



Anti-inflammatory effect of the ethanolic extract from *Bowdichia virgilioides* H.B.K stem bark

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ABSTRACT

Bowdichia virgilioides H.B.K stem bark (Fabaceae), locally known as “sucupira-preta”, is a reputed folk-remedy to treat some inflammatory disorders. To validate its traditional claim, the ethanolic extract from *B. virgilioides* was evaluated in several animal models of inflammation and nociception. The extract at oral doses of 100 to 1000 mg/kg body weight caused a significant inhibition of carrageenan-induced hind paw oedema, suppression of exudate volume and leukocyte immigration in rat pleurisy induced by carrageenan, and reduction of granuloma weights in the model of subcutaneous granulomas promoted by cotton pellets. In addition, the plant extract significantly inhibited the vascular permeability increase induced by intraperitoneal acetic acid. It also showed marked antinociceptive effect in acetic acid-induced writhing test and in the second phase of formalin test in mice. These findings evidence the anti-inflammatory potential of *Bowdichia virgilioides* bark and supports its traditional use in inflammatory conditions.

Key words: *Bowdichia virgilioides*, Fabaceae, stem bark extract, anti-inflammatory activity.

INTRODUCTION

Bowdichia virgilioides H.B.K (Fabaceae), popularly known as “sucupira-preta”, is a large tree that widely grows in the hilly and forest regions of north, northeastern and central parts of Brazil. Its bark and seeds are extensively employed in folk medicine in the form of infusion for the treatment of diarrhoea, gout, diabetes, bronchitis, hypertermia, inflammatory conditions of uterus, rheumatism, as body tonic and digestion facilitator (Sanguinetti 1989, Berg 1993, Pott and Pott 1994). Brazil, with the greatest biodiversity, has been and will be a potential source for important pharmacological discoveries to serve mankind and native population by providing some information related to these discoveries. Therefore, scientific investigations on *B. virgilioides* are part

of an important strategy linked to the conservation of biodiversity, discovery of new medicines, and the bettering of the life quality of poor rural communities.

Previous studies described the hypoglycemic activity of bark extract in normal as well as diabetic rats (Leôncio et al. 1994), and antimalarial activity *in vitro* (IC₅₀ = 1 μg/mL) and *in vivo* (51% at 100 mg/kg). Phytochemical studies carried out on the bark and root materials have revealed the presence of tannins and lupeol (Calle et al. 1983), alkaloids and terpenoids (Torrenegra et al. 1985, Marinho et al. 1994), volatile constituents and flavonoids (Arriaga et al. 1998, Veloso et al. 1999) and dihydrobenzofuran (Melo et al. 2001). There were also reports of a novel alkaloid named bowdichine and two new isoflavonoids from the stem bark of this plant (Barbosa-Filho et al. 2004, Juck et al. 2006). To the best of our knowledge, no scientific reports on the anti-

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inflammatory or analgesic effects of this plant were so far available. Being part of a programme to find new compounds with anti-inflammatory and antinociceptive activities, the stem bark of *B. virgilioides* was extracted with 70% of ethanol and evaluated in animal models of inflammation.

MATERIALS AND METHODS

ANIMALS

Male albino rats (170-250g) and male Swiss mice (25-30g) were used. The animals were kept in propylene cages at $22 \pm 2^\circ\text{C}$ in a 12 h light-dark cycle, with free access to standard pellet chow and water. Groups of eight to ten animals were used for experimentation. The animals were starved for 18 h before use. The experimental protocols were approved by the Institutional Animal Care and Use Committee in accordance with the international principles.

PLANT MATERIAL AND EXTRACTION

Bowdichia virgilioides bark was collected at the municipality of Chapada dos Guimarães, state of Mato Grosso, Brazil, after its identification and authentication by Prof. Germano Guarin Neto of the Department of Botany and Ecology, Federal University of Mato Grosso, Brazil. A voucher specimen (# 22591) was deposited at the Herbarium of this university. The collection was authorized by the Brazilian Institute of Environment and Renewable Natural Resources. The finely powdered stem bark (1.8 kg) was macerated in 70% of ethanol for a period of 7 days. After this period, the macerate was filtered through a filter paper (no 170 g), the filtrate was evaporated using a rotary evaporator ($45 \pm 1^\circ\text{C}$) under reduced pressure (625 mmHg) to get an yield of 16.91%. For the experiments, this ethanol extract of *Bowdichia virgilioides* (EEBv) was further diluted in distilled water to reach the necessary concentrations. Controls received the same volume of distilled water as the vehicle.

PHYTOCHEMICAL SCREENING

In order to verify the presence of different chemical classes, EEBv was subjected to standard screening test (Matos 1988). Conventional protocol for detecting the presence of alkaloids, flavonoids, saponins, tannins,

xanthenes, steroids, anthraquinones, triterpenes, etc., was used.

CARRAGEENAN-INDUCED PAW OEDEMA

The method of Winter et al. (1962) was followed. Paw oedema was induced by subplantar injection of 0.1 mL of lambda carrageenan (1% w/v in 0.9% of saline) into the plantar aponeurosis of the left hind paw in male rats 200-220g). An equal volume of vehicle was injected into the contralateral paw. The volume of both hind-paws up to the ankle joint was measured with a plethysmometer (model 7150, Ugo Basile) immediately before (0) and 30, 60, 120, 180, and 240 min after carrageenan. The difference in the volumes between the hind-paws was a measure of the oedema (mL). The plant extract (100, 300 and 1000 mg/kg), and the reference drug, indomethacin (5 mg/kg, diluted in 2% sodium bicarbonate), or the vehicle (10 mL/kg of distilled water), were given orally 60 min before the subplantar injection of phlogestogen.

VASCULAR PERMEABILITY TEST

The method of Whittle (1964) was used to evaluate the effect of EEBv on vascular permeability in adult albino male mice. Briefly, one hour after oral administration of vehicle, EEBv (100, 300 and 1000 mg/kg), and dexamethasone (0.5 mg/kg) or indomethacin (5 mg/kg), 0.1 mL/10g b.w. of Evans blue (2% w/v, in normal saline) was intravenously injected through the penial venous plexus. After 10 min, 0.4 mL of 0.5% (v/v) of acetic acid solutions was injected in the animals by intraperitoneal route. Twenty minutes later, the mice were killed by cervical dislocation and the abdominal cavity was washed with 0.9% of saline (6-8 mL). The washings were pooled, and the absorbance of the solution was measured spectrophotometrically at 590 nm. The amount of dye leakage in the supernatant was calculated from the absorbance measurements.

CARRAGEENAN-INDUCED PLEURISY

Pleurisy was induced in rats (200-220g) by intrapleural injection of 0.1 mL/rat of a 2% w/v of lambda carrageenan suspension in normal saline as described by Vinegar et al. (1978). The plant extract (100, 300 and 1000 mg/kg), dexamethasone (0.5 mg/kg) or vehicle (10 mL/kg) were given orally 60 min before the injection.

tion of phlogestogen. Six hours later, the rats were killed by excess ether, the thoracic cavity was opened, and the exudate was collected by aspiration. Exudate volume was measured (mL) and the mobilized leukocytes number in the exudate was quantified using an improved Neubauer haemocytometer.

COTTON PELLET-INDUCED GRANULOMA

Cotton pellets weighing 50 mg each were sterilized (autoclaved at 120°C for one hour) and implanted subcutaneously in rats through a skin incision on their backs, one on each side of the subscapular region (Winter and Porter 1957). Following the implantation, animals were treated orally with the vehicle (10 mL/kg), plant extract (100, 300, and 1000 mg/kg) or dexamethasone (0.5 mg/kg) once a day on six consecutive days. On day 7, the animals were sacrificed with excess ether, the granulomas were dissected and their wet and dry weights (at 60°C for 24 h) were established.

CHEMICAL NOCICEPTION INDUCED BY ACETIC ACID AND FORMALIN

In the acetic acid test, male mice were treated orally with the vehicle, *EEBv* (100, 300 and 1000 mg/kg) or indomethacin (5 mg/kg) one hour before the intraperitoneal injection of 0.1 mL/10g of 0.6% v/v of acetic acid (Koster et al. 1959). The number of writhings was counted for a 30 min period. In formalin test, groups of male mice were treated with the vehicle, *EEBv* (100, 300 and 1000 mg/kg), indomethacin (10 mg/kg) or meperidine (Dolantin[®], 25 mg/kg s.c) and, one hour later (meperidine group), nociception was induced by subplantar injection of 25 μ L of 2.5% of formalin in normal saline (Hunskar and Hole 1987) in each animal. The time spent by the animals on licking the injected hind paw was considered as an index of pain and was measured during the first 5 min (0-5 min, the first phase), and again between 20 and 30 min (the second phase).

THERMAL NOCICEPTION IN HOT-PLATE TEST

The hot-plate test was used as the thermal pain model (Eddy and Leimbach 1953). Vehicle, *EEBv* (100, 300 and 1000 mg/kg p.o.) or meperidine (25 mg/kg s.c.) were administered, respectively, 60 and 15 min before submitting the mice to hot plate (56 \pm 1°C). The antinociceptive

response was evaluated after 15, 30, 60, 120 and 180 min by measuring the latency period preceding the animal reaction of licking its hind paw or jump. The cut-off time was set at 45 s to prevent tissue injury.

STATISTICAL EVALUATION

All parametric values are given as the means \pm s.e.m. and were analysed by one-way ANOVA followed by Student-Newman-Keul's test. A *P* value less than 0.05 was considered statistically significant.

RESULTS

CARRAGEENAN-INDUCED PAW OEDEMA

The time-course of paw oedema in rats treated with the vehicle or test drugs is shown in Table I. In vehicle-treated control animals, intraplantar injections of carrageenan provoked an oedema response that was more pronounced at the time periods of 180 and 240 min. The plant extract inhibited the rat paw oedema in a dose-related manner. Paw oedema response was found to be significantly less in rats that received 300 and 1000 mg/kg of plant extract. Indomethacin pretreated group of animals demonstrated greater inhibitions on the paw oedema development. *EEBv* (300 and 1000 mg/kg) inhibited the carragenin-induced inflammation with the highest activity at 120 and 240 min, with 19% ($p < 0.01$) and 20% ($p < 0.01$) inhibition, and 36% ($p < 0.001$) and 36% ($p < 0.001$), respectively. Indomethacin (5 mg/kg) showed 56% ($p < 0.001$) and 52% ($p < 0.01$) inhibition at the same time point.

VASCULAR PERMEABILITY

It is well established that the intraperitoneal injection of acetic acid greatly enhances the vascular permeability and permits vascular leakage of Evans blue dye. The amount of extravasated Evans blue dye following the acetic acid injection (0.4 mL, i.p.) in control mice was found to be 107 \pm 8 μ g. Pretreatment with *EEBv* caused significant inhibition ($p < 0.01$) of vascular permeability and, consequently, of the dye leakage by 32%, 27%, and 39% at the respective doses of 100, 300 and 1000 mg/kg, when compared to the untreated control. Indomethacin (5 mg/kg) and dexamethasone (0.5 mg/kg) treated positive controls demonstrated much greater inhibitions of dye leakage by 49% and 58%, respectively.

TABLE I
Effect of ethanolic extract of *Bowdichia virgilioides* bark (EEBv) on carrageenan-induced hind paw oedema in rats.

Treatment	Dosage (mg/kg)	Paw oedema (mL)				
		(mean \pm S.E.M.)				
		Time (h)				
		0.5	1	2	3	4
Vehicle	—	0.21 \pm 0.05	0.36 \pm 0.04	0.52 \pm 0.05	0.86 \pm 0.03	1.00 \pm 0.02
<i>B. virgilioides</i>	100	0.16 \pm 0.03	0.26 \pm 0.03	0.45 \pm 0.03	0.78 \pm 0.02	0.92 \pm 0.02
	300	0.22 \pm 0.03	0.30 \pm 0.05	0.36 \pm 0.06	0.70 \pm 0.05**	0.80 \pm 0.03**
	1000	0.24 \pm 0.03	0.33 \pm 0.04	0.37 \pm 0.04**	0.55 \pm 0.03***	0.64 \pm 0.02***
Indomethacin	5	0.14 \pm 0.03	0.24 \pm 0.04	0.27 \pm 0.04**	0.38 \pm 0.04***	0.48 \pm 0.04**

Values are expressed as the mean \pm S.E.M. of 8 animals. The drugs were administered orally one hour before the subplantar injection of 0.1 mL of lambda carrageenan (1% w/v in 0.9% of saline). Asterisks indicate significant difference from the corresponding control. ** $p < 0.01$; *** $p < 0.001$ (One-way ANOVA followed by Student Newman-Keul's test).

CARRAGEENAN-INDUCED RAT PLEURISY

As shown in Figure 1, the plant extract produced significant ($p < 0.01$) inhibition on the carrageenan-induced pleuritic exudate volume (39%) only at a dose of 1000 mg/kg. However, the leukocyte migration was significantly blocked at all the used doses, reaching the maximum level (55%, $p < 0.001$) at 1000 mg/kg. Dexamethasone (0.5 mg/kg), the reference drug used in this study, produced 70% and 61% of inhibitions, respectively, of pleuritic exudate and leukocyte numbers.

COTTON PELLETT-INDUCED GRANULOMA

The plant extract as well as the reference drug, dexamethasone, showed significant inhibitions on the wet and dry weights of granulomas (Fig. 2). Compared to dexamethasone, the plant extract demonstrated less pronounced inhibitions on the development of granulomas. The wet and dry weights of granulomas were reduced by 14 ($p < 0.05$) and 24%, ($p < 0.01$) and 14 ($p < 0.05$) and 19% ($p < 0.01$), respectively, at doses of 300 and 1000 mg/kg of EEBv as against 50% and 50% ($p < 0.001$) of inhibition observed in dexamethasone (0.5 mg/kg) pretreated group. At similar doses, the dry weights of granulomas were reduced by 17 and 24% in plant extract treated groups as against 56% of dexamethasone treatment.

CHEMICAL NOCICEPTION INDUCED BY ACETIC ACID AND FORMALIN

The effect of EEBv on nociception induced by acetic acid and formalin are shown on Table II and III, respec-

tively. The mean number of observed writhes following intraperitoneal injection of 0.6% of acetic acid in vehicle-treated control mice were 46 ± 2 , whereas animal groups that received the plant extract at doses of 100, 300 and 1000 mg/kg or the indomethacin (5 mg/kg) pretreatments had shown a significantly decreased number of writhes (28, 48, 70, and 87%, respectively). In the formalin test, vehicle-treated animals showed the mean licking times (s) of 59 ± 5 in the first phase, and 161 ± 11 in the second phase. Pretreatment with the extract showed no significant effect on the first phase pain response, but significantly inhibited the second phase (inflammatory pain) response by 24, 45, and 50% at the doses of 100, 300 and 1000 mg/kg, respectively. Indomethacin (10 mg/kg), a known nonsteroidal anti-inflammatory agent (NSAID), also produced an inhibition of 69% only on the second phase. However, meperidine at a dose of 25 mg/kg, s.c. produced inhibitions at both phases (97 and 98%, respectively).

THERMAL NOCICEPTION (HOT PLATE TEST)

In the hot-plate test, the plant extract (100, 300, 1000 mg/kg) showed no significant analgesia in the tested doses where the synthetic opioid meperidine (25 mg/kg, s.c.) demonstrated significant antinociception at 60, 90, 120 and 180 min (data not shown).

DISCUSSION

Results of this study clearly point out to the anti-inflammatory activity of the bark extract from *Bowdichia virgilioides* in acute inflammatory conditions. It could

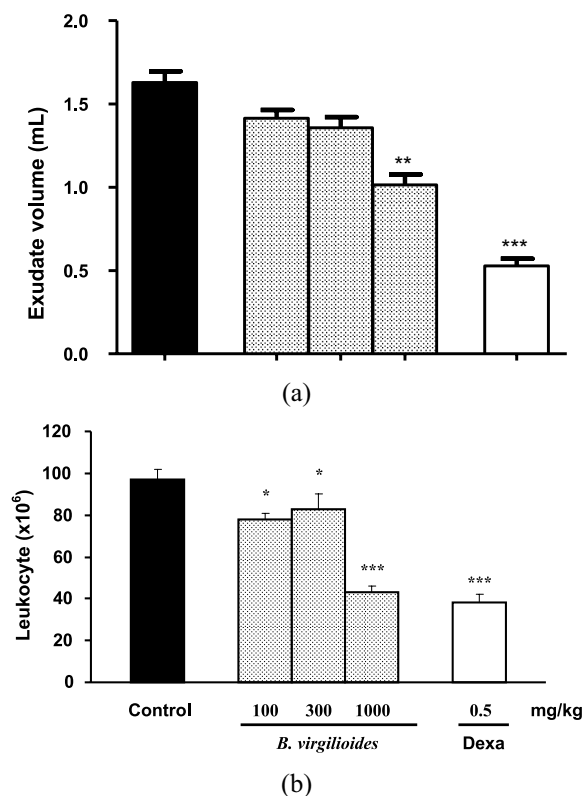


Fig. 1 – Effect of ethanolic extract of *B. virgilioides* on exudate volume (a) and leukocyte (b) accumulation in carrageenan-induced pleurisy in rats. Vehicle, *Bowdichia virgilioides*, 100, 300, 1000 mg/kg and dexamethasone 0.5 mg/kg. Each column represents the mean \pm S.E.M. of 9-10 animals. * $p < 0.005$; ** $p < 0.01$, *** $p < 0.001$ (One-way ANOVA followed by Student-Newman-Keul's test).

effectively inhibit the acute carrageenan-induced rat hind paw oedema and also the inflammatory exudate and leukocyte migration promoted by carrageenan in rat pleurisy test. In these tests, the effect of plant extract is comparatively less pronounced than that of indomethacin and dexamethasone, the respective reference drugs used in these models that are well known for their inhibitory effects on prostaglandin biosynthesis and leukocyte migration (Ackerman et al. 1980, Miyasaka and Mikami 1982, Wallner et al. 1986). Inflammatory oedema induced by intraplantar injection of carrageenan is believed to result from the action of locally released proinflammatory agents like histamine and serotonin in the initial phase (1 h), and prostaglandins, the cyclooxygenase products, at a later phase (over 2 h). The link between these two phases seems to be provided by

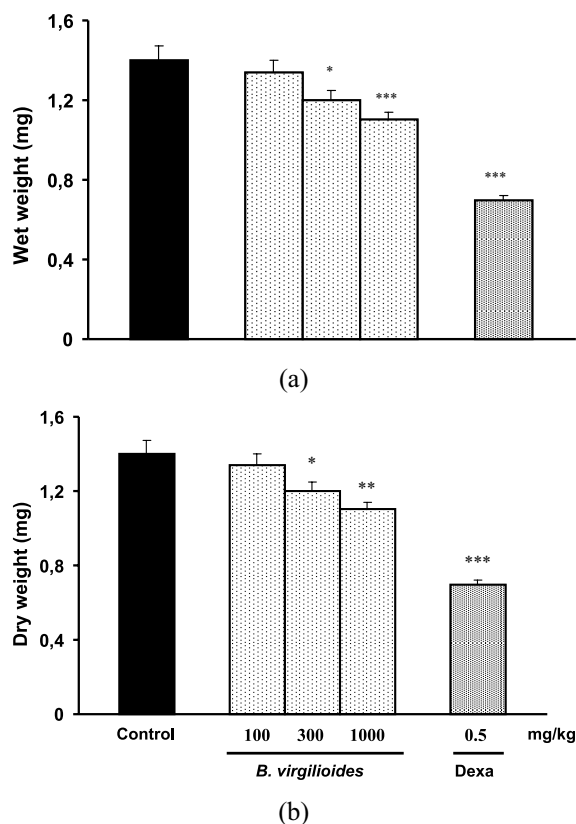


Fig. 2 – Effects of ethanolic extract of *Bowdichia virgilioides* on cotton pellets – induced granuloma (a) wet weight and (b) dry weight in rats. Vehicle, *B. virgilioides*, 100, 300, 1000 mg/kg and dexamethasone 0.5 mg/kg. Each column represents the mean \pm S.E.M. of 11 animals. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. (One-way ANOVA followed by Student-Newman-Keul's test).

tachykinins that include bradykinin (Winter et al. 1962, Vinegar et al. 1987). More recent studies demonstrated a role for the renin-angiotensin system (RAS) and proinflammatory cytokines in the development of carrageenan-induced paw oedema (Raghavendra and Kulkarni 2000, Lai et al. 2009). All doses of EEBv used in this study showed significant reduction of paw oedema at 2 h or more after carrageenan injection, suggesting that it produces an anti-oedematous effect during the later phase, similarly to indomethacin, a known cyclooxygenase inhibitor. It implies that EEBv exerts its anti-inflammatory action by either the inhibition of the synthesis, release or the action of mainly the prostaglandins.

Cotton pellet granuloma is a model of non-immunological type of inflammation mediated by the activation of mostly kinins, in particular B₁-receptor (Farmer and

TABLE II
Effect of orally administered ethanolic extract of *Bowdichia virgilioides* on nociception induced by acetic acid in mice.

Treatment	Dosage (mg/kg)	Number of writhings	Inhibition (%)
Vehicle	—	46 ± 2	—
<i>B. virgilioides</i>	100	33 ± 4***	28
	300	24 ± 2***	48
	1000	14 ± 1***	70
	Indomethacin	5	6 ± 1***

Values are expressed as the mean ± S.E.M. of 8 animals. The drugs were administered orally one hour before the intraperitoneal administration of 0.1 mL/10g of 0.6% of acetic acid (writhing test). Asterisks indicate significant difference from the corresponding control. ***p<0.001 (One-way ANOVA followed by Student Newman-Keul's test).

TABLE III
Effect of orally administered ethanolic extract of *Bowdichia virgilioides* on nociception induced by formalin in mice.

Treatment	Dosage (mg/kg)	Licking (s)	
		Early phase (0-5 min)	Late phase (20-25 min)
Vehicle	—	59 ± 5	161 ± 11
<i>B. virgilioides</i>	100	46 ± 3	89 ± 6***
	300	47 ± 5	82 ± 5***
	1000	49 ± 5	79 ± 11***
	Indomethacin	5	48 ± 6
Meperidine	25	3 ± 1***	0.4 ± 0.3***

Values are expressed as the mean ± S.E.M. of 10 animals. ***p<0.001 (One-way ANOVA followed by one-way Student Newman-Keul's test).

Burch 1992). In this model, the *EEBv* demonstrated antioedema as well as antiproliferative effects as evidenced by significant inhibitions of wet and dry weights of granuloma. Thus, it is found to be effective against acute as well as chronic inflammatory conditions, which reflected its efficacy in inhibiting the increase in the number of fibroblasts and synthesising collagen and mucopolysaccharides during the granuloma formation (Recio et al. 1995).

Besides its anti-inflammatory activity, the plant extract demonstrated a significant inhibition on the vascular permeability increase induced by intraperitoneal acetic acid, and an antinociceptive effect in experimental models of acetic acid-induced writhing test and in the second phase of formalin test in mice. Hot-plate test

and the first phase nociception in formalin test detect antinociceptive compounds of opioid type that act at the supraspinal sites (Besson and Chaouch 1987). Intraperitoneal injection of acetic acid causes inflammatory pain by inducing capillary permeability (Amico-Roxas et al. 1984) and liberating endogenous substances (histamine, serotonin, prostaglandins and cytokines) that excite pain nerve endings (Raj 1996). The second phase nociceptive response in formalin test seems to depend on the local inflammatory reaction, activation of *N*-methyl-D-aspartate (NMDA) and non-NMDA receptors, and the nitric oxide cascade (Davidson and Carlton 1998) and functional changes in the dorsal horn of the spinal cord (Abbott et al. 1995). Thus, the observed antinociceptive effect of *EEBv* seems to be a peripheral one since it was found to be inactive in suppressing thermal nociception in the hot-plate test.

Preliminary phytochemical studies carried out on *EEBv* demonstrated the presence of triterpenoids, tannins, flavonoids, alkaloids and athraquinones. These different classes of metabolites possess several pharmacological effects that includes an anti-inflammatory activity (Allcarz and Jiménez 1988, Safayhi and Sailer 1997, Buniatian et al. 1998, Kumar et al. 1998 and Ivanovska et al. 1999). Although the exact nature of phytochemical constituent (s) responsible for anti-inflammatory action remains unclear, the data seem to correlate well with the traditional indication of *Bowdichia virgilioides* in inflammatory conditions. However, further investigations are necessary to explore the mechanism(s) involved in its anti-inflammatory action.

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RESUMO

A casca do caule de *Bowdichia virgilioides* H.B.K. (Fabaceae), conhecida localmente como sucupira-preta, é um remédio popular muito utilizado para tratar inflamações. Com o objetivo de validar sua crença popular, o extrato etanólico de *B. virgilioides* foi avaliado em vários modelos experimentais de inflamação e

nocicepção. O extrato administrado, via oral, em doses de 100 a 1000 mg/kg de peso corporal produziu inibição significativa no edema de pata induzido por carragenina, no aumento na permeabilidade vascular induzido por ácido acético, no volume de exudato e na migração leucocitária no teste de pleurisia induzida por carragenina, bem como no peso de granulomas induzidos por pelotas de algodão, em ratos. Em camundongos, o EEBv reduziu o número de contorções abdominais induzidas por ácido acético e a lambadura da pata na segunda fase do teste da formalina. Esses resultados validam o potencial anti-inflamatório da casca de *Bowdichia virgilioides* e referendam seu uso tradicional em condições inflamatórias.

Palavras-chave: *Bowdichia virgilioides*, Fabaceae, extrato da casca, atividade anti-inflamatória.

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