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

Daisy Carbajal, PhD, Maria de Lourdes Arruzazabala, PhD, Rosa Más, PhD, Vivian Molina, PhD, Eduardo Rodríguez, MSc, and Victor González, PhD

Centre of Natural Products, National Center for Scientific Research, Havana, Cuba

ABSTRACT

Background: Benign prostatic hypertrophy is the nonmalignant, uncontrolled growth of prostatic epithelial cells and stroma that, left untreated, may lead to difficult urination and other complications. A common treatment of BPH is lipid extract from saw palmetto fruit, and lipid extract from Cuban Royal palm (a palm of the same family) fruit is being studied for this use. One study found that the latter, D-004, at 100 to 400 mg/kg daily prevented prostatic hypertrophy (PH) induced with testosterone (T) in a rat model.

Objectives: This study comprised 2 experiments in a rat model. The first assessed the effects of different doses of D-004 on T-induced PH; the second investigated the effects of D-004 on PH induced with dihydrotestosterone (DHT).

Methods: In experiment 1, rats were distributed in 6 groups of 10 rats each. One group received an SC injection of soy oil and oral treatment with Tween 65/water vehicle (negative control). The other 5 groups received an SC injection of T 3 mg/kg daily and oral treatment with vehicle (positive control) or D-004 at 50, 200, 400, or 800 mg/kg daily suspended in vehicle. In experiment 2, rats were distributed in 3 groups of 10 rats each. A negative control group received treatment as in experiment 1. Positive controls received an SC injection of DHT 1.5 mg/kg and vehicle orally. The third group received an SC injection of DHT and oral treatment with D-004 at 800 mg/kg suspended in vehicle. All treatments were given for 14 days. At sacrifice, prostates were removed and weighed. Mean prostatic weights and prostatic/body weight ratios were calculated.

Results: In experiment 1, in the groups receiving D-004 at 200, 400, or 800 mg/kg daily, prostatic weight was significantly lower compared with the positive control group ($P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively); this effect was not seen in the group receiving 50 mg/kg daily. In the groups receiving D-004 at 400 and 800 mg/kg daily, prostatic/body weight ratio was significantly lower compared with positive controls (both, $P < 0.05$); this effect was not seen in the groups receiving 50 or 200 mg/kg daily. In experiment 2, prostatic weight and prostatic/body weight ratio

in the group receiving D-004 were similar to those of positive controls. Body weight was not affected in any of the groups receiving D-004.

Conclusions: This study of rats with T- or DHT-induced PH suggests that D-004 at 200 to 800 mg/kg daily administered orally prevents T-induced PH, and that D-004 at 800 mg/kg daily does not prevent DHT-induced PH. (*Curr Ther Res Clin Exp.* 2004;65:505–514) Copyright © 2004 Excerpta Medica, Inc.

Key words: benign prostatic hypertrophy, testosterone, prostatic enlargement, dihydrotestosterone.

INTRODUCTION

Benign prostatic hypertrophy (BPH) is the nonmalignant, uncontrolled growth of the epithelial cells and stroma in the prostate gland. As BPH progresses, it may produce urethral compression and obstruction, causing difficult urination. Other complications of BPH include infection, bladder stones, and chronic prostatitis.^{1–3} Although BPH generally is not life-threatening, it affects quality of life and, left untreated, can lead to bladder or kidney damage.^{1–4} BPH is one of the most common disorders affecting men aged >50 years, and its prevalence increases with age. One study showed that >80% of men aged >80 years had BPH.⁵

Although the causes of BPH are not fully understood, the disorder has been associated with hormonal changes that occur as men age. For instance, prostatic 5 α -reductase causes the conversion of testosterone (T) to dihydrotestosterone (DHT) in prostatic cells. The increased DHT may accumulate, leading to BPH.^{6,7} Furthermore, as men age, prostatic estrogen levels rise, resulting in increased activity of substances stimulating cell growth, possibly causing BPH.

The symptoms of BPH (frequent urination, urgency, pain or a burning sensation on urination, and the feeling that the bladder is not empty after urination) are influenced by the increased tone in the bladder and in the smooth muscles of the prostate, a result of muscle contraction mediated by alpha₁-adrenergic receptors.^{3,8} Management of BPH depends on symptom severity. It is known that many patients having mild symptoms do not require intervention. Those with moderate symptoms may receive nonsurgical treatment, including pharmacotherapy with a 5 α -reductase inhibitor and/or an alpha₁-adrenergic receptor blocker (ARB). Patients with severe symptoms may undergo transurethral surgery, considered the most effective treatment for BPH and its complications.² However, transurethral surgery poses some risks, especially in older individuals (aged >65 years).^{2,4,8} Common complications of transurethral surgery include immediate postoperative hyponatremia, urinary incontinence, stricture, urinary tract infection, need for blood transfusion, and sexual dysfunction.²

Pharmacotherapy is the most common treatment option for BPH. It is generally accepted that 5 α -reductase inhibitors usually decrease prostatic size only with long-term therapy (6–12 months),³ and have little impact on BPH symptoms.^{1,3} It has been reported that long-term treatment (≥ 10 years) with

finasteride, a 5 α -reductase inhibitor, is associated with few adverse events.⁹ ARBs provide faster (3–6 weeks), more effective symptom relief by relaxing the smooth muscle, but sustained clinical benefit requires long-term treatment, increasing the risk for drug-related adverse events, especially orthostatic hypotension.¹⁰

The results of the Prospective European Doxazosin and Combination Therapy (PREDICT) trial¹¹ showed that the addition of finasteride to doxazosin therapy did not provide additional benefits to those achieved with a doxazosin regimen alone.¹¹ However, other results have shown that combination therapy with inhibitors of prostate 5 α -reductase and ARBs produces additive benefits in the management of BPH.¹⁰ Thus, this combination was the best option for providing symptom relief and reducing the risk for urinary complications resulting from prostatic enlargement. Patients most likely to benefit from such combined treatment appear to be those at high risk for BPH regression at baseline.^{12,13}

Lipid extract from saw palmetto, a palm of the Arecaceae family, contains fatty acids—mainly oleic, lauric, myristic, palmitic, linoleic, capric, stearic, and caprylic acids (>1.0%)—and their esters, whereas plant sterols and higher aliphatic alcohols are minor components.¹⁴ Phytotherapy with this extract has been used for treating BPH.^{4,15–23}

Evidence-based medicine supports that this extract is more effective in relieving BPH symptoms than placebo, and that the effects of saw palmetto and finasteride are comparable.¹⁵ The mechanisms of action of saw palmetto are thought to be related to the inhibition of 5 α -reductase and antagonism of α_1 -adrenergic receptors.¹⁷

Because, like saw palmetto, Cuban Royal palm (*Roystonea regia*) is a member of the Arecaceae family, one might suppose that its fruit lipid extract, D-004, could be used to treat BPH. D-004 primarily contains a reproducible mixture of fatty acids (oleic, palmitic, lauric, linoleic, and myristic acids) and their esters, and lesser concentrations of palmitoleic, caprylic, capric, and stearic acids.²⁴

Thus, the rationale to study whether D-004, like saw palmetto, could prevent prostatic hyperplasia, was supported,²⁵ and we initiated the evaluation of D-004 in experimental models of prostatic hyperplasia.

A previous study showed that D-004 at 100 to 400 mg/kg daily was effective in preventing prostatic hypertrophy (PH) induced with T in castrated and noncastrated rodents (rats and mice), and that the effects obtained in mice were more pronounced than those in rats, probably because mice are more sensitive to the preventive effects of D-004 on PH.²⁵ On the other hand, 5 α -reductase inhibitors have not been found to be effective in preventing PH induced with an exogenous supply of DHT.²⁶

The present study comprised 2 experiments in a rat model. The first assessed the effects of different doses of D-004 on T-induced PH; the second investigated the effects of D-004 on PH induced with DHT.

MATERIALS AND METHODS

Animals

Young (age, 2–3 months) adult male Sprague-Dawley rats weighing 250 to 270 g were purchased from the National Center for Laboratory Animals Production, Cenpalab, Havana, Cuba. Animals were adapted to laboratory conditions (temperature, $25^{\circ}\text{C} \pm 3^{\circ}\text{C}$; relative humidity, $60\% \pm 5\%$; light/dark cycle, 12 hours) for 7 days. Food (rodent chow obtained from Cenpalab) and water were provided ad libitum. Animal handling was conducted in accordance with the Cuban regulations for the use of laboratory animals and the ethical principles of animal management.

Study Drug Administration and Dose

T propionate 3 mg/kg daily (provided by Cuban Medical Pharmaceutical Industry [IMEFA], Havana, Cuba) and DHT 1.25 mg/kg daily (CH-9471, provided by Fluka Chemie GmbH, Buchs, Switzerland) were diluted in soy oil. Each drug was administered as an SC injection, as described previously.^{26,27}

D-004 was extracted from *Roystonea regia* fruit using hexane extraction conducted at the Centre of Natural Products, National Center for Scientific Research, Havana, Cuba. The composition and purity of the D-004 were verified using gas chromatography. D-004 was suspended in Tween 65/water (2%) vehicle and administered orally by gastric gavage (1 mL/kg). After verification of their stability, suspensions were prepared immediately before use.

To assess the effects of D-004 on T-induced PH (experiment 1), rats were randomly distributed, using a computer-generated table of codes based on baseline body weight, in 6 groups of 10 rats each (Table I). Two groups received SC injections of DHT (1.5 mg/kg). One group received Tween 65/water vehicle orally (positive control). Another group received oral treatment with D-004 at 800 mg/kg suspended in vehicle, and a third group received only vehicle treatment (negative control). All treatments were administered for 14 days.

To assess the effects of D-004 on DHT-induced PH (experiment 2), rats were randomly distributed, using a computer-generated table of codes based on baseline body weight, in 3 groups of 10 rats each (Table I). All 3 groups received SC injections of DHT 1.5 mg/kg daily. One group received no other treatment (negative control). One group received Tween 65/water vehicle orally (positive control). The other group received oral treatment with D-004 at 800 mg/kg suspended in vehicle. All treatments were administered for 14 days.

This study is part of the initial research on *Roystonea regia* fruit lipid extract.²⁵ Thus, the rationale for the low and moderate doses investigated in the 2 experiments was to determine the efficacy of doses slightly lower than those reported as effective (200 mg/rat)¹⁷ for *Serenoa repens* fruit lipid extract, the standard of phytotherapy for BPH.^{17,28} The study duration was selected to determine whether preventive effects were evident after short-term treatment. In experiment 2, we selected the highest dose used in exper-

Table I. Summary of treatment groups included in each experiment.

Treatment Group	Treatment*	
	SC Injection (n = 10)	Oral† (n = 10)
Experiment 1‡		
Negative control	Soy oil	Tween 65/water vehicle
Positive control	T	Tween 65/water vehicle
T + D-004 at 50 mg/kg daily	T	D-004 at 50 mg/kg daily
T + D-004 at 200 mg/kg daily	T	D-004 at 200 mg/kg daily
T + D-004 at 400 mg/kg daily	T	D-004 at 400 mg/kg daily
T + D-004 at 800 mg/kg daily	T	D-004 at 800 mg/kg daily
Experiment 2§		
Negative control	Soy oil	Tween 65/water vehicle
Positive control	DHT	Tween 65/water vehicle
DHT + D-004 at 800 mg/kg daily	DHT	D-004 at 800 mg/kg daily

T = testosterone; DHT = dihydrotestosterone.

*All treatments were administered for 14 days.

†Oral treatment was given by gastric gavage; D-004 was suspended in Tween 65/water vehicle (1 mL/kg).

‡The objective of this experiment was to investigate the effects of different doses of D-004 on prostatic hyperplasia (PH) induced with T.

§The objective of this experiment was to investigate the effects of D-004 on PH induced with DHT.

iment 1, because if the highest dose failed to inhibit DHT-induced PH, then it would be rational to expect that a lower dose would fail also, especially if the effects are dose dependent.

Body and Prostatic Weights

Body weight was measured the day before treatment was started (baseline) and at 14 days of treatment. At study completion (day 14), animals were anesthetized with ether and sacrificed. The prostates were immediately removed and weighed. Mean body weights and prostatic/body weight ratios were calculated in each group. Personnel involved in these procedures were blinded to treatment assignment.

Statistical Analysis

The study was designed to detect 50% inhibition of the increase in prostatic weight in the group receiving the highest dose of D-004 versus controls, with a power of 80% at an α level of 0.05. We calculated that increases in prostatic weight should be at least 300 mg more than those found in the negative control groups, based on cumulative historical records. Based on this assumption, it was calculated that a group size of 10 rats each would be sufficient.

Comparisons between groups were performed using the 2-sided nonparametric Mann-Whitney *U* test. The a priori level of significance was $P \leq 0.05$. All analyses were performed using StatSoft version 4.2 (StatSoft, Inc., Tulsa, Oklahoma).

RESULTS

Tables II and III show the results of experiments 1 and 2, respectively. In the positive control groups in the 2 experiments, mean prostatic weights and prostatic/body weight ratios were significantly increased after 14 days of injections with T (Table II) or DHT (Table III) compared with negative controls (experiment 1: both, $P < 0.001$; experiment 2: $P < 0.01$ and $P < 0.05$, respectively).

Table II summarizes the effects of D-004 at 50, 200, 400, and 800 mg/kg daily on PH induced with T. In the treatment groups receiving T and D-004 at 200, 400, or 800 mg/kg daily, the mean prostatic weights were significantly less than that in the positive control group at 14 days ($P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively); this effect was not seen with the dose of 50 mg/kg daily. The mean prostatic/body weight ratios in the groups receiving D-004 at 400 and 800 mg/kg daily were significantly lower than that of the positive control group at 14 days (both, $P < 0.05$); this effect was not seen with D-004 at 50 or 200 mg/kg daily. The effect of D-004 on prostatic weight was found to be dose dependent (34.1%, 56.2%, and 64.7% in the groups receiving 200, 400, and 800 mg/kg daily, respectively).

Table II. Effects of oral treatment with D-004 on prostatic hypertrophy induced with testosterone (T) 3 mg/kg daily in a rat model (experiment 1). Values are mean (SE).

Treatment Group	Body Weight, g		Prostatic Weight, mg	Prostatic/Body Weight Ratio, $\times 10^{-3}$
	Baseline	Day 14		
Negative control (no active treatment)	269.2 (6.2)	354.7 (6.9)	576.0 (51.4)*	1.60 (0.15)*
Positive control (T only)	252.4 (5.8)	339.5 (6.5)	916.1 (34.4)	2.70 (0.12)
T + D-004 at 50 mg/kg daily	251.2 (7.5)	332.5 (10.2)	895.4 (34.6)	2.65 (0.11)
T + D-004 at 200 mg/kg daily	260.5 (5.9)	335.3 (6.4)	800.0 (30.9)†	2.40 (0.09)
T + D-004 at 400 mg/kg daily	265.3 (6.0)	342.4 (6.4)	725.0 (35.4)‡	2.10 (0.11)†
T + D-004 at 800 mg/kg daily	267.0 (6.1)	345.0 (6.3)	696.1 (33.6)*	2.00 (0.12)†

* $P < 0.001$ versus positive control group (Mann-Whitney *U* test).

† $P < 0.05$ versus positive control group (Mann-Whitney *U* test).

‡ $P < 0.01$ versus positive control group (Mann-Whitney *U* test).

Table III. Effects of treatment with D-004 on prostatic hypertrophy induced with dihydrotestosterone (DHT) 1.25 mg/kg daily in a rat model (experiment 2). Values are mean (SE).

Treatment Group	Body Weight, g		Prostatic Weight, mg	Prostatic/Body Weight Ratio, $\times 10^{-3}$
	Baseline	Day 14		
Negative control (no active treatment)	262.6 (11.5)	370.0 (17.4)	790.0 (63.0)	2.2 (0.15)
Positive control (DHT only)	260.1 (12.0)	372.0 (9.5)	1264.0 (67.9)*	3.3 (0.11)†
DHT + D-004 at 800 mg/kg daily	256.8 (11.2)	364.4 (13.7)	1246.3 (57.6)*	3.2 (0.15)†

* $P < 0.01$ versus negative control group (Mann-Whitney U test).

† $P < 0.05$ versus negative control group (Mann-Whitney U test).

Table III summarizes the effects of D-004 on PH induced with DHT. In the group receiving D-004 at 800 mg/kg daily, the mean prostatic weight and prostatic/body weight ratio were similar to those in the positive control group at 14 days.

In both experiments, body weight was statistically similar between all of the treatment groups at 14 days (**Tables II and III**).

DISCUSSION

The results of the present study suggest that D-004, a lipid extract from the fruit of the Cuban Royal palm (*Roystonea regia*), at doses of 200 to 800 mg/kg daily inhibits PH induced with an exogenous supply of T in a rat model.

As expected, in the positive control groups in the 2 experiments, SC injection of T or DHT increased prostatic weight and prostatic/body weight ratio compared with negative controls, with the effects being comparable to those reported by other investigators.²⁷

However, the etiology of BPH in humans is heterogeneous, and no other species shows the same complexity of this disorder. Animal models of PH studied to date do not appear to fully mimic the stromal and epithelial changes with BPH in humans.²⁹ Therefore, in vitro and animal models are of limited value for the study of BPH events.²⁹ Spontaneous animal models are limited to nonhuman primates and canines (hormone-induced BPH in canines appears to be an especially replicative model of human BPH²⁹), but ethical and economic problems have reduced the applicability of these models.²⁹ Furthermore, a novel in vitro system and a transgenic mouse model have been conceived for the study of BPH,^{30,31} but their use also has been limited. In particular, PH induced with T or DHT does not reproduce

all findings of BPH in humans because the pathogenesis of BPH is dependent on a functional androgenic signal involving several components (eg, T synthesis in the testes, conversion of T to DHT, transportation of DHT to target prostate tissues, binding of DHT to androgenic receptor and the subsequent gene modulation).^{6,32,33} BPH in humans also involves prostatic estrogens and α_1 -adrenergic receptors not fully reproducible in other models.

Thus, the model used in the present study has limitations in predicting the effects of any treatment in the management of BPH in humans. Nevertheless, the effects of T and DHT on prostatic growth in rodents have previously been documented and used to assess the effects of drugs used for BPH treatment, including saw palmetto fruit lipid extract.^{17,28}

In the present study, in rats injected with T, prostatic weight and prostatic/body weight ratio were decreased after 14 days of oral treatment with D-004 with respect to positive controls. Because D-004 at any of the doses studied did not affect body weight, its effect on the prostatic/body weight ratio was likely a result of preventive effects of D-004 on PH. Furthermore, the results presented here suggest that D-004 at 200 to 800 mg/kg daily (but not 50 mg/kg daily) prevented PH in a dose-dependent manner. These results are consistent with those obtained in a previous study conducted in castrated and noncastrated rodents.²⁵ Although the effect achieved with the daily dose of 800 mg/kg was marked, we cannot conclude that it is the maximal effective dose of D-004 in this model because no plateau values were obtained, and higher doses were not studied.

Furthermore, although D-004 at 800 mg/kg daily seemed to prevent T-induced PH, the same effect was not found in DHT-induced PH, suggesting that the drug may inhibit prostatic 5α -reductase activity. The mechanism of action of D-004 in inhibiting T-induced PH may be multifactorial, as occurs with saw palmetto fruit lipid extract, which has been shown not only to inhibit prostatic 5α -reductase activity but also to antagonize prostatic α_1 -adrenergic receptors.¹⁷

Because the mechanisms of action of D-004 are unclear, they should be confirmed by further investigation, including *in vitro* and *ex vivo* studies of the effects of D-004 on prostatic 5α -reductase activity.

CONCLUSIONS

In this study of T- and DHT-induced PH in a rat model, in rats receiving D-004 at 200 to 800 mg/kg daily administered orally, prostatic weight and prostatic/body weight ratio were similar to those of positive controls despite simultaneous dosing with T. However, this effect was not found in rats receiving DHT and D-004 at 800 mg/kg daily. Further studies are needed to confirm and expand the present results, including the mechanism involving such effects.

REFERENCES

1. Irani J, Brown CT, van der Meulen J, Emberton M. A review of guidelines on benign prostatic hyperplasia and lower urinary tract symptoms: Are all guidelines the same? *BJU Int.* 2003;92:937–942.
2. Oesterling JE. Benign prostatic hyperplasia. Medical and minimally invasive treatment options. *N Engl J Med.* 1995;332:99–109.
3. Barry MJ, Roehrborn CG. Benign prostatic hyperplasia [published correction appears in *BMJ.* 2002;324:775]. *BMJ.* 2001;323:1042–1046.
4. Bhargava S, Canda AE, Chapple CR. A rational approach to benign prostatic hyperplasia evaluation: Recent advances. *Curr Opin Urol.* 2004;14:1–6.
5. Djavan B, Remzi M, Erne B, et al. The pathophysiology of benign prostatic hyperplasia. *Drug Today (Barc).* 2002;38:867–876.
6. Carson C III, Rittmaster R. The role of dihydrotestosterone in benign prostatic hyperplasia. *Urology.* 2003;61(Suppl 1):2–7.
7. Bartsch G, Rittmaster RS, Klocker H. Dihydrotestosterone and the role of 5 alpha-reductase inhibitors in benign prostatic hyperplasia [in German]. *Urologe A.* 2002;41:412–424.
8. 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.
9. 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.
10. Roehrborn CG, Schwinn DA. Alpha₁-adrenergic receptors and their inhibitors in lower urinary tract symptoms and benign prostatic hyperplasia. *J Urol.* 2004;171:1029–1035.
11. Kirby RS, Roehrborn CG, Boyle P, et al. Efficacy and tolerability of doxazosin and finasteride, alone or in combination, in treatment of symptomatic benign prostatic hyperplasia: The Prospective European Doxazosin and Combination Therapy (PREDICT) trial. *Urology.* 2003;61:119–126.
12. McConnell JD, Roehrborn CG, Bautista OM, et al. The long-term effect of doxazosin, finasteride and combination therapy on the clinical progression of benign prostatic hyperplasia. *N Engl J Med.* 2003;18:2387–2398.
13. Desgrandchamps F. Who will benefit from combination therapy? The role of 5 alpha-reductase inhibitors and alpha blockade: A reflection from MTOPS. *Curr Opin Urol.* 2004;14:17–20.
14. Andro MC, Riffaud JP. *Pygeum africanum* extract for the treatment of patients with benign prostatic hyperplasia: A review of 25 years of published experience. *Curr Ther Res Clin Exp.* 1995;56:796–817.
15. 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 1098 patients. *Prostate.* 1996;29:231–240.
16. 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;22:43–51.
17. Bombardelli E, Morazzoni P, Small JK. *Serenoa repens* (Bartram). *Fitoterapia.* 1997;68:99–113.
18. Plosker GL, Brogden RN. *Serenoa repens* (Permixon). A review of its pharmacology and therapeutic efficacy in benign prostatic hyperplasia. *Drugs Aging.* 1996;9:379–395.

19. Wilt TJ, Ishani A, Stark G, et al. Saw palmetto extracts for treatment of benign prostatic hyperplasia: A systematic review. *JAMA*. 1998;280:1604–1609.
20. Gordon AE, Shaughnessy AF. Saw palmetto for prostate disorders. *Am Fam Physician*. 2003;67:1281–1283.
21. Goepel M, Hecker U, Krege S, et al. Saw palmetto extracts potently and non-competitively inhibit human alpha 1-adrenoceptors in vitro. *Prostate*. 1999;38:208–215.
22. Marks LS, Partin AW, Epstein JI, et al. Effects of a saw palmetto herbal blend in men with symptomatic benign prostatic hyperplasia. *J Urol*. 2000;163:1451–1456.
23. Carbin BE, Larsson B, Lindahl O. Treatment of benign prostatic hyperplasia with phytosterols. *Br J Urol*. 1990;66:639–641.
24. Laguna A, Rodríguez E, González V, et al, inventors; Laboratorios Dalmer SA, assignee. Pharmaceutical composition and procedure for the prevention and treatment of prostate hyperplasia and prostatitis obtained from the fruits of *Roystonea regia* (Cuban royal palm). Cuba patent PCT/CU/2004/00004 (pending).
25. Arruzazabala ML, Carbajal D, Mas R, et al. Preventive effects of D-004, a lipid extract from Cuban royal palm (*Roystonea regia*) fruits, on prostate hyperplasia induced with testosterone on intact and castrated rodents. *Drugs Exptl Res*. In press.
26. Nakayama O, Hirosumi J, Chida N, et al. FRI46687, a novel steroid 5 alpha-reductase inhibitor: In vitro and in vivo effects on prostates. *Prostate*. 1997;31:241–249.
27. Pandita RK, Persson K, Hedlund P, Andersson KE. Testosterone-induced prostatic growth in the rat causes bladder overactivity unrelated to detrusor hypertrophy. *Prostate*. 1998;35:102–108.
28. Paubert-Braquet M, Richardson FO, Servent-Saez N, et al. Effect of *Serenoa repens* extract (Permixon) on estradiol/testosterone-induced experimental prostate enlargement in the rat. *Pharmacol Res*. 1996;34:171–179.
29. Mahapokai W, Van Sluijs FJ, Schalken JA. Models for studying benign prostatic hyperplasia. *Prostate Cancer Prostatic Dis*. 2000;3:28–33.
30. Habib FK, Ross M, Bayne CW. Development of a new in vitro model for the study of benign prostatic hyperplasia. *Prostate Suppl*. 2000;9:15–20.
31. Tutrone RF Jr, Ball RA, Ornitz DM, et al. Benign prostatic hyperplasia in a transgenic mouse: A new hormonally sensitive investigatory model. *J Urol*. 1993;149:633–639.
32. Deslypere JP, Young M, Wilson JD, McPhaul MJ. Testosterone and 5 α -dihydrotestosterone interact differently with the androgen receptor to enhance transcription of the MMTV-CAT reporter gene. *Mol Cell Endocrinol*. 1992;88:15–22.
33. Zhou ZX, Lane MV, Kemppainen JA, et al. Specificity of ligand-dependent androgen receptor stabilization: Receptor domain interactions influence ligand dissociation and receptor stability. *Mol Endocrinol*. 1995;9:208–218.

Address correspondence to: Rosa Más, PhD, Center of Natural Products, National Center for Scientific Research, Avenue 25 and 158th Street, Post Box 6990, Havana, Cuba. E-mail: clinica@enet.cu