PHYTOTHERAPY RESEARCH
Phytother. Res. **15**, 377–381 (2001)
DOI: 10.1002/ptr.884

Neurophysiological Effects of an Extract of *Eschscholzia californica* Cham. (*Papaveraceae*)

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An aqueous alcohol extract of *Eschscholzia californica* (Ec) has been evaluated for benzodiazepine, neuroleptic, antidepressant, antihistaminic and analgesic properties, in order to complete the study of the sedative and anxiolytic effects previously demonstrated.

The plant extract did not protect mice against the convulsant effects of pentylenetetrazol, and did not cause muscle relaxant effects but appeared to possess an affinity for the benzodiazepine receptor: thus, flumazenil, an antagonist of these receptors, suppressed the sedative and anxiolytic effects of the extract. The Ec extract induced peripheral analgesic effects in mice but did not possess antidepressant, neuroleptic or antihistaminic effects.

Keywords: Eschscholzia californica extract; anxiolytic; sedative; anticonvulsant; antipsychotic; antidepressant; analgesic activities.

INTRODUCTION

An ethnopharmacological study of *Eschscholzia californica* Cham. (Ec) was previously undertaken to record its traditional therapeutic indications and to evaluate its pharmacological properties with respect to the traditional uses and present phytotherapeutic medicine (Rolland, 1988), its chemical composition has also been reviewed (Fleurentin *et al.*, 1996).

In a previous paper we reported that an aqueous extract of Ec displayed dose-dependent sedative properties in mice at high doses, by decreasing behavioural parameters measured in familiar environment tests (two compartment test) and in non-familiar environment tests (staircase test). These sedative properties were confirmed using the sleep-induction test in mice. The extract also showed anxiolytic effects in mice at lower doses with an increase of behavioural parameters measured in the staircase test and in the light/dark choice situation test (Rolland *et al.*, 1991).

Similar results (anxiolytic and sedative effects) were obtained in mice with an aqueous alcohol (60°) extract of Ec (Rolland *et al.*, 1991). The aqueous alcohol extract (i.p. and p.o.) and the aqueous extract (i.p.) did not induce any acute toxic effects the LD_{50} being over 5000 mg/kg.

The purpose of the present study was: (1) to determine if the sedative and anxiolytic effects of Ec were linked to benzodiazepine receptors by using flumazenil, an antagonist at these receptors, in the open-field test and in the light/dark choice situation test, (2) to research putative anticonvulsant properties with the pentylenetetrazol test and muscle relaxant effects, in the suspension test and in the rota-rod test, (3) to evaluate possible neuroleptic properties by studying the effects of Ec on dexamphetamine-induced group toxicity and stereotypies and through catalepsy and hypothermia tests, (4) to study possible antidepressant properties by measuring the influence of Ec on reserpine and cholinergic effects, (5) to research putative antihistaminic effects on guinea-pig isolated ileum, and (6) to confirm the traditional analgesic effects reported by the rural population of California (Cheney, 1963) by studying the effect of the extract in the writhing test and in the hot plate test.

MATERIALS AND METHODS

Plant extract. The aqueous alcohol (60%) extract was provided by laboratory Vernin (Titrex, France) prepared from aerial parts of *E. californica*. 1 g of dried extract corresponded to 5 g of dried plant material. The yield of extraction was 20%.

Characterization of the extract was limited to qualitative chemical identification in thin layer chromatography of different substances such as alkaloids, amino acids, tannins and reducing compounds, according to the literature (Rolland, 1988). In this paper, all doses are expressed in terms of dried plant material (mg/kg of body weight).

Animals. Male Swiss mice (Janvier, Le Genest, France) weighing 30–35 g (8–9 weeks age) and male rats OFA

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Received 4 November 1998
Accepted 7 December 1999

(Iffa Credo, L'Arbresle, France) weighing 200–300 g were used for these studies.

Chemicals. The chemicals were obtained from the following sources: acetic acid solution (Prolabo, France); acetylsalicylic acid (Aspegic, Synthelabo, France); amitriptyline (Aldrich Europe B); atropine (Aguettant); pentylenetetrazole (Cardiazole, Aldrich Europe B); chlorpromazine (Largactil, Specia, France); dexamphetamine (Maxiton, Delagrange, France); clorazepate dipotassium (Tranxene, Clin-Midi, France), flumazenil (Hofmann-Laroche, CH); histamine (Merck, France); imipramine (Tofranil, Ciba-Geigy, France); midazolam (Hypnovel, Produits Roche, France); reserpine (Sarget, France); morphine sulphate; Tween 80 (Prolabo, France); oxotremorine (Aldrich Europe, B); paracetamol (Pro-Dafalgan, Upsa, France).

Effects of flumazenil on sedative and anxiolytic activities of *Ec*. The methods used have been previously described (Lanhers *et al.*, 1996). In the open-field test mice received i.p. plant extract (200 mg/kg), midazolam (2 mg/kg), flumazenil (10 mg/kg), a combination of plant extract and flumazenil, a combination of midazolam and flumazenil, or distilled water, with a drop of Tween 80.

In the light/dark choice situation test, the amount of time spent in a lighted box was recorded for 5 min after the first entry in the dark box. Mice received i.p. plant extract (25 mg/kg), clorazepate dipotassium (2 mg/kg); flumazenil (1 mg/kg), a combination of plant extract and flumazenil, a combination of clorazepate dipotassium and flumazenil, and distilled water, with a drop of Tween 80.

Anticonvulsant and muscle relaxant properties. The methods used have been previously described (Lanhers *et al.*, 1996). For the *anticonvulsant effects*, plant extract (25, 200 and 800 mg/kg), chlorazepate dipotassium (1, 10 and 40 mg/kg), or solvent (NaCl 0.9%) were injected i.p. 30 min before the i.p. administration of pentylenetetrazol (125 mg/kg).

For the suspension test the plant extract was injected i.p. at doses of 100, 200 and 800 mg/kg. Clorazepate dipotassium was used at 1, 10 and 20 mg/kg.

For the rotarod test 30 min before testing, the plant extract (100, 200, 400 and 800 mg/kg), clorazepate dipotassium (1, 10 and 20 mg/kg) and NaCl 0.9% solution (control) were injected i.p.

Neuroleptic properties. The methods used have been previously described (Lanhers *et al.*, 1996). For the group toxicity test mice received the plant extract (25 and 400 mg/kg), a reference neuroleptic chlorpromazine (20 mg/kg) and NaCl 0.9% solution (control group) by i.p. route. Mortality was recorded 4 h after dexamphetamine injection.

For the dexamphetamine-induced stereotyped behaviour test, rats received the plant extract (200 and 800 mg/kg), chlorpromazine (20 mg/kg) and NaCl 0.9% solution i.p. Thirty min after treatment each animal received an intraperitoneal injection of dexamphetamine (5 mg/kg). The stereotypy rating scale was that adopted by Simon and Chermat (1972). For each group, the summed quotation for a 210 min period was calculated. The general total was expressed as a percentage of the control group.

For catalepsy and hypothermia tests the plant extract (200 and 800 mg/kg), chlorpromazine (20 mg/kg) and NaCl 0.9% solution (control) were injected i.p.

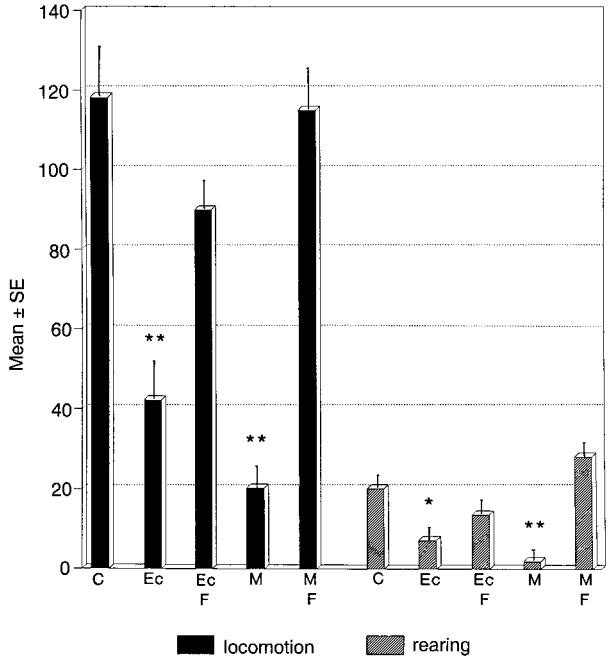

Figure 1. Influence of flumazenil on sedative effects of *E. californica* extract in the open-field test in mice. Behavioural parameters: locomotion (number of crossed square units) and rearing (number of rears against the walls). C, control; Ec, *E. californica* (200 mg/kg); M, midazolam (2 mg/kg); Ec-F, combination of *E. californica* (200 mg/kg) and flumazenil (10 mg/kg); M-F, combination of midazolam (2 mg/kg) and flumazenil (10 mg/kg), * $p < 0.05$. ** $p < 0.01$.

Antidepressant properties. For the antireserpine test mice received the plant extract (50, 100, 200, 400 and 800 mg/kg), NaCl 0.9% solution (control) or amitryptilline 12 mg/kg (reference antidepressant) by i.p. route 4 h after reserpine injection (2.5 mg/kg) (Rubin *et al.*, 1967).

For the anticholinergic test the mice received the plant extract (200 and 800 mg/kg), NaCl 0.9% solution (control) or imipramine 5 mg/kg (reference antidepressant) by i.p. route. Thirty min after these treatments the mice received oxotremorine (0.5 mg/kg) on i.p. route.

Antihistaminic effects. Segments of ileum about 4 cm long were suspended in 20 mL organ baths containing Tyrode's solution, bubbled with oxygen and maintained at 37° ± 1 °C. An isometric recording method was used and the results were recorded on a Narco biosystem recorder. Atropine (0.5 mg/L) was present in the bathing fluid to block muscarinic cholinergic receptors. The ED_{50} of histamine was determined by testing doses from 2×10^{-5} to 2×10^{-10} mol/L. The plant extract (6.25, 25, 50, 160 and 320 mg/L) was administered 2 min before the ED_{50} dose of histamine (2×10^{-8} mol/L) at 0.1 mL/bath. Each test was conducted in triplicate.

Analgesic properties. The methods used have been previously described (Lanhers *et al.*, 1991. For the Writhing test the plant extract was tested at 200, 400 and 800 mg/kg. Acetylsalicylic acid, a reference peripheral analgesic compound, was used at 68 mg/kg (ED_{50}). For the hot plate test, the plant extract was tested at 200, 400,

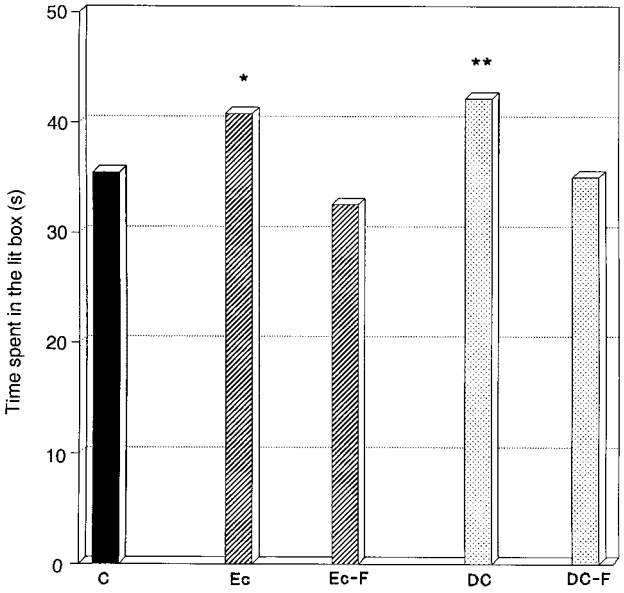

Figure 2. Influence of flumazenil on anxiolytic effects of *E. californica* extract in the light/dark choice situation test in mice. Behavioural parameters: time spent in the lit box. C, control; Ec, *E. californica* (25 mg/kg); DC, dipotassium chlorazepate (2 mg/kg); Ec-F, combination of *E. californica* (25 mg/kg) and flumazenil (10 mg/kg); DC-F, combination of dipotassium chlorazepate (2 mg/kg) and flumazenil (10 mg/kg). * $p < 0.05$. ** $p < 0.01$.

800 and 1600 mg/kg. Acetylsalicylic acid was used at 68 mg/kg and morphine sulphate at 9.2 mg/kg.

Statistics tests. Methods have been previously described (Lanhers *et al.*, 1991, 1996). According to different tests, the statistics were a combined analysis of variance and Newman–Keuls's method, the χ^{r2} test and a non-parametric test, Mann–Whitney–Wilcoxon's test.

RESULTS

Effects of flumazenil on sedative and anxiolytic activities of Ec

Open-field test. As can be seen in Fig. 1, Ec (200 mg/kg) significantly reduced the locomotor activity (−64%, versus control group) and rearing in mice (−65%); such sedative effects were partly antagonized by flumazenil used at 10 mg/kg (respectively −24% and −33%). Similar effects to Ec were obtained with midazolam (2 mg/kg) which also reduced the locomotor activity (−83%) and rearing (−91%); flumazenil also antagonized these effects (−2% and + 39%).

Light/dark choice situation test. As can be seen in Fig. 2, Ec (25 mg/kg) significantly increased the time spent by mice in the lit box; such anxiolytic effects were antagonized by flumazenil used at 1 mg/kg. Similar effects were obtained with clorazepate dipotassium used at 2 mg/kg; flumazenil also antagonized these effects.

Anticonvulsant and myorelaxant effects

The plant extract did not cause any protection against pentylenetetrazol effects, when it was tested at 25, 200 and 800 mg/kg (100% of tonic convulsions and mortality). Clorazepate dipotassium (reference benzodiazepine) possessed anticonvulsant effects from 10 mg/kg (0% of tonic convulsions and mortality).

The plant extract was inactive in the suspension test and in the rota-rod test from 100 to 400 mg/kg. A slight effect was observed at 800 mg/kg in the suspension test (50% of atony, $p < 0.05$). Clorazepate dipotassium induced a dose-dependent significant effect from 10 to 40 mg/kg in both experiments.

Neuroleptic effects

Group toxicity test. Dexamphetamine (40 mg/kg) produced mortality in all grouped animals. The plant extract (25 and 400 mg/kg) did not protect grouped mice from the toxic effects of dexamphetamine. On the other hand, chlorpromazine (20 mg/kg) exerted a protective effect (80%).

Dexamphetamine-induced stereotyped behaviour test. According to the results presented in Table 1, Ec significantly increased the stereotyped behaviour effects of dexamphetamine (+69% with 200 mg/kg; +79% with 800 mg/kg). Chlorpromazine (20 mg/kg) significantly decreased these stereotyped behaviour effects (−37%).

Catalepsy test. The plant extract (200 and 800 mg/kg) was inactive in the five tests, while chlorpromazine (20 mg/kg) was effective in all experiments.

Influence of chlorpromazine and Ec on the rectal temperature of the rats. Ec induced only a weak and transient hypothermia when it was tested at 200 mg/kg (−1.3 °C, 1 h after the treatment) and at 800 mg/kg (−0.9 °C and −1.7 °C respectively 1 and 2 h after the treatment). On the other hand, the rectal temperature was

Table 1. Influence of *E. californica* extract and chloropromazine on dexamphetamine-induced stereotypies in rats

Group	Cumulative stereotype score after 210 min	Variation (%)	*n*
Control	99	–	4
Chlorpromazine 20 mg/kg	62[a]	−37	4
E. californica 200 mg/kg	167[a]	+69	4
E. californica 800 mg/kg	177[b]	+79	4

Significantly different from control: [a] $p < 0.01$; [b] $p < 0.001$.
n: number of rats per group.

Table 2. Influence of *E. californica* extract and amitriptylline on reserpine effects (ptosis, akinesia and hypothermia) in mice

Group	Ptosis 2 h	Akinesia 2 h	Temperature °C 4 h
Control	5.7 ± 1.1	100	−6.4
E. californica 50 mg/kg	5.7 ± 10.6	100	−7.2
E. californica 100 mg/kg	4.7 ± 0.8	100	−7.1
E. californica 200 mg/kg	4.0 ± 0.9	100	−7.6
E. californica 400 mg/kg	5.8 ± 0.7	100	−7.2
E. californica 800 mg/kg	5.7 ± 0.6	100	−6.9
Amitriptylline 12 mg/kg	2.8 ± 1.1[a]	100	−2.6[b]

Significantly different from control: [a] $p < 0.05$; [b] $p < 0.01$.

progressively reduced by chlorpromazine (20 mg/kg) up to 3 h (−4.8 °C), it remained stable until 5 h, and then increased until 24 h.

Antidepressant properties

Antireserpine test. As can be seen in Table 2, Ec had no effect against reserpine-induced ptosis, akinesia or hypothermia, when it was tested at 50, 100, 200, 400 and 800 mg/kg; on the other hand, amitryptilline (12 mg/kg) significantly reduced the ptosis and hypothermia.

Anticholinergic test. Ec (200 and 800 mg/kg) did not antagonize hypothermia (Table 3) and shivers, lachrymation and salivation (results not shown) induced by oxotremorine (0.5 mg/kg)

Imipramine (5 mg/kg) was effective on all parameters, from 30 min after oxotremorine injection (0.5 mg/kg) and until 120 min.

Antihistaminic effects

The plant extract from 12.5 to 320 mg/L had no action in antagonizing the contractile response of histamine on guinea-pig ileum.

Analgesic properties

Writhing test. As can be seen in Fig. 3 Ec significantly reduced the number of writhes and stretches induced in mice by acetic acid 1.2% solution, with a dose of 200 mg/kg, the percentage of protection being 70%. This dose-dependent effect reached 85% with a dose of 800 mg/kg. The peripheral analgesic compound acetylsalicylic acid tested at 68 mg/kg, exerted a significant protective effect, inducing a protection of 60%.

Table 3. Influence of *E. californica* extract and imipramine on hypothermia induced by oxotremorine) in mice

Group	Temperature °C 30 min	Temperature °C 24 h
Control	−4.8	−6.8
Imipramine	−2.8[b]	−5.3[a]
E. californica 200	−5.2	−6.9
E. californica 800	−5.9	−7.1

Significantly different from control: [a] $p < 0.05$; [b] $p < 0.001$.

Hot plate test. The results presented in Table 4 show that Ec (200, 400, 800 and 1600 mg/kg) did not significantly increase the number of positive mice (percentages of positive mice: 20% to 30%). Acetylsalicylic acid was also inactive (0% of positive mice). On the other hand, morphine sulphate (9.2 mg/kg) was efficient (50% of positive mice).

DISCUSSION AND CONCLUSIONS

This study aimed at investigating the psychotropic effects of *Eschscholzia californica* (Ec) extract, in order to complete the pharmacological profile of an aqueous alcohol extract of Ec, along with the sedative and anxiolytic properties previously demonstrated (Rolland *et al.*, 1991: Rolland, 1988, 1991). The present results

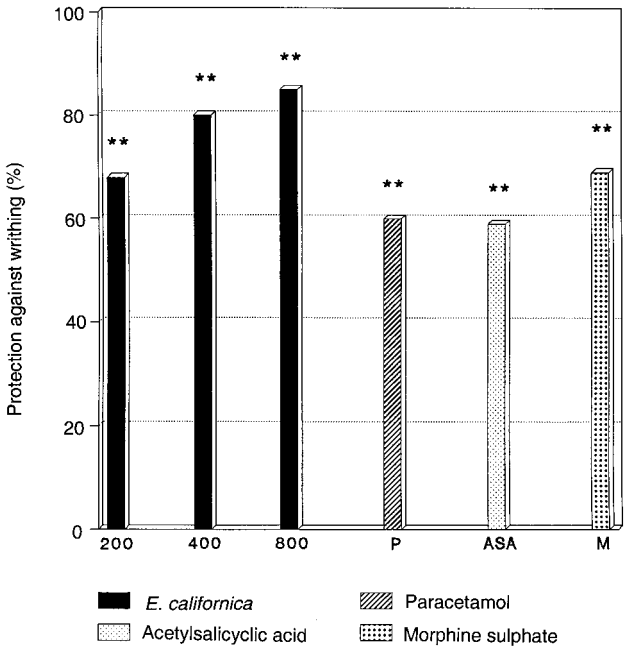

Figure 3. Influence of *E. californica* extract, paracetamol, acetylsalicyclic acid and morphine sulphate on the writhing and stretching induced in mice by acetic acid 1,2% solution (writhing test), 200, 400, 800, *E. californica* (200, 400 and 800 mg/kg); P, paracetamol (68 mg/kg); ASA acetylsalicyclic acid (68 mg/kg); M, morphine sulphate (1.15 mg/kg). ** $p < 0.01$.

Table 4. Influence of *E. californica* extract, acetylsalicylic acid and morphine sulphate on thermal stimulus-induced painful effects in mice (hot plate test)

Group	$T_b \pm SE$	$T_a \pm SE$ (%)	Variation animals (%)	Positive	*n*
E. californica 200 mg/kg	5.8 ± 0.4	6.0 ± 0.6	3	0	10
E. californica 400 mg/kg	5.3 ± 0.3	7.3 ± 0.8	38	20	10
E. californica 800 mg/kg	5.2 ± 0.8	8.9 ± 0.9	71	30	10
E. californica 1600 mg/kg	5.0 ± 0.4	7.7 ± 0.8	54	20	10
Acetylsalicylic acid 68 mg/kg	5.3 ± 0.3	4.7 ± 0.4	−11	0	10
Morphine 9.2 mg/kg	5.1 ± 0.3	9.4 ± 0.4	84	50[a]	10

T_b time before stimulus; T_a, time after stimulus.
Significantly different from control: [a] $p < 0.01$.

confirmed the sedative (reduction of behavioural parameters) and anxiolytic (increase of behavioural parameters) properties in two behavioural tests, the open field test and the light/dark compartment test, respectively. As the sedative and the anxiolytic effects of Ec were antagonized by the benzodiazepine receptor antagonist flumazenil, we suggest that benzodiazepine receptors may be involved in the sedative and anxiolytic properties of this extract.

However, unlike benzodiazepines, this plant extract was devoid of anticonvulsant properties against pentetylenetetrazole. It also lacked muscle relaxant activity in the suspension test and the rota rod test.

Ec was devoid of antipsychotic properties, since it did not protect mice against dexamphetamine-induced group toxicity, it did not induce catalepsy in rats and it failed to protect rats against dexamphetamine-induced stereotypy; on the contrary, Ec increased stereotyped behaviour. Such an action can be obtained with various compounds such as benzodiazepines and antidepressants (Treit, 1985). Ec induced a weak and transient hypothermia compared with the reference product chlorpromazine. Ec did not possess antidepressant-like properties since it had no effect against reserpine-induced ptosis, akinesia or hypothermia and against oxotremorine-induced shivers, lachrymation and hypothermia.

Since no antihistaminic effects were observed on the guinea-pig isolated ileum test, the sedative effect of Ec could not be correlated with the side effects of some antihistaminic properties.

However, dose-dependent peripheral analgesic effects of Ec were demonstrated from a dose of 200 mg/kg in the writhing test; the lack of effects in the hot plate test indicated that Ec did not possess a central analgesic action.

In conclusion, the sedative and anxiolytic effects of *E. californica* have been largely demonstrated; these effects of the extract were most likely linked to benzodiazepine receptor activation.

Peripheral analgesic effects were also shown. These results validate the traditional therapeutic use of this species and also justify the use of *E. californica* as a sedative phytomedicine according to French regulations. (Agence du Médicament, 1998).

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