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Original Article

Antioxidant effects of D-004, a lipid extract from the *Roystonea regia* fruit, on the plasma of healthy men

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Abstract

The aim of this study was to conduct a randomized, double-blind and placebo-controlled study to investigate the effects of D-004, a lipid extract of the *Roystonea regia* fruit that prevents testosterone- and phenylepinephrine-induced prostate hyperplasia in rodents, on plasma oxidative markers in healthy men. We enrolled male volunteers (20–55 years) in good health and without lower urinary tract symptoms. Thirty-four eligible participants were randomized to placebo or D-004 (320 mg) capsules administered daily for 6 weeks. An interim check-up and a final visit were conducted after 3 and 6 weeks of therapy, respectively. Physical examinations were performed at each visit, and laboratory tests were performed at baseline and at treatment completion. Oxidative variables included plasma malondialdehyde (MDA), total hydroxyperoxides (TOH), sulphydryl (SH) groups and total antioxidant status (TAS). We assessed treatment compliance and addressed adverse experiences (AEs) at weeks 3 and 6. At week 6, with D-004, the mean reductions of plasma MDA (26.7%), TOH (18.8%) and SH groups (31.6%), and the mean increase of TAS (35.3%) were significantly different from those of placebo ($P < 0.001$ for plasma TAS, $P < 0.0001$ for all other comparisons). D-004 did not differ from the placebo in safety indicators. There were two withdrawals (both in the D-004 group), with one due to dyspepsia (the only AE during the trial). In conclusion, D-004 displayed antioxidant effects on plasma oxidative markers in healthy men, which was consistent with findings from laboratory experimental studies.

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1 Introduction

Oxidative stress arises when the balance between oxidant factors and cellular antioxidant mechanisms is shifted in favour of the former [1]. Oxygen-free radicals and other chemical species, often referred to as reactive oxygen species (ROS), are recognized as mediators of cell injury that are thought to be involved in the pathogenesis of several disorders [2–9]. Male infertility, benign prostate hyperplasia (BPH) and prostate cancer are among the pathological conditions linked with increased oxidative stress [7–9].

ROS induce tissue damage by several mechanisms, such as lipid peroxidation, protein peroxidation, DNA damage, decrease of cellular thiols and increased release of pro-inflammatory cytokines. The defensive cellular capacity is determined by a dynamic interaction between antioxidant enzymes (superoxide dismutase, glutathione peroxidase) and antioxidant molecules (glu-

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tathione, α-tocopherol, ascorbic acid) [10].

BPH is a growth of the prostate gland that commonly produces lower urinary tract symptoms (LUTS) [11, 12]. In BPH, increased lipid peroxidation [7–9] and decreased levels of superoxide dismutase and antioxidant molecules (α-tocopherol, ascorbate and β-carotene) have been documented [7–9, 13, 14].

Herbal extracts, such as black tea and mango extracts, have been shown to reduce oxidative stress in testosterone (T)-treated rats and mice, respectively [15, 16], and an extract from the cactus flower displayed antioxidant effects and inhibited the 5α-reductase activity in rat prostate homogenates [17]. In addition, *Prunus africana* bark and saw palmetto extracts, which are commonly used to treat BPH, have shown antioxidant effects when given orally [18, 19].

D-004 is a lipid extract of the mature *Roystonea regia* fruit obtained from the basic hydrolysis and additional hexane extraction. It contains a mixture of free fatty acids, the most abundant of which are oleic, lauric, palmitic and myristic acids; fats such as caprylic, capric, palmitoleic, stearic, linoleic and linolenic acids are found in lower proportions. D-004, given orally, has been shown to decrease T-induced prostate hyperplasia in rodents [20–22] and to inhibit rat prostate 5α-reductase *in vitro* [23]. In addition, D-004 antagonized α1-adrenoreceptor-mediated responses [24, 25] in rats and reduced the oxidation of LP and protein in prostate tissue of both normal and T-treated rats [26, 27]. However, the antioxidant effects of D-004 have not yet been shown in humans.

Thus, this study investigated the effects of D-004 (320 mg day^{-1}) on plasma oxidative markers in healthy men without evidence of LUTS.

2 Materials and methods

2.1 Study design

This double-blind, placebo-controlled, randomized study was carried out at the Medical Surgical Research Centre (Havana City, Cuba). The study protocol was approved by the centre's institutional ethics committee, and the study was conducted in accordance with the Declaration of Helsinki.

Written consent was obtained from participants after they were given a verbal and written explanation of the objectives and risks of the trial. After providing consent, male volunteers (20–55 years) in good health were enrolled (visit 1). On this first visit, participants were screened for eligibility with an interview, they underwent physical examinations, and participants without LUTS were instructed to obtain laboratory tests in the following week. On the second visit, participants were randomized to receive placebo or D-004 (320 mg) capsules once daily for 6 weeks. They were allowed to continue their usual dietary habits. Interim check-up (visit 3) and final (visit 4) visit occurred after completing 3 and 6 weeks of therapy, respectively.

Physical examinations were administered at each visit, whereas assessments of treatment compliance and adverse experiences (AEs) were conducted only at visits 3 and 4. Laboratory tests (oxidative variables, blood safety indicators) were conducted at baseline and after the completion of 6 weeks of therapy.

As this study was aimed at investigating whether D-004 produces antioxidant effects in healthy men, we chose a double-blind design to reduce the possible influence of subjective biases from investigators or participants. Thus, a placebo group was included so that any changes of response variables could be attributed to the treatment and not external factors.

2.2 Eligibility criteria

The eligibility criteria for enrolment in the trial included being male, aged 20–55 years and having good health based on medical history and physical examination. To be eligible for randomization, enrolled men had to have an International Prostate Symptom Score (IPSS) < 4 (confirming that they were free of LUTS) and had to be free of any of the exclusion criteria summarized below.

Exclusion criteria included being diagnosed with chronic diseases (such as diabetes, hypertension, and thyroid, hepatic, renal or neoplastic diseases), having abnormal values of major blood indicators of health (prostate-specific antigen [PSA] ≥ 4 ng mL^{-1}, total cholesterol [TC] > 5.2 mmol L^{-1}, triglycerides [TG] ≥ 2.3 mmol L^{-1}, alanine amino transferase [ALT] > 55 UI, fasting glucose > 7 mmol L^{-1}, creatinine >130 μmol L^{-1}), and having a history of myocardial infarction, unstable angina, stroke, ischaemic transient attacks or major surgery before the trial.

Reasons for premature withdrawals included experiencing any AE justifying withdrawal, unwillingness to come in for follow-up visits and major violations (failure to take treatments for ≥ 5 days during the trial and/or consuming steroidal drugs or supplements or medicines with antioxidant effects).

2.3 Treatments and randomization

The capsules of D-004 were provided by the Chemistry Department of the Centre of Natural Products (Havana City, Cuba). The free fatty acid composition of the batch of D-004 capsules, as assessed by a validated gas chromatography method, was 0.4% caprylic, 0.6% capric, 28.9% lauric, 10.8% myristic, 10.0% palmitic, 0.3% palmitoleic, 2.3% stearic, 30.3% oleic, 9.4% linoleic and 0.1% linolenic. The purity (total content of free fatty acids) was 91.1%. The composition of the capsules in the batch complied with substance specifications.

Placebo (sunflower oil) and 320 mg D-004 capsules were identical in appearance and were placed in identical containers that were codified and given to the participants according to their sequential order. Randomization was computer-generated using balanced blocks and an allocation ratio of 1:1.

D-004 has a composition similar, although not identical, to that of saw palmetto (a lipid extract of *Serenoa repens* used to treat BPH at doses from 160 to 320 mg day^{-1}) [28, 29]. Experimental data have shown that D-004 extracts were more effective than saw palmetto extracts in reducing prostate enlargement [20–22, 27] and that the doses effective in eliciting such an effect were also able to reduce oxidative markers in rat plasma and prostate [26, 27]. In light of this, we expected that the oral administration of D-004 at a dose similar to a therapeutic dose of saw palmetto would produce antioxidant effects in plasma oxidative markers of healthy men. Thus, although higher doses of D-004 (960 mg day^{-1}) for 3 weeks were well tolerated by healthy men in an earlier trial [28], we preferred to test the potential antioxidant effect of D-004 at the lowest effective dose. On the basis of experimental data [24, 25], D-004 was given for 6 weeks, which was a treatment duration that was likely to show the antioxidant effects of D-004 in humans, if any.

Participants were advised to bring all unused medications to each visit. Compliance with study treatments was assessed at visits 3 and 4 by counting the remaining tablets and interviewing the participants. At study completion, the tablets that were not consumed were recovered. Compliance was considered good if the participants took at least 90% of the tablets from the earlier visit.

Consumption of medications and/or supplements with recognized antioxidant effects was not allowed during the entire trial, and participants who had a history of taking such products became eligible for randomization only if they had discontinued such consumption from at least 4 weeks before the trial.

2.4 Response variables

The primary measure of efficacy was a change in plasma malondialdehyde (MDA) concentrations, with regard to which efficacy was defined as a 20% greater reduction compared with placebo. Secondary efficacy measures were reductions in plasma total hydroxyperoxides (TOH) and sulphydryl (SH) groups, and increase in total antioxidant status (TAS).

2.5 Safety and tolerability

The information from physical examinations, laboratory safety indicators and AEs was included for the analyses of safety and tolerability. Any undesirable event experienced by a participant during the trial, regardless of the cause, was considered an AE if it was a new occurrence during the study.

On the basis of their intensity, AEs were classified as mild, moderate or serious. Mild AEs did not require suspension of study capsules and/or specific treatment; moderate AEs required the termination of therapy and/or specific treatment; and serious AEs required hospitalization and/or included deaths.

2.6 Assessment of oxidative variables

Oxidative variables were assessed on the same day of blood draw. Venous blood samples were drawn after an overnight fast of at least 10 h and were collected in two Eppendorf tubes. For each participant, an aqueous solution of 10% ethylene diamine tetra acetic acid was added to one tube (final blood concentration of 0.1%), and 5 μL of 5 000 UI mL^{-1} sodium heparin was added to the other tube, containing a blood aliquot of 1 mL.

Plasma was separated from red blood cells by centrifugation at 3 000 × *g* for 10 min. Suitable portions of the plasma were used to assess oxidative variables. Whole-blood and serum samples were used for determining other indicators. Serum and plasma samples were frozen at −70ºC for the remaining analyses, which were carried out within the next 48 h.

2.6.1 Materials

All chemicals were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Measurements were conducted using an Utrospec-Plus spectrophotometer from LKB (Pharmacia LKB Biotechnology, Uppsala, Sweden).

2.6.2 Effects on plasma lipid peroxides

Plasma MDA was measured using thiobarbituric acid (TBA) assay [30]. Briefly, 0.5 mL of plasma was added to the TBA reagent (0.2 mL of 8.1% sodium dodecyl sulphate, 1.5 mL of 20% acetic acid, pH 3.5, and 1.5 mL of 0.8% TBA), and distilled water was added to make the total volume 4 mL. This mixture was incubated at 95ºC for 45 min. Fifty microliters of butylated hydroxytoluene (1 mmol L^{-1}) was added, and the mixture was then cooled. Immediately after this, 1 mL of distilled water and 5 mL of *n*-butanol:piridine (15:1, v/v) were added. The preparation was stirred and centrifuged (20 min at 1 660 × *g*). The organic layer was separated and the absorbance was measured at 534 nm. The concentrations of TBA-reactive substances (TBARS) were calculated from a standard calibration curve generated with known amounts of freshly diluted MDA bis(dimethyl acetal). Values were expressed as MDA (μmol mL^{-1}).

2.6.3 Effects on plasma TOH

Plasma TOH values were determined according to the method described by Jiang *et al.* [31]. In brief, 0.1 mL of heparinized plasma was mixed with 0.9 mL of Fox reactive (88 mg of butylated hydroxytoluene, 7.6 mg of orange xylenol, 9.8 mg of ammonium sulphate were dissolved in a 9:1 mixture of methanol:sulphuric acid). This mixture was incubated at 37ºC for 30 min, then cooled. The absorbance was measured at 560 nm. The concentrations of TOH were calculated from a standard calibration curve generated with cumene hydroperoxide. TOH values were expressed as mol mg^{-1} of tissue protein.

2.6.4 Effects on plasma SH groups

Plasma SH groups were quantified according to a modification of the Miao-Lin Hu method [32]. Briefly, 950 μL of 10 mmol L^{-1} dithionitrobenzene (DTNB) was added to plasma aliquots of 50 μL, and this mixture was incubated for 20 min at 25ºC. A blank with DTNB was run in parallel. The absorbance of the supernatant was measured at 412 nm. The numbers of SH groups were estimated using an absorptivity of 13 600 cm^{-1} mol^{-1} and expressed in nmol.

2.6.5 Effects on plasma TAS

For TAS quantification, a commercial kit (NX2332; Randox, Ltd., Crumlin, UK) was used. Briefly, 2,2′-azino-di-(3-ethylbenzthiazoline sulphonate) was incubated with metamyoglobin and hydrogen peroxide to produce the radical cation $ABTS^{+}$, which has a relatively stable blue-green colour that was measured at 600 nm. Depending on the concentration, antioxidants will cause a suppression of the colour production [23–25]. TAS was expressed in mmol L^{-1}.

All the assays described above were carried out in triplicate.

2.7 Safety indicators

Safety indicators included physical (body weight, pulse rate and blood arterial pressure), haematological (haemoglobin, haematocrit, red cells and white cell counts) and blood biochemistry (ALT, creatine phosphokinase, glucose, creatinine, TC, TG) parameters. Haematological indicators were automatically determined in the Haematological Complex equipment, and blood biochemistry indicators were assessed with enzymatic routine methods using reagent kits (Roche, Switzerland) in the Hitachi 719 autoanalyser (Hitachi, Tokyo, Japan) of the Medical Surgical Research Centre (Havana City, Cuba). PSA levels were determined by the immunoenzymatic method (Cobas reagent kit).

2.8 Statistical analysis

A sample size of 15 participants per treatment group was expected to provide 80% power to detect a 20% between-group difference in the mean percent change from baseline in plasma MDA. We used intention-to-treat analyses, including all randomized participants, regardless of adherence to the study medication. Assuming 10% premature withdrawals, a sample size of 33 participants was necessary.

Comparisons of continuous variables were carried out using the Wilcoxon test for paired samples (within-group comparisons) and the Mann-Whitney *U*-test (between-group comparisons). Comparisons of categorical variables were done with the Fisher's exact test. All tests were two tailed. For statistical significance, α was set at 0.05. Comparisons were carried out with the Statistics software for Windows (Release 4.2, Copyright StatSoft, Tulsa, OK, USA).

3 Results

3.1 Baseline characteristics of study participants

Of the 37 men who were assessed for initial eligibility, 34 were qualified to be randomized to D-004 (n = 17) or placebo (n = 17). Three men were not eligible

Table 1. Main baseline characteristics of study participants.

Characteristics	Placebo (n = 17)	D-004 (320 mg day^{-1}) (n = 17)	P-values	Total (n = 34)
Age (years) (mean ± SD)	36 ± 9	37 ± 13	0.9587[a]	37 ± 11
Age (years) (95% CI)	31.6–40.8	30.4–43.6	—	32.8–40.4
Smokers (%)	3 (17.6)	5 (29.4)	0.6880[b]	8 (23.5)
Body mass index (kg m^{-2}) (mean ± SD)	23.2 ± 2.3	24.0 ± 3.6	0.5239[a]	23.6 ± 3.0
Diastolic blood pressure (mmHg)	71.18 ± 7.81	73.53 ± 6.79	0.5353[a]	73.23 ± 7.16
Systolic blood pressure (mmHg)	112.35 ± 9.70	117.06 ± 10.47	0.2557[a]	115.59 ± 9.27
Prostate serum antigen (ng mL^{-1})	0.94 ± 0.69	1.06 ± 0.79	0.8009[a]	1.00 ± 0.74
Total cholesterol (mmol L^{-1})	4.30 ± 0.59	4.39 ± 0.74	0.5352[a]	4.35 ± 0.66
Triglycerides (mmol L^{-1})	1.09 ± 0.44	1.28 ± 0.47	0.2086[a]	1.18 ± 0.46
Glucose (mmol L^{-1})	4.56 ± 0.52	4.51 ± 0.56	0.5290[a]	4.54 ± 0.53
Creatinine (μmol L^{-1})	84.41 ± 8.52	88.00 ± 10.11	0.3095[a]	86.21 ± 9.39
IPSS	1.35 ± 1.50	1.59 ± 1.40	0.5128[a]	1.47 ± 1.40

Abbreviations: CI, confidence interval; IPSS: International Prostate Symptom Score.
[a]Mann-Whitney *U*-test.
[b]Fisher Exact Probability test.
None of the comparisons was significant.

because their values of PSA and TC (1 participant), TC, TG and ALT (1 participant) or TG (1 participant) were above the eligibility criteria. Thirty-two of the 34 qualified men completed the study.

There were no statistically significant differences in baseline characteristics between D-004 and placebo groups. Baseline characteristics showed that participants met all the eligibility criteria (Table 1).

3.2 Effects of D-004 on oxidative variables

Treatment compliance was good and similar in both groups.

Table 2 shows the effects on oxidative variables, which were well matched in both groups at baseline. No significant changes occurred in the placebo group. At 6 weeks, the mean reduction (26.7% *vs.* baseline, 21.9% *vs.* placebo) of plasma MDA in the D-004 group was significantly larger than that in the placebo group ($P < 0.0001$). In addition, the reduction of this variable met the definition of efficacy. The mean reductions of plasma TOH (18.8%) and SH groups (31.6%) in the D-004 group were also significantly larger than those in the placebo group ($P < 0.0001$), and the mean increase in plasma TAS (35.3%) was also significant ($P < 0.0001$ compared with placebo).

Table 2 Effects of D-004 (320 mg day^{-1}) on oxidative variables in healthy men.

Treatment	Baseline	6 weeks	Percent changes
TBARS (MDA μmol mL^{-1}) (mean ± SD)			
Placebo	0.42 ± 0.14	0.40 ± 0.11	–4.8
D-004	0.45 ± 0.14	0.33 ± 0.11$^{***,+}$	–26.7^{++++}
Total hydroxyperoxides (nmol mg^{-1} prot) (mean ± SD)			
Placebo	49.67 ± 17.58	50.04 ± 16.74	+0.7
D-004	56.98 ± 23.18	46.28 ± 17.45$^{***,+}$	–18.8^{++++}
Sulphydryl groups (mmol mg^{-1} prot) (mean ± SD)			
Placebo	0.71 ± 0.09	0.66 ± 0.19	–7.0
D-004	0.76 ± 0.16	0.52 ± 0.05$^{***,+++}$	–31.6^{++++}
TAS (mmol L^{-1}) (mean ± SD)			
Placebo	0.56 ± 0.14	0.58 ± 0.16	+3.6
D-004	0.51 ± 0.13	0.69 ± 0.14$^{***,+}$	+35.3^{+++}

Abbreviations: MDA, malondialdehyde; TAS, total antioxidant status; TBARS, thiobarbituric acid-reactive substances;
$^{**}P < 0.01$; $^{***}P < 0.001$ (compared with baseline [Wilcoxon test for matched samples]).
$^{+}P < 0.05$; $^{+++}P < 0.001$; $^{++++}P < 0.0001$ (compared with placebo [Mann-Whitney U-test]).
Values were means of triplicates.

3.3 Safety and tolerability

The oral treatment with D-004 (320 mg day^{-1}) for 6 weeks was well tolerated. No significant changes of physical or blood safety indicators (including PSA values) between treated and placebo groups were found (Table 3). In addition, individual values remained within the normal range.

There were two study withdrawals, both in the D-004-treated group. One participant withdrew because

Table 3 Effects on safety indicators (mean ± SD).

Group	Baseline	3 weeks	6 weeks
Physical safety indicators			
Weight (kg)			
Placebo	70.38 ± 9.27	70.59 ± 9.51	70.59 ± 9.37
D-004	71.03 ± 10.30	69.16 ± 8.14	68.63 ± 8.28
Pulse rate (beats min^{-1})			
Placebo	72.94 ± 4.48	72.53 ± 4.90	73.88 ± 3.71
D-004	73.18 ± 5.20	70.75 ± 5.65	73.60 ± 4.08
Diastolic arterial pressure (mmHg)			
Placebo	71.18 ± 7.81	68.23 ± 8.83	71.18 ± 7.81
D-004	73.53 ± 6.79	71.87 ± 5.44	72.00 ± 5.61
Systolic arterial pressure (mmHg)			
Placebo	112.35 ± 9.70	112.06 ± 9.53	111.18 ± 8.57
D-004	117.06 ± 10.47	115.00 ± 6.01	114.67 ± 6.40
Blood indicators			
PSA (ng mL^{-1})			
Placebo	0.94 ± 0.69		1.06 ± 0.79
D-004	1.06 ± 0.79		0.99 ± 0.66
Total cholesterol (mmol L^{-1})			
Placebo	4.30 ± 0.59		4.38 ± 0.60
D-004	4.39 ± 0.74		4.49 ± 0.52
Triglycerides (mmol L^{-1})			
Placebo	1.09 ± 0.44		0.99 ± 0.52
D-004	1.28 ± 0.47		1.20 ± 0.52
ALT (U L^{-1})			
Placebo	19.47 ± 9.75		21.12 ± 7.93
D-004	18.76 ± 7.96		17.87 ± 3.78
CPK (U L^{-1})			
Placebo	158.59 ± 79.12		174.94 ± 74.94
D-004	158.18 ± 48.89		167.50 ± 55.09
Glucose (mmol L^{-1})			
Placebo	4.56 ± 0.52		4.45 ± 0.49
D-004	4.51 ± 0.56		4.29 ± 0.56
Creatinine (μmol L^{-1})			
Placebo	84.41 ± 8.52		87.35 ± 9.69
D-004	88.00 ± 10.11		88.00 ± 8.66
Haemoglobin (g 100 mL^{-1})			
Placebo	14.24 ± 0.82		14.28 ± 1.01
D-004	14.43 ± 0.74		14.61 ± 0.84
Haematocrit (%)			
Placebo	43.03 ± 2.29		43.30 ± 2.03
D-004	43.15 ± 2.30		44.21 ± 2.11
Red blood cells ($\times 10^3$)			
Placebo	4.83 ± 0.36		4.79 ± 0.31
D-004	4.72 ± 0.33		4.75 ± 0.36
White blood cells ($\times 10^3$)			
Placebo	5.83 ± 1.28		5.66 ± 1.31
D-004	5.69 ± 1.27		5.77 ± 1.20

Abbreviations: ALT, alanine amino transferase; CPK, creatine phosphokinase; PSA, prostate-specific antigen.

of dyspepsia (unspecific gastric sensation of fullness) that lasted for 3 days; this was the only AE that occurred during the trial. As the participant had not experienced such a sensation before, he decided to discontinue the trial. This AE disappeared 2 days after stopping the therapy, without requiring any additional action.

4 Discussion

The results of this study show that D-004 (320 mg day^{-1}) administered for 6 weeks significantly reduced plasma concentrations of MDA (the primary efficacy variable), TOH and SH groups, and significantly increased plasma TAS compared with placebo. Although D-004 has earlier been shown to reduce oxidative stress in rat plasma, brain, liver and prostate [26, 27], this is the first report of the antioxidant effects of D-004 in humans.

The baseline characteristics of the participants were similar in both groups, and this confirms that the two groups were comparable and that the effects observed with D-004 were treatment related. Participants were men in good health according to predefined variables, including being free of LUTS and having a PSA < 4 ng mL^{-1}. Nevertheless, as only three participants were ≥ 50 years, we re-assessed PSA values according to age-specific reference ranges and found that all randomized men younger than 50 (31 of 34) had PSA values < 1.5 ng dL^{-1}. As most participants were under 50 years of age, were asymptomatic and had normal age-matched PSA values, we did not consider it necessary to carry out digital rectal prostate examinations. The ages of the study participants ranged from 20 to 55 years, but the age distributions in the two groups were comparable, on the basis of the comparison of mean ± SD and 95% confidence limits.

Although smoking increases OS and lowers the concentration of some antioxidants [33], we did not exclude smokers from the trial because we wanted to determine whether D-004 produced antioxidant effects in a sample of men that was representative of the population of males that came to our clinics, who, unfortunately, include a high proportion of smokers. As the frequency of smokers was similar in both groups, the antioxidant effects found here cannot be attributed to any disparities in smoking frequency.

In this study, we assessed the effects of D-004 on plasma oxidative markers as they provide a reasonable index of the general oxidative stress status [34, 35]. D-004 was found to produce antioxidant effects

in humans, specifically in healthy men, consistent with experimental results [26, 27].

Plasma MDA, the most abundant aldehyde generated by the attack of free radicals on polyunsaturated fatty acids of cell membranes, represents a non-invasive biomarker of oxidative stress in BPH [9]. Thus, the reduction of this indicator was selected as the primary measure of efficacy in the study. The reduction of plasma MDA (26.7% *vs.* baseline, 21.9% *vs.* placebo) achieved the efficacy criterion (20.0% reduction *vs.* placebo). The decrease of TOH (18.8% *vs.* baseline, 19.5% *vs.* placebo) was roughly similar, which indicates that the responses of both LP markers to D-004 treatment were consistent. It should be noted that TOH are not only passive markers of oxidizing stress but also cytotoxic products that could modify DNA and proteins [10].

The decrease in plasma SH groups, a marker of protein oxidation (31.6% *vs.* baseline, 24.6% *vs.* placebo), and the increase in plasma values of TAS (35.3% *vs*. baseline, 31.7%) were more accentuated. As TAS measures plasma antioxidant capacity, these results suggest that the decrease in LP and protein oxidative variables induced by D-004 is associated with the increase in whole-plasma antioxidant capacity.

A limitation of this trial, however, was that plasma levels of defensive antioxidant enzymes, which should provide more information on the mechanisms by which D-004 produces antioxidant effects, were not assessed. The effects of D-004 on oxidative variables could be attributed, at least in part, to the content of myristic acid (one of the most abundant fatty acids in D-004), as fractions and extracts from *Prunus africana* bark that contained high levels of myristic acid have been shown to inhibit iron-induced stimulation of LP in rabbit liver microsomal preparations [18]. Similarly, recent studies have shown that saw palmetto extracts (which also contain myristic acid) produced antioxidant effects [19].

D-004, given orally, has been shown to reduce prostate enlargement [20–22, 27], to antagonize α1-adrenoreceptor-mediated responses *ex vivo* [24, 25], and to produce anti-inflammatory effects in rodents [36] and antioxidant effects in rat prostate [26, 27]. All of these effects are potentially beneficial for men with BPH, especially in light of the finding that it was the rat prostate in which there was the greatest accumulation of D-004, in a radioactivity distribution study of an oral dosing with ^{3}H-labelled-oleic acid mixed with D-004 (400 mg kg^{-1}) [37]. The potential usefulness of D-004 in men with BPH/LUTS, however, has not been shown clinically.

The data presented here show evidence of the antioxidant effects of D-004 that can be observed in humans, and this investigation merely represents a first step in the full assessment of the clinical effects of D-004. Whether D-004 also produces antioxidant effects in men with BPH/LUTS and whether such effects may have therapeutic implications deserve extensive further investigation.

Consistent with earlier experimental toxicological [38–40] and clinical [28] data, D-004 was well tolerated and did not produce significant changes in any of the variables studied (including PSA) compared with placebo. Only one paticipant, who voluntarily discontinued the trial, reported an adverse effect (dyspepsia) during the study.

In coclusion, D-004 displayed antioxidant effects on plasma oxidative markers in healthy men, which was consistent with findings from laboratory experimental studies. The treatment was well tolerated. Further studies should investigate whether D-004 has antioxidant effects in men with BPH/LUTS.

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