



Antinociceptive and anti-inflammatory properties of the hydroalcoholic extract of stems from *Equisetum arvense* L. in mice

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Abstract

In this study, antinociceptive and anti-inflammatory effects of hydroalcoholic extract of stem from *Equisetum arvense* in mice were evaluated. The extract (10, 25, 50 and 100 mg kg⁻¹, i.p.), reduced the writhing induced by acetic acid in 49, 57, 93 and 98%, respectively. In the formalin test, 50 and 100 mg kg⁻¹ (i.p.) extract, reduced in 80 and 95% the licking activity in the first phase, but in the second phase only the latter dose diminished the licking time (35%). In both phases, naloxone failed to revert the analgesic effect of the extract. In the hot-plate test, the extract at 100 and 200 mg kg⁻¹ does not change the latency to licking or jumping. In the carrageenan-induced paw oedema, the extract at 50 mg kg⁻¹, reduced the paw oedema 2 h (25%) and 4 h (30%) after carrageenan administration. The dose of 100 mg kg⁻¹ caused reduction of the paw oedema (29%) only 4 h after carrageenan administration. These results indicate that this extract exhibits an antinociceptive effect in chemical models of nociception which is not related to the opioid system, as well as anti-inflammatory properties. © 2003 Elsevier Ltd. All rights reserved.

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1. Introduction

Equisetum arvense (Equisetaceae, traditional name: “horsetail”) is a plant showing aerial stems, branched with regular verticillies 2–23 mm in diameter, terminal strobile in the branches and in the main stem 10-mm long and 4 mm in diameter. It grows in several regions of Europe and North, Central and South America [1]. Several studies showed a hypoglycemic [2,3] and diuretic activity [4–8] of some species of horsetail. The plant present a popular use as an anti-inflammatory agent in bathing for skin disease in Europe, Asia and America, as well as antiseptic in Turkey and America [12–14].

Therefore, the purpose of the present study was to evaluate the antinociceptive and anti-inflammatory effects of hydroalcoholic extract of stems from *E. arvense* (HAE).

2. Materials and methods

2.1. Plant material and preparation of hydroalcoholic extract

The plant was collected in Santa Catarina State, South of Brazil, during the summer of 2002. Botanical material was classified by Dr. Claudete Schrage Nuernberg (Department of Agricultural Botanic, State University of Santa Catarina, Lages, Brazil). A voucher sample has been deposited at the Herbarium of the Medicinal Plants of the State University of Santa Catarina. The dried stem of *E. arvense* were minced and extracted with 50% ethanol–water, being stirred and macerated at room temperature (21 ± 3 °C) for 15 days. The ethanol was evaporated and the extract was stored in the concentration of 5% at –20 °C, until use. The extract was suspended in 0.9% NaCl solution to the desired concentration just before use. The phytochemical analysis of the HAE from *E. arvense* showed the presence of pirogalic tannins, sterols, saponins and flavonoids.

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2.2. Drugs

Morphine (Dimorph[®], Cristália, Brazil), naloxone (Sigma, USA), acetyl salicylic acid (Aspirina[®], Bayer, Brazil), lambda carrageenan type IV (FMC Corporation, USA), paraformaldehyde (Alpha Aesar, USA), acetic acid (Synth, Brazil).

2.3. Animals

Male Swiss mice weighing 25–30 g were used. The animals were housed in standard environmental conditions ($22 \pm 1^\circ\text{C}$, humidity $60 \pm 5\%$ and a 12 h/12 h dark/light cycle—light on at 7.00 a.m.), with food and water available ad libitum. Experiments were performed during the mid-light phase of the dark/light cycle and the animals were maintained in accordance to the Guide for the Care and Use of Laboratory Animals, US Department of Health and Human Services, 1985.

2.4. Acetic acid-induced writhing

The abdominal constriction was induced in mice by intraperitoneal injection of acetic acid (0.6%), as described by Collier et al. [15]. Animals were pre-treated with the HAE (10, 25, 50 and 100 mg kg^{-1} , i.p.), 30 min before acetic acid administration. Control animals received a similar volume of saline solution (10 ml kg^{-1}). The number of abdominal constrictions (full extension of both hind paws) was cumulatively counted over a period of 20 min.

2.5. Formalin test

The observation chamber was a glass cylinder of 20 cm diameter on an acrylic transparent plate floor. Beneath the floor, a mirror was mounted at a 45° angle to allow clear observation of the paws of the animals. The mice were treated with 0.9% saline solution (i.p.), morphine (10 mg kg^{-1} , s.c.) or HAE (10, 25, 50 and 100 mg kg^{-1} , i.p.) or acetyl salicylic acid (100 mg kg^{-1} , i.p.) 30 min before formalin injection. Other two groups received naloxone (2 mg kg^{-1} , i.p.) 10 min before both morphine (10 mg kg^{-1} , s.c.) and HAE (100 mg kg^{-1} , i.p.) Each animal was placed in the chamber for 5 min before treatment in order to allow acclimatization to the new environment. The formalin test was carried out as described by Hunskaar and Hole [16]. Twenty microlitres of a 2.5% formalin solution (0.92% formaldehyde) in phosphate buffer were injected intraplantarly in the right hind paw. The animal was then returned to the chamber and the amount of time that it spent licking the injected paw was considered as indicative of pain. Two distinct phases of intensive licking activity were identified: an early acute phase and a late or tonic phase (0–5 and 15–30 min after formalin injection, respectively).

2.6. Hot-plate test

The hot-plate test was used to measure response latencies according to the method described by Eddy and Leimback [17]. The mice were treated with saline solution, morphine (10 mg kg^{-1} , s.c.) or HAE (100 and 200 mg kg^{-1} , i.p.) placed individually on a hot plate (Ugo Basile, Italy) maintained at $56 \pm 1^\circ\text{C}$ and the time between placement of the animal on the hot plate and the occurrence of either the licking of the hind paws, shaking or jumping off from the surface was recorded as response latency. Mice with baseline latencies of more than 20 s were eliminated from the study and the cut-off time for the hot-plate latencies was set at 30 s. The hot-plate paw-licking latencies were measured 0, 30, 60 and 90 min, but the animals were treated 30 min before of the assay.

2.7. Carrageenan-induced paw inflammation assay

Paw oedema was induced in mice by sub-plantar injection of $100\ \mu\text{l}$ of 1% sterile carrageenan lambda in saline into right hind paw. The animals were treated with saline solution, or hydroalcoholic extract of stems from *E. arvense* (25, 50 and 100 mg kg^{-1} , i.p.) or acetyl salicylic acid (100 mg kg^{-1} , i.p.). The volume (ml) of the paw was determined with a plethysmometer (Ugo Basile), as describe by Winter et al. [18] in the followed periods: just before administration of carrageenan (initial volume), 1, 2, 4 and 24 h after carrageenan injection (final volume). The paw oedema was determined using the following formula: oedema = final volume – initial volume.

2.8. Statistical analysis

All data were expressed as mean \pm S.E.M., and the statistical significance was determined used a two-way analysis of variance ANOVA followed by Student–Newman–Keuls multiple comparison test. Values were considered significantly different at $P < 0.05$. The percent of inhibition was determined for each experimental group using the following formula: inhibition (%) = $100 \times [(\text{control} - \text{experiment}) / \text{control}]$.

3. Results

The results obtained with acetic acid-induced writhing are shown in Table 1. All dose administered (10, 25, 50 and 100 mg kg^{-1} , i.p.) had a significant effect on the number of abdominal constrictions, promoting 49, 57, 93 and 98% inhibition, respectively, as compared with the control group treated with saline. Acetyl salicylic acid at dose of 100 mg kg^{-1} (i.p.) used as control shown 93% inhibition.

Table 2 shows the results obtained with the formalin test. During the first phase (0–5 min), the hydroalcoholic extract at dose of 100 mg kg^{-1} reduced the licking activity

Table 1
Effect of the hydroalcoholic extract of stems from *E. arvense* in acetic acid-induced writhing response in mice^a

Groups (n = 10)	Writhing response	Percent
Control	17.6 ± 4.0	–
HAE 10	9.0 ± 2.3*	49
HAE 25	7.5 ± 1.6*	57
HAE 50	1.3 ± 0.3*	93
HAE 100	0.3 ± 0.2*	98
ASA	0.6 ± 0.3*	97

HAE: hydroalcoholic extract of *E. arvense*; ASA, acetyl salicylic acid (100 mg kg⁻¹). ANOVA followed by Student–Newman–Keuls post hoc.

^a The writhing response was expressed as mean ± S.E.M.

* *P* < 0.01.

by 35%. As a comparison, the administration of morphine (10 mg kg⁻¹, s.c.), reduced the licking time by 91% and this effect was totally antagonized by naloxone (2 mg kg⁻¹, i.p.). Nevertheless, naloxone failed to revert the analgesic effect of hydroalcoholic extract. In regard to the second phase (15–30 min), the doses of 50 and 100 mg kg⁻¹ of hydroalcoholic extract diminished the licking time by 80 and 95%, respectively, as compared with control group. Indeed, the late dose has a similar effect to the standard drug, acetyl salicylic acid (100 mg kg⁻¹, i.p.), which reduced the licking activity by 94%.

In the hot-plate test, hydroalcoholic extract at doses of 100 and 200 mg kg⁻¹ (i.p.) failed to change the latency time to licking or jumping, when compared with control animals (Table 3). Morphine at dose 10 mg kg⁻¹ (s.c.) produced a significant effect when compared to control group.

In the carrageenan-induced paw oedema test, the extract at 50 mg kg⁻¹ promoted a reduction of 25 and 29% in the paw

Table 2
Effect of the hydroalcoholic extract of stems from *E. arvense* in the early (0–5 min) and late (15–30 min) phases of the formalin test in mice^a

Group (n = 10)	Licking time (s)		Inhibition (%)	
	Early phase	Late phase	Early phase	Late phase
Control	82.1 ± 4.4	113.7 ± 8.7	–	–
HAE 25	58.1 ± 6.1	92.2 ± 10.5	29	19
HAE 50	66.1 ± 2.9	22.3 ± 7.4**	19	80
HAE 100	53.6 ± 7.2*	6.1 ± 2.2**	35	95
ASA	83.8 ± 6.2	6.8 ± 2.8**	NA	94
MOR	7.5 ± 2.7**	9.7 ± 3.3**	91	91
MOR + NAL	83.3 ± 10.4 [#]	82.5 ± 7.2 [#]	NA	27
EHA 100 + NAL	47.5 ± 5.6**	11.6 ± 2.0**	42	90

HAE: hydroalcoholic extract of *E. arvense*; ASA, acetyl salicylic acid (100 mg kg⁻¹); MOR: morphine (10 mg kg⁻¹); MOR + NAL: morphine (10 mg kg⁻¹) + naloxone (2 mg kg⁻¹); EHA 100 + NAL: hydroalcoholic extract of *E. arvense* (100 mg kg⁻¹) + naloxone (2 mg kg⁻¹). NA: not available, because the control value is greater than value of the group in question. ANOVA followed by Student–Newman–Keuls.

^a The licking time in both early and late phase was expressed as mean ± S.E.M.

* *P* < 0.05, compared with control group.

** *P* < 0.01, compared with control group.

[#] *P* < 0.01, compared with morphine group.

Table 3
Effect of the hydroalcoholic extract of stems from *E. arvense* in hot-plate test in mice^a

Group (n = 10)	Latency (s)			
	0 min	30 min	60 min	90 min
Control	15.6 ± 2.8	14.2 ± 3.4	14.7 ± 2.3	14.9 ± 2.1
HAE 100	12.9 ± 1.8	14.7 ± 1.5	14.6 ± 2.5	13.2 ± 2.1
HAE 200	16.1 ± 1.4	15.5 ± 1.8	15.1 ± 2.1	16.6 ± 2.8
MOR	13.9 ± 1.4	30 ± 0*	29 ± 0.9*	25.7 ± 1.4*

The animals were pre-treated 30 min before of the assay. HAE: hydroalcoholic extract of *E. arvense*; MOR: morphine (10 mg kg⁻¹). ANOVA followed by Student–Newman–Keuls post hoc.

^a The latency for licking of the hind paws, shaking or jump off from the surface is expressed as mean ± S.E.M.

* *P* < 0.01, compared with control group.

oedema, respectively, 2 and 4 h after carrageenan administration. Whereas, the dose of 100 mg kg⁻¹ reduced the paw oedema (30%) only 2 h after carrageenan administration.

4. Discussion

According to our findings, the hydroalcoholic extract of stems from *E. arvense* produced an antinociceptive effect when assessed in chemical models of nociception, including acetic acid-induced writhing and formalin tests; however it failed to exhibit effect in a thermal model of nociception, the hot-plate test.

In acetic acid-induced writhing, a dose-related antinociceptive effect of the extract was seemed. Collier et al. [15] proposed that the acetic acid acts indirectly by inducing the release of endogenous mediators which stimulate the nociceptive neurons sensitive to non-steroidal anti-inflammatory drugs (NSAIDs) and opioids.

As in the acetic acid-induced writhing, the extract exhibited an antinociceptive action in the formalin test. This model produces a distinct biphasic nociception. The first phase starts immediately after the formalin injection and continues for 5 min, after which nociception appears to diminish. The second phase is characterized by a return to high levels of nociception beginning 15–30 min after the formalin injection and continued until 60 min [16]. These phases have different properties and are very useful tools, not only for assessing the potency of analgesic, but also for elucidating the mechanisms of pain and analgesia. The action of analgesic is different in the early (neurogenic) and late (inflammatory) phase. Drugs such as narcotics which primarily act centrally, inhibit both phases equally [19,20], but peripherally acting drugs such as NSAIDs and dexamethasone only inhibit the second phase of formalin-induced nociception [20,21]. These drugs attenuate the pain by inhibition of cyclooxygenase in arachidonic acid pathways [22].

Since the hydroalcoholic extract of *E. arvense* inhibited both phases of the formalin test, a central action can be suggested for it and seems to act independently from the opioid

Table 4
Effect of the hydroalcoholic extract of *E. arvense* in carrageenin-induced hind paw oedema in mice^a

Group (n = 10)	Hind paw oedema (ml)				Inhibition (%)			
	1 h	2 h	4 h	24 h	1 h	2 h	4 h	24 h
Control	0.6 ± 0.13	1.4 ± 0.08	1.4 ± 0.11	1.4 ± 0.16	–	–	–	–
HAE 25	0.8 ± 0.11	1.1 ± 0.11	1.4 ± 0.08	1.3 ± 0.08	NA	22	NA	09
HAE 50	0.8 ± 0.13	1.0 ± 0.12*	1.0 ± 0.10*	1.3 ± 0.07	NA	25	29	09
HAE 100	0.8 ± 0.15	1.0 ± 0.15*	1.3 ± 0.12	1.3 ± 0.13	NA	30	04	05
ASA	0.7 ± 0.07	0.9 ± 0.08*	1.2 ± 0.12	0.5 ± 0.10**	NA	38	14	61

HAE: hydroalcoholic extract of *E. arvense*; ASA: acetyl salicylic acid (100 mg kg⁻¹). NA: not available, because the control value is greater than value of the group in question. ANOVA followed by Student–Newman–Keuls.

^a The hind paw oedema was expressed as mean ± S.E.M.

* *P* < 0.05 compared with control group in the same period.

** *P* < 0.01 compared with control group in the same period.

system, but how the extract affects these phases is not clear. On the other hand, the extract failed to promote antinociceptive effect in the hot-plate model of analgesia, which measures nociception induced by central mechanisms. Obviously, a sedative effect of the extract would have diminished the licking activity, especially in the dose of 100 mg kg⁻¹. However, a pilot study from our laboratory indicates that the extract shows sedative effects only at doses higher than 200 mg kg⁻¹. Thus, the reduced licking activity observed in this work is likely due to an analgesic effect.

In addition, as showed in Table 4, the extract has significant anti-inflammatory effects. Because inflammation is a peripheral process, this suggests that the extract also presents peripheral effects. In fact, the licking activity in the formalin test was strongly diminished in the second phase, whereas this reduction was more discreet in the first phase. In this view point, the extract seems to act by both by central and peripheral mechanisms.

Phytochemical analysis of the hydroalcoholic extract of stems from *E. arvense* performed in our laboratory revealed the presence of tannins, saponins, flavonoids and sterols. D'Agostino et al. [23] showed which sterol fraction of hydroalcoholic extract from *E. arvense* contains β-sitosterol (60%), campesterol (32.9%) and isofucosterol (5.9%). These compounds have well-known anti-inflammatory effects [10,11,24–26]. Gomez et al. [27] and Navarro et al. [28] speculated that the anti-inflammatory activity of these compounds is markedly influenced by the inhibition of neutrophil migration into inflamed tissue. Indeed, Ali and Houghton [29] verified a lipoxygenase inhibitory activity from β-sitosterol. Otherwise, Santos et al. [30] obtained antinociceptive effect of β-sitosterol isolated from *Phyllanthus corcovadensis*. Thus, we suggest that β-sitosterol may be, at least in part, responsible for the effect observed with our extract in this work.

Broudiscou and Lassalas showed that the flavonoids portion of hydroalcoholic extract of *E. arvense* contain basically isoquercitrin [31]. This compound displayed a significant antioxidant effect [32–37]. Chanh and colleagues [9] observed that isoquercitrin inhibits both the biosynthesis and the release of PG-like substances. Indeed, isoquercitrin increases

brain cGMP levels and potentiates electroacupuncture analgesia [38]. Thus, this compound presents both central and peripheral action.

To summarize, the hydroalcoholic extract of stems from *E. arvense* possesses analgesic effect against chemical models of nociception, but not in the thermal models. This action is both central and peripheral, but the exact mechanism remains in question. It is likely that the opioid system does not play a role in the antinociceptive effects of extract. Indeed, the extract presents a clear anti-inflammatory effects. Flavonoids, sterols and others compounds (saponins and tannins) can be related, at least in part, to the analgesic and anti-inflammatory effects of hydroalcoholic extract of *E. arvense*. Obviously, further studies will be necessary to understand the mechanisms of action underlying the effects of the extract and their active compounds.

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