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Journal of Ethnopharmacology 88 (2003) 1–4
Journal of ETHNO-PHARMACOLOGY
www.elsevier.com/locate/jethpharm

Central antinociceptive effect of a hydroalcoholic extract of *Dioclea grandiflora* seeds in rodents

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Received 28 November 2002; received in revised form 1 March 2003; accepted 1 March 2003

Abstract

The acute treatment of rats and mice with a hydroalcoholic extract from the seeds of *Dioclea grandiflora* (EHDg) at doses of 250 and 500 mg/kg, by intraperitoneal or oral administration, produced a significant antinociceptive effect in the tail flick and hot plate tests, an effect which was inhibited by naloxone. EHDg given to mice daily for 30 days at a dose of 500 mg/kg, did not cause any observable toxic effect nor any alteration in the pattern of antinociceptive response by the tail immersion test during the course of this treatment. These results suggest that EHDg has a central antinociceptive action devoid of tolerance effect typical of opioid drugs.

Keywords: *Dioclea grandiflora*; Hydroalcholic extract; Morphine; Naloxone; Analgesia; Tolerance

1. Introduction

Medicinal plants have been used extensively by the Brazilian people to treat bodily ailments (Almeida, 1993; Almeida et al., 1999). A case in point is *Dioclea grandiflora* Mart. ex. Benth (Leguminosae), a plant popularly known as "mucunã," "mucunã-de-caroço" and "olho-de-boi" or bull's eye, which thrives in the Caatinga and Serrado regions of Northeastern Brazil. The seeds and root bark of *Dioclea grandiflora* are widely used in folk medicine for diseases of the kidneys and prostate (Batista, 1993). Initial chemical and pharmacological studies were carried on this plant by Batista in 1993. In a subsequent investigation, Bhattacharyya et al. (1995), isolated and identified dioclein, a flavonone obtained from the roots of this plant which, was shown to have analgesic effect in rodents (Batista et al., 1995). Mattei et al. (1995) examined the central pharmacological action of the seeds of *Dioclea grandiflora* and detected a possible anxiolytic activity. Bhattacharyya et al. (1997) isolated and identified dioclenol in 1997 and in the following year dioflorin from the root bark of this plant (Bhattacharyya et al., 1998). Subsequently, Jenkins et al. (1999) isolated and identified agrandol, paraibanol and diosalol from the root bark of the same plant. In that same year Lemos et al. (1999) reported a potent vasorelaxant endothelium dependent effect in rat aorta for dioclein. Almeida et al. (2000) later demonstrated the pharmacological activity for dioclenol and dioflorin in two experimental models of analgesia. The aim of the present work was to examine the possible central analgesic activity of EHDg using the tail flick and hot plate tests, and to determine the persistence of the antinoceptive effect during long-term treatment for 30 days using a dose of 500 mg/kg (i.p.) of EHDg.

2. Materials and methods

2.1. Plant material

Seeds of *Dioclea grandiflora* were collected in the region around Santa Rita, Paraiba, Brazil, during January 1997. The plant was identified by Prof. Maria de Fátima Agra of the Federal University of Paraiba, and a voucher specimen was deposited at the Lauro Pires Xavier Herbarium (JPB) (4440-JPB, MO).

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doi:10.1016/S0378-8741(03)00180-6

2.2. Preparation of EHDg

Seeds of *Dioclea grandiflora* were dried in an oven at 40 °C, and subsequently pulverized. The plant material was extracted with 70% ethanol/water (v/v, %) for 72 h in a Soxhlet apparatus and the extract was concentrated using a rotary evaporator. A dry solid (48.3 g) was obtained corresponding to a yield of 10%.

2.3. Animals

The rodents used in experiments were Swiss albino mice of both sexes (20–30 g) and Wistar rats (150–250 g), obtained from the animal facility in the Laboratory of Pharmaceutical Technology of Federal University of Paraiba. The animals were kept at a controlled temperature of 23 ± 1 °C, with a light/dark period of 12 h, with the light period beginning at 06:00 h, and they were given food and water ad libitum. All experiments were conducted between 10:00 and 16:00 h.

2.4. Drugs

Morphine and cremophor were obtained from Sigma (USA) and naloxone from Research Biochemicals Inc. (USA). Morphine and naloxone were administered intraperitoneally in a volume of 0.1 ml/10 g body weight for mice and 0.1 ml/100 g for rats. Cremophor (0.1%) was utilized as an emulsifier in the preparation of suspensions of EHDg.

2.5. Statistical analysis

The data obtained in the various experiments were evaluated by a one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test or Student's *t*-test depending on the case. The results obtained were considered significant when $P < 0.05$.

2.6. Tail flick test

In this experiment, four groups of 10 rats each were utilized following the method described by Mayer and Liebeskind (1974). EHDg was administered at doses of 250 and 500 mg/kg (i.p.). Saline was used as vehicle at 0.1 ml/100 g (i.p.), and morphine at 6 mg/kg (i.p.). The measurements using the tail flick test apparatus DS 20 SOCREL (Ugo Basile) were conducted at 60, 120 and 180 min after administration of drugs and vehicle.

2.7. Hot plate test

Six groups of 10 mice each were used for these experiments according to the technique of Yeh and Mitchell (1971). The test groups received doses of 250 and 500 mg/kg (i.p. or p.o.) of EHDg 60 min before measurements. In the control group, saline was administered i.p., and morphine (6 mg/kg, i.p.) was given to the standard group. Each assessment was carried out for a period of 15 min and the tests were performed at 60, 120 and 180 min after the respective treatments.

2.8. Antagonism of the antinociceptive effect of EHDg by pre-treatment with naloxone

In these experiments, four groups of 10 mice each were used. Initially, all the animals was administered with naloxone at a dose of 2 mg/kg (i.p.). After 15 min the test groups were given doses of 250 and 500 mg/kg (i.p.) of EHDg. The control group received saline (i.p.) and the standard group was administered (i.p.) with morphine (6 mg/kg, i.p.). The assessments were conducted at 60, 120, and 180 min in accordance with the method proposed by Younos et al. (1990).

2.9. Effect of EHDg administered for 30 days determined by the tail immersion test in mice

In these experiments three groups of 15 mice each were used to study the chronic administration of EHDg (500 mg/kg, i.p.) and of morphine (6 mg/kg, i.p.). The control group received saline (0.1 ml/10 g, i.p.). Analgesia was assessed by the tail immersion test according to Janssen et al. (1963) before the start of treatment (for baseline values) and on days 7, 14, 21 and 30 after administration of drugs and saline.

3. Results

3.1. Effect of EHDg in the tail flick test

Table 1 shows that the administration of EHDg, at doses of 250 and 500 mg/kg (i.p.) and of morphine (i.p.) prolonged significantly the rat tail's time of permanence when the animal's tail was subjected to heat generated by the tail flick apparatus. Meanwhile, the response to heat stimulus in the control group was not altered during the period of assessment.

Table 1
Effect of EHDg in mice assessed by the tail flick test

Treatment	Dose (mg/kg, i.p.)	Reaction time (s), mean ± S.D.		
		60 min	120 min	180 min
Saline	–	3.8 ± 0.3	4.3 ± 0.3	4.4 ± 0.5
EHDg	250	11.7 ± 2.1*	15.4 ± 3.2**	16.5 ± 3.2**
EHDg	500	11.3 ± 2.5*	16.8 ± 2.4**	17.8 ± 2.8**
Morphine	6	13.6 ± 1.6**	18.2 ± 1.2**	24.0 ± 1.0**

$n = 10$.
* $P < 0.05$ compared to control group values.
** $P < 0.01$ compared to control group values.

Table 2
Effect of EHDg and naloxone in mice assessed by the hot plate test

Treatment (mg/kg)	Reaction time (s), mean ± S.D.				
	Basal	30 min	60 min	90 min	120 min
Saline (–)	7.3 ± 0.9	4.2 ± 0.5	2.9 ± 0.5	3.9 ± 0.5	3.3 ± 0.4
EHDg (250) i.p.	8.2 ± 0.4	20.0 ± 1.6*	19.3 ± 2.7*	14.8 ± 2.0*	8.8 ± 1.7*
EHDg (250) p.o.	6.8 ± 1.0	11.6 ± 1.4'	8.0 ± 0.4'	8.0 ± 0.7'	8.3 ± 1.0'
EHDg (500) i.p.	7.8 ± 0.9	14.3 ± 1.2*	15.5 ± 3.0*	10.6 ± 0.7*	14.1 ± 1.6*
EHDg (500) p.o.	7.2 ± 0.8	8.4 ± 0.9*	12.8 ± 1.4*	16.0 ± 2.5*	13.2 ± 2.7*
Morphine (6) i.p.	7.5 ± 0.7	15.3 ± 1.1'	14.5 ± 1.3'	13.6 ± 0.8'	10.8 ± 0.3'
Naloxone (2) + saline (–)	7.2 ± 0.5	6.8 ± 0.5	5.8 ± 0.4	6.2 ± 1.0	5.5 ± 0.3
Naloxone (2) + EHDg (250)	6.5 ± 0.5	6.9 ± 0.6	6.6 ± 0.4	4.8 ± 0.3	7.3 ± 0.8
Naloxone (2) + EHDg (500)	7.1 ± 0.5	5.1 ± 0.7	6.0 ± 0.5	6.9 ± 0.6	5.9 ± 0.5
Naloxone (2) + morphine (6)	5.1 ± 0.3	5.7 ± 0.5	5.0 ± 0.5	6.0 ± 0.5	5.0 ± 0.5

$n = 10$.
* $P < 0.01$, compared to saline group values.

Table 3
Effect of EHDg administration for a period of 30 days in mice assessed by tail immersion test

Treatment (mg/kg, i.p.)	Reaction time (s), mean ± S.D.				
	Basal	7th day	14th day	21st day	30th day
Saline	3.2 ± 0.3	4.3 ± 0.5	3.7 ± 0.6	3.9 ± 0.8	4.1 ± 0.7
EHDg (500)	3.9 ± 0.6	8.2 ± 0.3*	7.4 ± 0.2*	9.0 ± 0.2*	8.7 ± 0.2*
Morphine (6)	3.2 ± 0.3	17.2 ± 1.9*	8.3 ± 1.2*	5.6 ± 0.8	4.2 ± 0.5

$n = 15$.
* $P < 0.01$, compared to control group values.

3.2. Effect of EHDg in the hot plate test

The results presented in Table 2 show that EHDg at doses of 250 and 500 mg/kg (p.o. and v.o.) produced a significant antinociceptive action when compared to the control group independent of the route of administration and similar to that of morphine at 6 mg/kg (i.p.).

3.3. Inhibition of the antinociceptive action of EHDg by naloxone in mice assessed using the hot plate apparatus

The antinociceptive action of EHDg or morphine was blocked by naloxone (2 mg/kg, i.p.), as shown in Table 2.

3.4. Effect of daily administration of EHDg for 30 days in mice assessed by the tail immersion test

EHDg at a dose of 500 mg/kg (i.p.) demonstrated an antinociceptive action during the entire period of treatment in contrast to morphine (6 mg/kg, i.p.) in which the antinociceptive effect was no longer significant, after day 21, as displayed in Table 3.

4. Discussion

Medicinal plants are widely used in Brazil, and have been a substantial source of ethnopharmacological information for the identification of phytochemical substances of therapeutic potential, as in the case of seeds of *Dioclea grandiflora* used for the treatment of kidney and prostate ailments. The analgesic action presented by EHDg involves supraspinal as well as spinal components as demonstrated by the utilization of the hot plate (Yaksh and Rubi, 1976) and tail flick (Mayer and Liebeskind, 1974) tests, respectively. The results suggest that EHDg has a central analgesic effect, as evidenced by the prolonged delay in response when rats were subjected to a nociceptive stimulus in the tail flick test and also by the increase in reaction time of mice in the hot plate test. This central analgesic action was confirmed by the blocking effect of naloxone, a specific antagonist of morphinomimetic receptors (Belvisi et al., 1998; Quock et al., 1999; Mundey et al., 2000). Moreover, EHDg did not produce any acute toxicity, and did not show classic tolerance, characteristic of morphine and other opioid substances, probably because it acts at the level of the μ-opioid receptor regulated by ganglioside GM1 linked to G_s protein (Crain and Shen, 1998; Pan, 1998), thus showing an opioid activity without the classic effect of opioid drugs. This is in agreement with results obtained by Batista et al. (1993, 1995) and Almeida et al. (2000), suggesting that the nature of the constituents present in the roots of *Dioclea grandiflora* are either the same or similar to the ones responsible for the antinociceptive action in the seeds. This antinociceptive effect of EHDg may be related to the reduction in Ca^{2+} influx at the axon terminal of the afferent nerve inducing a decrease in adenylyl cyclase activity, which results in decreased levels of cyclic AMP and efflux

of K^+ ions. The latter lead to hyperpolarization of the nerve and finally an apparent antinociceptive effect (Dickinson and Fleetwood-Walker, 1998; Yaksh, 1999; Grubb, 1998).

Acknowledgements

E. R. Almeida is a fellowship recipient, CAPES/PICDT.

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