ARTÍCULO ORIGINAL

Preclinical validation of antinociceptive, antiinflammatory, and antipyretic activities of *Cordia martinicensis* leave decoction

Validación preclínica de las actividades antinociceptiva, antiinflamatoria y antipirética de decocción de hojas de *Cordia martinicensis*

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ABSTRACT

Introduction: leave decoction of *Cordia martinicensis (*Jacq.) Roem. & Schult. is commonly used in Dominica, Martinique and St. Lucia to relieve thoracic pain and fever.
Objectives: to validate the antiinflammatory, antinociceptive (analgesic), and antipyretic ethnobotanical uses of *Cordia martinicensis* in experimental animal models.
Methods: 30 % aqueous extract of *Cordia martinicensis* dry leaves was prepared just before use. Analgesic activity was assayed by writhing and tail flick, and antiinflammatory activity by the ear oedema test, both in mice. Finally, antipyretic activity was tested by inducing pyrexia with brewer's yeast in rats.
Results: decoction of *Cordia martinicensis* significantly decreased the number of abdominal stretchings by 44.4 %, but it didn't produce a significant antinociceptive response to thermal stimuli. It also displayed strong antiinflammatory activity, the percentage of inhibition was near 60 %, and a dose of 5 mg/kg showed significant antipyretic activity. *Cordia martinicensis* reacted positively to alkaloids, flavonoids, tannins, and anthocyanidines, and did not show any signs of toxicity.
Conclusions: this is the first report on the activity of *Cordia martinicensis* directly related to its popular use, and it provides pharmacological validation for the relief of

fever, inflammation and pain. Further studies should be done to identify the active principles responsible for the biological activity of the plant.

Key words: *Cordia martinicensis,* analgesic, antiinflammatory, antipyretic.

RESUMEN

Introducción: la decocción de hojas de *Cordia martinicensis* (Jacq.) Roem. & Schult. muestra un uso tradicional significativo para el alivio del dolor torácico y la fiebre, por parte de la población de Dominica, Martinica y Santa Lucía.
Objetivos: validar el uso etnobotánico de *Cordia martinicensis* en esta región, como antiinflamatorio, antinociceptivo (analgésico) y antipirético, en modelos experimentales en animales.
Métodos: el extracto acuoso al 30 % de hojas secas de *Cordia martinicensis* se preparó justo antes de ser usado. La actividad analgésica fue estudiada mediante el modelo de contorciones abdominales y retirada de la cola en ratones. El efecto antiinflamatorio se evaluó sobre el edema en la oreja del ratón inducido por aceite de *Croton*. Finalmente, la actividad antipirética del extracto se evaluó mediante la inducción de fiebre en ratas por levadura de cerveza.
Resultados: la decocción de *Cordia martinicensis* disminuyó significativamente el número de contorciones abdominales en 44.4 %, pero no la respuesta al estímulo térmico; en edema en la oreja inhibió la inflamación 60 %, tanto tópico como oral. Resultó capaz de disminuir la fiebre a dosis de 5 mg/kg. *Cordia martinicensis* contiene alcaloides, flavonoides, taninos, y antocyanidinas y no mostró señales de toxicidad.
Conclusiones: constituye el primer estudio de validación del uso tradicional de *Cordia martinicensis*, avalando el uso farmacológico en procesos de fiebre, dolor e inflamación. Es necesario continuar los estudios fitoquímicos para determinar los principios activos responsables de su actividad biológica.

Palabras clave: *Cordia martinicensis,* analgesia, antiinflamatorio, antipirético.

INTRODUCTION

Cordia is a genus of trees or shrubs, sometimes subscandent in the borage family Boraginaceae. About 300 species have been identified, generally in tropical regions, frequently growing in gardens with beautiful flowers. It has been used as a wound healing agent, as astringent, antiinflammatory, anthelminthic, antimalarial, diuretic, febrifuge, appetite suppressant, cough suppressant, and to treat urinary infections, lung diseases, and leprosy in traditional medicine.[1,2]

An ethnobotanical survey conducted as part of the Program for Applied Research and Diffusion of Traditional Plant Uses in the Caribbean (TRAMIL), found that leave decoction of *Cordia martinicensis (*Jacq.) Roem. & Schult. was commonly used by local populations to relieve thoracic pain and fever. This species is native to the Lesser Antilles and is distributed in Dominica, Martinique and St. Lucia. It is popularly known as Black sage, *mahaut noir*, *maho nwe*, *mahot noir*.[3,4]

The aim of the present research was to validate the antiinflammatory, antinociceptive (analgesic), and antipyretic ethnobotanical uses in this region by means of experimental animal models.

METHODS

Analgesic and antiinflammatory activity was assayed using two antinociception models (writhing and tail flick tests) and an acute inflammation model (ear oedema test) in mice. Finally, antipyretic activity was tested by inducing pyrexia with brewer's yeast in rats.

Plant materials

*Cordia martinicensis (*Jacq.) Roem. & Schult. leaves were collected from *Le Prêcheur*, Martinique, and authenticated by Dr. Emmanuel Nossin in Martinique. A voucher, under number LN-14, was deposited at *Herbier l'Association pour la Valorisation des Plantes Médicinales de la Caraïbe* (HAVPMC).

Preparation of the extract

Leaves were carefully washed and disinfected. They were dried in a naturally ventilated room in the shade. Aqueous extracts (decoction) of *Cordia martinicensis* were prepared just before use, boiling dry leaves in distilled water on a low flame for 10 min, followed by fast paper filtration.

Phytochemical screening

Phytochemical analysis was performed on 30 % decoction through various tests, mainly chemical reaction identification by color change or precipitated formations, determining the presence of secondary metabolites: ûavonoids (*Shinoda*), phenolic and tannin compounds (ferric chloride $FeCl_3$), alkaloids (*Dragendorff* and *Mayer*), triterpenes and steroids (*Liebermann-Buchard*), quinones (*Bortrager*), lactonic compounds and coumarins (*Baljet*), amino acids (ninhidrine), anthocyanidines (*Rosenhein*) and reducing sugar (*Fehling)*, saponines (foam), starch (lugol).[5]

Animals

Animals were obtained from the National Center for Experimental Animals Production (CENPALAB, Havana, Cuba). They were kept under controlled conditions, 23 ± 0.5 ^{o}C, relative humidity 50-60 %, in a 12 h:12 h alternate light-dark cycle, food and water ad libitum. All experimental protocols were approved by the Institutional Animal Care and Ethical Committee at the Central Pharmacological Research Unit of the Medical Sciences University of Havana, following the "Principles of Laboratory Animal Care". Prior to each experiment animals were fasted for 12 h with free access to water.[6,7]

Acute toxicity tests

Acute toxic class method

Six non isogenic Wistar rats (3 per sex) weighing between 200-250 g were used in the study. Aqueous leave extract of *Cordia martinicensis* (50 %) was administered by orogastric gavages. Due to the low concentrations of total solids in the decoction

(2.6 %), animals were administered the maximum allowable volume (equivalent to 520 mg/kg of body weight). After a single dose animals were constantly observed during the first 24 h, and then once a day, recording the weight and any clinical sign.[8,9]

Acute oral repeated dose toxicity

Decoction of *Cordia martinicensis* (50 %), dose 1 000 mg/kg, was administered during five consecutive days to 10 Wistar rats (5 per sex) weighing between 200-250 g. *Cordia martinicensis* was given in 2 administrations which were separated 4 h. Animals were constantly observed during the first 12 h and every 24 h for 14 days, recording the weight and any clinical sign.

In both methods, on day 14 the animals were sacrificed by inhalation of ether and a macroscopic study of entire organs was performed.[10]

Analgesic activity

Analgesic effect of *Cordia martinicensis* decoction (30 %) was evaluated using two different models: writhing and tail-flick test. Ten non-isogenic male OF-1 mice (20-25 g) were used in each group. In both test, animals were treated 1 h before the trial by orogastric means with doses of 0.5, 1, and 5 g/kg of *Cordia martinicensis*, while the negative control group received distilled water. The positive control group received indomethacin (10 mg/kg) dissolved in sodium bicarbonate 4 %.[11]

Writhing test

The test was performed with some modifications according to the method described by *Koster* et al. One hour after receiving the corresponding treatment, acetic acid 0.75 % was injected (0.1 mL/10 g i.p). The number of writhes or stretches was counted during 15 min.[11]

Tail flick test

Nociceptive stimuli were induced by a heat stimulus (water at 55 °C) on the tail as previously described by *Janssen* et al. After 60 min of receiving the corresponding treatment, animals were placed in a box with the tail sticking out and the latter proceeded to be immersed in the water (55 °C). The animal's reaction to the heat was quantified as latency of the tail flick response. Time was measured with a chronometer in seconds.[11]

Antiinflammatory activity

Croton oil-induced ear oedema

The procedure was conducted according to *Schiantarelli* et al. Ear edema was provoked by topical application of Croton oil. Eight non-isogenic male OF-1 mice (20-25 g) were divided into groups of treatment. Each mouse received 10 µL of Croton oil dissolved in 2.5 mL of acetone (75 µg/per ear). 10 µL of the irritant were applied to the anterior and posterior surfaces of the right ear. The left ear (control) received the same volume of solvent. 4 h after administration, animals were killed by cervical dislocation and discs 6 mm in diameter were removed from each ear and weighed.[11] Edema was expressed as the difference between the right and left ears. Consequently, the percentage of inflammation inhibition due to the

administration of extracts was expressed as a reduction of edema with respect to the control group, according to the expression:

% Inhibition = (ΔPc - ΔPt) / 100 x ΔPc

where: ΔPc — mean weight variation in the control group; ΔPt — mean weight variation in the treated group.

Topical activity

Immediately after administration of irritant and solvent, different doses (6, 3, 1.5 mg/ear) of aqueous extract of *Cordia martinicensis* were applied in the right ear. Dexamethasone (0.5 mg/ear)[12] was used as reference drug.

Oral activity

One hour before the procedure, animals were administered decoction of *Cordia martinicensis* (10, 5, 1 g/kg p.o.). Indomethacin (10 mg/kg p.o.) was used as reference drug. The negative control group received distilled water.

Antipyretic activity

Non-febrile male Sprague Dawley rats weighing between 200-300 g were used in the study. Antipyretic activity of *Cordia martinicensis* was measured by slightly modifying the method described by *Winder* et al. Rats were fasted overnight with water before the experiments. Rectal temperature of each rat was measured with a digital thermometer before inducing pyrexia by subcutaneously injecting 15 % (1 mL/100 g s.c.) brewer's yeast suspension into the animal's dorsum region. Eighteen hours after the injection rats that showed an increase in temperature of at least 0.8 ^{o}C were used in the experiments.[13] Test agent, cosolvent and positive control (acetaminophen, 150 mg/kg)[14] were administered orally and the temperature was measured 1, 2, 3, and 4 h after drug administration.

Statistical analysis

Data were expressed as mean ± SD. Statistical significance of the results was determined using one-way analysis of variance. Differences between two mean values were compared using unpaired Student's *t*-test. Data were considered different at the level of $p < 0.05$.

RESULTS

Phytochemistry

Phytochemical analysis of 30 % decoction of *Cordia martinicensis* found a positive reaction to alkaloids, flavonoids, tannins, and anthocyanidines (table 1).

Table 1. Phytochemical screening of decoction of dry leaves of *Cordia martinicensis*

Compounds	*Cordia martinicensis* (dry leaves)
Dragendorff's test (alkaloids)	+
Shinoda's test (flavonoids)	+
Phenolic and Tannin compounds	+
Lactonic compounds and coumarins	-
Quinones	-
Liebermann-Bouchard's test (triterpenes and steroids)	-
Fehling´s test Reducing sugars	-
Ninhidrina´s test Amino acids	-
Anthocyanidins and leucoanthocyanidins	+
Saponines	-
Starch	-
Total solids (%)	2,39

-: negative reaction, +: positive reaction.

Toxicity

Administration of *Cordia martinicensis* did not show any signs or symptoms of toxicity. Twenty-four hours later a softening of grounds was observed, but it disappeared the next day. Body weight behaved according to the growth curve for this species and no abnormalities appeared in the organs studied.

Analgesic activity

Writhing test

The acetic acid induced writhing test was used for detecting peripheral analgesia. Administration of *Cordia martinicensis* significantly reduced the number of writhes after acetic acid (0.75 %) injection, in all doses. The highest dose produced the most significant ($p < 0.05$) effect, decreasing the number of abdominal stretching to 14.31 ± 4.49, representing an inhibition of 44.4 %.

Tail flick test

Cordia martinicensis administration at all doses did not produce a significant antinociceptive response to thermal stimuli compared to the control group. Data from both experiments are shown in table 2.

Table 2. Analgesic effect of *Cordia martinicensis* on acetic acid induced abdominal stretching and the tail flick test in mice

	Abdominal stretching (15 min)	Time response in the tail flick test (60 min)
Control (water)	25,73 ± 8,11	1,69 ± 0,5
Extract (g/kg)		
0,5	17,12 ± 7,15*	1,77 ± 0,4
1	18,94 ± 7,88*	2,07 ± 0,3
5	14,31 ± 4,49*	1,92 ± 0,4
Indomethacin (10 mg/kg)	5,25 ± 4,64*	1,82 ± 0,56

Data are expressed as mean ± SD; * statistical significance ($p < 0,05$).

Antiinflammatory activity

Croton oil-induced ear oedema

Antiinflammatory activities of *Cordia martinicensis* extracts are shown in table 3. They were measured in mice's ears using Croton oil as oedema inducer. Results show a significant inhibition ($p < 0.05$) of oedema compared to the control when the extract was administered orally at 10 and 5 g/kg, with a percentage of inhibition of 60.9 and 56.9 %, respectively. However, topical administration of *Cordia martinicensis* extract only had a significant anti-inflammatory effect at 6 mg/ear with 62.9 % of inhibition.

Antipyretic activity

Hyperthermia induced by brewer's yeast developed during the eighteen hours following injection of the suspension. The dose of *Cordia martinicensis* extracts (5 mg/kg) showed significant antipyretic activity ($p < 0.05$) from hour 1 to hour 4, similar to the control with acetaminophen (table 4).

Table 3. Topical and oral antiinflammatory activity of *Cordia martinicensis* on croton oil induced ear edema in mice

	Weight edema (mg ± SD)	Inhibition (%)
Topical administration		
Irritant (*Croton* oil)	15,1 ± 3,8	
Extract (mg/ear)		
6	5,6 ± 1,5 *	62,9
3	7,8 ± 1,3	48,3
1,5	8,8 ± 7,3	41,7
Dexamethasone (0,5 mg/ear)	2,2 ± 0,3 *	79,62
Oral administration		
Extract (g/kg)		
10	5,9 ± 2,9 *	60,9
5	6,5 ± 3,5 *	56,9
1	14,0 ± 4,1	7,2
Indomethacin (10 mg/kg)	3,4 ± 2,8 *	77,4

Data are expressed as mean ± SD; * statistical significance (p< 0,05).

Table 4. Antipyretic activity of *Cordia martinicensis* on brewer's yeast-induced pyrexia in rats

Treatment	Average rectal temperature (ºC)					
	-18 h	0 h	1 h	2 h	3 h	4h
Extract 5 g/kg	37,1±0,38	38,3±0,26	37,6±0,32*	37,4±0,2*	37,6±0,2*	37,5±0,1*
Acetaminophen 150 mg/kg	36,8±0,33	38,0±0,5	37,0±0,46*	37,3±0,32*	37,4±0,43*	36,8±0,5*

Data are expressed as mean ± SD; * statistical significance (p< 0,05).

DISCUSSION

The aim of the present research was to validate the ethnobotanical uses of *C. martinicensis* as an analgesic, antiinflammatory, and antipyretic agent.

Cordia martinicensis extracts do not show acute oral toxicity even at high doses, so they are potentially safe for consumption. Effects on the softening of grounds may be related to activity on the digestive system attributed to other species like *Cordia latifolia* Roxb., *Cordia globosa (*Jacq.) Kunth, and *Cordia dichotoma* G. Forst.,[1] which could be present in *C. martinicensis*.

The writhing and tail flick tests are widely used for analgesic screening to study a possible pain mechanism. The writhing test is used to determine the peripheral

activity of analgesic drugs, especially non-steroidal drugs. Acetic acid administration produced pain by releasing endogenous substances such as serotonin, histamine, prostaglandins, bradykinins and substance P, which stimulate nerve endings and local peritoneal receptors.[14] However, the tail flick test acts via the central nervous system along a pathway at spinal level.[15] 30 % decoctions of *Cordia martinicensis* inhibited abdominal constriction response statistically at all doses used in the study, but not the thermic painful stimuli. These results suggest that the antinociceptive action of the active compounds in decoction of *Cordia martinicensis* may be mediated through peripheral but not central mechanisms, in a way similar to AINES drugs.

Decoction of *Cordia martinicensis* also displayed strong anti-inflammatory activity. The croton oil-induced ear oedema test triggers many enzymatic cascades and stimulates vascular permeability, vasodilation and polymorphonuclear leukocyte migration. This is one of the most commonly used models of acute inflammation.[16-18] We tested two types of administration: topical and oral. In both *Cordia martinicensis* decoction was able to inhibit inflammation processes at higher doses, 6 mg/ear (62.9 %) and 10 mg/kg (60.9 %).

Fever induced by yeast in rats may enhance the formation of cytokines which increase the synthesis of prostaglandin E_2, and thus the hypothalamus will raise body temperature.[19] *Cordia martinicensis* decoction lowered the fever induced by yeast, and was able to keep the temperature low during the 4 h of the experiment, in the same way as acetaminophen. Therefore, it is possible that active metabolites for antipyretic action may inhibit some mediators involved in the generation of fever.

Phytochemical screening revealed the existence of tannins and flavonoids in the extract. These compounds are known to possess antiinflammatory, analgesic and antipyretic effects due to their inhibitory effect on enzymes involved in the production of mediators of inflammation activity.[20] Finally, the results of the study confirm the potent antiinflammatory and antipyretic activity of *Cordia martinicensis*, and show an analgesic activity similar to AINES. *C. martinicensis* has been studied before as analgesic and antiinflammatory by *Ficarra R*. et al., without positive results[21]. This is the first report on the activity of *C. martinicensis* directly related to its popular use and provides pharmacological validation of its use to relieve fever, inflammation and pain. There is a need for further studies to isolate the active ingredients in the plant which are responsible for its biological activity, and to elucidate the mechanism of action of these active ingredients.

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