Latin American Journal of Pharmacy
(formerly *Acta Farmacéutica Bonaerense)*
Lat. Am. J. Pharm. **27** (1): 41-5 (2008)

Trabajos originales

Recibido el 18 de septiembre de 2007
Aceptado el 24 de noviembre de 2007

Different Ripening Stages of *Roystonea regia* Fruits Influence their Effects on Testosterone-induced Prostate Enlargement in Rats

María L. ARRUZAZABALA *, Vivian MOLINA, Daisy CARBAJAL, Rosa MÁS, Victor GONZÁLEZ, Eduardo RODRÍGUEZ & David MARRERO

Centro de Productos Naturales, Centro Nacional de Investigaciones Científicas, Avenida 25 y 158, P.O 6880, Cubanacán, Ciudad Habana, Cuba

SUMMARY. The effects of lipid extracts from *Roystonea regia* fruits at different ripening stages on prostate hyperplasia induced with testosterone in rats were compared . Ripe, medium ripe and unripe fruits were collected and processed to obtain lipid extracts whose fatty acid contents were determined by gas chromatography. Lauric acid increased while oleic acid concentrations decreased, as per fruit ripening, while other fatty acids had little changes. Higher concentrations of lauric acid reduced prostate enlargement, meanwhile batches with low concentrations were ineffective.

RESUMEN. "Diferentes Estados de Maduración de los Frutos de *Roystonea regia* afectan su Efecto sobre el Aumento del Tamaño de la Próstata inducido por Testosterona en Ratas". En este trabajo se han comparado los efectos de extractos lipídicos obtenidos de los frutos de *Roystonea regia* de diferentes estados de maduración sobre la hiperplasia prostática inducida por testosterona en ratas. Frutos maduros, pintones y verdes fueron colectados y procesados para la obtención de extractos lipídicos, cuyo contenido de ácidos grasos fue analizado por cromatografía gaseosa. A medida que los frutos maduran la concentración de ácido láurico aumenta y el oleico disminuye, mientras que los otros ácidos presentaron pocos cambios en sus concentraciones. Los extractos con mayores concentraciones de ácido láurico fueron más efectivos en reducir el incremento del tamaño de la próstata inducido por testosterona.

INTRODUCTION

Benign prostate hyperplasia (BPH) is the uncontrolled growth of both glandular and stromal elements of prostate gland that may urethral obstruction and difficult urinating, very common in the aging man [1-4]. The mechanisms triggering BPH are not totally elucidated, but hormonal changes that occur with age seems to be a pivotal factor [4-6], associated to the increased conversion of testosterone (T) in dihydrotestosterone (DHT), its highly active metabolite, catalyzed by the prostate 5-α reductase enzyme. DHT binds to androgenic receptors and promotes protein synthesis and cellular growth, but with aging, it increasingly accumulates in the prostate and produces hyperplasia 5-6, therefore, prostate 5 α-reductase inhibitors are indicated to treat BPH, improving the enlarged prostate and associated complications, although their impact on symptoms is modest [7-9]. BPH, however, also involves non-hormonal factors, like the increased tone of prostate smooth muscle through the stimulation of α1-adrenergic receptors [10], and 1-adrenoreceptors blockers are frequently used for improving BPH symptoms [11].

Phytotherapy, and mainly the lipid extracts of the fruits of saw palmetto *(Serenoa repens)* palm (Arecaceae) are commonly used to treat BPH [12-17], although some placebo-controlled trials have found negative results [18-19], perhaps influenced by differences in the composition and formulation of the extracts.

Lipid extracts from saw palmetto fruits contain fatty acids, mainly oleic ($C_{18:1}$), lauric ($C_{12:0}$), mirystic ($C_{14:0}$), palmitic ($C_{16:0}$), linoleic ($C_{18:2}$), among others, their esters, and plant

KEY WORDS: D-004, Lauric acid, Prostate hyperplasia, Prostate 5α-reductase activity, *Roystonea regia*.
PALABRAS CLAVE: Ácido láurico, Actividad 5α-reductasa prostática, D-004, Hiperplasia prostática, *Roystonea regia*.

* Author to whom correspondence should be addressed. *E mail:* lourdes.arruzazabala@cnic.edu.cu

sterols and higher aliphatic alcohols as minor components [12, 20]. Saw palmetto extracts lipid extracts, as well as lauric (LA), myristic and oleic acids, have shown to inhibit prostate 5 α-reductase, while palmitic and stearic acids, esterified fatty acids, alcohols and sterols, have been ineffective [21-26]. Consequently, these extracts prevent T-induced prostate hyperplasia (PH) in rodents [27]. One study, however, reported that saw palmetto extract has no effect on enzyme activity [28].

D-004 is a lipid extract of royal palm *(Roystonea regia)* fruits that contains a mixture of free fatty acids, like lauric, oleic, palmitic and myristic acids, while linoleic, palmitoleic, linolenic, caprylic, capric and stearic acids are at lower concentrations. D-004 has shown to inhibit 5 α-reductase in vitro [29], and to prevent prostate hyperplasia (PH) induced with T [30-33], not with DHT [31], in rodents *in vivo*.

Since fruit fatty acid composition is influenced by fruit ripening [34-35], the composition of D-004, mainly obtained from ripe fruits, could change with fruit ripening, and because of lauric, oleic and myristic acids inhibit prostate 5 α-reductase activity [21-26] such changes could influence the effects of the extracts.

Given the common variation in the quality of herbal products, control of the identity of the active ingredients, and of the aspects influencing this matter, should be imperative to ensure reproducible effects and safety. In light of these matters, this study investigated the effects of lipid extracts from *Roystonea regia* fruits of at different ripening stages, but obtained through a same manufacturing process, on the PH induced with T in rats.

MATERIALS AND METHODS

Preparation of lipid extracts from Roystonea regia

Roystonea regia fruits of different ripening stages were collected of the West of Havana City. Ripening stage was classified in three options assessed through the appearance of the fruits, in ripe (R, red/orange), medium ripe (MR, green/red spots) and unripe (UR, totally green) fruits were dried at room temperature for 15 days, grounded and homogenized independently to obtain separated pools of R, MR and NR dry fruits. Dry fruits were saponified first and extracted with hexane later to obtain the respective lipid extracts namely the batches: 021104 and 030306 (R fruits), 190606 (MR fruits), 020206 and 211004 (UR fruits). Samples of these batches were taken for chemical analysis and pharmacological evaluation.

Gas chromatography analysis

The fatty acid content of the samples was assessed by a gas chromatography method, in a Shimadzu GC14-B chromatograph, with flame-ionization detector for quantification. The fatty acid methyl esters of the lipid extract were prepared by methylation using acetyl chloride-methanol (10:90, v/v) according to a validated method (INA Method 108.003), using a BP225 column (30 m x 0.53 mm, 0.5 µm Df, SGE, Australia). The oven was heated from 80 to 182 °C at 20°C/min, and from 182 to 220 °C at 2 °C/min. The injector/detector was set at 220 °C. H2 at a flow of 4 mL/min was the carrier gas. The identities of the FID peaks were confirmed *versus* authentic standards (Sigma Chemical Co., St. Louis, Missouri, USA) and concentrations were calculated from peak areas using tridecanoic acid as internal standard.

Effects of the extracts on PH induced with T in rats

Animals

Young adult male SD rats, weighing 250-270 g, were acquired at the National Centre for Laboratory Animals Production (CENPALAB, Havana, Cuba), and adapted to laboratory conditions (temperature 25 ± 3 °C, relative humidity 60 ± 5 %, light/dark cycles of 12 h) for 7 days. Food (rodent chow obtained from CENPALAB) and water were provided *ad libitum*. An independent ethical board approved animal use. Animal handle conducted according with the Cuban Regulations for the use and ethical management of laboratory animals.

Administration and dosage

The lipid extracts of *Roystonea regia* fruits were suspended in a 2% Tween 65/H2O vehicle. After corroborating their homogeneity and stability, suspensions were prepared immediately before use and administered orally through gastric gavage (1 mL/rat).

Testosterone-propionate (Cuban Medical Pharmaceutical Industry, Cuba) was diluted in soy oil, and injected subcutaneously (sc, 3 mg/kg) for inducing PH, as described by Pandita *et al.* [36].

PH induced in rats

Rats were randomly distributed in 7 groups (10 rats/group). A negative control injected sc with soy oil and orally treated with Tween

65/water vehicle, and six groups injected sc with T (3 mg/kg): one orally treated with the vehicle (positive control) and five others with the different extracts (batches: 021104, 030306, 190606, 020206 and 211004) of *Roystonea regia* fruits, all at 400 mg/kg. Treatments were administered daily (6 days a week) for 14 days.

Bodyweight was controlled the day before starting the treatment (baseline) and weekly thereafter. At study completion, animals were anaesthetized with ether and sacrificed.

The whole prostates were immediately removed and weighed. Personnel involved in these procedures were blind to the treatment allocation of the rats. The extracts were considered as effective if significantly reduced prostate enlargement induced with T with respect to the positive control.

STATISTICAL ANALYSIS

This study was planned expecting about a 50% inhibition *versus* the positive control group. To detect such effect, with $\alpha = 0.05$, and a test power of 0.80, 10 rats/group should be sufficient. Comparisons between groups were done using the two-side non-parametric Mann-Whitney. The relation the relative concentration of the acids and the effects were determined by test of Correlation matrices. All analyses were performed using the software Statistics for Window (Release 4.2; Stat Soft, Inc., USA).

RESULTS

Table 1 shows variations in the relative concentrations of individual fatty acids in the different extracts of *Roystonea regia* fruits. LA increased, while oleic acid concentrations decreased, according to fruit ripening, while meanwhile the relative proportions of the other acids were rather stable.

Table 2 summarizes the effects on T-induced prostate enlargement in rats. The batches obtained from R (021104, 030306) and MR (190606) fruits, not from UR (020206 and 211004) fruits significantly prevented the increase of prostate weight induced with T. LA concentrations, not of other individual acids, directly and highly correlated (r =1.00) with the inhibition of prostate enlargement, so that batches with higher concentrations of LA (30.2%, 21.6% and 16.0%, respectively), reduced prostate enlargement by 64.0, 56.0 and 49.7%, respectively, with respect to the positive control, whereas batches with concentrations ≤ 15% were ineffective. Although the concentration of oleic acid seemed to present an inverse relation, regression analysis did not show significant results or negative slopes (r = 0.47).

Not any extract changed significantly the body weight compared with the positive control (Table 3).

DISCUSSION

The present study demonstrates that the concentration of LA and oleic acid of lipid extracts of *Roystonea regia* fruits change according to fruit ripening, despite the manufacturing process was the same, and that the LA content of these extracts is relevant to effectively prevent T-induced prostate enlargement in rats.

Fatty acids	Batch 021104	Batch 030306	Batch 190606	Batch 020206	Batch 211004
	Ripening stages				
	R	R	MR	UR	UR
Caprilic ($C_{8:0}$)	0.8	0.6	0.3	0.3	0.2
Capric ($C_{10:0}$)	1.0	0.7	0.4	0.4	0.3
Lauric ($C_{12:0}$)	30.2	21.6	17.0	15.0	14.8
Myristic ($C_{14:0}$)	10.4	8.9	8.7	9.1	8.8
Palmitic ($C_{16:0}$)	7.7	10.8	11.3	9.0	8.0
Palmitoleic ($C_{16:1}$)	0.1	0.5	0.3	0.2	0.1
Stearic ($C_{18:0}$)	2.2	2.0	2.2	2.5	2.1
Oleic ($C_{18:1}$)	29.7	29.5	40.8	40.4	43.3
Linoleic ($C_{18:2}$)	9.5	9.7	13.7	13.3	10.5
Linolenic ($C_{18:3}$)	0.1	2.1	0.2	0.3	0.1
Total	91.7	86.3	93.9	91.1	88.2

Table 1. Composition of fatty acids in fractions obtained from fruits of Cuban royal palm. Composition is expressed as percents in the whole lipid extracts. **R** ripe, **MR** medium ripe, **UR** unripe (Details in Material and Methods).

Treatment	Prostate weight (mg)	Inhibition (%)
Negative control	445.6 ± 25.4	-
Positive control	922.6 ± 42.5++	-
Lipid extracts from *Roystonea regia* fruits		
Batch 021104 (400 mg/kg) (R)	620.0 ± 44.4 ***	64.0
Batch 030306 (400 mg/kg) (R)	655.0 ± 42.7 **	56.0
Batch 190606 (400 mg/kg) (MR)	686.2 ± 43.6*	49.7
Batch 020206 (400 mg/kg) (UR)	730.3± 42.3	40.2
Batch 211004 (400 mg/kg) (UR)	740.3± 43.3	38.2

Table 2. Effects of lipid extracts from Roystonea regia fruits at different ripening stages on prostate weight increase (mean ± SD) induced with T in rats. SD standard deviation, **T** testosterone, **R** ripe, **MR** medium ripe, **UR** unripe (Details in Material and Methods). * $p< 0.05$; ** $p< 0.01$. Comparison with positive control, ++ $p <0.001$ Comparison with negative control, (Mann-Whitney U test).

Treatment	Baseline	After treatment
Negative control	228.2 ± 11.5	350.8 ± 8.08
Positive control	236.3 ± 7.75	340.9 ± 8.92
Lipid extracts from *Roystonea regia* fruits		
Batch 021104 (400 mg/kg)	236.0 ± 7.2	345.5 ± 9.0
Batch 030306 (400 mg/kg)	237.1 ± 10.0	335.7 ± 12.8
Batch 190606 (400 mg/kg)	235.0 ± 7.8	339.2 ± 8.7
Batch 020206 (400 mg/kg)	238.0 ± 9.1	340.7 ± 10.7
Batch 211004 (400 mg/kg)	236.2 ± 8.9	338.7 ± 10.2

Table 3. Effects on body weight (mean ± SD) of study animals. **SD** standard deviation.

Fruit lipid extracts from *Roystonea regia* [12,20] show similarities in their fatty acid composition, including LA among the most abundant compounds, which can be affected by different factors, as fruit ripening, since changes on the fatty acids composition in fruit extracts of other Arecacea, like *Cocos nucifera,* have been reported [34].

Consequently, the lipid extracts of *Roystonea regia* and saw palmetto fruits show common effects, like to prevent T-induced prostate enlargement in rodents [28-33]. Free lauric, myristic and oleic acids, not palmitic and stearic acids, have shown to inhibit prostate 5 α-reductase activity [23-26], therefore, compositional changes of individual acids could affect the effects of these lipid extracts.

Taking into account that *in vivo* effects include the contribution of drug absorption and distribution in target tissues, predicting better the effects of a substance in the man, we studied the free fatty acid composition of the lipid extracts of *Roystonea regia* fruits of different ripening stage and their effects on prostate enlargement induced with T *in vivo*.

As per fruit ripening, lipid extracts of royal palm fruits of same palm trees and obtained through the same manufacturing method, LA concentrations increased, and oleic acid concentrations decreased, while the other fatty acids were little affected.

The lipid extracts of R and MR fruits, which contained the higher LA concentrations, not the lipid extracts of UR fruits, were effective to inhibit the prostate enlargement induced with T, and the LA concentrations highly correlated with the efficacy of the extracts on this model, consistent with previous data of the effects of LA on prostate 5 α-reductase activity [23-26] . Nevertheless, although oleic acid has been reported to inhibit the enzyme activity *in vitro* [26], their increased concentrations in UR extracts did not compensate the fall in LA to achieve the efficacy criterion on this model. Since the compositional changes in other acids were minor, the key role of LA in the efficacy of *Roystonea regia* fruit extracts seems to be evident.

Since no extract changed significantly body weight values, the effects on prostate enlarge-

ment presented should not be influenced by changes in bodyweight, and actually depend of the effects of the extracts on prostate, as target organ.

A limitation of the present study, however, is that results were obtained with fruits collected from *Roystonea regia* trees of a same area and during a same season. Therefore, in which extension the changes here presented are representative of fruits ripening deserves future research, assessing the fatty acid content in fruits at different ripening stages, from different sites and through different seasons.

CONCLUSIONS

The fruit ripening of the royal palm *Roystonea regia* influences on the composition of lipid extracts obtained through a same manufacturing method. LA concentrations in the extracts increased with fruit ripening, and positively correlated with the inhibition of T-induced prostate enlargement. Therefore, LA concentration limits should be among the quality specifications of these extracts to ensure reproducible quality and meaningful effects. These results indicate that the extracts should not be prepared from UR fruits, and that the minimal concentration of these extracts should be above 15%, although further studies should corroborate the definitive specification limits.

Acknowledgement. This study was conducted through a research grant of the West Havana Scientific Pole.

REFERENCES

1. Barry, M.J. & C.G. Rochrborn (2001) *Brit. Med. J.* **323**: 1042-6.
2. Djavan, B., M. Remzi, B. Erne & M. Marberger (2002) *Drugs Today (Barc).* **38**: 867-76.
3. Thorpe A & D. Neal (2003) *Lancet* **19**: 1359-67.
4. Bhargava, S., A.E. Canda & C.R. Chapple (2004) *Curr. Opin. Urol.* **14**: 1-6.
5. Bartsch, G., R.S. Rittmaster & H. Klocker. (2000) *Eur. Urol.* **37**: 367-80.
6. Carson, C. & R. Rittmaster (2003) *Urology* **61** (Suppl 1): 2-7.
7. Bartsch, G., R.S. Rittmaster & H. Klocker (2002) *Urologe A* **41**: 412-24.
8. Lam, J.S., N.A. Romas & F.C. Lowe (2003) *Urology* **61**: 354 -8.
9. Sandhu, J.S. & A.E. Te (2004) *Curr. Urol. Rep.* **5**: 274-9.
10. Michel, M.C., K. Taguchi, R.S. Schafers, T.J. Williams, D.E. Clarke & A.P. Ford (1998) *Adv. Pharmacol.* **42**: 394-8.
11. Olke, M., K. Hofner, R.R. Berges & U. Jonas (2002) *Urologe A* **41**: 425-41.
12. Plosker, G.L & R.N. Brodgen (1996) *Drugs Aging* **9**: 379-95
13. Lowe, F.C, K. Dreikorn, A. Borkowski, J. Braeckman, L. Denis, P. Ferrari, G. Gerber, R. Levin, P. Perrin & T. Senge (1998) *Prostate* **37**: 187-93.
14. Wilt, T., A. Ishani, R. Mac Donald, C. Mulrow & J. Lau. (2000) *Cochrane Database Syst. Rev.* **3**: CD001423Alaiev, I.G., A.Z. Vinarov, K.L. Lokshin & L.G. Spivak. (2002) *Urologia* **1**: 23-5.
15. Alaiev IG, AZ Vinarov, KL Lokshin & LG Spivak. (2002) *Urologia* **1**: 23-25.
16. Carraro, J.C., J.P. Raynaud, G. Koch, G.D. Chirsholm, F. Di Silverio, P. Teillac, F.C. Da Silva, J. Cauquil, D.K. Chopin, F.C. Hamdy, M. Hanus, D. Hauri, A. Kalinteris, J. Marencak, A. Perier & P. Perrin (1996) *Prostate* **29**: 231-40.
17. Debruyne, F., G. Koch, P. Boyle, F.C. Da Silva, J.G. Gillenwater, F.C. Hamdy, P. Perrin, P. Teillac, R. Vela-Navarrete, J.P. Raynaud, Groupe d' étude PERMAL (2002) *Prog. Urol.* **3**: 384-92.
18. Willetts, K.E., M.S. Clements, S. Champion, S. Ehsman & J.A. Eden (2003) *Brit. J Urol. Int.* **92**: 267-70.
19. Bent, S., C. Kane, K. Shinohara, J. Neuhaus, E.S. Hudes, H. Goldberg, & A.L. Avins (2006) *New Engl. J. Med.* **354**: 557-66.
20. INA (Institute for Nutraceutical Advancement) Method 108.003. Fatty Acid Content in Saw Palmetto by GC. http://www.nsf.org/busines/ina/fattyacids.asp.
21. Bayne, C.W., F. Donnelly, M. Ross & F.K. Habib (1999) *Prostate* **40**: 232-41.
22. Habib, F.K., M. Ross, C.K.H. Ho, V. Lyons & K. Chapman. (2005) *Int. J. Cancer* **114**: 190-4.
23. Niederprum, H.J., H.U. Schweikert & K.S. Zanker (1994) *Phytomedicine* **1**: 127-33.
24. Weisser, H., S .Tunn, B. Behnke & M. Krieg (1996) *Prostate* **28**: 300-6
25. Raynaud, J.P., H. Cousse & P.M. Martin. (2002) *J. Steroid. Biochem.* **82**: 233-239.
26. Liang, T. & Liao, S. (1992) *J. Biochem.* **285**: 557-62.
27. Paubert-Braquet, M. & F.O. Richardson (1996) *Pharmacol. Res.* **34**: 171-9.
28. Rhodes, C.W., R.L. Priomka, C. Berman, G.Vergult, M. Gabriel, M. Pierre-Malice & B. Gibelin (1993) *Prostate* **20**: 43-51.
29. Pérez, Y., R. Menéndez, R. Más & R. González (2006) *Curr. Ther. Res.* **67**: 396-405.
30. Arruzazabala, M.L., D. Carbajal, R. Más, V. Molina, V. González & E. Rodríguez (2004) *Drugs Exp. Clin. Res.* **30**: 227-33.
31. Carbajal, D., M.L. Arruzazabala, R. Mas, V. Molina, E. Rodríguez & V. González (2004) *Curr. Ther. Res. Clin. E.* **65**: 505-14.
32. Carbajal, D., V. Molina, R. Mas & M.L. Arruzazabala (2005) *Drugs Exp. Clin. Res.* **31**: 193-7.
33. Noa, M., M.L. Arruzazabala, D. Carbajal, R. Mas, V. Molina (2005) *Int. J. Tissue Reaction* **32**: 203-11.
34. Akpan, E.J., O.E. Etim, H.D. Akpan & I.F. Usoh (2006) *Pakistan J. Nutr.* **5**: 106-9.
35. Salvador, M.D., F. Aranda & G. Fregapane (2001) *Food Chemistry* **73**: 45-53.
36. Pandita, R.K., K. Persson, P. Hedlund & K.E. Andersson (1998) *The Prostate* **35**: 102-8.