NPC Natural Product Communications

2008
Vol. 3
No. 12
2099 - 2102

Antihyperalgesic Effect of *Eschscholzia californica* in Rat Models of Neuropathic Pain

Elisa Vivoli[a]*, Anna Maidecchi[b], Anna Rita Bilia[c], Nicoletta Galeotti[a], Monica Norcini[a] and Carla Ghelardini[a]

[a]*Dipartimento di Farmacologia, Università di Firenze, Viale G. Pieraccini 6, 50139 Firenze, Italy*

[b]*ABOCA SpA Società Agricola, loc. ABOCA 20, 52037 Sansepolcro, (AR) Italy*

[c]*Dipartimento di Scienze Farmaceutiche, Università di Firenze, Via U. Schiff 6, 50019, Sesto Fiorentino, (FI) Italy*

elisa.vivoli@unifi.it

Received: June 11th, 2008; Accepted: October 23rd, 2008

Eschscholzia californica Cham. (Papaveraceae) is traditionally used by the Indians as a medicinal plant for its anxiolytic, anticonflict, analgesic and sedative properties. The mechanisms of action for the sedative and anxiolytic activities have not been clearly established and so to further investigate the pharmacological profile of *E. californica* in some painful conditions, a 70% v/v ethanol extract, DER_{native}=5:1, was tested in rat models of neuropathy induced by chronic constriction injury of the sciatic nerve (CCI), with chemotherapeutic oxaliplatin, and osteoarthritis caused by intrarticular injection of monoiodoacetate. In the CCI model evaluated in the rat paw-pressure test, the examined extract (100 mg kg^{-1} p.o.) showed an antihyperalgesic effect. *Eschscholzia* extract, after single injection at a dose of 100-300 mg kg^{-1} p.o., produced also a statistically significant decrease of pain perception on hyperalgesia induced by oxaliplatin and osteoarthritis, while in the same condition gabapentin did not display any antihyperalgesic effect. Furthermore, in the range of antihyperalgesic doses, the extract was efficacious in the hot-plate (thermal stimulus) and carrageenan tests (inflammatory model) without producing any behavioral impairment, as evaluated by the Irwin test. The analgesic effect exhibited by *Eschscholzia* extract in the mouse hot-plate test was not antagonized by naloxone, indicating that opioid neurotransmission is not involved in the effect.

The above reported results suggest that a 70% (v/v) ethanol dried extract (DER_{native}=5:1) of *E. californica* might represent a promising product for the therapy of acute and chronic pain.

Keywords: *Eschscholzia californica* Cham., Papaveraceae, 70% v/v ethanol extract (DER_{native}=5:1), antihyperalgesic effect, acute and chronic pain.

Eschscholzia californica Cham. (California poppy, Papaveraceae) is an annual plant found throughout California and traditionally used by the Indians as a medicinal plant for its anxiolytic, anticonflict, analgesic and sedative properties [1]. The mechanisms of action for the sedative and anxiolytic activities have not been clearly established, although the involvement of several receptors have been observed and recently it has been reported that a 70% (v/v) ethanol extract of California poppy was able to bind to 5-HT(1A) and 5-HT(7) receptors at 100 μg/mL [2]. To further investigate the pharmacological profile of *E. californica* in some painful conditions, a 70% v/v ethanol extract, DER_{native}=5:1, was used for the tests in rat models of neuropathy induced by chronic constriction injury of the sciatic nerve (CCI), repeated treatment with the chemotherapeutic agent oxaliplatin, and osteoarthritis caused by intrarticular injection of monoiodoacetate and inflammation caused by carrageenan plantar injection.

In the CCI model evaluated in the rat paw-pressure test, the examined extract (100 mg kg^{-1} p.o.) showed an antihyperalgesic activity (Figure 1). The anti- hyperalgesic effect induced by 300 mg kg^{-1} did not differ from that obtained with a dose three folds lower (data not shown). California poppy

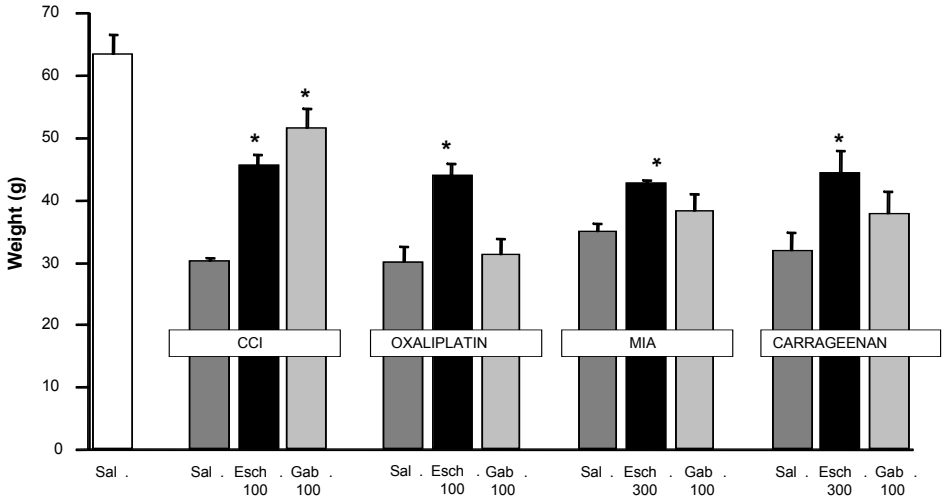

Figure 1: Effect of *Eschscholzia californica* extract (ECE) in comparison with gabapentin (Gab.) in rat models of hyperalgesia induced by Chronic Constriction injury (CCI) and by the administration of Oxaliplatin, Monoiodoacetate (MIA) and Carrageenan. Tests were performed 30 min after administration. Doses are expressed as mg kg^{-1} p.o. *P<0.05 vs saline treated rats.

extract, after single injection at a dose of 100 mg kg^{-1} and 300 mg kg^{-1} p.o., respectively, produced also a statistically significant decrease of pain perception on hyperalgesia induced by oxaliplatin and osteoarthritis, while in the same condition gabapentin did not display any antihyperalgesic effect (Figure 1). Particularly, the extract peaked 30 min after administration (Figure 1). Similarly, in the painful condition caused by intraplantar injection of carrageenan, California poppy extract reduced hyperalgesia at the dose of 300 mg kg^{-1} p.o., 30 min after administration (Figure 1). Furthermore, in the same range of doses, the extract was efficacious in the mouse hot-plate test in the presence of a thermal stimulus. The analgesic effect exhibited by California poppy extract in the mouse hot-plate test was of an intensity comparable to that exhibited by gabapentin and it was not antagonized by naloxone (1 mg kg^{-1} i.p.) indicating that opioid neurotransmission is not involved in the effect (Table 1).

The examined extract, at the dose which was effective in relieving acute and persistent pain, was tested in order to assess its effect on mouse behaviour. At the highest dose employed, the extract did not induce any alteration of either mouse gross behaviour or show any other side effect, as observed in the Irwin test (data not shown). Moreover, mice treated with California poppy extract were evaluated for motor coordination by using the rota-rod test. The endurance time, evaluated before and 15, 30 and 45 minutes after the beginning of the rota-rod test showed the lack of any impairment in the motor coordination of the treated mice (Figure 2).

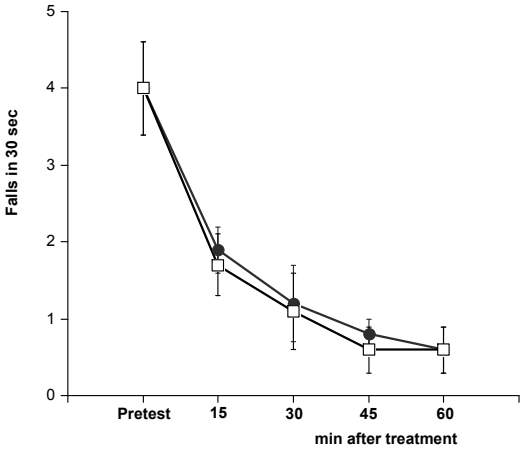

Figure 2: Effect of *Eschscholzia californica* extract on mouse rota-rod test: empty square is saline, filled circle is *E. californica* at a dose of 1000 mg kg^{-1}.

Table 1: Effect of *Eschscholzia californica* extract (ECE) in mouse hot plate test.

	Licking latency (s)	
Treatment	Pre-test	30 min after treatment
SALINE	14.4±0.5	14.6±0.4
ECE 100 mg kg^{-1} p.o	14.4±0.7	19.6±0.9*
ECE 300 mg kg^{-1} p.o	14.7±0.7	18.6±0.7*
GABAPENTIN 300 mg kg^{-1} p.o	15.2±0.8	18.6±1.3*
NALOXONE 1 mg kg^{-1} i.p.	14.8±0.5	15.4±0.6
NALOXONE + ECE 100 mg kg^{-1} p.o.	14.3±0.6	19.7±0.8*

*P<0.05 vs saline-treated mice

The above reported results suggest that the freeze-dried extract (DER_{native}=5:1) of *E. californica* might represent a promising material for the therapy of acute and chronic pain.

Experimental

***Plant material*:** *Eschscholzia californica* (California poppy) was cultivated at Aboca's Organic Farms in 2007 (Aboca, San Sepolcro (AR), Italy). After harvest, the drying process was carried out using special equipment, air-heated ovens, with cells in which forced air is circulated at a temperature and level of humidity that are carefully controlled (the temperature is kept between 32° and 40°C). After this, the desired plant material was separated from any extraneous bodies present (weeds, insects, seeds) based on specific weight, using aero-separators and densimeters, and cut, to reduce the herb to the proper dimensions according to its intended use (for herbal tea, extraction, micronized powder).

Preparation of plant extract: *Eschscholzia californica* freeze-dried extract was produced by Aboca Spa (Sansepolcro, AR) and was obtained according to the extraction procedure described below. The dried ground tops were extracted by percolation with a hydro ethanolic solution (70% v/v) and herb/solvent ratio of 1:10.

After 8-10 h, the plant extract was filtered to remove the exhausted herb and concentrated under vacuum to remove the ethanol. The extract was freeze-dried under vacuum, without the use of either heat or excipients, at temperatures less than -50°C. The Drug Extract Ratio (DER) was 5:1 and the batch number was n. 7B1150. The content of protopin in this batch was 0.22%, analyzed by a HPLC method.

Chemicals: Protopin was purchased from Sigma (Sigma-Aldrich S.r.l., Milan, Italy). All the solvents used for the extraction and HPLC analysis (EtOH, MeOH, diethanolamine and acetonitrile) were HPLC grade from Merck (Darmstadt, Germany). Water was purified by a Milli-Q_{plus} system from Millipore (Milford, MA). The following drugs were used in the pharmacological experiments: Naloxone hydrochloride (Sigma, St. Louis, USA), Oxaliplatin (Sequoia Research Products Ltd, Pangbourne, UK), Sodium iodoacetate (Sigma-Aldrich, Germany). Other chemicals were of the highest quality commercially available.

Table 2: Mobile phases used for HPLC analysis.

Time(min)		B %	Flow (mL/min)
0.00	67	33	1.500
30.00	50	50	1.500
50.00	50	50	1.500
65.00	67	33	1.500

HPLC-DAD system: The HPLC analyses were performed using a HP 1100 L Palo Alto, CA, USA) equipped with a HP 104iquid Chromatograph (Agilent Technologies, 0 Diode Array Detector (DAD), an automatic injector, an auto sampler, a column oven and managed by a HP 9000 workstation (Agilent Technologies, Palo Alto, CA, USA). Separations were performed on a reversed phase column prodigy 5 µm ODS (3) 100 Å (250.0 × 4.6 mm) column fitted with a 4.0 × 3.0 mm i.d. guard column, both from Phenomenex (Torrance, CA). The eluents were A: SDS 10 mM + DEA 0.1 M in water adjusted to pH 2.5 by H_3PO_4; B: acetonitrile. The mobile phase is reported in Table 2. The system was operated with an oven temperature of 40°C. Before HPLC analysis, each sample was filtered through a cartridge-type sample filtration unit with a polytetrafluoroethylene (PTFE) membrane [d=13 mm, porosity 0.45 µm (Lida Manufacturing Corp.)] and immediately injected (20 µL).

Chromatograms were recorded between 200 and 450 nm. DAD spectra were stored for all peaks exceeding a threshold of 0.1 mAu and detection was performed at 280 nm.

Calibration curves: A calibration curve was obtained from an 80% MeOH solution (containing 4% of diluted HCl) of protopin in the range between 0.025 and 0.00625 mg/mL.

Pharmacological assays: Drugs were either dissolved in isotonic (NaCl 0.9%) saline solution or dispersed in 1% carboxymethylcellulose sodium salt (CMC, Fluka Chemie GmbH, Steinheim, Germany) immediately before use. Drug concentrations were prepared so that the necessary dose could be administered in a volume of 10 mL kg^{-1} by i.p. and p.o. injection.

Animals: Male Sprague-Dawley albino rats (180-200 g) from Harlan (S.Piero al Natisone, Italy) and male Swiss albino mice (24-26 g) from Morini (San Polo d'Enza, Italy) were used. Four rats and 10 mice were

housed per cage. For acclimatization, the cages were placed in the experimental room 24 h before the test. The animals were fed a standard laboratory diet and tap water *ad libitum* and kept at 23±1°C with a 12-h light/dark cycle. All experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) for experimental animal care. All efforts were made to minimize the number of animals used and their suffering.

Chronic constriction injury: A peripheral mono neuropathy was produced in adult rats by placing loosely constrictive ligatures around the common sciatic nerve, according to the method described by Bennet and Xie [3].

Monoiodoacetate injection: Joint damage was induced by a single intra-articular injection of 2 mg of sodium monoiodoacetate into the left knee joint of anaesthetized rats in a total volume of 25 μL. The dose of iodoacetate was chosen based on previous literature [4] and in-house dose response data using 0.5, 1 and 2 mg.

Oxaliplatin injection: Hyperalgesia was induced by 15 injections for 5 consecutive days every week for 3 weeks of Oxaliplatin, 2.4 mg kg^{-1} (15 i.p. injections- cumulative dose 36 mg kg^{-1}) [5].

Paw-pressure test: The instrument exerts a force which is applied at a constant rate (32 g per second) with a cone-shaped pusher on the upper surface of the rat hind paw. The force is continuously monitored by a pointer moving along a linear scale. The pain threshold is given by the force that induces the first struggling from the rat. Pretested rats which scored below 40 g or over 80 g during the test before drug administration (25%) were rejected. An arbitrary cut off value of 250 g was adopted.

Hot plate test: Mice were placed inside a stainless steel container, which was set thermostatically at 52.5±0.1°C in a precision waterbath from KW Mechanical Workshop, Siena, Italy. Reaction times (s) were measured with a stopwatch before and 15, 30, 45 and 60 min after administration of the drug. The endpoint used was the licking of either the forepaws or hind paws. Those mice scoring less than 12 and more than 18 s in the pretest were rejected (30%). An arbitrary cutoff time of 45 s was adopted.

Carrageenan test: Rat paw volumes were measured using a plethysmometer (Ugo Basile, Varese, Italy). Rats received the investigated extract 30 min after a 0.1mL injection of 1.0% carrageenan in the right hind paw. Four h after the injection of carrageenan, the pain threshold of the right hind paw was measured and compared with saline/carrageenan-treated controls.

Statistical analysis: All experimental results are given as the mean ± S.E.M. An analysis of variance (ANOVA), followed by Fisher's Protected Least Significant Difference procedure for post-hoc comparison, were used to verify significance between two means. Data were analyzed with the StatView software for the Macintosh. P values of less than 0.05 were considered significant.

References

[1] Rolland A, Fleurentin J, Lanhers MC, Younos C, Misslin R, Mortier F, Pelt JM. (**1991**) Behavioural effects of the American traditional plant *Eschscholzia californica*: sedative and anxiolytic properties. *Planta Medica*, ***57***, 212-216.

[2] Gafner S, Dietz BM, McPhail KL, Scott IM, Glinski JA, Russell FE, McCollom MM, Budzinski JW, Foster BC, Bergeron C, Rhyu M-R, Bolton JL. (**2006**) Alkaloids from *Eschscholzia californica* and their capacity to inhibit binding of [^{3}H]8-hydroxy-2-(di-*N*-propylamino)tetralin to 5-HT_{1A} receptors *in vitro*. *Journal of Natural Products*, ***69***, 432-435.

[3] Bennett GJ and Xie YK (**1988**) A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. *Pain*, ***33***, 87-107.

[4] Guingamp C, Gegout-Pottie P, Philippe L, Terlain B, Netter P, Gillet P. (**1997**) Mono-iodoacetato-induced experimental osteoarthiritis: a dose-response study of loss of mobility, morphology and biochemistry. *Arthritis and Rheumatism*, ***40***, 1670-1679.

[5] Cavaletti G, Tredici G, Petruccioli MG, Dondè E, Tredici P, Marmiroli P, Minoia C, Ronchi A, Bayssas M, Etienne GG. (**2001**) Effects of different schedules of oxaliplatin treatment on the peripheral nervous system of the rat. *European Journal of Cancer*, ***37***, 2457-2463.