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Analgesic properties of *Epilobium angustifolium*, evaluated by the hot plate test and the writhing test☆

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Abstract

The analgesic properties of *Epilobium angustifolium* (Ea), a plant containing flavonoids with anti-inflammatory activity, have not been sufficiently studied so far. Thus, we decided to evaluate, by the classical hot plate test and the writhing test, the analgesic effect of a dry extract of Ea obtained by evaporating a commercially available mother tincture. In the former assay, the effect of Ea (380 mg/kg) was slightly lower than that of morphine (10 mg/kg s.c.). In the writhing test, which is more sensitive for non-steroidal analgesics, the effect of Ea was already significant ($P < 0.05$) at 95 mg/kg while at doses ≥ 190 mg/kg, its activity was similar to that of lysine acetylsalicylate (300 mg/kg). The LD_{50} of this dry extract of Ea was 1.4 ± 0.1 g/kg. Further studies are necessary for the identification of the active principles and the elucidation of their mechanism of action. © 2001 Éditions scientifiques et médicales Elsevier SAS

Keywords: Epilobium angustifolium; Analgesic activity; Flavonoids; Hot plate; Writhing test

1. Introduction

The genus *Epilobium* (family Onagraceae) consists of over 200 species, the most common being *E. angustifolium* (Ea). Traditional medical uses include the treatment of prostate, gastrointestinal, and menstrual disorders [1], which have been partially justified in the last two decades by the discovery that the plant is rich in flavonoids (myricitrin, isoquercitrin, quercitrin, guaiaverin, quercetin-3-*O*-β-D-glucuronide, etc.) and steroids (β-sitosterol and different esters) [1,2]. In particular, Ea appeared very active in inhibiting the carrageenan-induced rat paw edema [3,4] probably due to the action of the flavonoid myricetin-3-*O*-β-D-glucuronide [5].

Although it has been suggested that the anti-inflammatory activity of Ea is due to the inhibition of the synthesis of prostaglandins [3,5], which is the well-known mechanism of action of the aspirin-like analgesics [6], surprisingly, the analgesic properties of Ea have not been studied so far. Thus, we decided to evaluate the analgesic effects of Ea using classical pharmacological assays.

2. Materials and methods

2.1. Reagents

The *Epilobium angustifolium* ethanolic tincture (EaT, 10% w/v) was obtained from Boiron Laboratories (Sainte-Foy-lès-Lyon, France). Morphine (M) chloride was obtained from Salars (Italy). Lysine acetylsalicylate (ASL) was obtained from Sanofi Winthrop Pharmaceuticals (Morrisville, PA, USA). Acetic acid (glacial) and propylene glycol were obtained from Sigma Chemical

Abbreviations: ASL, lysine acetylsalycylate; Ea, *Epilobium angustifolium*; EaT, *Epilobium angustifolium* tincture; EaDE, *Epilobium angustifolium* dry extract; i.p., intraperitoneally; LD50, lethal dose 50; M, morphine; s.c., subcutaneously.

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Co. (St. Louis, MO, USA). Swiss ICR (CD1) male mice (about 30 g) were obtained from Harlan (Italy) and housed for one week at 22°C on a 12-hr light/12-hr dark cycle with free access to food and water.

2.2. Sample preparation

EaT (150 ml) was evaporated under vacuum at 45°C and the dry extract (EaDE) was resuspended in a proper volume of 3% propylene glycol in water in order to administer the same volume (0.01 ml/g) for all tested doses.

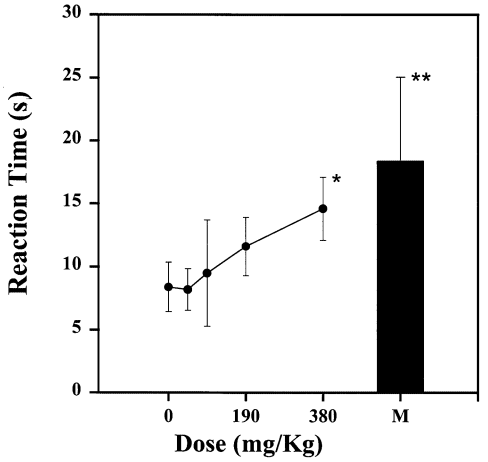

Fig. 1. Hot plate test. The animals ($n = 5$) were treated with M (10 mg/kg s.c.) or the dry extract of *Epilobium angustifolium* (0, 47.5, 95, 190, and 380 mg/kg s.c.), and after 30 min were put on a hot plate heated at 55°C. The time necessary for the response to the painful stimulus (elevation of the paws, licking, etc.) was recorded. The cut-off time was 30 s. $*P < 0.05$; $**P < 0.01$ (ANOVA and Dunnett's tests).

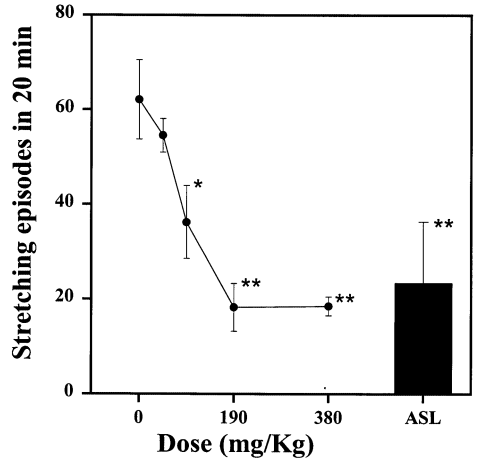

Fig. 2. Writhing test. The animals ($n = 5$) were treated with ASL (300 mg/kg s.c.) or the dry extract of *Epilobium angustifolium* (0, 47.5, 95, 190, and 380 mg/kg s.c.), and after 30 min were injected i.p. with 0.6% acetic acid (0.01 ml/g). The stretching episodes were recorded for 20 min. $*P < 0.05$; $**P < 0.01$ (ANOVA and Dunnett's tests).

2.3. Hot plate test

The assay was performed according to the classical hot-plate technique of Eddy and Leimbach [7]. Briefly, the animals ($n = 5$) were treated with M (10 mg/kg s.c.) or EaDE (0, 47.5, 95, 190, and 380 mg/kg s.c.); 30 min later, they were put on a hot plate (Basile, Varese, Italy) heated at 55°C and the time necessary for the response to the painful stimulus (elevation of the paws, licking, etc.) was recorded. The cut-off time was 30 s.

2.4. Writhing test

The assay was performed according to the classical technique of Koster et al. [8]. Briefly, the animals ($n = 5$) were treated with ASL (300 mg/kg s.c.) or EaDE (0, 47.5, 95, 190, and 380 mg/kg s.c.). After 30 min, they were injected i.p. with 0.6% acetic acid (0.01 ml/g) and the stretching episodes were recorded for 20 min.

2.5. LD_{50}

The animals ($n = 5$) were treated with EaDE (0, 0.76, 1.14, 1.71, and 2.47 g/kg s.c.) and observed for 24 h.

2.6. Statistical methods

Statistical analysis was performed by parametric (ANOVA and Dunnett's) tests using the software Sigma-Stat® version 2.3 for Windows 98™ (SPSS, Chicago, IL, USA). Data with $P < 0.05$ were considered statistically significant. The logistic fitting of the mortality versus dose curve was performed with the software Sigma-Plot® version 6 for Windows 98™ (SPSS, Chicago, IL, USA).

3. Results

3.1. Hot plate test

The s.c. injection of EaDE increased, after 30 min, the reaction time of the mouse to the hot plate painful stimulus (Fig. 1). In this test, the analgesic effect of the highest dose of EaDE (380 mg/kg) appeared lower than that of M (10 mg/kg s.c.) (Fig. 1).

3.2. Writhing test

The subcutaneous injection of EaDE (47.5, 95, 190, and 380 mg/kg s.c.) reduced, after 30 min, the number of stretching episodes induced by the intraperitoneal injection of acetic acid (0.6%, 0.01 ml/g) (Fig. 2). In this test, the analgesic effects of doses ≥ 190 mg/kg of EaDE were higher than that of ASL (300 mg/kg s.c.) (Fig. 2).

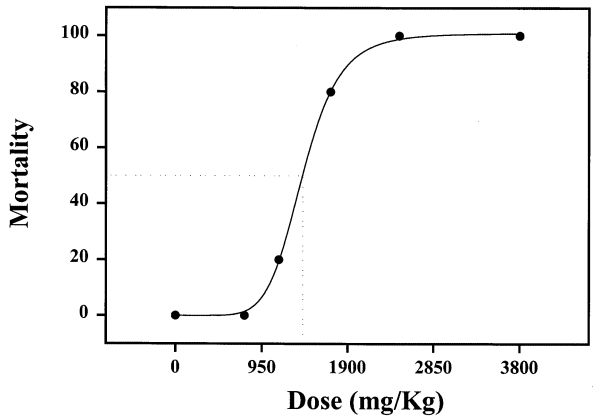

Fig. 3. LD_{50}. The animals ($n = 5$) were treated with the dry extract of *Epilobium angustifolium* (0, 0.76, 1.14, 1.71, and 2.47 g/kg s.c.) and the animals were observed for 24 h. The logistic fitting of the curve was performed with the software Sigma-Plot® version 6 for Windows 98™ (SPSS, Chicago, IL, USA).

3.3. LD_{50}

By plotting the mortality (after 24 h) of mice treated with EaDE versus the corresponding dose, the curve shown in Fig. 3 was obtained. The logistic fitting of this curve yielded the LD_{50} of EaT (1.4 ± 0.1 g/kg).

4. Discussion

By both hot plate and writhing tests, we showed that Ea has marked analgesic properties (Figs. 1 and 2). In the former assay, even at the highest dose (380 mg/kg) the effect was lower than that of M (10 mg/kg s.c.) used as reference compound [9] (Fig. 1). In the writhing test, which is more sensitive for non-steroidal analgesics [10], the effect of Ea was already significant ($P < 0.05$) at 95 mg/kg, while at doses ≥ 190 mg/kg its activity was similar to that of ASL, a water-soluble derivative of acetylsalicylic acid and parenterally administrable analgesic that we used at the reference dose of 300 mg/kg [11] (Fig. 2). Finally, we determined, for the first time to our knowledge, the LD_{50} of Ea, which resulted as 1.4 ± 0.1 g of dry extract per kilogram (Fig. 3).

In conclusion, we described the analgesic activity of Ea, although further studies are necessary for the identification of the active principles and the elucidation of their mechanism of action, even if we speculate that they could be the previously described anti-inflammatory flavonoids [4,5] as suggested by the analgesic properties of structurally related compounds [12–16].

References

[1] J. Gruenwald, T. Brendler, C. Jaenicke (Eds.), PDR for Herbal Medicines, 1st ed., Medical Economics Data, 1998 (ISBN 1563632926).
[2] I. Slacanin, A. Marston, K. Hostettmann, N. Delabays, C. Darbellay, Isolation and determination of flavonol glycosides from *Epilobium* species, J. Chromatogr. 557 (1991) 391–398.
[3] A. Hiermann, H. Juan, W. Sametz, Influence of *Epilobium* extracts on prostaglandin biosynthesis and carrageenin induced oedema of the rat paw, J. Ethnopharmacol. 17 (1986) 161–169.
[4] H. Juan, W. Sametz, A. Hiermann, Anti-inflammatory effects of a substance extracted from *Epilobium angustifolium*, Agents Actions 23 (1988) 106–107.
[5] A. Hiermann, M. Reidlinger, H. Juan, W. Sametz, Isolation of the antiphlogistic principle from *Epilobium angustifolium*, Planta Med. 57 (1991) 357–360.
[6] S.H. Ferreira, Prostaglandins, aspirin-like drugs and analgesia, Nat. New Biol. 240 (1972) 200–203.
[7] N.B. Eddy, D. Leimbach, Synthetic analgesics II. Dithienylbutenyl and dithienylbutylamines, J. Pharmacol. Exp. Ther. 45 (1953) 339.
[8] R. Koster, M. Anderson, E.J. De Beer, Acetic acid for analgesic screening, Fed. Proc. 18 (1959) 412.
[9] S. Kayan, L.A. Woods, C.L. Mitchell, Morphine-induced hyperalgesia in rats tested on the hot plate, J. Pharmacol. Exp. Ther. 177 (1971) 509–513.
[10] J. Cochin, Methods for the appraisal of the analgesic drugs for addiction liability, in: A. Burger (Ed.), Selected pharmacological testing methods, Marcel Dekker, New York, 1968.
[11] S. Hunskaar, Similar effects of acetylsalicylic acid and morphine on immediate responses to acute noxious stimulation, Pharmacol. Toxicol. 60 (1987) 167–170.
[12] M. Rylski, H. Duriasz-Rowinska, W. Rewerski, The analgesic action of some flavonoids in the hot plate test, Acta Physiol. Pol. 30 (1979) 385–388.
[13] P. Thirugnanasambantham, S. Viswanathan, S. Ramaswamy, V. Krishnamurthy, C.I. Mythirayee, S. Ramachandran, L. Kameswaran, Involvement of calcium in flavonoid analgesia, Eur. J. Pharmacol. 152 (1988) 367–369.
[14] M. Picq, S.L. Cheav, A.F. Prigent, Effect of two flavonoid compounds on central nervous system, analgesic activity, Life Sci. 49 (1991) 1979–1988.
[15] L.C. Cardenas, J. Rodriguez, M.C. Villaverde, R. Riguera, R. Cadena, J.A. Otero, The analgesic activity of *Hedyosmum bonplandianum*: flavonoid glycosides, Planta Med. 59 (1993) 26–27.
[16] E.M. Galati, M.T. Monforte, S. Kirjavainen, A.M. Forestieri, A. Trovato, M.M. Tripodo, Biological effects of hesperidin, a citrus flavonoid. (Note I): Antiinflammatory and analgesic activity, Farmaco 40 (1994) 709–712.