

Artículo Original | Original Article

Inhibition of cyclooxygenase (COX) and 5-lipoxygenase (5-LOX) by D-005 (A lipid extract of *Acrocomia crispera* fruits)

[Inhibición de la ciclooxigenasa (COX) y la 5-lipooxigenasa (5-LOX) por el D-005 (extracto lipídico del fruto de la *Acrocomia crispera*)]

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Abstract: This study was aimed to investigate whether the a lipid extract from *Acrocomia crispera* fruits (D-005) inhibits COX and 5-LOX enzyme activities in vitro. This study demonstrates that D-005 inhibits markedly and in a dose dependent manner COX-2 and 5-LOX activities. The dual inhibition of COX-2 and 5-LOX supports further research on the potential anti-inflammatory effect of D-005.

Keywords: *Acrocomia crispera*, anti-inflammatory, COX, dual inhibition, 5-LOX

Resumen: El objetivo de este estudio fue investigar si el extracto lipídico de los frutos de *Acrocomia crispera* (D-005) inhibe in vitro las actividades de las enzimas COX y 5-LOX. Este estudio demuestra que el D-005 inhibe marcadamente y de manera dosis dependiente las actividades de la COX-2 y 5-LOX. La inhibición dual de la COX-2 y 5-LOX soportan futuras investigaciones sobre el potencial efecto anti-inflamatorio del D-005.

Palabras clave: *Acrocomia crispera*, anti-inflamatorio, COX, inhibición dual, 5-LOX

Recibido | Received: August 24, 2016

Aceptado | Accepted: December 8, 2016

Aceptado en versión corregida | Accepted in revised form: January 9, 2017

Publicado en línea | Published online: May 30, 2017

Declaración de intereses | Declaration of interests: A los financiadores de esta investigación: Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET) y al proyecto ANPCyT/FONCyT Pict 1001.

Este artículo puede ser citado como / This article must be cited as: Y Pérez, A Oyarzábal, R Sierra, R Mas, V Molina, S Jiménez, V González. 2017. Inhibition of cyclooxygenase (COX) and 5-lipoxygenase (5-LOX) by D-005 (A lipid extract of *Acrocomia crispera* fruits). **Bol Latinoam Caribe Plant Med Aromat** 16 (3): 319 – 328.

INTRODUCTION

Benign prostatic hyperplasia (BPH) is a common urological disease in aging men and it is frequently associated with troublesome lower urinary tract symptoms (LUTS), such as weak urinary stream, frequency and urgency (Roehrborn, 2011).

Phytotherapeutic alternatives have been used to manage BPH for decades, their efficacy being based in multiple mechanisms, such as the inhibition of 5 α -reductase activity, antagonism of α -adrenoreceptors (α -ADR), as well as on anti-inflammatory and antioxidant effects (Sun & Zhang, 2014; Pagano *et al.*, 2014; Allkanjari & Vitalone, 2015). Lipid extracts from the fruits of saw palmetto palm represent the main phytotherapy for BPH (Pharmacopeial Convention, 2005). Despite some negative results (MacDonald *et al.*, 2012), several clinical studies and popular use document that the efficacy and safety of saw palmetto (Sinescu *et al.*, 2011; Giulianelli *et al.*, 2012), recent evidence supports that saw palmetto soft capsules it improves not only BPH symptoms, but erectile sexual dysfunction in men with both entities (Suter *et al.*, 2013). Experimental studies have demonstrated that 5 α -reductase enzyme inhibition, α -ADR antagonism, antioxidant and anti-inflammatory effects are included among the different mechanisms that support the efficacy of saw palmetto (Belostotskaia *et al.*, 2006; Minciullo *et al.*, 2014). The palms, quite abundant in Cuba, represent a relevant source for ethnomedicine of the American continent (Sosnowska & Balslev, 2009).

Moreover, D-004, a lipid extract from the Cuban royal palm (*Roystonea regia*) fruits has been effective on model experimental prostatic hyperplasia (Carbajal *et al.*, 2004; Noa *et al.*, 2005; Carbajal *et al.*, 2005; Arruzazabala *et al.*, 2005; Arruzazabala *et al.*, 2006; Pérez *et al.*, 2006) and in patients with (BPH) (Pérez *et al.*, 2008; López *et al.*, 2009; Guzmán *et al.*, 2013a; Guzmán *et al.*, 2013b). Evidence support that the efficacy of D-004 involves the inhibition of prostate 5 α -reductase activity, and the antagonism of α 1-ADR -mediated responses (Carbajal *et al.*, 2005; Pérez *et al.*, 2006). Antioxidant and anti-inflammatory effects, however, may also contribute to D-004 efficacy in BPH (Menéndez *et al.*, 2007; Pérez *et al.*, 2008; López *et al.*, 2009). Experimental studies have proven that D-004 inhibits both COX and 5-LOX activities, which supports the anti-inflammatory action of D-004 (Menéndez *et al.*, 2006; Menéndez *et al.*, 2007; Oyarzábal *et al.*, 2014).

The COX and 5-LOX enzymes are involves in high levels of arachidonic acid (AA) produced by the action of phospholipase A2 on membrane phospholipids, which is then metabolized through the COX and LOX pathways to produce prostaglandins (PG), thromboxanes, prostacyclins, and inflammatory leukotrienes (LT). (Menéndez *et al.*, 2006).

Based on the exiting research it is significant to search for of potential pharmacological effects, useful for manage BPH or other pathological entities, in the extracts of the fruits of other palm species. *Acrocomia crispa* (Cuban belly palm, Corajo palm), endemic to Cuba (Henderson *et al.*, 1995; Govaerts & Dransfield, 2005), after a literature review (2000 - 2015) evidence associated to any pharmacological effect was not found (Entrez PubMed, search the June 2000 to June 2015).

A lipid extract from *Acrocomia crispa* fruits (D-005) obtained in our centre shows a reproducible mixture of fatty acids, but different from that of D-004 and saw palmetto extracts. This extract contains a mixture of fatty acids, mainly oleic, palmitic, lauric, linoleic, and myristic acids. Previous studies had demonstrated that saturated and unsaturated fatty acids, such as myristic, stearic, palmitic, oleic and several oils extracts containing fatty acids inhibit COX and LOX, *in vitro* (Naidu, 1995; Chan *et al.*, 1996; Henry *et al.*, 2002; Zhang *et al.*, 2002; Menéndez *et al.*, 2007)

Previous studies demonstrated the non-toxicity of D-005 following acute oral administration to mice and rabbits (Gutierrez *et al.*, 2016a; Gutierrez *et al.*, 2016b).

Therefore, this study was aimed to investigate whether D-005 inhibits COX and 5-LOX enzyme activities *in vitro*.

MATERIALS AND METHODS

Animals

Male Wistar rats (180 - 200g), from the Center for Laboratory Animals Production (CENPALAB, Habana, Cuba) were adapted for 7 days to laboratory conditions: controlled temperature 25 \pm 2 $^{\circ}$ C, relative humidity 60 \pm 5% and 12 hours light/dark cycles. Food (rodent pellets from CENPALAB) and water were provided *ad libitum*. After a 12 hour fast rats were anaesthetized in ether atmosphere, sacrificed by exsanguinations.

Animal handle was conducted according to the Cuban Code for the Use of Laboratory Animals and ethical principles for animal management N $^{\circ}$

39/04. An independent ethical board approved the study protocol and use of the animals for such aim (Cuban Guidelines for the laboratory animals care, N° 39/2004).

Materials

All chemicals were purchased from Sigma-Aldrich Co. (St Louis, MO), except 2, 2 azo-bis-2-amidinopropane hydrochloride (ABAP), obtained from Polyscience (Warrington, PA). Ultracentrifuge was from Beckman (Beckman Instruments, Inc. Palo Alto, CA) and Ultraspec-Plus spectrophotometer from LKB (Pharmacia LKB Biotechnology, Uppsala, Sweden). Standards of gas chromatography, Ácidos: octanoico (Caprílico, C_{8:0}), decanoico (Cáprico, C_{10:0}), dodecanoico (Láurico, C_{12:0}), tridecanoico (C_{13:0}), tetradecanoico (mirístico, C_{14:0}), hexadecenoico (cis-9-palmitoléico, C_{16:1}), hexadecanoico (palmítico, C_{16:0}), octadecanoico (esteárico, C_{18:0}) y octadecenoico (cis-9-oleico, C_{18:1}), (p.a., Sigma, EUA).

Administration and dosage

D-005 consisted of a lipid extract obtained from the dried mature fruits of *Acrocomia crispera* collected at the north shore of west Havana, being duly authenticated by the Cuban Botanic Garden (Havana, Cuba) voucher number 1982-1031. Plant material was powdered and passed through mesh of size 236 mm and then subjected to extraction and purification in hexane and basic hydrolysis with KOH. D-005 was obtained from the Chemistry Department of the Centre of Natural Products (Havana, Cuba), its composition and purity being controlled by gas chromatography with standards. The fatty acids content of the tested batch was as follows (w/w, %): lauric (35.8%), oleic (28.4%), myristic (14.2%), palmitic (8.9%), stearic (3.3%), capric (1.9%), caprylic (1.2%), and palmitoleic (0.05%). Purity (total content of these free fatty acids) was 93% (Rodríguez, 2013).

D-004, supplied by the Plants of Natural Products (Havana, Cuba), had the following fatty acids composition, which was controlled by gas chromatography (w/w, %): lauric (23.6%), oleic (41.9%), myristic (10.6%), palmitic (11.4%), stearic (2.9%), capric (0.6%), caprylic (0.3%), and palmitoleic (0.3%). Purity (total content of these fatty acids) was 91% (Sierra *et al.*, 2014).

For the experiments, D-004 and D-005 were suspended in 2% Tween 65/water vehicle. INDO

(Cuban Pharmaceutical Industry -QUIMEFA-) was dissolved in 5% sodium bicarbonate.

Cytosolic microsomal preparations from rat platelet rich plasma (PRP) and rat seminal vesicles were used for assessing COX-1 and COX-2 enzyme activities, respectively, whereas the cytosolic fraction of polymorphonuclear leukocytes (PMNL) was used for determining 5-LOX activity.

Preparation of the rat platelets microsomal fraction

The effects on COX-1 activity were assessed by using microsomal preparations from rat platelets. Briefly, venous blood samples were collected in tubes containing sodium citrate (3.8%) (9:1, v/v). The tubes were centrifuged at 160 x g for 10 min at 10° C and the supernatant was centrifuged again at 2100 x g for 10 min at 10° C. The pellet was re-suspended in Tris-HCl EDTA (50 mol/l, pH 7.4, 1 mol/l EDTA) and ammonium oxalate (2%) (1:20, v/v) and centrifuged at 2100 x g for 10 min at 4° C. The pellet was re-suspended the same Tris-HCl EDTA buffer, sonicated (3 cycles of 30 sec, sub-maximal potency) and centrifuged at 15.000 x g for 20 min at 4° C. Finally, the supernatant was centrifuged at 100 000 x g for 2 hours at 4° C. The pellet (platelets microsomal fraction) was re-suspended in 0.05 mol/l Tris/HCl buffer (pH = 8.4) containing 0.01% Triton X-100 (1:9, w/v) and frozen at -20° C until use (Boyum, 1983).

Preparation of the rat seminal vesicles microsomal fraction

The effects on COX-2 activity were assessed by using microsomal preparations from rat seminal vesicles. In brief, seminal vesicle slices were homogenized in 0.05 mol/l Tris/HCl buffer (pH = 8.4) containing 0.01% Triton X-100 (1:9, p/v) with a potter. The homogenates were centrifuged at 15.000 x g for 15 min and the supernatant was centrifuged again at 100 000 x g for 1 hour, all operations being carried out at 4° C, the pellet (microsomal fraction) was frozen at -20° C until use (Neeraja *et al.*, 2005).

Preparation of the PMNLs cytosolic fraction

Effects on 5-LOX activity were assessed by using enzyme preparations from the from the cytosolic fraction of freshly isolated blood was obtained the PMNLs. Briefly, venous blood samples were collected in tubes containing EDTA (10%), and then diluted in 0.9% of saline solution (NaCl 0.9%) to 10 ml. Six (6) ml of diluted blood were then gently

layered over 3 ml of 14.1% Nycodenz (density 1.077 g/ml, 20° C) prepared in 0.44% NaCl and 5 mmol/l Tris HCl buffer (pH = 7.2), and centrifuged at 800 x g for 30 min. at 20° C. After centrifugation, the mononuclear cells formed as band at the Nycodenz-plasma interface were removed with a Pasteur pipette, washed with 50 mmol/l phosphate buffer/1mmol/l EDTA (pH = 7.4), and centrifuged at 400 x g for 10 min. The pellet was washed again in buffer, re-suspended in the same buffer and then used as the crude enzyme preparation. For obtaining the cytosolic fraction, PMNL were sonicated (3 cycles of 30 sec, sub-maximal potency), and centrifuged at 2000 x g for 10 min at 0° C. The supernatant was centrifuged at 100 000 x g for 1 hour at 4° C, and then the fraction was frozen at -20° C until the activity test (Boyum, 1983).

Effects of D-005 on COX enzyme activity

COX activity was measure accordance to Abad *et al.*, (1994). The reaction mixture contained 2 mmol/l AA; microsomal fraction (1 mg/ml); 5.8 mmol/l L-epinephrine and 0.05 mol/l Tris HCl buffer (pH = 8.4). Tubes containing the vehicle, D-005 (0.9, 3.9, 15.6, 62.5, 250, 500, or 1000 µg/ml), or INDO (0.4 µg/ml) (reference inhibitor of COX) and D-004 500 µg/ml (dual inhibitor of COX and 5-LOX) were run. Then, mixture reactions were preincubated with L-epinephrine for 4 min and then AA at 37° C was added. The changes of absorbance at 480 nm were measured for 10 min in the spectrophotometer. The enzyme activity was expressed in OD 480 nm changes/mg of protein.

Each experiment was run in triplicate and the results averaged. The concentration producing a 50% inhibition (IC₅₀) was calculated from the outline of the inhibition percentages as a function of the concentrations of D-005. The effects on COX reaction rates were assessed as the increase in the substrate (AA) concentrations (7.8, 31.2, 62.5, 125, 250 mmol/l).

Once the substrate was added, increase in absorbance was measure at 234 nm every min for 10 min. The enzyme activity was expressed as µmol of conjugated dienes/min/mg protein. The initial reaction rate was determined from the slope of the straight line portion of the curve and the percentage inhibition of the enzyme activity was calculated by comparing with the control samples.

Effects of D-005 on 5-LOX enzyme activity

Lipoxygenase is known to catalyse the oxidation of unsaturated fatty acids containing 1-4 diene structures. The conversion of linoleic acid to 13-hydroperoxy linoleic acid was followed spectrophotometrically by the appearance of a conjugate diene at 234 nm. In brief, the enzyme preparation (1 ml, final volume) that contained the cytosolic fraction (50µg of protein) dissolved in 50 mmol/l phosphate buffer/1mmol/l EDTA (pH = 7) was pre-incubated for 5 min prior to the addition of the substrate (linoleic acid 250 µmol/l in ethanol). Tubes containing the vehicle, D-005 (0.9, 3.9, 15.6, 62.5, 250, 500, or 1000 µg/ml), INDO 0.4 µg/ml (COX, not LOX inhibitor), or D-004 500 µg/ml (dual inhibitor of COX and 5-LOX) were run. Once the substrate was added, the increase of absorbance at 234 nm was measured every min for 10 min in the spectrophotometer. The enzyme activity was expressed as µmol of conjugated dienes/min/mg protein. The initial reaction rate was determined from the slope of the straight line portion of the curve and the percentage inhibition of the enzyme activity was calculated by comparing with the control samples (Tateson *et al.*, 1988).

Each experiment was tested in triplicate and the results averaged; the concentration that gave 50% inhibition (IC₅₀) was calculated from the outline of the inhibition percentages as a function of the inhibitor concentration. The effects of D-005 on the initial rate of 5-LOX reaction (V_{max}) were assessed as the increase in the substrate concentration (linoleic acid 7.8, 31.2, 62.5, 125, and 250 mmol/l).

Statistical analyses

All the analyses were carried out in triplicate and the data were expressed as the mean ± standard deviation. Comparisons between treated and control groups were performed with the Mann-Whitney U and the ANOVA tests. Statistical significance was chosen for $\alpha = 0.05$. Dose-effect relationships were assessed by using a linear regression and correlation test. Regression analysis was used to calculate IC₅₀, defined as the concentration of inhibitor necessary for 50% inhibition of the enzyme reaction. Data were processed with the Statistics Software for Windows (Release 4.2 Stat Soft Inc, Tulsa OK, US).

RESULTS**Effects of D-005 on COX-1 and COX-2 activities**

Table 1 lists the effects on COX-1 activity. The addition of D-005 (0.9-1000 µg/ml) did not modify significantly the enzyme activity, although an

apparent reduction of almost 30% as compared to the control was noted. INDO 0.4 µg/ml, not D-004 500 µg/ml, produced a significant ($P < 0.01$) and marked inhibition of COX-1 activity (94.8%).

Table 1
Effects of D-005 on COX-1 enzyme activity in rat platelets microsomes

Groups	Concentrations (µg/ml)	Enzyme activity (ΔOD/min/mg protein)	Inhibition (%)
Control	0	0.350 ± 0.01	-
D-005	0.9	0.342 ± 0.04	2.2
D-005	3.9	0.282 ± 0.01	19.4
D-005	15.6	0.270 ± 0.08	22.8
D-005	62.5	0.245 ± 0.06	30.0
D-005	125	0.243 ± 0.05	30.5
D-005	250	0.243 ± 0.05	30.5
D-005	500	0.242 ± 0.06	30.8
D-005	1000	0.242 ± 0.06	30.8
D-004	500	0.316 ± 0.01	9.7
Indomethacin	0.4	0.018 ± 0.01**	94.8

(Mean ± SD) ** $P < 0.01$ Comparison with the control (Mann Whitney U test)

All added substances inhibited significantly COX-2 activity (Table 2). D-005 (0.9-1000 µg/ml) produced a significant, dose-dependent ($r = 0.968$; $P < 0.001$) and marked (95.1%) inhibition of COX-2 activity

($IC_{50} = 6.17\mu\text{g/ml}$). In addition, INDO 0.4 µg/ml and D-004 500 µg/ml inhibited significantly ($P < 0.01$ and $P < 0.05$, respectively) COX-2 by 97.1% and 85.0%, respectively.

Table 2
Effects on COX-2 activity in rat seminal vesicle microsomes

Groups	Concentrations (µg/ml)	Enzyme activity (ΔOD/min/mg protein)	Inhibition (%)
Control	0	0.351 ± 0.01	-
D-005	0.9	0.288 ± 0.23	17.9
D-005	3.9	0.191 ± 0.15*	45.5
D-005	15.6	0.147 ± 0.05*	58.1
D-005	62.5	0.063 ± 0.01*	82.0
D-005	125	0.044 ± 0.01*	87.4
D-005	250	0.035 ± 0.01**	90.0
D-005	500	0.019 ± 0.01**	94.5
D-005	1000	0.017 ± 0.01**	95.1
D-004	500	0.053 ± 0.02*	85.0
Indomethacin	0.4	0.010 ± 0.01**	97.1

(Mean ± SD) * $P < 0.05$, ** $P < 0.01$ Comparison with the control (Mann Whitney U test)

The inhibition of COX-2 activity by D-005 involved the modification of both kinetic parameters (V_{max}

and K_m) (Figure 1, Lineweaver-Burk plots), so that the inhibition was uncompetitive.

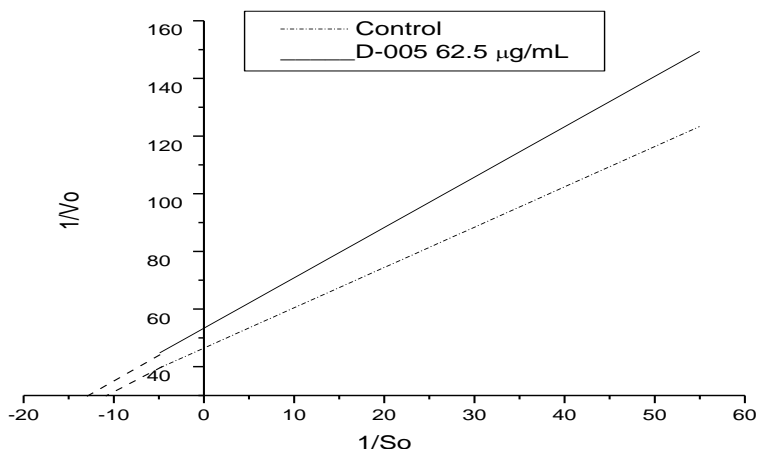


Figure 1

Lineweaver-Burk plot ($1/v_0$ versus $1/[S]_0$) of the effect of D-005 (62.5 $\mu\text{g/ml}$) on the initial rate of the enzyme reaction measured in front of increasing concentrations of the substrate (arachidonic acid 7.8, 31.2, 62.5, 125 and 250 mmol/l). D-005 modified the values of both kinetic parameters K_m ($-1/K_m$, intercept with abscise axis) and V_{max} ($1/V_{max}$, intercept with the ordinate axis) of COX-2 enzyme activity.

Effects of D-005 on 5 LOX activity

Table 3 summarizes the effects on 5-LOX activity. D-005 (0.9-1000 $\mu\text{g/ml}$) addition to PMNL preparations significantly, dose-dependently ($r = 0.909$; $P < 0.002$) and markedly inhibited 5-LOX

activity (94.5%) ($IC_{50} = 140.7 \mu\text{g/ml}$). D-004 500 $\mu\text{g/ml}$ inhibited significantly ($P < 0.01$) 5-LOX activity by 91.4 %, while INDO was ineffective modifying the enzyme activity.

Table 3

Effects of D-005 on 5-LOX activity in cytosolic fraction of polymorphonuclear leukocytes (PMNL)

Groups	Concentrations ($\mu\text{g/ml}$)	Enzyme activity ($\Delta\text{OD}/\text{min}/\text{mg protein}$)	Inhibition (%)
Control	0	11.00 ± 0.01	-
D-005	0.9	10.70 ± 2.50	2.7
D-005	3.9	9.70 ± 1.10	11.8
D-005	15.6	9.40 ± 1.80	14.5
D-005	62.5	8.80 ± 1.90	20.0
D-005	125	$6.30 \pm 1.40^*$	45.7
D-005	250	$1.85 \pm 1.25^{**}$	83.1
D-005	500	$0.95 \pm 0.35^{**}$	91.3
D-005	1000	$0.60 \pm 0.01^{**}$	94.5
D-004	500	$0.95 \pm 0.35^{**}$	91.4
Indomethacin	0.4	11.1 ± 2.20	0

(Mean \pm SD) * $P < 0.05$, ** $P < 0.01$ Comparison with the control (Mann Whitney U test)

The addition of D-005 modified both kinetic parameters (V_{max} and K_m) of COX and 5-LOX enzyme activities (Figure 2, Lineweaver-Burk plots),

so that 5-LOX inhibition by D-005 was uncompetitive.

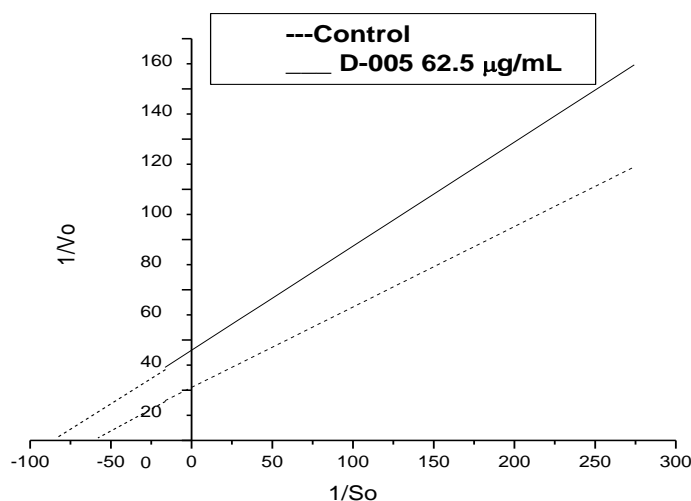


Figure 2

Lineweaver-Burk plot ($1/v_0$ versus $1/[S]_0$) of the effect of D-005 (62.5 $\mu\text{g/ml}$) on the initial rate of the enzyme reaction measured in front of increasing concentrations of the substrate (linoleic acid 7.8, 31.2, 62.5, 125 and 250 mmol/l). D-005 modified the values of both kinetic parameters K_m ($-1/K_m$, intercept with abscise axis) and V_{max} ($1/V_{max}$, intercept with the ordinate axis) of 5-LOX enzyme activity.

DISCUSSION

In this study we demonstrated that the addition of D-005 (0.9 - 1000 $\mu\text{g/ml}$) inhibited significantly, dose-dependently and markedly (about 95% in both cases) COX-2 activity in the microsomal fraction of rat seminal vesicles, and 5-LOX activity in the cytosolic fraction of rat PMNL *in vitro*, without modifying the activity of COX-1 in rat platelets microsomal fraction.

Despite the observation that D-005 produced an apparent decrease of COX-1 activity versus the control, such reduction was not significant, its magnitude was modest to moderate (about 30%) and constant across a wide concentration range (62.5 to 1000 $\mu\text{g/ml}$) we acknowledge that D-005 failed to inhibit COX-1 in this study.

INDO inhibited significantly and markedly COX-1 (94.8%) and COX-2 activities (97.1%) leaving unaffected 5-LOX activity, as expected of a non-selective COX inhibitor (Martel-Pelletier *et al.*, 2003); while D-004 inhibited significantly COX-2 (85%) and 5-LOX (91.4%), consistently with previous reports (Menéndez *et al.*, 2006; Menéndez

et al., 2007). These facts validate the assessment of enzyme activities under our experimental conditions and the results.

The addition of D-005 inhibited markedly COX-2 and 5-LOX (maximal inhibitions of about 95% in both cases) rat seminal vesicles preparations and rat PMNL, respectively. Nevertheless, D-005 appears to be more potent to more potent inhibitor of COX-2 ($IC_{50} = 36.08 \mu\text{g/ml}$) than 5-LOX ($IC_{50} = 140.7 \mu\text{g/ml}$), which suggests that D-005 has a higher affinity for COX-2 than for 5-LOX enzyme.

The nature of the inhibitions of COX-2 and 5-LOX by D-005 was uncompetitive, since in both cases D-005 addition modified the two kinetic parameters (V_{max} and K_m) of COX-2 activity. These results suggest that D-005 does not interact directly with the active site of these enzymes to curtail the enzyme reaction, but with a site near to it. This interaction, however, should be strong since inhibitions of COX-2 and 5-LOX achieved with D-005 were not just significantly, but actually meaningful ($\geq 90\%$ in both cases). In the case of COX-2 this statement is reinforced by the fact that

INDO, an effective unspecific COX inhibitor, produced a COX-2 inhibition of about 97.0%, near to that caused by D-005. In turn, at similar concentrations (500 µg/ml) the inhibition of COX-2 by D-005 was slightly superior (about 95%) than that reached with D-004 (about 85%). Nevertheless in the present study, we cannot reach to conclusions about the potency and efficacy of both substances (D-005 and D-004) on this model because we did not compare concentration versus effects relationships of both treatments, but only tested the effect of one concentration of D-004.

According to the present results, D-005 acts as a dual COX/5-LOX inhibitor. It is known that dual acting anti-inflammatory drugs, able to inhibit COX and 5-LOX, seem to retain the activity of non-steroidal anti-inflammatory drugs (NSAIDs) while avoiding their main adverse effects. NSAIDs display their anti-inflammatory action mainly through inhibition of COX, thus interfering with the production of gastroprotective prostaglandins and then, displacing the AA metabolism towards the increase of the production of pro-inflammatory, bronchoconstrictive and gastrototoxic leukotrienes (LTs). Although D-005 inhibits markedly COX-2, its inhibitory action on 5-LOX should prevent the switch to the increased production of LTs (Leone *et al.*, 2007; Van Wauwe & Goossens, 2009). Previous studies had demonstrated that saturated and unsaturated fatty acids, such as myristic, stearic, palmitic, oleic and several oils extracts containing fatty acids inhibit COX and LOX, *in vitro* (Naidu, 1995; Chan *et al.*, 1996; Henry *et al.*, 2002; Zhang *et al.*, 2002; Menéndez *et al.*, 2007). These fatty acids had demonstrated anti-inflammatory effect *in vivo* and clinical assay (Menéndez *et al.*, 2006; Zhang *et al.*, 2008; Ravelo *et al.*, 2011).

The present results add knowledge on the pharmacological effects of lipid extracts obtained from palm fruits, in particular obtained from *Acrocomia crispa*, specie endemic to Cuba which hasn't been studied enough as researched subject. The present results encourage the investigation of the effects of D-005 on experimental models of acute and chronic inflammation *in vivo*.

CONCLUSIONS

This study demonstrates that D-005, a lipid extract obtained from *Acrocomia crispa* fruits, inhibited COX-2 and 5-LOX enzyme activities, with highest affinity for COX-2. The dual inhibition of COX-2

and 5-LOX suggests that D-005 could produce anti-inflammatory effects.

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