selective inhibitors. *J Steroid Biochem Mol Biol.* 1995;54:273–279.
24. Bayne CW, Donnelly F, Ross M, Habib FK. *Serenoa repens* (permixon): A 5alpha-reductase types I and II inhibitor—new evidence in a coculture model of BPH. *Prostate.* 1999;40:232–241.
25. Habib FK, Ross M, Ho CK, et al. *Serenoa repens* (permixon) inhibits the 5alpha-reductase activity of human prostate cancer cell lines without interfering with PSA expression. *Int J Cancer.* 2005;114:190–194.
26. Raynaud JP, Cousse H, Martin PM. Inhibition of type 1 and type 2 5alpha-reductase activity by free fatty acids, active ingredients of permixon. *J Steroid Biochem Mol Biol.* 2002;82:233–239.

27. Rhodes L, Primka RL, Berman C, et al. Comparison of finasteride (proscar), a 5 alpha reductase inhibitor, and various commercial plant extracts in in vitro and in vivo 5 alpha reductase inhibition. *Prostate*. 1993;20:43–51.
28. Arruzazabala ML, Carbajal D, Mas R, et al. Preventive effects of D-004, a lipid extract from Cuban royal palm (*Roystonea regia*) fruits, on testosterone-induced prostate hyperplasia in intact and castrated rodents. *Drugs Exp Clin Res*. 2004;30:227–233.
29. Carbajal D, Arruzazabala ML, Mas R, et al. Effects of D-004, a lipid extract from Cuban royal palm fruit, on inhibiting prostatic hypertrophy induced with testosterone or dihydrotestosterone in a rat model: A randomized, controlled study. *Curr Ther Res Clin Exp*. 2004;65:505–514.
30. Noa M, Arruzazabala ML, Carbajal D, et al. Effect of D-004, a lipid extract from Cuban royal palm fruit, on histological changes of prostate hyperplasia induced with testosterone in rats. *Int J Tissue React*. 2005;27:203–211.
31. Carbajal D, Molina V, Mas R, Arruzazabala ML. Therapeutic effect of D-004, a lipid extract from *Roystonea regia* fruits, on prostate hyperplasia induced in rats. *Drugs Exp Clin Res*. 2005;31:193–197.
32. George FW, Russell DW, Wilson JD. Feed-forward control of prostate growth: Dihydrotestosterone induces expression of its own biosynthetic enzyme, steroid 5 alpha-reductase. *Proc Natl Acad Sci U S A*. 1991;88:8044–8047.
33. Kitahara S, Higashi Y, Takeuchi S, Oshima H. Relationship of changing steroid 5α-reductase activity to iododeoxyuridine uptake during regeneration of involuted prostates. *Biol Rep*. 1989;40:793–799.
34. Marxwell MA, Haas SM, Beiber LL, Tolbert NE. A modification of the Lowry procedure to simplify protein determination in membrane lipoprotein samples. *Anal Biochem*. 1987;87:206–209.
35. Russell DW, Wilson JD. Steroid 5-alpha reductase: Two genes/two enzymes. *Annu Rev Biochem*. 1994;63:25–61.
36. Bombardelli E, Morazzoni P, Small JK. *Serenoa repens* (bartram). *Fitoterapia*. 1997;68: 99–113.
37. Saw palmetto extract. In: Expert Committee. United States pharmacopeial forum: (DSB) dietary supplement: Botanicals. Vol. 28. No. 2. Rockville, Md: Pharmacopeial Convention; 2005:425. Publication USP28-NF23.

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