

Comparative Effects of *Roystonea Regia* (D-004) and Saw Palmetto Lipid Extracts On Blood Oxidative Variables in Men with Benign Prostate Hyperplasia (BPH)

Raúl Guzmán¹, José Illnait², Rosa Mas², Yohani Perez², Lilia Fernández,² Sarahí Mendoza,² Ambar Oyarzábal,² Julio Fernández,² Meilis Mesa,³ Lisete Borrero,³ Pablo Reyes.⁴

¹Dr. Salvador Allende Hospital, Havana City, Cuba.² Centre of Natural Products, National Centre for Scientific Research, Havana City, Cuba.³ Medical Surgical Research Centre, Havana City, Cuba.

⁴ Software and Database Group from the National Centre for Scientific Research, Havana City, Cuba.

ABSTRACT:

Background: Lipid extracts of *Roystonea regia* (D-004) and saw palmetto (SP) fruits have been shown to prevent experimentally-induced prostate hyperplasia in rodents, and to produce antioxidant effects in experimental and clinical studies.

OBJECTIVE: To compare the effects of D-004 and SP extracts on the International Prostate Symptoms Score (IPSS) and plasma oxidative variables in men with benign prostate hyperplasia (BPH)

METHODS: This randomized, double-blind study was conducted in patients with moderate BPH. Forty-eight eligible subjects (average age: 65 years) were randomised to D-004 (320 mg/day) or SP (320 mg/day) capsules for 8 weeks. Decrease on IPSS was the primary efficacy variable. Oxidative markers were secondary outcomes. Data were analysed as per Intention to treat.

RESULTS: D-004 and SP significantly decreased mean IPSS values by 33.9% ($p < 0.0001$) and 24.4% ($p < 0.001$), respectively, as compared to baseline: D-004 ($p < 0.0001$) reduced plasma malondialdehyde (MDA) (32.6%), protein-linked carbonyl groups (CG) (25.2%) and increased ($p < 0.0001$) catalase (CAT) activity. SP treatment lowered ($p < 0.0001$) MDA (28.2%), CG (23.4%) and raised ($p < 0.0001$) CAT activity. Effects on oxidative variables were similar in both groups. D-004, not SP, significantly lowered ($p < 0.05$) prostate specific antigen (PSA) values. Both treatments were well tolerated. Only 2 SP-treated patients withdrew from the study. No adverse experiences were reported.

CONCLUSIONS. Treatment with D-004 or SP (320 mg/day) for 8 weeks decreased significantly IPSS values in patients with moderate BPH, the effect of D-004 being the better, but further studies should confirm this result. Both treatments favourably and similarly modified plasma MDA (lipid peroxidation marker), GC (protein oxidation marker) and CAT activity.

KEY WORDS: antioxidant, benign prostate hyperplasia, D-004, lipid peroxidation, *Roystonea regia*, saw palmetto

I. INTRODUCTION

Accumulating evidences suggest that oxidative stress (OS), resulting from the imbalance of reactive oxygen species (ROS) production and cellular antioxidant defensive systems, is a key aging-associated factor on prostate diseases [1,2]. Cumulative ROS effect may contribute to lipids, proteins, and DNA damage. Prostate gland is prone to OS probably due to inflammation and hormonal deregulation processes and epigenetic modifications, frequently occurring in such target, which may contribute to develop benign prostate hyperplasia (BPH) and associated lower urinary tract symptoms (LUTS) [3,4]. The link between increased OS and BPH is underlined by the efficacy of antioxidant phytotherapy agents in experimentally-induced prostate hyperplasia and in BPH, as well [5,6]. Despite some recent negative results [7,8], other studies and views support the use of the lipid extracts of saw palmetto (SP) (*Serenoa repens*) fruits, widely used for decades, as the main phytotherapeutic agent to treat BPH [9-12]. SP extracts contain a mixture of fatty acids, mainly oleic, lauric and myristic acids [13]. The efficacy of saw palmetto in BPH is associated to a multifactorial mechanism including the inhibition of prostate 5α -reductase, the antagonism of $\alpha 1$ -adrenoreceptors, anti-inflammatory and antioxidant effects, as well [14-16]. D-004, a lipid extract of the *Roystonea regia* (Arecaceae fam) fruits also contains a mixture of fatty acids, oleic, lauric, palmitic and myristic being the most abundant. Experimental studies demonstrated that D-004 decreased testosterone (T)-induced prostate hyperplasia in rodents [17-20], inhibited rat prostate 5α -reductase in vitro [21], antagonized $\alpha 1$ -adrenoreceptors-mediated responses in vitro and in vivo [22-24] and produced antioxidant effects in the prostate tissue of normal and T-treated rats [25,26].

Oral D-004 (320 mg/day) treatment for 6-8 weeks has been shown to reduce significantly plasma malondialdehyde (MDA), total hydroxyperoxides (TOH) and sulphhydryl groups (SHG), and to increase total antioxidant status (TAS) and catalase (CAT) activity in healthy men [27,28], effects similar to those produced by the same dose of SP extract [28]. No previous trial, however, had investigated the antioxidant effects of D-004 in patients with BPH. In light of these issues, this study compared the effects of D-004 and SP on the International Prostate Symptoms Score (IPSS) and plasma oxidative variables in men with BPH.

II. MATERIALS AND METHODS

2.1 Study Design

This randomized, double-blinded, comparative study was conducted in Dr. Salvador Allende Hospital (Havana City, Cuba) in accordance with the Declaration of Helsinki, its protocol being approved by the Institutional Ethics Committee of the centre. After obtaining their informed written consent, BPH patients with an international prostate symptom score (IPSS) of 7 or more, but < 19, were enrolled and underwent clinical history and physical examination for screening their eligibility for randomization (visit 1). On the second visit, they were randomized to D-004 or SP (320 mg) soft gel capsules once daily for 8 weeks and continued on their usual dietary habits. Interim check-up (visit 3) and final (visit 4) visits were done after complete 4 and 8 weeks on therapy, respectively. Subjects underwent a physical examination at each visit. Treatment compliance and adverse experiences (AE) were controlled at visits 3 and 4. Laboratory tests (oxidative variables, blood safety indicators) were conducted at baseline and after complete 8 weeks on treatment.

2.2 Study subjects

BPH patients aged 48 to 75 years were enrolled in the trial. To be eligible for randomization, enrolled men should have an IPSS value ≥ 7 , but <19 and should not show any of the exclusion criteria summarised below. Digital rectal prostate examination confirmed participants' eligibility for the study. Patients with any major prostate disease except BPH or those who had urogenital surgery, urinary retention or any complication were excluded from the study. Likewise, patients with arterial pressure >180/110 mmHg, diagnosed neoplasias, psychiatry problems that limited proper answers to the IPSS questionnaire, serious events (acute coronary syndromes, stroke, transient ischemic attacks, major surgery, among others) during the prior 6 months and/or receiving BPH/LUTS-related therapy (inhibitors of 5 α -reductase, α 1-adrenoreceptor inhibitors, or phytotherapy) were also excluded. Causes of premature discontinuations were to experience any AE justifying such a decision, unwillingness to continue on the trial and major violations (failure in taking study treatments for ≥ 5 days and/or to consume supplements or medicines with antioxidant effects).

2.3 Treatments

The free fatty acid composition of the capsules of D-004 and SP, provided by the Rainbow & Nature, Ltd (Sydney, Australia), assessed with a validated gas chromatography method, was as follows: D-004 capsules: caprylic 0.4 %, capric 0.6 %, lauric 21.9%, myristic 10.8%, palmitic 10.5%, palmitoleic 0.3%, stearic 2.3%, oleic + linoleic + linolenic 43.3%, with a purity (total of free fatty acids) of 90.1%. SP capsules: caprylic 1.5%, capric 2.5%, lauric 29.9%, myristic 10.8%, palmitic 8.2%, palmitoleic 0.2%, stearic 1.9%, oleic + linoleic + linolenic 33.3%, with a purity of 88.4%. The daily dosage (320 mg/day) was selected taken into account that D-004 (320 mg/day) administered for short-term reduced plasma MDA, TOH, SHG and increased TAS in healthy males [27,28], and the most common dose of SP used in different clinical studies [9-12]. D-004 and SP capsules, identical on appearance and packaged in identical codified containers, were given to the subjects according to their serial progressive inclusion. Randomisation was computer-generated using balanced blocks and allocation ratio 1:1. Participants were advised to bring all unused treatment to each visit. At visits 3 and 4 treatment compliance was assessed by counting the remainder capsules and interviewing the subjects. Compliance was considered as good if the subjects taken at least 80% of the capsules scheduled from the previous visit, and very good if consumption was over 90%. Medications and/or supplements with known effects on BPH/LUTS or/and antioxidant effects were not allowed during the study. Subjects who were taking some of them were eligible for randomisation only if they discontinued consumption for at least 3 months prior to the trial.

2.4 Efficacy variables

A significant reduction of IPSS was the primary efficacy variable. IPSS was assessed using a standard questionnaire form, composed with seven questions, each measured on a scale, to which patients respond on a scale of 0 (the best) to 5 (the worst) [29-31]. Decreases of MDA, plasma carbonyl groups (CG) and increase of CAT activity were secondary variables.

2.5 Safety and tolerability

Data from physical examination, laboratory safety indicators and adverse experiences (AE) were analysed. All undesirable events occurred to a subject during the trial, disregarding the cause, should be considered as AE, whenever they newly appeared during the trial.

In accordance with their intensity, AE were classified as mild, moderate or serious. Mild AE should not require suspension of study capsules and/or specific treatment of the AE, moderate AE should require stopping therapy and/or specific treatment of the AE, serious AE should lead to hospitalisation and/or deaths.

In addition, at study completion effects on sexual performance were assessed by a simple 3 score questionnaire as unchanged, worsened or improved.

2.6 Assessment of oxidative variables

Blood venous samples, drawn after an overnight fast of 8-12 hours, were collected in two groups of Eppendorf tubes. Aqueous solution of EDTA 10% was added to a group (final blood concentration of 0.1%) and 5 μ L of sodium heparin 5000UI/mL were added to the others containing 1 mL of blood aliquots. Plasma was separated from red blood cells by centrifuging at 3000 x g for 10 min and suitable portions were taken to assess oxidative variables. Whole blood and serum samples were used for assessing other indicators. Determinations were conducted in an Utrospec-Plus LKB spectrophotometer (Pharmacia LKB Biotechnology, Uppsala, Sweden). Oxidative markers were assessed in the same day of blood drawing. All assays were done in triplicate. Plasma samples were frozen at -70°C for the other analyses, which were done within the next 48 hours.

Plasma MDA concentrations were analyzed with a reagent kit (NWK-MDA01, NWLSSTM, Canada) based on the reaction of MDA with thiobarbituric acid (TBA) forming an MDA-TBA adduct that absorbs strongly at 532 nm [32]. The values of TBA-reactive substances (TBARS), expressed as MDA ($\mu\text{mol/L}$), were calculated from a standard calibration curve generated with known amounts of freshly diluted malondialdehyde bis (dimethyl acetal).

Protein oxidation was measured as the content of CG using the 2,4-dinitrophenylhydrazine (DNPH) assay [33]. After centrifugation at $600 \times g$ for 10 min, plasma aliquots equivalent to 50 mg of protein were allowed to react for 1 hour with 4 mL of 10 mmol/L DNPH dissolved in 2.5 mmol/L HCl. The mixture was vigorously stirred, placed in the darkness for 1 hour, precipitated with 5 mL of 10% trichloroacetic acid, and centrifuged at 3000 rpm for 15 min. The protein pellet was washed thrice with a mixture of ethanol: ethyl acetate (1:1), dissolved in 2 mL of 6 mol/L guanidine HCl in 20 mmol/L potassium phosphate (pH 2.3), centrifuged. The carbonyl content of the supernatant was measured at 362 nm ($\epsilon = 22000\text{M}^{-1}\text{cm}^{-1}$) and reported in nmol/mg of protein.

CAT enzymatic activity, expressed as UI/min x mg protein, was quantified with a modification of Aebis (1974) method [34]. Briefly, 0.1 mL of hydrogen peroxide was added to 2.89 mL of potassium-phosphate buffer (50 mmol/L, pH=7.4) containing 10 μ L of plasma (25°C). CAT activity was calculated by assessing the molar coefficient extinction (43.6×10^{-3}) for 5 min at 240 nm and reported in UI/min/ mg of protein.

2.7 Safety indicators

Safety indicators included physical (body weight, pulse rate and blood arterial pressure), haematological (haemoglobin, hematocrit, platelets, red cells and white cell counts) and blood biochemistry safety indicators (alanine amino transferase –ALT-, aspartate amino transferase –AST-, glucose, creatinine, total cholesterol (TC) triglycerides (TG)). Haematological indicators were automatically determined in the Haematological Complex equipment. Blood biochemistry indicators were assessed using reagent kits (Roche, Switzerland) in the Hitachi 912 autoanalyser (Tokyo, Japan) of the Medical Surgical Research Centre (Havana City, Cuba). PSA levels were determined by immunoenzymatic method (Cobas reagent kit).

2.8 Statistical analysis

A sample size of 20 subjects/treatment group was expected to provide 80% power to detect a 20.0% between-group difference in the mean percent change from baseline in IPSS. Data analyses were performed in accordance to intention to treat (ITT), including all randomized subjects, regardless of study treatment compliance. Assuming a 10% of premature withdrawals, approximately 45 patients should be enrolled, so that 50 subjects were enrolled.

Comparisons of continuous variables were performed using the t test for paired (within group comparisons) and the t test for independent samples (between group comparisons). Categorical variables were compared with the two tailed Fisher's Exact Test. A value of $\alpha = 0.05$ was assumed for statistical significance. Comparisons were done with the Statistics software for Windows (USA).

III. RESULTS

3.1 Baseline characteristics of study subjects

Of 50 enrolled patients 48 were randomised to D-004 (n=24) or SP (n=24), while 2 were not eligible because of their IPSS values were above exclusion criteria. Forty-six (46) of 48 randomized subjects (95.8%) completed the study. The two withdrawals were due to protocol violations.

Both study groups were well balanced at baseline (Table 1). Main co-morbid diseases were hypertension (56.2%), diabetes (25.0%), dyslipidemia (22.9%) and obesity ($\text{kg/m}^2 \geq 30$) (10.4%). Undesirable lifestyle habits like smoking and alcoholism accounted for 25% and 12.5%, respectively. The frequency of concomitant medications, well matched in the two groups, was high (83.3%), the most frequent being antihypertensive drugs (angiotensin converting enzyme inhibitors –ACEI-, diuretics, β -blockers), followed by oral hypoglycemic, lipid-lowering and antiplatelet drugs.

Treatment compliance was very good (>95%) and similar in both groups.

3.2 Effects on IPSS

IPSS values in both groups were significantly reduced after 8 weeks of treatment as compared to baseline (table 2). IPSS of D-004 group was decreased ($p < 0.0001$) by 4.5 points (33.9% reduction), while the IPSS of SP group was reduced ($p < 0.001$) by 3.1 points (24.4% decrease). Differences between groups were not significant. At study completion, the frequency of D-004-treated patients with IPSS reductions ≥ 3 points (17/24, 70.8%) was apparently, but not significantly, greater than that in SP group (12/24, 50%).

3.3 Effects on plasma oxidative and inflammatory variables

Table 3 shows the effects on plasma oxidative variables. After 8 weeks on treatment D-004 reduced significantly ($p < 0.0001$) plasma MDA (32.6%), CG (25.2%) and increased ($p < 0.0001$) CAT activity. Also, treatment with SP lowered ($p < 0.0001$) MDA (28.2%), CG (23.4%) and raised ($p < 0.0001$) CAT activity. Effects on oxidative variables were similar in both groups. Treatment with D-004, not with SP, significantly lowered ($p < 0.05$) PSA values.

3.4 Safety and tolerability

Both treatments were well tolerated. No significant impairment of physical or blood safety indicators were found, all individual values remaining within normal range (values not shown for simplicity).

Only two patients, both SP-treated, withdrew from the study, none due to adverse experiences. No adverse experiences were reported.

At study completion no study patient referred a worst sexual performance, meanwhile 12/24 (50%) and 11/24 (45.8%) of D-004- and SP-treated patients, respectively, reported a better sexual activity. Unchanged sexual activity was declared by 12 D-004 and 13 SP-treated patients.

IV. DISCUSSION

The results of this study demonstrate that D-004 and SP, administered at 320 mg/day for 8 weeks, improved LUTS, assessed by the changes on IPSS values, and produced beneficial changes on plasma oxidative variables of patients with BPH, significantly reducing plasma MDA, CG, and increasing CAT activity. The present results are consistent with previous reports of the antioxidant effects of D-004 [25-28] and SP [28], and demonstrate, for the first time, that D-004 may decrease IPSS and to produce antioxidant effects in men with BPH that were comparable to those of SP. Both groups had similar baseline characteristics, so that they were homogeneous for comparisons. Study subjects were men (average age: 65 years) with moderate BPH (IPSS values ≥ 7 , but < 19), and with several co-morbidities (hypertension diabetes, dyslipidemia and obesity) characteristic of this population [35,36]. Concomitant medications were consistent with the personal history of the patients.

Despite smoking increases the extent of OS [37,38] and may contribute to BPH risk, although not clearly [39], we did not exclude smokers because we wanted to compare the effects of D-004 and SP in conditions near to routine practice, in which smoking unfortunately this habit is common. The frequency of smokers was relatively high (12 of 48 randomized subjects, 25%), but similar in both groups, so that this factor had no impact on the comparative results. Here we compared the effects of both treatments on IPSS values and plasma oxidative variables. D-004 and SP decreased IPSS by 33.9% and 24.4%, respectively. Although there are no previous references of the effect of D-004 on IPSS, the effect of SP (320 mg/day, once-a-day) administered for only 8 weeks seems to be coherent with the 37% decrease found for a similar dosage given for a longer (6 months) period [40]. We did not find significant differences between the groups, so that we should conclude that D-004 was as effective as SP for ameliorating LUTS on these patients. Indeed, these results suggest that the 20% difference between treatments that we expected for the sample size calculation was

overestimated, so that the present results should be confirmed in studies conducted in larger sample sizes.

Another limitation of this study, however, is that we did not test the effects of the treatments on other classical BPH/LUTS outcomes like prostate volume, post void residual volume and maximal urinary flow, but since the present study was just an introductory pilot study, we pretended to determine whether D-004 was able to modify LUTS, a cornerstone of BPH/LUTS therapy. The present results merit further extensive research on the effects of D-004 on all the urodynamic profile of patients with BPH.

Plasma MDA, a key marker of lipid peroxidation, and the most abundant aldehyde generated by the attack of free radicals on polyunsaturated fatty acids of cell membranes, is a non-invasive biomarker of OS in BPH [41,42]. Reductions of plasma MDA with D-004 (32.5%) and SP (28.2%) achieved the efficacy criterion (20.0% decrease versus baseline). Despite the effect of D-004 was apparently greater than with SP, the lack of between group significant difference lead to conclude that the effects of both treatments were similar, although we cannot discard that similar results in a study conducted in a larger population could have found a significant difference. In turn, the significant reductions of plasma GC, a marker of protein oxidation, with D-004 (25.2%) and SP (23.4%) were similar, and the same was true for the significant increase of CAT activity. The antioxidant effects of D-004 and SP in patients with BPH may be interpreted as an additional potential benefit of both substances on BPH, beyond their effects on 5 α -reductase enzyme and α 1-adrenoreceptors. Indeed, lipid peroxides and protein oxidation are increased in patients with BPH, who have a decreased antioxidant defence system [42]. D-004 and SP not only reduced lipid peroxidation (MDA) and protein oxidation (GC) markers in men with BPH, but increased CAT activity, which indicates an stimulating effect on antioxidant defence system, important target for prostate health. Treatment with D-004, not with SP, decreased PSA. Serum PSA has been reported to be increased in BPH, prostate cancer and prostatitis and others [43]. Despite the current controversy of the real value of PSA as a marker of prostate cancer [44], it is widely used in routine practice due to its simplicity and usefulness to follow the evolution of prostate diseases [45]. The effect of D-004 on this variable, however, should not be interpreted as a proof of efficacy, as recent reports show controversial results of the use of this marker. The lack of effect of SP on this variable agrees with other reports [46]. The treatments were well tolerated, consistent with previous data [25-28,40]. Safety indicators and sexual performance were not impaired by the treatments, which agree with previous reports on SP [11,12,46], and being the first report on this matter for D-004. Also, no adverse experiences were reported.

V. CONCLUSIONS

Treatment with D-004 or SP (320 mg/day) for 8 weeks decreased significantly IPSS values in patients with moderate BPH, the effect of D-004 being the better, but further studies should confirm this result. Both treatments favourably and similarly modified plasma MDA (lipid peroxidation marker), GC (protein oxidation marker) and CAT activity.

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Table 1. Main baseline characteristics of study subjects

Characteristics	Saw palmetto (n = 24)	D-004 (n = 24)	Total (n = 48)
Age (years) (mean \pm SD)	65 \pm 7	65 \pm 6	65 \pm 6
Body mass index (kg/m ²) (mean \pm SD)	24.4 \pm 4.1	25.7 \pm 4.2	25.1 \pm 4.1
IPSS	12.8 \pm 3.5	13.1 \pm 3.5	13.0 \pm 3.4
Prostate serum antigen (ng/mL)	2.26 \pm 1.41	2.23 \pm 1.79	2.25 \pm 1.60
Personal history (n)			
Hypertension	13	14	27
Diabetes	7	5	12
Smoking	5	7	12
Hypercholesterolemia	5	6	11
Alcoholism	4	2	6
Concomitant therapy (n)^a			
Any	20	20	40
ACEI	8	10	18
Diuretics	6	9	15
Oral hypoglycemic drugs	7	5	12
Lipid-lowering drugs	4	3	7
β -blockers	3	3	6
Antiplatelet drugs	3	3	6

N: numbers. IPSS: International Prostate Symptom Score. ACEI: Angiotensin converting enzyme inhibitors

^a Consumed by ≥ 5 patients. All comparisons were not significant.

Continuous variables (t test for independent samples), categorical variables (Fisher Exact Probability test)

Table 2. Effects of treatments on IPSS values in patients with BPB (X ± SD)

Treatment	Baseline	8 weeks	%
D-004	13.12 ± 3.47	8.67 ± 4.16**	-33.9
Saw palmetto	12.79 ± 3.49	9.67 ± 4.09*	-24.4

X: mean, SD: standard deviation

*p < 0.001, **p < 0.0001, Comparison with baseline (t test for paired samples)

Table 3. Effects on oxidative and inflammatory variables in men with BPH

Treatment	Baseline	8 weeks
TBARS (MDA μmol/L) (X ± SD)		
D-004	0.43 ± 0.08	0.29 ± 0.09**
Saw palmetto	0.39 ± 0.11	0.28 ± 0.09**
CG (nmol/mg of protein) (X ± SD)		
D-004	1.39 ± 0.33	1.04 ± 0.28**
Saw palmetto	1.24 ± 0.27	0.95 ± 0.23**
CAT (IU/min/mg of protein) (X ± SD) ($\times 10^{-1}$)		
D-004	0.09 ± 0.03	0.20 ± 0.07**
Saw palmetto	0.08 ± 0.02	0.18 ± 0.07**
PSA (ng/mL)		
D-004	2.26 ± 1.41	1.82 ± 1.35*
Saw palmetto	2.23 ± 1.79	1.89 ± 2.21

X: mean, SD: standard deviation, TBARS: thiobarbituric acid reactive substances, MDA: malondialdehyde, CG: carbonyl groups, CAT: catalase (Values were means of triplicates), PSA: prostate specific antigen.

* p < 0.05; ** p < 0.0001. Comparison with baseline (t test for paired samples)