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D-004, a lipid extract from royal palm fruit, exhibits antidepressant effects in the forced swim test and the tail suspension test in mice

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ABSTRACT

D-004, a lipid extract of *Roystonea regia* fruits, has been shown to reduce Testosterone, but not dihydrotestosterone-induced prostate hyperplasia in rodents. Inhibition of prostate 5α-reductase seems to explain these effects of D-004. Finasteride, an inhibitor of 5α-reductase used to treat benign prostate hyperplasia (BPH), has been shown to produce drug-induced depression and to increase mouse immobility in the forced swim test (FST). In this study, therefore, we investigated the effect of D-004 on the immobility in the FST and the tail suspension test (TST) in mice. Also, its effects on other behavioural tests (grip strength, open field activity and rotarod test) were investigated. Mice were randomized into five groups: three groups orally treated with D-004 (250, 500 and 1000 mg/kg) or vehicle (control group), and a fifth group that received intraperitoneally (IP) imipramine 20 mg/kg for 30 days. In the FST, D-004 (250, 500 and 1000 mg/kg) produced a statistically significant reduction in immobility (51, 58, and 65%, respectively, versus the control group), whereas imipramine reduced FST immobility by 69%. In the TST, D-004 (250 and 500 mg/kg) significantly, but modestly (21%) reduced the immobility versus the control group, although less than imipramine (50%). The lowest dose of D-004 (50 mg/kg), however, was ineffective. D-004 did not alter the results of other behavioural tests. In conclusion, D-004 (250–1000 mg/kg) administered orally for 30 days reduced the immobility in the FST and the TST in mice, and had no effect on other behavioural tests in mice.

1. Introduction

Benign prostate hyperplasia (BPH) is the growth of the prostate gland, and as BPH advances, it may press on the urethra and obstruct the urine outflow, producing lower urinary tract symptoms (LUTS) in aging men (Bhargava et al., 2004; Nix and Carson, 2007; Roehrborn, 2008). Currently, BPH and depression, together with coronary heart disease and erectile dysfunction are the major non-cancer diseases that adversely affect males over the age of 50 (Zakaria et al., 2001). In particular, depression is a common, debilitating, life-threatening illness that seriously impairs the quality of life of sufferers (Rapaport et al., 2005; Andrews, 2008; Rouillon, 2008; Blumenthal, 2008).

The pharmacological treatment of BPH includes 5α-reductase inhibitors, α1-adrenoreceptor blockers and their combined therapy (Nix and Carson, 2007; Roehrborn, 2008). While the 5α-reductase inhibitors reduce the enlarged prostate size and modestly improve LUTS (Sandhu and Te, 2004), the α1-adrenoreceptor blockers relax prostate smooth muscle and effectively relieve LUTS (Schwinn and Roehrborn, 2008).

Finasteride, a 5α-reductase inhibitor used to treat BPH, has been shown to induce drug-induced depression due to the suppression of brain allopregnanolone (Lephart, 1995; Altomare and Capella, 2002; Rahimi-Ardabili et al., 2006; Finn et al., 2006) and to the decline in serum dihydroxytestosterone (DHT) (Rahimi-Ardabili et al., 2006). Also, regulatory authorities were worried that α1-adrenoreceptor blockers, another millstone of BPH treatment, could increase the risk of depression, but the results of a Practice Research Database study did not support such impression (Clifford and Farmer, 2002).

D-004 is a "lipid extract of *Roystonea regia* fruits" that contains a mixture of fatty acids wherein oleic, lauric, palmitic and myristic acids are the most abundant, and caprylic, capric, palmitoleic, stearic, linoleic and linolenic acids are in lower concentrations. Oral administration of rodents with D-004 has been shown to prevent prostate hyperplasia (PH) induced with Testosterone (T) (Arruzazabala et al., 2004; Carbajal et al., 2004, 2005; Noa et al., 2005), but not with dihydrotestosterone (DHT) (Carbajal et al., 2004), an effect associated with the inhibition of prostate 5α-reductase activity (Pérez et al., 2006). Also, D-004 antagonizes α1-adrenorecptor-mediated responses and phenylephrine-induced PH in the rat (Arruzazabala et al., 2005; 2006).

Since D-004 inhibits prostate 5α-reductase (Pérez et al., 2006), finasteride-induced depression has been documented (Lephart 1995; Altomare and Capella, 2002; Rahimi-Ardabili et al., 2006; Finn et al., 2006, (Beckley and Finn, 2007) and BPH frequently coexists with depression in aging man (Zakaria et al., 2001), we evaluated whether D-004 reduced immobility in the forced swimming test (FST) (Porsolt et al., 1978) and the tail suspension test (TST) (Steru et al., 1985), two animal models of depression.

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2. Materials and methods

2.1. Animals

Two hundred and fifty (250) male young adult Swiss OF1 mice (20 ± 5 g) were used in the study. These animals were obtained from the National Center for Laboratory Animal Production (CENPALAB, Havana, Cuba). Mice were housed in groups of four per polycarbonate cage for 2 weeks prior to the experiments, and adapted to laboratory conditions (25 ± 2 ° C, 60 ± 10% of relative humidity, 12-h light–dark cycle) with free access to standard rodent pellet diet acquired in CENPALAB and tap water. The experiments were performed in accordance with Cuban guidelines for the care and use of laboratory animals. The independent local ethics committee for animal use approved the protocol for these experiments. Animals were fasted for 12 h overnight before the experiments.

2.2. Test substances

The free fatty acid composition (w/w) of the D-004 batch (Chemistry Department of the Centre of Natural Products, Havana City, Cuba) used in the experiments, assessed with a validated gas chromatography method, was as follows: caprylic 0.7%, capric 1.1%, lauric 30.4%, myristic 10.2%, palmitic 7.5%, palmitoleic 0.3%, stearic 2.4%, oleic 29.9%, linoleic 9.4% and linolenic 0.2%. Purity (total content of free fatty acids) was 91.8%. D-004 was suspended in Tween 65/H_2O (2%) 1 h before use.

Imipramine (hydrochloride form) and diazepam (DZP) were purchased from the Medical Pharmaceutical Industry (IMEFA, Havana City, Cuba) and were dissolved in an acacia gum/water vehicle (1%).

2.3. Behavioural tests

All behavioural tests were conducted in quiet rooms at the same controlled conditions referred above and isolated from external noise.

Different groups of mice were used for each behavioural task. For each experiment, mice were randomized into five groups (10 mice per group): one control group treated with the vehicle, three groups treated with D-004 at 250, 500 and 1000 mg/kg, respectively, and a fifth drug reference group treated with imipramine 20 mg/kg (FST and the TST) or DZP 3 mg/kg (open field, rotarod, grip strength). Treatment with D-004 or vehicle was given orally (PO) via gastric gavage (1 mL/kg) for 30 days.

Intraperitoneal (IP) administration with imipramine and oral treatment with DZP were done 30 and 60 min before the tests, respectively, following the schemes of other authors (Tadano et al., 2000; Kulkami and Ashish, 2007; Griebel et al., 2001).

2.4. Forced swim test (FST)

This test was conducted according to the method of Porsolt et al. (1978). In brief, mice were placed individually in an acrylic cylinder filled with water at 25 ° C ± 3 ° C to a depth of 6 cm, without the possibility of escaping. The resulting anxiety produces vigorous swimming activity and attempts at escaping by diving or climbing the walls of the cylinder. After an initial 2 min period of vigorous activity, animals ceased all movements, except those necessary for survival (keeping the head above the water). Mice were judged immobile when floating motionless or making only those movements necessary to keep its head above water. The duration of immobility was measured during the next 4 min, as recommended (Porsolt et al., 1978; Shimamura et al., 2008) by observers blind to the treatment conditions. The changes in the duration of immobility of separate groups of mice were recorded.

Mice were used only once and water was changed after every swimming test to eliminate urine, excrement, and fur. After the swimming session, the mice were removed from the cylinder, dried with towels, and placed gently under a lighted bulb for 15–30 min.

Table 1
Effect of D-004 on the forced swim test (FST) (mean ± SEM) in mice.

Treatment	Dose (mg/kg)	Immobility time (s)	Inhibition (%)
Control	0	206.8 ± 28.3	
D-004	250	100.9 ± 25.9*	51
D-004	500	87.1 ± 28.4*	58
D-004	1000	72.9 ± 20.3*	65
Imipramine	20	64.7 ± 11.0[y]	69

*$p<0.0008$ compared with control animals (ANOVA followed by Dunnett's Multiple Comparison test).
[y]$p<0.001$ compared with control animals (ANOVA).

Table 2
Effect of D-004 on tail suspension test (TST) (mean ± standard error) in mice.

Treatment	Dose (mg/kg)	Immobility time (s)	Inhibition (%)
Control	0	155.6 ± 9.50	
D-004	250	135.8 ± 15.20	13
D-004	500	123.0 ± 7.97*	21
D-004	1000	122.8 ± 7.09*	21
Imipramine	20	78.2 ± 11.10**	50

*$p<0.05$; **$p<0.01$; comparisons with the control (Mann Whitney U test).

2.5. Tail suspension test (TST)

The TST was performed according to the method described by Steru et al. (1985). Mice were individually suspended 7.5 cm above the surface of a table 70 cm above the floor with an adhesive tape placed 1 cm away from the tip of the tail. Immobility duration was recorded for 6 min by observers blind to the treatment conditions. Mice were considered immobile only when they hung passively and were completely motionless.

2.6. Exploratory activity

The open field was an acrylic round device (10 cm diameter, 30 cm height) with a central circle (10 cm diameter). Mice were individually placed in the centre of the open field device. During the next 6 min the number of crossing through the central circle (C) and the number of rears (R) (number of times seen standing on hind legs or on the wall) were recorded (Fernández et al., 2005).

2.7. Rotarod test

A rotarod apparatus (Ugo Basile, Varese, Italy) was used in the experiment. Before starting the treatment mice were evaluated in order to select those able to walk on the rotating bar under the same conditions used on the test. Only those able to walk in the rotarod for at least 2 min were used in the experiment.

The equipment had a non-slippery plastic rod (3 cm in diameter, 13 cm over the base) that rotated at 10 rpm. Mice were observed for 5 min. The numbers of mice that fell from the rotating rod and the time (s) to the first fall (latency) were recorded.

2.8. Grip strength

In brief, mice were placed on a grip strength apparatus (Ugo Basile, Varese, Italy) with the surface of a plate disposed in front of a grip trapezium. When pulled by the tail, mice grasp the trapeze movement until pulling force overcomes the grip strength. After the animal lost the grip, the preamplifier stored the peak pull force achieved by the forelimbs. The grip strength was thus recorded by the apparatus.

2.9. Statistical analysis

Continuous data were analyzed with the non parametric Mann Whitney U test. Effects of D-004 on FST were re-assessed with the one-

way analysis of variance (ANOVA), followed by Dunnett's Multiple Comparison test. Categorical variables were compared with the Fisher Exact Probability test. An alpha level of 0.05 was accepted for statistical significance. Data were analyzed by Statistics software for Window (Release 4.2; Stat Soft, Inc, USA) and presented as means ± SEM values.

3. Results

D-004 oral treatment did not affect body weight of the animals in any group, as compared with the controls (data not shown).

3.1. Effect of D-004 on the FST

The overall effects of the oral administration of D-004 on the FST are summarized in Table 1. D-004 (250, 500 and 1000 mg/kg, PO) significantly ($p = 0.006$) reduced mice immobility time by 51, 58 and 65%, respectively, when compared with the vehicle-treated control group. One-way ANOVA revealed significant effects of the treatment on mice immobility $F(3,30) = 5.20$; $p < 0.005$. Subsequent Dunnett's test demonstrated that D-004 produced a significant and dose-dependent decrease in mice immobility. The decrease in immobility achieved with the highest dose of D-004 (1000 mg/kg) was comparable to that of the imipramine (20 mg/kg ip) treatment group.

3.2. Effect of D-004 on the TST

Compared with the vehicle-treated control group, immobility time in the TST in mice was significantly ($p = 0.0014$) shortened (50%) after imipramine treatment. D-004 at 500 and 1000 mg/kg, not at 250 mg/kg, significantly ($p = 0.024$ and $p = 0.030$ respectively), but modestly (21%) reduced mice immobility when compared with the vehicle-treated group (Table 2). The effect of imipramine was greater than that of D-004.

3.3. Effects of D-004 on the open field, rotarod and grip strength tests

Table 3 summarizes the effects of D-004 on the other behavioural tests. Treatment with D-004 (250–1000 mg/kg PO) did not result in overt behavioural changes or motor impairment in the open field test (Table 3a). The numbers of crossings and rearings in the D-004-treated groups were not statistically different from the control group. Oral treatment with DZP, however, significantly decreased both components in this test.

Also, the number of mice that were maintained on the rod and the time of walking on the rotating rod were unaffected by D-004 (250–1000 mg/kg PO). However, DZP significantly decreased the time on the rotarod, since 100% of the mice fell from the rod. (Table 3b) Likewise, D-004 did not affect the grip strength of mice compared with the vehicle control, whereas DZP significantly reduced such values (Table 3c).

Table 3
Effect of D-004 on other behavioural tests (mean ± standard error) in rodents.

a. Effect on the open field test in mice			
Treatment	Dose (mg/kg)	Crossings	Rearing
Control	0	123.6 ± 15.3	22.4 ± 3.9
D-004	250	129.3 ± 19.8	32.5 ± 4.4
D-004	500	102.8 ± 11.3	25.0 ± 3.1
D-004	1000	107.5 ± 9.5	26.1 ± 3.6
Diazepam	3	26.4 ± 3.0**	10.8 ± 1.6**
b. Effect on the rotarod test in mice			
Treatment	Dose (mg/kg)	Fallen mice (n)	Time (s) on the rod
Control	0	2	299.0 ± 16.1
D-004	250	3	299.2 ± 13.7
D-004	500	2	298.8 ± 11.3
D-004	1000	2	298.4 ± 14.8
Diazepam	3	10++	105.5 ± 7.0***
c. Effect on the grip strength in mice			
Treatment	Dose (mg/kg)	Grip strength (g)	
Control	0	11.0 ± 4.4	
D-004	250	10.7 ± 3.9	
D-004	500	10.4 ± 5.7	
D-004	1000	10.3 ± 3.1	
Diazepam	3	7.1 ± 4.7***	

**$p < 0.01$ comparisons with the control group (Mann Whitney U test).
***$p < 0.001$ comparisons with the control (Mann Whitney U test).
++$p < 0.01$ comparisons with the control (Fisher Exact Probability test).

4. Discussion

This study demonstrates that oral administration with D-004 for 30 days decreased mice immobility times in FST and TST, two well known animal models of depression (Porsolt et al., 1978; Steru et al., 1985), when compared with the control group.

D-004 (250 to 1000 mg/kg) orally given for 30 days reduced the FST immobility time in a dose-dependent manner, and the effect of the highest dose (1000 mg/kg) was comparable to that of imipramine 20 mg/kg IP, a classic tricyclic antidepressant. Also, D-004 decreased significantly the immobility time in the TST, but this effect did not increase with the doses and at 500 mg/kg achieved just a modest ceiling inhibition of 21%. Then, while imipramine at 20 mg/kg IP (single dose) was equally effective in both tests, D-004 was more effective in the FST. In the TST, however, the effect of D-004 was actually modest and lower than that of imipramine.

Although these animal models may be measuring slightly different constructs, the potential antidepressant-like effect of D-004 should be modest to moderate, since in addition to its effect on TST immobility was modest, only the highest dose (1000 mg/kg) given for 30 days produced an effect similar to that of imipramine 20 mg/kg IP given acutely.

The purpose of this study was to evaluate the potential depressive-like effect of D-004 using behavioural animal models based in the fact that D-004 has been found to reduce testosterone-induced PH (Arruzazabala et al., 2004; Carbajal et al., 2004, 2005; Noa et al., 2005) and to inhibit prostate 5α-reductase (Pérez et al., 2006), and that drug-induced depression has been reported for finasteride (an inhibitor of 5α-reductase used in BPH patients) (Lephart 1995; Altomare and Capella, 2002; Rahimi-Ardabili et al., 2006; Finn et al., 2006). Indeed, finasteride has been found to alter 5α-reductase activity in some brain regions (Steru et al., 1985; Lephart, 1995, Lephart et al., 1996) and to inhibit the transformation of dihydroprogesterone into allopregnanolone, a neuroactive steroid that modulates GABA-A receptors (Lephart, 1995; Stoffell-Wagner, 2001; Rahimi-Ardabili et al., 2006; Finn et al., 2006). Also, joint administration of finasteride 100 mg/kg and progesterone for 3 days increased FST immobility and reduced brain allopregnanolone in female mice, like progesterone withdrawal (Beckley and Finn, 2007). In comparison, we can affirm that D-004 administered at doses effective for reducing testosterone-induced PH in rodents (Arruzazabala et al., 2004, 2005, 2006; Carbajal et al., 2005) did not elicit a depressive-like effect.

There were some limitations in the assessment of our initial hypothesis. First, an enhancement of the immobility time in FST and TST should confirm a depressive-like effect of D-004, but a negative result should not discard such possibility completely, since greater doses could induce such effect. In such regard, since finasteride was more effective than D-004 for preventing Testosterone-induced PH (Molina et al., 2007), we included the highest dose of D-004 (1000 mg/kg) and a treatment duration ten times greater than that at which finasteride (100 mg/kg for 3 days) increased FST immobility (Beckley and Finn, 2007). Considering these facts and that we found that D-004 produced an antidepressant-like effect, the opposite effect should not be expected.

Second, the FST immobility of the control group was high (207 of the 240 s), consistent with results of other authors (Mantovani et al., 1999). These results, however, make difficult to observe any further significant increase. Indeed, although we used the test conditions of other authors (Mantovani et al., 1999; Dinesh and Amandeep, 2005; Bourin et al.,

2004), the FST model has been validated for assessing the antidepressant rather than depressive-like effects of substances. Then, maybe other FST conditions should be more suitable for our objectives. Also, daily administration with the vehicle by gastric gavage could have contributed to an additional increase in the stress-induced immobility in control mice. Nevertheless, since D-004 (not imipramine or DZP) were administered similarly to the vehicle, the decrease of FST immobility in D-004-treated mice should be a treatment-related effect.

The explanation of how D-004 produces antidepressant-like effects in both tests was beyond the objective of this study, which merely examined whether D-004 would enhance the mice immobility caused by stressing conditions like the forced swimming or tail suspension.

Treatment with D-004, however, did not produce other behavioural changes as assessed in the open field (exploratory activity), rotarod and grip strength tests, which differed from the effects of some anxiolytics, sedatives and psychostimulants on these rodent behavioural patterns (Aragao et al., 2006). These results indicate, therefore, that the reduction in FST and TST immobility times after 30 days administration of D-004 should be attributed to an antidepressant-like effect.

In an attempt to explain the present results, we have speculated that the antidepressant-like effect of D-004 might be due to the presence of lauric and oleic acid in its composition. Thus, high serum concentrations of lauric acid, one of the most abundant components of D-004, were related to a low level of depression in psychiatric inpatients according to the Bipolar Depression Index (BDI) and the Hamilton score (Irmisch et al., 2006). Also, palmitoleic and oleic acid concentrations seem to be relevant for sleep disturbances in depressive subjects, maybe due to their function as precursors of the sleep inducing oleamide, (Irmisch et al., 2007) a fatty acid amide that has shown to produce antidepressant-like effects (Akanmu et al., 2007). Further studies comparing the effects of D-004, lauric and oleic acid in these tests should help to confirm or reject such hypothesis.

On the other hand, since oxidative stress may be associated with the progress of depression, the antioxidant effects of antidepressant three herb extracts (*Hypericum perforatum*, *Ginkgo biloba* L, *Apocynum venetum* L) have been considered to beneficially contribute to depression management (Shirai et al., 2005). Nevertheless, although the antioxidant effects of D-004 (Menéndez et al., 2007, Pérez et al., 2008) could contribute to its potential antidepressant effect, from the present results we cannot conclude anything in such regard.

In conclusion, the present data indicate that D-004 orally administered for 30 days at pharmacologically effective doses (250–1000 mg/kg) reduces, rather than enhances, mice immobility in the FST and TST models and that it does not affect novelty and motor behaviours in open field, rotarod and grip strength tests. Further studies, however, should confirm if the antidepressant effect of D-004 is reproducible and to explore the underlying causes of such effect.

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