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Journal of Ethnopharmacology 99 (2005) 153–156
Journal of ETHNO-PHARMACOLOGY
www.elsevier.com/locate/jethpharm

Ethnopharmacological communication

Analgesic and anti-inflammatory properties of *Nelsonia canescens* leaf extract

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Received 10 January 2005; received in revised form 25 January 2005; accepted 4 February 2005
Available online 14 March 2005

Abstract

An ethanolic extract of the dried leaves of *Nelsonia canescens* was investigated for anti-inflammatory and analgesic activities in rat. In the test for anti-inflammatory activity, the extract at the doses of 50–200 mg/kg significantly ($P < 0.05$) inhibited carrageenan-induced paw oedema and cotton pellet granuloma. Likewise, at the same doses the extract exhibited analgesic activity in both the hot plate latency assay (hot plate maintained at 55 °C) and on the early and late phases of formalin-induced paw licking in rats. The result of the present study confirm that *Nelsonia canescens* has analgesic and anti-inflammatory activities. These findings also justify the traditional use of the plant for treating pain.

Keywords: *Nelsonia canescens* (Lam.) spreng; Anti-inflammatory; Analgesic properties

1. Introduction

Nelsonia canescens (Lam.) spreng. (family Acanthaceae) commonly called blue pussy leaf is a small perennial herb with soft decumbent villous branches. It has erect spike and small pink or purple flower, (Vollesen, 1994; Wasshausen and Wood, 2004) the stem is wooly and the plant grow fast in western part of Nigeria during the dry season (Hutchinson and Dalziel, 1963). There is little or no information about the medicinal properties of this plant in the literature, however oral reports from herbal medical practitioner indicate that the extract of the plant is usually prepared and given to patients to drink (especially children) for the treatment of fever, pain, chicken pox and to reduce the effects of ulcers. The present study was therefore undertaken to investigate some of the folkloric claims especially the use of the plant as an analgesic. Furthermore, the desire to investigate the biological activity of this plant was also due to the report of Dalziel (1937), which showed that *Nelsonia campestria*, a closely related species to *Nelsonia canescens*, is effective in the treatment of yellow fever and eye inflammation.

2. Materials and methods

2.1. Animals

Male wistar rats weighing 190–230 g were used for this study. The animals were bred and housed in the pre-clinical animal house of the Faculty of Basic Medical Sciences, College of Medicine, University of Ilorin. They were provided with standard mouse cubes (Bendel Feeds, Ilorin) and water ad libitum. The research was conducted in accordance with the ethical rules on animal experimentation, approved by Ethical Committee, Faculty of Basic Medical Sciences, College of Medicine, University of Ilorin.

2.2. Plant materials

The plant (*Nelsonia canescens*) used for this study was collected within Ilorin town (Nigeria) in January 2003 and

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doi:10.1016/j.jep.2005.02.003

identified by Mr. T.K. Odewo of the Forestry Research Institute of Nigerian (FRIN). A Voucher specimen (FHI196534) has also been deposited in the herbarium of the Institute. The air-dried leaves of the plant were cleaned and reduced to powdery form with mortar and pestle, after which, 120 g of the powdered sample was exhaustively extracted with 2.5 l of ethanol (analytical grade) for 3 days (by maceration). The solvent was removed at 45 °C in a water bath to give a dark solid extract weighing 4.3 g. The extract was stored in a refrigerator at 4 °C and dilutions of the extract were made in normal saline for the various studies. Preliminary phytochemical screening was carried out on the extract using the standard screening method of Trease and Evans (1983).

2.3. Anti-inflammatory and analgesic tests

The anti-inflammatory effects of the extracts were assessed using the carrageenan and cotton pellet models in rats, while the analgesic tests were by the hot plate test and the formalin-induced paw licking tests. The animals used for each of the tests were divided into five groups with each group containing five rats. The control group (A) and the reference group (E) received normal saline (10 ml/kg) and indomethacin (5 mg/kg, Shanghai, China) respectively. While the test groups (B, C and D) were treated with 50, 100 and 200 mg/kg of the extract, respectively. Saline, extract and indomethacin were all administered orally.

Carrageenan-induced pedal inflammation was produced according to the method described by Winter et al. (1962). An injection of 0.1 ml of 1% carrageenan suspension was made into the right hind foot of each rat under the subplantar aponeurosis. The control, reference and test groups were treated orally with saline, indomethacin and the extract 1 h before carrageenan injection. Measurement of paw size was carried out by wrapping a piece of cotton thread round the paw and the length of the thread corresponding to the paw circumference was determined using a meter rule (Hess and Miloning, 1972; Olajide et al., 2000). Measurement was done immediately before and 1–5 h following carrageenan injection. The inhibitory activity was calculated according to the following formula (Olajide et al., 2000):

$$\text{Percentage inhibition} = \frac{(C_t - C_0)_{\text{control}} - (C_t - C_0)_{\text{treated}}}{(C_t - C_0)_{\text{control}}} \times 100$$

where C_t = paw circumference at time t, C_0 = paw circumference before carrageenan injection and $C_t - C_o$ = oedema.

The cotton pellet-induced granuloma formation was carried out as described by Mossa et al. (1995). A sterilized cotton pellet weighing 30 mg was introduced subcutaneously into the groin region of rats after the rats have been anaesthetized with ether. Following the implantation of the cotton pellet, the animals in the control, test group and reference groups were treated (once daily) for 4 days with saline, extract and indomethacin, respectively. All the animals were sacrificed on the fifth day with an overdose of ether and the pellet surrounded by granuloma tissue were dissected out carefully and dried overnight in an oven at 60 °C to a constant weight. The mean weight of the granuloma tissue formed in each group was obtained and the percentage inhibition was determined by comparing the mean weight in the test and reference groups with the mean weight in the control group.

The hot plate latency assay was based on the method of Eddy et al. (1950). The extracts, saline and indomethacin were given to the animals orally after a 12 h fast. All the animals in each group were placed on a hot plate (maintained at 55 ± 0.5 °C) 30 min after the administration of extract, reference drug and saline. The time taken (latency) for the animal to lick the foot or jump off the hot plate was noted and the mean of the latency for each group was determined. The same procedure was repeated for all the groups at 60 and 90 min after the administration of extracts, saline and indomethacin. The latencies of groups B–E was compared to that of group A.

The formalin-induced paw licking was studied in rats (groups A–E) using the method of Hunskaar and Hole (1997). In this method, 100 μl of 3% formalin was injected into the subcutaneous tissue on the planter surface of the left hind paw of rats 1 h after oral administration of the extracts, normal saline or indomethacin. The rats in groups B, C and D were given oral doses of the extracts 50, 100 and 200 mg/kg respectively 1 h before formalin injection. The rats in groups E and A were given oral doses of indomethacin (5 mg/kg) and an equivalent amount of normal saline (10 ml/kg), respectively 1 h before the injection. The time spent on licking the injected paw by each rat was observed as soon (0–5 min, post-injection) as the formalin was injected and later (late phase 20–30 min, post-injection). The mean of the time spent on licking the injected paw in each group was determined (Table 4).

2.4. Statistical analysis

In this study, recorded values are expressed as mean ± standard error of mean (S.E.M.). Statistical significance was determined using the Student's *t*-test. Values with $P < 0.05$ compared with control were considered significant.

3. Results

In the anti-inflammatory tests, the result show that oral pretreatment of animals with *Nelsonia canescens* extract (50–200 mg/kg body weight) and indomethacin (5 mg/kg) significantly ($P < 0.05$) inhibited carrageenan-induced paw oedema (Table 1) and granuloma tissue formation (Table 2).

In the analgesic studies, the results from hot plate test show that at 30 min the oral doses of *Nelsonia canescens* and indomethacin increased the reaction time from 6.8 ± 0.37 to 8.4 ± 0.6 s likewise, at 60 and 90 min the reaction time were significantly increased compared to the control (Table 3). In