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ScienceDirect
Journal of Ethnopharmacology 107 (2006) 406–411
Journal of ETHNO-PHARMACOLOGY
www.elsevier.com/locate/jethpharm

Study on the antinociceptive effects of *Thymus broussonetii* Boiss extracts in mice and rats

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Received 21 October 2005; received in revised form 12 March 2006; accepted 29 March 2006
Available online 7 April 2006

Abstract

In Morocco, *Thymus broussonetii* is widely used in folk medicine for the treatment of a variety of diseases including gastroenteric and bronchopulmonary disorders and to relieve dolorous process. The antinociceptive effect of the aqueous, butanolic and ethyl acetate extracts of this species was examined in rats and mice using chemical and thermal models. The results obtained showed that aqueous and butanolic extracts exerted an antinociceptive activity in the two phases of formalin (50–300 mg/kg), tail immersion and writhing tests. Whereas, the ethyl acetate extract reduced the nociceptive response only in the second phase of formalin (100–300 mg/kg) and writhing tests. The aqueous extract, which is the most effective, contains active analgesic principles acting both centrally and peripherally. Furthermore, this antinociceptive effect has been avoided by naloxone at a dose of 1 mg/kg in the first phase of formalin and hot plate tests indicating that this extract acts partly through an opioid-mediated mechanism. The present results demonstrated that *Thymus broussonetii* contains active constituents which possess antinociceptive activity justifying its popular use to relieve some pains.

Keywords: *Thymus broussonetii*; Rats and mice; Antinociceptive activity; Opioid system

1. Introduction

For centuries, the genus *Thymus* from the labiatae family is known in several countries as a spice and food preservative as well as a protective and curative remedy for many ailments. It has been reported that thyme possesses numerous biological activities including antispasmodic (Van Den Broucke and Lemli, 1983), antimicrobial (Marino et al., 1999), antioxidant (Miura et al., 2002) and antifungal (Soliman and Badeaa, 2002) activities.

The genus *Thymus* is one of the most popular herbs used in Moroccan folk medicine because of its numerous medicinal properties. It is widely distributed and found in several areas in Morocco (Jahandiez and Maire, 1934). This availability and the relatively low cost are a precious advantage for popular using. *Thymus broussonetii* Boiss subsp. *hanonnis* Maire, an endemic species, grows spontaneously at the regions of Essaouira-Agadir (Morocco) (Tahiri, 1996). It is popularly known as '*Za'tar es-swirî*'. The leaves and stem barks of this plant were used as powder, decoction or infusion form to treat digestive disorders, diarrhoea, fever, coughs, cold and numerous infected areas. Moreover, this plant is widely used as a remedy of dolorous events (Bellakhdar, 1997; Sijelmassi, 2000). Previous pharmacological essays conducted on this species had reported its anti-inflammatory (Ismaili et al., 2002) and antimicrobial (Lattaoui et al., 1993) activities. Moreover, we have evoked in our previous paper the immunological and behavioural effects of this species (Elhabazi et al., 2006). Furthermore, phytochemical assays revealed the presence of triterpens and flavonoids, such as luteolin, eriodictyol and thymonin in the *Thymus broussonetii* extracts (Ismaili et al., 2002).

Considering the popular use of this plant to relieve some pains, we focused in this report to investigate the antinociceptive effect of aqueous, butanolic and ethyl acetate extracts using chemical and thermal nociception models. On the other hand, an attempt was conducted to determine the participation of the opi-

Abbreviations: ASA, acetyl salicylic acid; b.w., body weight; i.m., intramuscular; i.p., intraperitoneally; p.o., per os; s.c., subcutaneously

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doi:10.1016/j.jep.2006.03.029

oid system in the antinociceptive effect of these extracts using naloxone (a non-selective opioid antagonist). In addition, preliminary phytochemical screening was conducted in order to determine the presence of the main classes of phytoconstituents in the tested extracts.

2. Materials and methods

2.1. Animal models and habituation

Male Sprague Dawley rats and male mice, weighing 180–230 and 25–30 g, respectively, were used in this study. Animals were housed in groups of three rats or six mice per standard makrolon cage, on 12-h light/12-h dark cycle; and air temperature was maintained at 22 ± 2 °C. They were offered food and water ad libitum.

Experiments reported in this study were carried out in accordance with current guidelines for the care of laboratory animals and the ethical guidelines for investigation of experimental pain in conscious animals (Zimmermann, 1983).

2.2. Plant material and preparation of extracts

The plant was collected in March 2002, from the Essaouira region, Morocco. It was identified and classified by Prof. A. Ouyahya from the Scientific Institute (Rabat). A voucher specimen (no. 65870) was deposited at the herbarium of the Scientific Institute, Mohamed V University, Rabat, Morocco.

The *Thymus broussonetii* leaves and their stem barks were dried at 40 °C and triturated in order to obtain a powder. Then, 1 l of ethanol was added to this powder (200 g) to obtain ethanolic extract, using Soxhlet apparatus. This ethanolic extract was concentrated and then partitioned successively by a series of increasing polar solvents (hexane, chloroform, ethyl acetate and butanol). The fractions thus obtained were concentrated with a rotaevaporatory, and dissolved and made up to appropriate volume with 0.9% NaCl just before the start of experiments. The w/w extraction yield was 3.8, 2.44 and 1.0% for the aqueous, butanol and ethyl acetate extract, respectively.

2.3. Antinociceptive tests

2.3.1. Formalin test

The method used in the present study was similar to that described previously by de Miranda et al. (2001) with slight modifications. It consists briefly of injecting subcutaneously 20 μl of 2% formalin into the right posterior paw of mice placed in a transparent enclosure. Throughout 5 min prior to this procedure, each mouse is allowed to adapt the testing box and left freely moving and exploring (habituation). The formalin-induced licking of the paw was considered as indicative of the nociceptive behaviour. Using a chronometer, the total time spent in licking and biting the injected paw is recorded, quantifying thus the nociceptive behaviour. However, as the formalin test in rodent consists of two successive phases (Hunskaar and Hole, 1987; Tjolsen et al., 1992), the amount of time spent licking the injected paw is subsequently noted during the two relevant periods: the first one over the 10 min after the formalin injection and the second during the following 50 min.

In this test, thyme extracts (i.p.) and acetyl salicylic acid (ASA, i.m.) were administered at 50, 100, 200 and 300 mg/kg of the animal body weight (b.w.) 30 min before formalin injection. Control group received isotonic saline 0.9%. On the other hand, to investigate the participation of opioid system in the antinociceptive effect of the thyme extracts, animals were pre-treated with naloxone (1 g/kg, s.c.) 15 min before administration of thyme extracts according the method indicated in Abdel-Fattah et al. (2000).

2.3.2. Tail immersion test

The procedure consists of immersing the base of the animal's tail in heated water that is under a constant 55 °C temperature (Aboufatima et al., 2002). The nociceptive response to this thermal stimulation is indicated by the reflex withdrawal of the animal's tail. Using a chronometer, the latency time was recorded before ASA, isotonic saline or extracts treatment and at 30, 60, 90, 120, 180 or 240 min after treatment. Rats were treated with thyme extracts (i.p.) or ASA (i.m.) at 200 mg/kg b.w. Control group received 0.9% NaCl solution i.p.

2.3.3. Writhing test

This test is performed in mice according to the method described by Ferreira et al. (2000) with slight modifications. Thus, 0.6% acetic acid solution (10 ml/kg, b.w.) was injected intraperitoneally. Animals were pre-treated with thyme extracts i.p. or ASA i.m. (200 mg/kg, b.w.) 30 min prior to the peritoneal irritation. Control animals received the same volume of 0.9% NaCl solution i.p. The resulting writhes and stretching were observed and counted over a period of 60 min after acetic acid injection.

2.3.4. Hot plate test

In this test, animals were placed in a glass cylinder on a heated metal plate maintained at 55 ± 2 °C. The latency of nociceptive responses such as licking or shaking one of the paws or jumping was recorded as the reaction time. Treated rats received orally three doses of aqueous extract (100, 200 and 300 mg/kg, b.w.). Control group received orally water at 10 ml/kg. Morphine was administered intraperitoneally at 10 mg/kg (b.w.). The latency to nociceptive response was recorded before treatment and at 30, 60, 90, 120 and 180 min after intraperitoneal administration of morphine and at 60, 90, 120 and 180 min after oral treatment with aqueous extract. In order to determine the involvement of the opioid system on the antinociceptive effect, naloxone (1 mg/kg, s.c.) was administered 15 min before treatment with morphine (10 mg/kg, i.p.) or aqueous extract (200 mg/kg, p.o.).

2.3.5. Phytochemical screening

Phytochemical screening of the tested extracts was performed to detect the eventual presence of different classes of constituents, such as alkaloids, flavonoids, quinons, saponins, terpenes and tannins using specific reactions.