Oral administration of D-005, a lipid extract from Corojo palm (*Acrocomia crispa*) fruits, attenuates testosterone induced prostate enlargement and increased oxidative stress in rats

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**ABSTRACT**

**Background:** Benign prostatic hyperplasia (BPH), common in older men, leads to lower urinary tract symptoms. Lipid extracts from palm (saw palmetto, *Roystonea regia*) fruits are effective on experimental prostate hyperplasia (PH) and in BPH patients, which encourage the search of similar effects on other palms. Effects of *Acrocomia crispa* (AC) (corojo palm), endemic to Cuba, remained unexplored.

**Objective:** We investigated the effect of D-005, a lipid extract of AC fruits, on testosterone (T)-induced PH in rats.

**Materials and methods:** Rats were randomized into seven groups: a negative control and six T-injected: a positive control, four D-005 (5, 25, 50 and 200 mg/kg), one D-004 (400 mg/kg). Treatments were given for 14 days. Effects on bodyweight (BW), prostate weight (PW), PW/BW ratio and prostate oxidative variables (malondialdehyde – MDA, sulfhydryl groups – SHG) were assessed.

**Results:** T injection increased (p<0.01) PW, PW/BW ratio, and oxidative variables in the positive controls versus the negative control group. D-005 (5-200 mg/kg) inhibited significantly (p<0.05), dose-dependently and markedly PW (63.7 – 72.4%), PW/BW ratio (61.6 – 73.2%) and (p<0.05) prostate oxidative variables versus the positive control. D-005 (5-200 mg/kg) lowered MDA (53.3% - 95.6%) but only 25 to 200 mg/kg lowered SHG (59.5% - 70.7%). D-004 (400 mg/kg) lowered PW (75.4%), PW/BW (75.6%), MDA (98.3%) and SHG (78%) concentrations.

**Conclusion:** Effects of D-005 200 mg/kg and D-004 400 mg/kg were similar. D-005 reduced prostate enlargement and prostate oxidative variables in rats with T-induced PH. This is the first report of a pharmacological activity of AC.

**Key words:** D-005, *Acrocomia crispa*, Cuban belly palm, Cuban corojo palm, prostate hyperplasia, lipid peroxidation, protein oxidation

**INTRODUCTION**

Benign prostatic hyperplasia (BPH), a chronic progressive disease, consists in prostate gland enlargement secondary to hyper-proliferation of stromal and glandular cells (Djavan et al., 2011). BPH is common in older men, with an age-dependent incidence that is associated with lower urinary tract symptoms (LUTS), such as frequent urination, nocturia, urgency, hesitancy and weak urine stream (Hollingsworth and Wilt, 2014). Treatment options include watchful waiting, life-style modification, pharmacologic treatment, and surgery. 5α-reductase inhibitors lower the production of dihydrotestosterone (DHT) within the prostate, which results in decreased prostate volume. In turn, α-adrenoreceptor (ADR) blockers effectively lower LUTS and...
increase urinary flow rates in men with symptomatic BPH. For patients with moderate to severe symptoms and a large prostate, combination therapy with both α-blockers and 5-ADT improve clinical efficacy. Nowadays, phosphodiesterase-5 inhibitors or antimuscarinic agents may be added. All these options, however, are not exempt of adverse side effects, some of them impairing men’s sexual performance (Djavan et al., 2011; Bechis et al., 2014; Truish et al., 2014; Sun and Zhang, 2014).

On its side, phytotherapy is increasingly used as an alternative or complement to the conventional medication. Although conventional drug therapy and surgery seem to be most effective for patients with moderate-severe BPH, phytomedicines are successfully used in patients with mild-moderate symptoms (Sun and Zhang, 2014; Pagano et al., 2014; Allkanjari and Vitalone, 2015).

Lipid extracts from *Serenoa repens*—saw palmetto—fruits, which mainly contains free fatty acids such as oleic, lauric, myristic, palmitic, linoleic, caprylic, caprylic, linolenic and stearic acids, are the most documented (Pharmacopeial Convention, 2005). Despite some negative results (MacDonald et al., 2012), several clinical studies and routine use documented its efficacy and safety (MacDonald et al., 2012; Sinescu et al., 2011; Giulianelli et al., 2012), and recently evidences support that it improves not only BPH symptoms, but erectile sexual dysfunction in men with both entities (Suter, 2013). On the basis of preclinical studies several mechanisms of action have been postulated for its efficacy, including 5 α-reductase inhibition, α-ADR antagonism, DHT and estrogen receptor inhibition (Pharmacopeial Convention, 2005), and also its antioxidant effects (Belostotskaia et al., 2006), since oxidative stress plays an important role in the pathology of BPH (Mincuillo et al., 2014).

Likewise, D-004, a lipid extract from *Roystonea regia* fruits that contains a mixture of fatty acids, mainly oleic, palmitic, lauric, linoleic, and myristic acids, has been shown to be effective on experimental prostate hyperplasia (PH) (Carbalj et al., 2004; Noa et al., 2005; Carbalj et al., 2005; Pérez et al., 2006; Arruzazabala et al., 2005; Arruzazabala et al., 2006; Menéndez et al., 2007; Pérez et al., 2008) and in patients with BPH (López et al., 2009; Guzmán et al., 2013 a,b). D-004 has demonstrated to prevent testosterone, not DHT, induced PH in rodents (Carbalj et al., 2004; Noa et al., 2005; Carbalj et al., 2005), to inhibit prostate 5α-reductase activity in vitro (Pérez et al., 2006), and to antagonize ADR-mediated responses (Arruzazabala et al., 2005; Arruzazabala et al., 2006). Also, D-004 exhibits antioxidant (Menéndez et al., 2007; Pérez et al., 2008) and anti-inflammatory (Menéndez et al., 2007) effects that may contribute to its efficacy. Clinical studies have demonstrated the antioxidant effects of D-004 (López et al., 2009; Guzmán et al., 2013a) and its ability to lower LUTS in patients with BPH (Guzmán et al., 2013 a,b).

The facts referred above encourage the search of similar effects on other palm species, as palms are important in the ethnomedicine of the American continent (Sosnowska and Balslev, 2009). *Acrocomia* is a genus of palms growing in Mexico, Central America, the Caribbean and South America. *Acrocomia crispa* (Cuban belly palm, Corojo Palm) is a palm species endemic to Cuba. Up to date, pharmacological effects of *Acrocomia crispa* (Cuban belly palm, Corojo Palm) have not been reported (Govaerts and Dransfield, 2005; Henderson, 1995). A lipid extract from *Acrocomia crispa* obtained in our center shows a reproducible mixture of fatty acids, but different from that of D-004 and saw palmetto extracts.

In light of these facts, this study investigated the effects of D-005 on T induced PH in rats and explore its potential antioxidant effects on this model.

**MATERIALS AND METHODS**

Young adult male Wistar rats (200-300g) supplied by Centre for Laboratory Animals Production (CENPALAB, Havana, Cuba), were adapted for 7 days to laboratory conditions (25 ± 3°C, 60 ± 5% relative humidity, and 12 light/dark cycles), with free access to tap water and standard rodent chow (CENPALAB). Animal handling was conducted in accordance with the Cuban Code for the Use of Laboratory Animals and ethical principles for animal management. An independent ethical board approved the study protocol and use of the animals for such aim.

D-005 consisted on a lipid extract obtained from the dried mature fruits of *A. crispa*. The fruits were obtained from the north shore of West Havana, and duly authenticated by the Cuban Botanic Garden (Havana, Cuba). Plant material was powdered and passed through mesh of size 2.36 mm and then subjected to extraction and purification in n-hexane plus further basic hydrolysis with KOH. The batch used in the study (150710) was provided by the Chemistry Department of the Centre of Natural Products (Havana, Cuba). The fatty acids present in this batch, assessed by gas chromatography, were in the following proportions (w/w, %): lauric (35.8%), oleic (41.9%), myristic (14.2%), palmitic (8.9%), stearic (3.3%), caprylic (1.2%), and palmitoleic (0.05%). Purity (total content of these free fatty acids) was 93%.

D-004 (batch 090609), the reference substance, supplied by the Plants of Natural Products (National Center for Scientific Research, Havana, Cuba), had the following composition: lauric (28.4%), oleic (28.4%), myristic (14.2%), palmitic (8.9%), stearic (3.3%), caprylic (1.9%), caprylic (1.2%), and palmitoleic (0.05%). Purity (total content of these free fatty acids) was 93%.

For dosing, D-005 and D-004 were suspended in 2% Tween 65/H2O vehicle. Suspensions were prepared immediately before use.

Testosterone propionate (Cuban Pharmaceutical Industry, Havana, Cuba) was dissolved in soy oil. All other chemicals were purchased from Sigma-Aldrich Co. (St Louis, MO).
Rats were randomized into seven experimental groups of 10 rats each: a negative control (injected subcutaneously s.c.) with soy oil and treated orally with vehicle, and six T-injected groups: a positive vehicle control, four treated with D-005 (5, 25, 50 and 200 mg/kg, respectively) and one with D-004, the reference substance, at 400 mg/kg, an effective dose on this model (Henderson et al., 1995; Hollingsworth and Wilt, 2014). Taking in mind the similarities in the botanical origin and composition of D-004 and D-005, we selected D-004 as reference substance in this study.

All treatments (vehicle, D-005, D-004) were given by gastric gavage (5 ml/kg), once daily (8:00–10:00 am), 6 days a week, for 14 days.

T was injected sc (3 mg/kg/day) for 14 weeks to induce prostate hyperplasia (Carbajal et al., 2004; Noa et al., 2005).

Body weight (BW) was measured in Mettler balance the day before starting the treatments (baseline) and at 14 days on treatment. Twenty-four (24) hours after treatment completion, and after 12 h overnight fast, rats were anaesthetized by injection of thiopental (40 mg/kg; i.p) and sacrificed by complete bleeding from the abdominal aorta.

The abdomen was opened by a ventral middle line incision; and prostates were immediately removed and weighed. Prostate weight (PW) was determined and the PW to BW ratio (PW/BW) was then calculated.

### Table 1. Effects on body weight (BW) (g), prostate weight (mg) and PW/BW ratios in rats with T-induced prostate hyperplasia.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Baseline BW</th>
<th>Final BW</th>
<th>PW</th>
<th>% I</th>
<th>PW/BW</th>
<th>% I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative vehicle control</td>
<td>278.1 ± 16.18</td>
<td>320.1 ± 14.03</td>
<td>349.2 ± 21.06**</td>
<td>-</td>
<td>1.09 ± 0.06**</td>
<td>-</td>
</tr>
<tr>
<td>Positive control (vehicle + T)</td>
<td>275.1 ± 13.96</td>
<td>327.0 ± 13.39</td>
<td>629.0 ± 46.56</td>
<td>-</td>
<td>1.95 ± 0.17</td>
<td>-</td>
</tr>
<tr>
<td>D-005 (5 mg/kg) + T</td>
<td>273.6 ± 19.79</td>
<td>318.2 ± 19.97</td>
<td>450.7 ± 38.32*</td>
<td>63.7</td>
<td>1.42 ± 0.10*</td>
<td>61.6</td>
</tr>
<tr>
<td>D-005 (25 mg/kg) + T</td>
<td>275.4 ± 14.41</td>
<td>321.7 ± 8.52</td>
<td>435.1 ± 28.15**</td>
<td>69.3</td>
<td>1.36 ± 0.10*</td>
<td>68.6</td>
</tr>
<tr>
<td>D-005 (50 mg/kg) + T</td>
<td>277.0 ± 13.19</td>
<td>322.5 ± 16.32</td>
<td>428.3 ± 24.65**</td>
<td>71.7</td>
<td>1.34 ± 0.08**</td>
<td>70.9</td>
</tr>
<tr>
<td>D-005 (200 mg/kg)+ T</td>
<td>273.4 ± 11.81</td>
<td>319.6 ± 14.30</td>
<td>426.4 ± 37.19*</td>
<td>72.4</td>
<td>1.32 ± 0.08**</td>
<td>73.2</td>
</tr>
<tr>
<td>D-004 (400 mg/kg)+ T</td>
<td>274.3 ± 12.18</td>
<td>319.5 ± 13.23</td>
<td>418.2 ± 25.56**</td>
<td>75.3</td>
<td>1.30 ± 0.08**</td>
<td>75.6</td>
</tr>
</tbody>
</table>

X mean, SE standard error, I Inhibition, T testosterone. *p < 0.05, **p < 0.01, comparison with positive control group (Mann-Whitney U test).

Lipid peroxidation (LP) assays

LP in prostate homogenates was estimated by measuring MDA concentrations (Ohkawa et al., 1979). Prostate tissue aliquots (equivalent to 200 mg) were taken and gently homogenized in 9 volumes of 150 mM tris/HCl buffer, pH 7.4, in ice cold bath, with an Ultra-Turrax homogenizer. The reaction mixture was treated with 0.2 ml of 8.1% SDS, 1.5 ml of 20% acetic acid (pH 3.5) and 1.5 ml of 0.8% thiobarbituric acid, and heated to 95°C for 1 h. Fifty (50) 50 ml of 1 mM BHT were then added, samples were cooled and 5 ml of a n-butanol:piridine (15:1 v/v) mixture were added, stirring vigorously with a vortex and centrifuged at 4000 rpm for 20 min. The organic layer was taken and the optical density was measured at 534 nm (final volume 1 ml, protein concentration 500 mg).

MDA concentrations were determined from a standard curve of malondialdehyde bis (dimethyl acetal) and reported as nmol MDA/mg protein. Protein concentration was assessed through a modification of the Lowry method (Marxwell et al., 1987). All assays were performed as triplicates.

### Effects on protein oxidation

Protein oxidation was assayed through the 5′5′-dithio-bis (2-nitrobenzoic acid) (DTNB) assay (Hu, 1994). Homogenate aliquots (200 μl) were treated with 600 μl of 20mM TRIS-EDTA buffer (pH 8.2), 40 μl of 10mM DTNB and 3.16ml of absolute ethanol. The mixture reaction was incubated to ambient temperature for 20 min and centrifuged at 3000xg for 10 min. The optical density of the supernatant was measured at 412 nm, using a 13,600 cm-1M-1 coefficient of absorptivity. The concentration of sulphydryl groups (SHG) was reported in mmol/L.

### Statistical analyses

Data were expressed as the mean ± SE. Comparisons among the groups were done with the Kruskal-Wallis test, paired comparisons between treated and control groups with the Mann-Whitney U test. The level of statistical significance was set at α = 0.05. Data were processed with the Statistics Software for Windows (Release 6.1 Stat Soft Inc, Tulsa OK, USA). Dose/effect relationship was assessed by using linear regression and correlation test using a Primer of Biostatistics program (Stanton A, Glantz; copyright (c) 1992, McGraw-Hill, Inc Version 3.01).

### RESULTS

BW values were similar in all groups at baseline and at study completion, which demonstrates that D-005 did not modify this parameter (Table 1). T injection increased...
significantly PW and PW/BW ratio (p<0.01 for both comparisons) in the positive controls as compared to the negative control group, indicating enlargement. Oral administration of D-005 (5 - 200 mg/kg) inhibited significantly, markedly (63.7 - 72.4%) and in a dose-dependent manner (r=0.943; p<0.05) prostate PW/BW ratio (61.6 - 73.2%) (r=0.972; p< 0.05) as compared to the positive control group. In turn, oral treatment with D-004 (400 mg/kg), the reference substance, reduced prostate enlargement and PW/BW ratio by 75.4 and 75.6%, respectively. The effect of D-005 200 mg/kg was statistically similar to that of D-004 400 mg/kg.

Table 2 shows the effects on MDA and SHG prostate concentrations, which were significantly increased in the positive control with regards to the negative control group. D-005 reduced significantly, markedly (53.3 - 95.6%) and dose-dependently (r = 0.970, p <0.05) prostate MDA concentrations. D-005 (25 - 200 mg/kg, not 5 mg/kg) produced similar effects, albeit less pronounced, on prostate SHG concentrations, which also decreased significantly, dose-dependently (r = 0.994, p <0.05) and markedly (58.5 - 70.7%). Oral administration of D-004 also reduced significantly and markedly prostate MDA and SHG levels (98.3 and 78%, respectively). These effects were indistinguishable of those of D-005 at 200 mg/kg.

**DISCUSSION**

The present study demonstrates that oral administration of D-005, a lipid extract obtained from the mature fruits of AC, inhibits prostate enlargement in the model of T-induced PH in rats, and also reduces prostate concentrations of MDA and SHG, markers of lipid peroxidation and protein oxidation, respectively (Fujii et al., 2003; Niki, 2008). Positive control rats injected with T and receiving orally the vehicle exhibited significant increases in PW and PW/BW ratio compared with the negative control animals, as expected. In addition, oral administration of D-004, the reference substance, was effective for decreasing all variables, such as PW (75.3%), PW/BW (75.6%), and prostate concentrations of MDA (98.3%) and SHG (78%) in T-treated rats (Menéndez et al., 2007). These results confer validity to the model in our experimental conditions.

Increased PW/BW ratio, also referred as prostatic index, is a relevant marker of PH development (Barry et al., 1992) since BPH involves epithelial and stromal prostate hyperplasia (Krieg et al., 1983) leading to an increased prostate size that may contribute to partial or complete urethral obstruction. For these reasons, experimental evaluation of substances with potential effects on BPH often starts by investigating their inhibitory effects on the prostatic index in the model of T-induced PH (Lee et al., 2012).

Our study demonstrates that oral administration of D-005 (5 - 200 mg/kg) for 14 days causes a significant decrease in the PW and prostatic index when compared with the positive control PH group. The reductions produced by D-005 were marked (about 60 - 73%) for both variables), being relevant that even the low dose investigated (5 mg/kg) produced decreases greater than 50% when compared with the positive vehicle control group.

Dose-effect relationship, albeit significant, reveals a soft increase as the difference in the effects between the lowest and highest dose tested was between 10 and 15%, and that the effect reached with 50 mg/kg was the ceiling effect. Further studies on this model should assess the effects of lower doses of D-005.

In light of the similarities between the composition of D-005 and D-004, the present results were not surprisingly, but expected. In addition, the fact that lauric and myristic acids, both components of D-005, have been effective to reduce the increase of PW and PW/BW ratio in rats with T-induced PH (Veerehs Babu et al., 2010), also supports the rationale of the present results.

This study was not focused to elucidate the mechanism of the potential efficacy of D-005 on this model. Nevertheless, the no inclusion of an experiment to evaluate the effects of this treatment on DHT-induced PH in rats remains as a
limitation for simple speculation on such topics. The assessment of the effects of D-005 on prostate oxidative variables should add some clues on this sense due to the association between oxidative stress and BPH (Minciullo et al., 2014). In line with this fact and with previous results (Pérez et al., 2008), injection of T increased prostate concentrations of both oxidative variables.

Oral administration of D-005 was effective for lowering MDA and SHG prostate concentrations. In this case, the differences between the effects of the lowest and highest doses were more than 40 and 20% for MDA (53.3 - 95.6%) and SHG (58.5 - 70.7%), respectively. We cannot confirm that 200 mg/kg is the maximal dose for lowering prostate MDA concentrations because no plateau effect was obtained and higher doses were not assessed. Nevertheless, since the effect was near 100%, such limitation lacks biological relevance. In contrast, we can affirm that the dose of 50 mg/kg was the maximal effective dose for reducing SHG. The reduction of SHG was less marked (about 70%) than that of MDA (about 95%) and also the lowest dose was not effective for reducing this parameter, which suggests that D-005 is effective for lowering lipid peroxidation and protein oxidation, but more effective on the former. Further experiments, however, should go deeper on this sense. Overall, the results on the oxidative variables suggest, but not demonstrate, that the effects may contribute to the efficacy of D-005 on this model.

CONCLUSIONS

In conclusion, the D-005, a lipid extract of AC fruits, was effective for lowering prostate enlargement and prostate oxidative variables in rats with T-induced PH. This is the first report of a pharmacological activity of this species.

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