Antinociceptive Effect of Heliopsis longipes Extract and Affinin in Mice

Article *in* Planta Medica · November 2010

DOI: 10.1055/s-0029-1240658

CITATIONS
36

READS
484

3 authors:

Myrna Deciga-Campos
IPN
81 PUBLICATIONS **1,457** CITATIONS
SEE PROFILE

Yolanda Rios Gomez
Universidad Autónoma del Estado de Morelos
134 PUBLICATIONS **2,186** CITATIONS
SEE PROFILE

Angelica Aguilar
Universidad Autónoma del Estado de Morelos
43 PUBLICATIONS **549** CITATIONS
SEE PROFILE

Planta Medica

Journal of Medicinal Plant and Natural Product Research

Publishers

Georg Thieme Verlag KG
Stuttgart · New York
Rüdigerstraße 14
D-70469 Stuttgart
Postfach 30 11 20
D-70451 Stuttgart

Thieme Publishers
333 Seventh Avenue
New York, NY 10001, USA
www.thieme.com

Antinociceptive Effect of *Heliopsis longipes* Extract and Affinin in Mice

Authors **Myrna Déciga-Campos**[1], **María Yolanda Rios**[2], **A. Berenice Aguilar-Guadarrama**[2]

Affiliations
[1] Sección de Estudios de Posgrado e Investigación, Escuela Superior de Medicina, Instituto Politécnico Nacional, Col. Santo Tomás, México, México
[2] Centro de Investigaciones Químicas, Universidad Autónoma del Estado de Morelos, Col. Chamilpa, Cuernavaca, Morelos, México

Key words
- *Heliopsis longipes*
- Compositae
- affinin
- antinociceptive activity
- capsaicin test
- writhing test

received July 29, 2009
revised October 24, 2009
accepted Nov. 2, 2009

Bibliography
DOI http://dx.doi.org/10.1055/s-0029-1240658
Published online November 30, 2009
Planta Med 2010; 76: 665–670

ISSN 0032-0943

Correspondence
Myrna Déciga-Campos, Ph.D.
Sección de Estudios de Posgrado e Investigación
Plan de San Luis y Díaz Mirón s/n
Colonia Santo Tomas
Delegación Miguel Hidalgo
11340 México D. F.
México
Phone: + 525 5 57 29 63 00 ext. 5729
mdeciga@ipn.com.mx

Correspondence
María Yolanda Rios, Ph.D.
Centro de Investigaciones Químicas
Avenida Universidad 1001
Colonia Chamilpa,
62209 Cuernavaca
Morelos
México
Phone: + 52 7 77 3 29 70 00 ext. 6024
myolanda@uaem.mx

Abstract

Heliopsis longipes is used as analgesic in Mexican traditional medicine. The present study assesses the possible antinociceptive effect of *Heliopsis longipes* and describes the pharmacological mechanism of action of the antinociceptive effect of affinin, identified as the one active principle in *Heliopsis longipes* acetone extract. Intraperitoneal administration of *H. longipes* extract and affinin produced a dose-dependent antinociceptive effect when assessed in mice submitted to acetic acid and capsaicin tests. Affinin-induced antinociception (30 mg/kg, *i.p.*) was blocked by naltrexone (1 mg/kg, *s.c.*), *p*-chlorophenylalanine (80 mg/kg, *i.p.*) and flumazenil (5 mg/kg, *s.c.*) suggesting that its pharmacological effect could be due to the activation of opiodergic, serotoninergic and GABAergic systems. In addition, the antinociceptive effect of affinin was attenuated by pretreatment with 1*H*-[1,2,4]oxadiazolo[1,2-*a*]quinoxalin-1-one (1 mg/kg, *s.c.*) and glibenclamide (10 mg/kg, *s.c.*) suggesting that the nitric oxide-K^+ channels pathway could be involved in its mechanism of action. These results suggest that affinin itself or its derivatives may have potential antinociceptive effects.

Introduction

Heliopsis longipes (A. Gray) Blake (Compositae), known in Mexico under the popular names of "chilcuague" or "chilcuan", is used to treat complaints associated with pain, particularly toothache [1]. This plant is still widely used as a condiment in the areas of its natural occurrence. *H. longipes* is distributed in Mexico in the states of Guanajuato, San Luis Potosí and Queretaro [2,3]. Affinin [*N*-isobutyl-2(*E*),6(*Z*),8(*E*)-decatrienamide (**Fig. 1**)], is the major lipidic component of the plant [4–6]. In a previous study, it was demonstrated that affinin has fungistatic (*Sclerotium rolfsii*, *Sclerotium cepivorum, Phytophthora infestans* and *Rhizoctonia solana)* and bacteriostatic (*Escherichia coli* and *Bacillus subtilis)* activities [7]. In Mexico, *H. longipes* is used as a local analgesic and anesthetic [1,5]. It produces intense numbness and a tingling sensation in the lips, tongue and mouth and it stimulates salivation [5]. The use of *H. longipes* for treating the symptoms of pain in folk medicine suggests the presence of compounds with analgesic and/or anti-inflammatory properties [1,8,9]. Accordingly, the *H. longipes* extract has shown to produce anti-hyperalgesic effects in carrageenan-induced thermal hyperalgesia [8]. This effect could be produced by affinin, by increasing γ-amino butyric acid (GABA) release in mice brain slices [9]. However, the effects of affinin as well as its possible mechanisms of action are at present unknown. Thus, the aim of the present research was to assess the antinociceptive effect of the acetone extract of *H. longipes* and a compound obtained from such extract, affinin, as well as the possible mechanisms of antinociceptive activity. Two well-known animal tests were used in this study to accomplish such an endeavor, the writhing and capsaicin tests.

Material and Methods

Animals

Experiments were performed on male *ICR* mice (body weight range, 25–30 g) from Centro Harlan (Harlan México). All experiments followed the Guidelines on Ethical Standards for Investigation of Experimental Pain in Animals [10] and their care was conducted in conformity with Mexican Official Norm for Animal Care and Handing (NOM-062-ZOO-1999). Efforts were made to

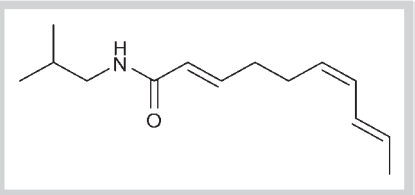

Fig. 1 Chemical structure of *N*-isobutyl-2(*E*),6(*Z*),8(*E*)-decatrienamide (affinin) obtained from the extract of *Heliopsis longipes*.

minimize animal suffering and to reduce the number of animals used. Animals were housed in groups of six per standard cage on climate controlled room (22 ± 2 °C) with a 12-h light/dark cycle. At the end of the experiment, animals were sacrificed in a CO_2 chamber.

Plant material

Fresh stems of *H. longipes* (Compositae) were collected in "Real de Xichu", Guanajuato, México, in July 2007, and identified by Ramiro Ríos Gómez, MSc, Universidad Nacional Autonóma de México. A voucher specimen (number 5904) was deposited at the FEZA Herbarium of the Facultad de Estudios Superiores Zaragoza, Universidad Nacional Autonóma de México, México.

Extraction and isolation

Fresh stems of *H. longipes* (4.725 kg) were extracted at room temperature with acetone (3 × 24 L, 48 h each). The extraction solvent was concentrated to dryness in vacuum to render 76.5 g of extract. Fractionation of the acetone extract by means of open column chromatography (silica gel, 100–230 mesh; Natland Int. Corp.; 11.5 × 50 cm) was performed with a step gradient of *n*-hexane-acetone 100 : 0 to 40 : 60, collecting 124 fractions of 250 mL each. The presence of affinin [*N*-isobutyl-2(*E*),6(*Z*),8(*E*)-decatrienamide] in the obtained fractions was monitored by TLC using one authentic sample of affinin (Rf = 0.33, *n*-hexane-acetone 80 : 20) as reference, visualizing with a UV lamp (affinin develops a dark gray spot at 254 nm), and by spraying with a 1% solution of $(NH_4)_4Ce(SO_4)_4 \cdot H_2O$ in 2 N H_2SO_4 (were affinin develops a brilliant yellow spot). On the basis of TLC the fractions 17–29 (4000–7250 mL, eluent *n*-hexane-acetone, 9 : 1), 30–73 (7251–18 250 mL, eluent *n*-hexane-acetone, 8 : 2 to 7 : 3), and 74–107 (18 251–26 750 mL, *n*-hexane-acetone, 6 : 4) were combined because they afforded practically only affinin. This compound was further purified from these fractions by means of flash column chromatography: fractions 17–29 (2.39 g, silica gel, 230–400 mesh; Natland Int. Corp.; 3 cm i.d. ×10 inch, eluent *n*-hexane-CH_2Cl_2 70 : 30, flow rate 2 mL/min) collecting 11 fractions of 25 mL each, yielding 1.97 g (2.58%) of pure affinin, fractions 30–73 (9.34 g, silica gel, 230–400 mesh; 6 cm i.d. ×10 inch, eluent *n*-hexane-CH_2Cl_2 70 : 30, flow rate 2 mL/min) collecting 33 fractions of 50 mL each, yielding 7.34 g (9.59%) of pure affinin, and fractions 74–107 (5.55 g, silica gel, 230–400 mesh; 4 cm i.d. ×10 inch, eluent *n*-hexane-CH_2Cl_2 70 : 30, flow rate 2 mL/min) collecting 21 fractions of 35 mL each, yielding 4.78 g (6.25%) of pure affinin. The total yield of pure affinin was 14.09 g (18.42%) with respect to extract weight. The structure of the compound, affinin, was established on the basis of its IR, UV, ^{1}H- and ^{13}C-NMR, DEPT, COSY, HSQC, HMBC, and MS data and their comparison with those previously reported [1]. The purity of affinin (96.15%) was established by means of NMR and by the comparison of its retention time (20.57 min) with respect to an authentic sample on GC-MS analysis (*vide infra*).

GC-MS analyses

GC-MS analyses were obtained using an Agilent 6890 GC System/5973 MSD chromatograph equipped with an HP-1 capillary column (length 30 m, i.d. 0.25 mm, 0.25 ím). The carrier gas was helium, and the linear gas velocity was 36 cm/s. The injector temperature was 250 °C, and the column temperature, initially at 45 °C, was gradually increased at a rate of 10 °C/min to 250 °C. For detection, a flame ionization detector at 280 °C, IE (scan 30–550 u), was used. The identification of affinin as total extract and as pure compound was based on the comparison of its retention time (20.57 min) with respect to an authentic sample, and the comparison of its mass spectrum with those contained in the N-15598 Mass Spectral Library. On GC-MS analysis, fresh stems of *H. longipes* extracted with acetone showed eight principal peaks at: 20.71 min (affinin, 80.307%), 20.81 min (affinin isomer, 1.591%), 20.91 min (2(*E*),6(*Z*),8(*E*)-decatrienoic acid bornyl ester, 0.523%), 21.54 min [*N*-(2-methylbutyl)-2(*E*),6(*Z*),8(*E*)-decatrienamide isomer, 3.396%], 21.61 min [*N*-(2-methylbutyl)-2(*E*),6(*Z*),8(*E*)-decatrienamide, 6.880%], 22.42 min (unknown, 3.489%), 22.85 min (unknown, 1.085%) and 23.31 min (unknown, 2.297%).

Chemicals and drugs

Affinin was isolated from the fresh stems of *H. longipes* by acetone extraction. 1*H*-[1,2,4]-oxadiazolo[4,2-*a*]quinoxalin-1-one (ODQ), glibenclamide, flumazenil, 4-chloro-DL-phenylalanine (PCPA), naltrexone, capsaicin, metamizol and carboxymethylcellulose were purchased from Sigma. Acetic acid and morphine were purchased from Baker and Laboratorios Pisa, respectively. All drugs were of the highest-purity grade (99.9%). *H. longipes* extract, affinin, and PCPA were suspended in carboxymethylcellulose 1%. Glibenclamide, morphine, naltrexone, ODQ, metamizol and flumazenil were suspended in saline solution (0.9%). Capsaicin was dissolved in vehicle (containing 10% ethanol, 10% Tween 20, and 80% phosphate buffered saline [pH 6.2]) as previously described by Sakurada et al. [11]. All drugs were freshly prepared each time and administered (subcutaneous or intraperitoneal) in a volume of 0.1 mL/10 g body weight. Capsaicin was administered in a volume of 20 µL in the right paw. Control animals received the same volume of vehicle (carboxymethylcellulose 1% in saline solution 0.9%).

Measurement of antinociceptive activity

Acetic acid-induced abdominal writhes: The abdominal writhes were performed as previously described by Koster et al. [12]. The total number of writhes following the intraperitoneal (*i.p.*) administration of 0.6% acetic acid (10 mL/kg) was assessed for 30 min after the injection. Logarithmic doses were administered of the extract of *H. longipes* (0.01–1 mg/kg, *i.p.*) and affinin (1–1.875 mg/kg, *i.p.*) 15 min before acetic acid injection. Control animals received a similar volume of vehicle (carboxymethylcellulose 1%) or the morphine as a positive control (log dose 1.875 mg/kg, *i.p.*). Animals were then placed in a Plexiglas observation chamber for 15 min to allow them to accommodate to their surroundings, then they were removed for algesic administration. Nociceptive behavior was induced by intraperitoneal acetic acid 0.6%. Mice were then returned to the chamber for observation. Nociceptive behavior was quantified as the number of writhes (abdominal constrictions) produced in the treated animals during 5-min periods up to 30 min after algesic injection.

Capsaicin-induced nociception: In an attempt to provide more direct evidence concerning the possible antinociceptive effects of

This is a copy of the author's personal reprint

an extract of *H. longipes* and affinin on neurogenic nociception, we also investigated whether these decrease capsaicin-induced nociception in the mouse paw. The procedure used was similar to that described previously by Sakurada et al. [11], with a minor modification. Before testing, the animals were placed individually in a transparent glass cylinder, 20 cm in diameter, serving as a observation chamber. After the adaptation period, 20 µL of capsaicin (1.6 µg/paw) were injected subcutaneously into the dorsal surface of the right hind paw with a 26-gauge needle. The animal was then returned to the chamber for observation and nociceptive behavior was observed immediately after capsaicin injection. A mirror was placed behind the chamber to enable unhindered observation. Nociceptive behavior was quantified as the amount of time spent licking the injected paw which was timed with a chronometer and was considered as being indicative of nociception. Logarithmic doses of the extract of *H. longipes* (0.01–1.75 mg/kg, *i.p.*) and affinin (1–1.875 mg/kg, *i.p.*) were administered 15 min before capsaicin injection. Control animals received a similar volume of vehicle (carboxymethylcellulose 1% in saline solution 0.9%) or morphine as a positive control (log dose 1.875 mg/kg, *i.p.*).

Analysis of the possible mechanism of action of affinin on acetic acid-induced writhes in mice: To investigate the participation of the GABAergic, opiodergic and serotoninergic mechanisms in the writhing test, animals were pretreated with: naltrexone (1 mg/kg, *s.c.*), an antagonist of opioid receptors, PCPA (80 mg/kg, *i.p.*, for four consecutive days), an inhibitor of serotonin synthesis, and flumazenil (5 mg/kg, *s.c.*), a benzodiazepine site antagonist at the $GABA_A$ receptor. These animals received affinin (30 mg/kg, *i.p.*) or vehicle (10 mL/kg, *i.p.*) 15 min before acetic acid (0.6%, *i.p.*) injection, and the antinociceptive effect was recorded as described above. We also investigated the possible participation of the nitric oxide pathway in the antinociceptive effect of affinin. For this, animals were pretreated 15 min before affinin administration (30 mg/kg, *i.p.*) with ODQ (1 mg/kg, *s.c.*), an inhibitor soluble guanylyl cyclase, and glibenclamide (10 mg/kg, *s.c.*), a blocker of ATP-sensitive K^+-channels. 15 min after affinin administration the animals were injected with acid acetic (0.6%, *i.p.*) and the number of writhes was quantified.

Statistical analysis

All experimental results are given as the mean ± S.E.M. of the data obtained in 8 animals per group. Analysis of variance followed by the Tukey test was used to test the significance of differences with three or more treatments. Differences with a $p < 0.05$ were considered significant.

Results

Intraperitoneal 0.6% acetic acid administration in mice produces writhes. Systemic administration of logarithmic doses of *H. longipes* extract (0.01–1 mg/kg, *i.p.*) and affinin purified from *H. longipes* extract (1–1.875 mg/kg, *i.p.*) significantly reduced ($p < 0.05$) the number of abdominal constrictions in a dose-dependent manner when tested in the writhing model (**Fig. 2A** and **B**).

Morphine, a standard opiate analgesic drug (used as a positive control) significantly reduced the number of writhes induced by acetic acid (**Fig. 2**). Linear logarithmic data showed that systemic administration of *H. longipes* extract ($DE_{50} = 2.2 \pm 0.2$ mg/kg, *i.p.*) was more potent than affinin ($DE_{50} = 36 \pm 5$ mg/kg, *i.p.*) in nociception induced by acetic acid.

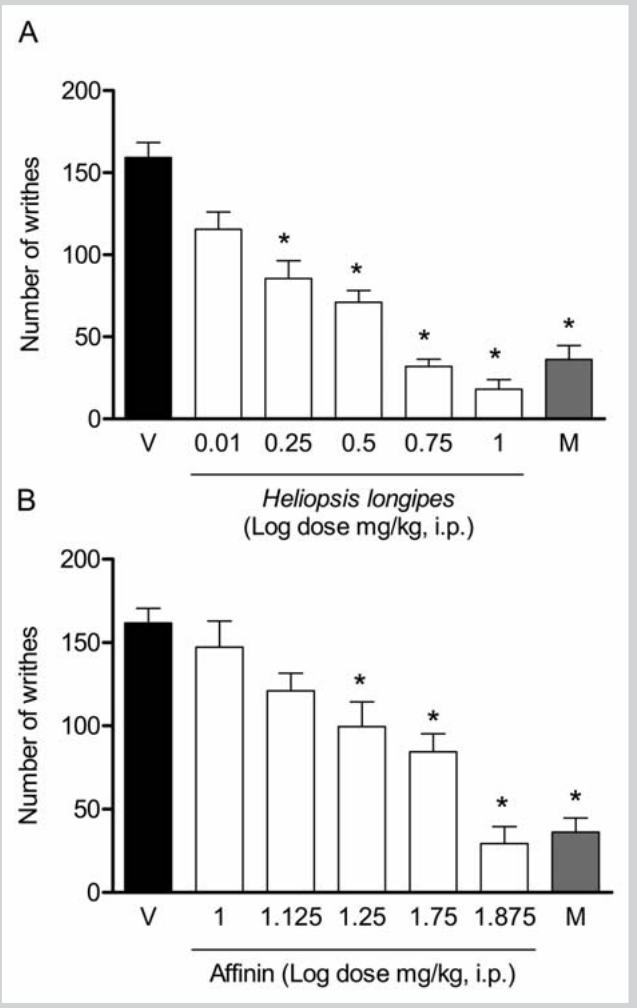

Fig. 2 Antinociceptive effect of *H. longipes* extract (**A**) and affinin (**B**) in the acetic acid-induced writhes. Morphine (M, log dose 1.875 mg/kg, *i.p.*), used as a positive control, was given 15 min before the noxious stimulation. Data are expressed as the total number of writhes counted over a 30-min period. Bars are the means of eight mice ± S.E.M. * Significantly different from the vehicle (V) group ($p < 0.05$), as determined by analysis of variance followed by the Tukey test.

Fig. 3A and **B** shows that intraperitoneal administration of logarithmic doses of *H. longipes* extract (0.01–1.75 mg/kg, *i.p.*) and affinin (1–1.875 mg/kg, *i.p.*) reduced nociceptive behaviors (licking) induced by capsaicin. The maximum antinociceptive effect observed with the extract was 65.8% (log dose 1.75 mg/kg, *i.p.*) whereas affinin showed a maximum antinociceptive effect of 46.67% (log dose 1.875 mg/kg, *i.p.*). Morphine (used as a positive control) significantly reduced the licking time in capsaicin-induced nociception (**Fig. 3**). Thus, the results clearly reveal that extract *H. longipes* is able to produce antinociception in two different nociceptive tests. One of the compounds involved in this effect is affinin that showed part of this antinociceptive effect.

Systemic pretreatment with naltrexone (1 mg/kg, *s.c.*), PCPA (80 mg/kg, *i.p.*, once a day for four consecutive days) and flumazenil (5 mg/kg, *s.c.*) before injection of the vehicle did not modify the behavior generated by acid acetic injection. However, when naltrexone, PCPA or flumazenil was administered before affinin (30 mg/kg, *i.p.*), all of them were able to partially reverse the anti-

This is a copy of the author's personal reprint

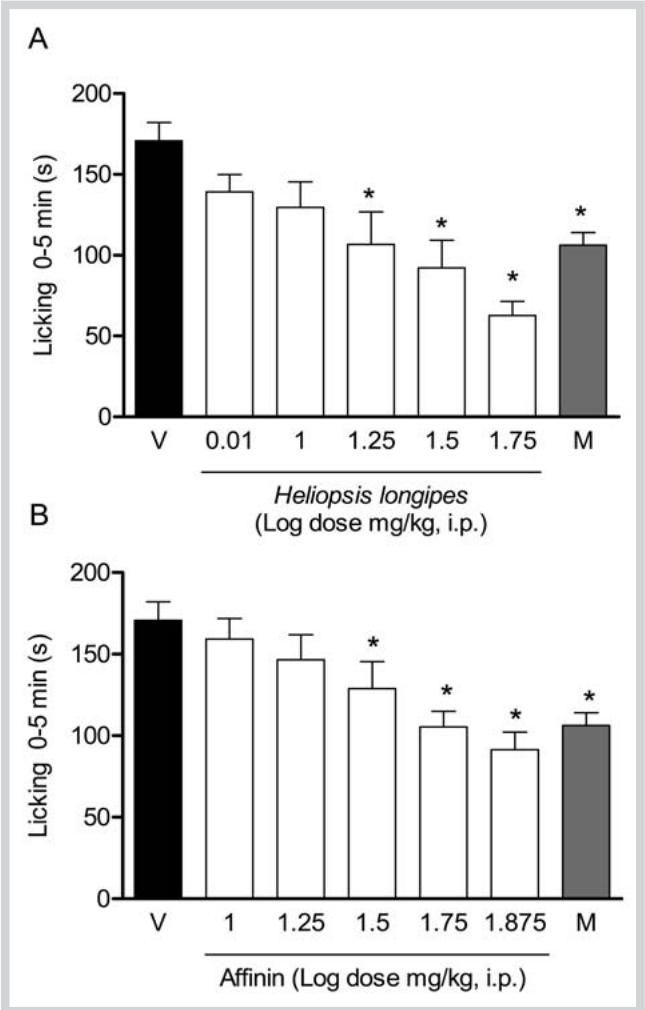

Fig. 3 Antinociceptive effect of *H. longipes* extract (**A**) and affinin (**B**) in capsaicin-treated mice. Morphine (M, log dose 1.875 mg/kg, *i.p.*), used as positive control, was given 15 min before the noxious stimulation. Data are expressed as the total licking time counted over a 5-min period. Bars are the means of eight mice ± S.E.M. * Significantly different from the vehicle (V) group ($p < 0.05$), as determined by analysis of variance followed by Tukey.

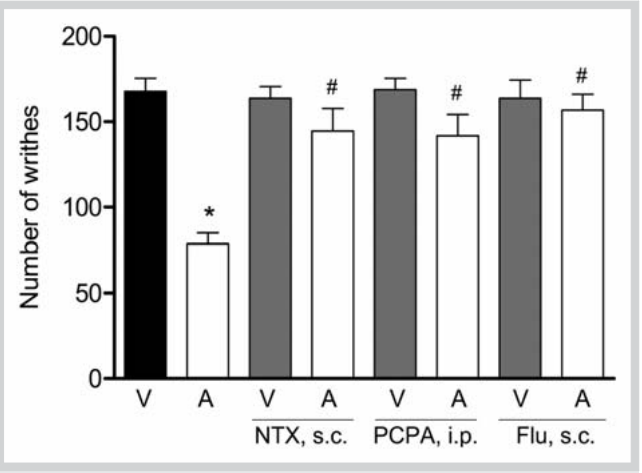

Fig. 4 Effect of naltrexone (NTX, 1 mg/kg, *s.c.*, −15 min), 4-chloro-DL-phenylalanine (PCPA, 80 mg/kg, *i.p.*, four consecutive days before affinin) or flumazenil (Flu, 5 mg/kg, *s.c.*, −15 min) on the antinociceptive effect of affinin (A, 30 mg/kg, *i.p.*) in the writhing test. Number of writhes was counted over a 30-min period following the injection of 0.6% acetic acid. Bars are the means of eight mice ± S.E.M. * Significantly different from the vehicle (V) and # significantly different from the affinin (A) group ($p < 0.05$), as determined by analysis of variance followed by the Tukey test.

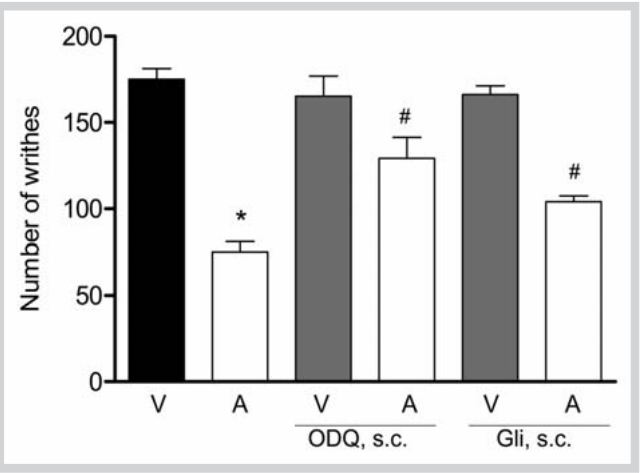

Fig. 5 Effect of ODQ (1 mg/kg, *s.c.*, −15 min) and glibenclamide (Gli, 10 mg/kg, *s.c.*, −15 min) on the antinociceptive effect of affinin (A, 30 mg/kg, *i.p.*) in the writhing test. Number of writhes was counted over a 30-min period following the injection of 0.6% acetic acid. Bars are the means of eight mice ± S.E.M. * Significantly different from the vehicle (V) group and # significantly different from the affinin (A) group ($p < 0.05$), as determined by analysis of variance followed by the Tukey test.

nociception caused by affinin when assessed on acid acetic-induced writhes in mice (▶ **Fig. 4**).

▶ **Fig. 5** shows that pretreatment of animals with ODQ (1 mg/kg, *s.c.*) or glibenclamide (10 mg/kg, *s.c.*), given 15 min prior to affinin (30 mg/kg, *i.p.*), slightly modifies the antinociceptive effect caused by affinin (30 mg/kg, *i.p.*) when assessed against the acetic acid-induced nociception. Neither glibenclamide nor ODQ modify the behavior of vehicle administration (saline solution 0.9%).

Discussion

Animal pain models have proven to be useful both as instruments to teach us about the basic biology of pain in addition to having proven predictive value for drug discovery. Acetic acid and capsaicin test were used because the administration of these irritants provokes a very stereotyped behavior in mice that represent a nocieptive effect. In this way, the antinociceptive effect can be measured as reversal of nociception to "normal" levels. The extracts of *H. longipes* and affinin were administered before algesic stimulus as preventive of behavior. Thus, the effect observed is characterized as prevention of nociception.

In the present experiments, systemic administration of the *H. longipes* extract and affinin, the main compound of the extract, produced significant antinociceptive effects against chemical nociception in mice induced by intraperitoneal acetic acid and subplantar capsaicin. To the best of our knowledge, this is the first report regarding the antinociceptive effect of affinin. Our results in the writhing test agree with previous observations in this model [13]. Therefore, our data confirm the antinociceptive effect of the

H. longipes extract in acetic acid-induced writhing. The acetic acid-induced writhing test has been widely used for the evaluation of antinociceptive activity. Transmission of visceral nociceptive information in this model is related with the spinal cord, parabrachial nucleus and certain hypothalamic nuclei [14]. Thus, it is likely that the *H. longipes* extract and affinin may exert their effects at these sites. Moreover, our data extend these observations in the writhing test by showing that the extract as well as affinin are also effective in mice previously injected with capsaicin. The later effect suggests that extract and affinin may reduce the release of neuropeptides in both central and peripheral afferents otherwise transmitting nociceptive signals to the spinal cord and brain as there is evidence that capsaicin promotes neurogenic inflammation, plasma protein extravasation, neuropeptide release and vasodilatation [15, 16]. Thus the data obtained in the capsaicin test suggest that the extract of *H. longipes* and affinin may produce antinociception suppressing neurogenic as well as the inflammatory nociception. In support of our data, it has been reported that the *H. longipes* extract is also able to reduce thermal hyperalgesia induced by carrageenan [8].

Our results showed that doses of the extract were lower than those of affinin to reduce nociception in both tests. This may be explained by the fact that the extract has a complex mixture of olefinic alkamides [6] that may synergize the antinociceptive effect. Of these, affinin was identified as the main alkamide present in the plant [4–6].

In order to asses the possible mode of action of affinin, its antinociceptive effect was determined in mice pretreated with different antagonists using the acid-acetic induced nociception. Pretreatment with naltrexone, a nonselected opioid receptor antagonist, significantly reversed the affinin-induced antinociception suggesting that affinin activates opioid receptors and/or increases the endogenous opioid system. Moreover, in the present study we were able to observe that pretreatment with PCPA significantly reversed affinin-antinociception in the writhing test. Since PCPA is an inhibitor of serotonin synthesis [17], our data suggest a role of the serotoninergic system in the antinociceptive effect of affinin. Likewise, flumazenil, a benzodiazepine site antagonist at the $GABA_A$ receptor [18], blocked the antinociceptive effect of affinin. This result suggests that $GABA_A$ receptors might participate in the antinociceptive effect of affinin. Accordingly, there is evidence that *H. longipes* increases GABA release in mice brain slices [9]. Taken together, these data suggest that affinin may activate three of the main mechanisms to produce antinociception. However, the way that affinin activates these mechanisms remains to be determined.

The antinociceptive effect is related to the elevated levels of cyclic GMP and NO activates guanylate cyclase and the subsequent production of cGMP. We confirm that cGMP is associated with affinin antinociception because pretreatment with ODQ, a soluble guanylyl cyclase inhibitor, decreased the antinociceptive effect. Thus, these results suggest the participation of the NO–cGMP pathway in the antinociceptive effect of affinin, as is the case for other NSAIDs [19–21]. On the other hand, NO can activate different types of K^+ channels in different types of tissues by an increase in cGMP [22]. It has been reported that glibenclamide, an ATP-sensitive K^+ channel blocker with no effect on Ca^{2+} or voltage dependent K^+ channels [22–26], reduces the antinociceptive effects of the NO donor, sodium nitroprusside [27], suggesting a link between activation of the L-arginine–NO–cGMP pathway and potassium channel opening. Thus this work showed that ODQ and glibenclamide were able to decrease affinin-induced antinociceptive effects. In this way, the antinociceptive effect of NO may be explained by its interaction with guanylyl cyclase and the subsequent production of cGMP; this latter, in turn, would activate ATP-sensitive K^+ channels. Hence, our data suggest that cyclic GMP and K^+ channels partially participate in the antinociceptive effect of affinin as proposed for several drugs [28–30].

The information generated in this study indicates that *H. longipes* and one of its major metabolites, namely affinin, have antinociceptive effects in the writhing and capsaicin tests in mice. The precise mechanisms and sites by which affinin induces antinociception are currently under investigation, but an interaction with the opioidergic, GABAergic and serotoninergic systems as well as the cyclic GMP-K^+ channel pathway has an important modulatory role in its antinociceptive action. Thus, *H. longipes* extract or its alkamides have antinociceptive effects in mice and rats. These results might support the popular use of the species in folk medicine for the treatment of painful complaints.

Acknowledgements

This work was financially supported by CONACyT (Project 48358-Q to AB A–G and 79584-Q to MYR) and ESM-IPN (Project C01–018 to MD-C).

References

1 *Correa J, Roquet S, Díaz E.* Multiple NMR analysis of the affinin. Org Magn Reson 1971; 3: 1–5
2 *Molina-Torres J, Salgado-Garciglia R, Ramírez-Chávez E.* Presence of the bornyl ester of deca-2E,6Z,8E-trienoic acid in *Heliopsis longipes* roots. J Nat Prod 1995; 50: 1590–1591
3 *Molina-Torres J, Salgado-Garciglia R, Ramírez-Chávez E, Del Río RE.* Purely olefinic alkamides in *Heliopsis longipes* and *Acmella* (Spilanthes) *oppositifolia*. Biochem Syst Ecol 1996; 24: 43–47
4 *Jacobson M, Acree F, Haller HL.* Correction of the source of "affinin" (N-isobutyl-2,6,8-decatrienoamide). J Org Chem 1947; 12: 731–732
5 *Jacobson M.* Constituents of *Heliopsis longipes* species. III. *Cis-trans* isomerism in affinin. J Am Chem Soc 1954; 76: 4606–4608
6 *Jacobson M.* Constituents of *Heliopsis longipes* species. IV. The total synthesis of *trans*-affinin. J Am Chem Soc 1955; 77: 2461–2463
7 *Molina-Torres J, Salazar-Cabrera CJ, Armenta-Salinas C, Ramírez-Chávez E.* Fungistatic and bacteriostatic activities of alkamides from *Heliopsis longipes* roots: affinin and reduced amidas. J Agric Food Chem 2004; 52: 4700–4704
8 *Acosta-Madrid II, Castañeda-Hernández G, Cilia-López VG, Cariño-Cortés R, Pérez-Hernández N, Fernández-Martínez E, Ortíz MI.* Interaction between *Heliopsis longipes* and diclofenac on the thermal hyperalgesia test. Phytomedicine 2009; 16: 336–341
9 *Rios MY, Aguilar-Guadarrama AB, Gutiérrez M del C.* Analgesic activity of affinin, an alkamide from *Heliopsis longipes* (Compositae). J Ethnopharmacol 2007; 110: 364–367
10 *Zimmerman M.* Ethical guidelines for investigations of experimental pain in conscious animals. Pain 1983; 16: 109–110
11 *Sakurada T, Katsumata K, Tan-No K, Sakurada S, Kisara K.* The capsaicin test in mice for evaluating tachykinin antagonists in the spinal cord. Neuropharmacology 1992; 31: 1279–1285
12 *Koster R, Anderson M, De Beer EJ.* Acetic acid for analgesic screening. Fed Proc 1959; 18: 412
13 *Ogura M, Cordell GA, Quinn ML, Leon C, Benoit PS, Soejarto DD, Farnsworth NR.* Ethnopharmacologic studies. I. Rapid solution to a problem – oral use of *Heliopsis longipes* – by means of multidisciplinary approach. J Ethnopharmacol 1982; 5: 215–219
14 *Cervero F, Laird JM.* Visceral pain. Lancet 1999; 353: 2145–2148
15 *Santos AR, Calixto JB.* Ruthenium red and capsazepine antinociceptive effect in formalin and capsaicin models of pain in mice. Neurosci Lett 1997; 235: 73–76
16 *Fusco M, D'Andrea G, Miccichè F, Stecca A, Bernardini D, Cananzi AL.* Neurogenic inflammation in primary headaches. Neurol Sci 2003; 24: S61–S64

This is a copy of the author's personal reprint

17 *Pini LA, Sandrini M, Vitale G.* The antinociceptive action of paracetamol is associated with changes in the serotoninergic system in the rat brain. Eur J Pharmacol 1996; 308: 31–40
18 *Sierralta F, Miranda HF.* Analgesic effect of benzodiazepines and flumazenil. Gen Pharmacol 1992; 23: 739–742
19 *Duarte IDG, Lorenzetti BB, Ferreira S.* Peripheral analgesia and activation of the nitric oxide-cyclic GMP pathway. Eur J Pharmacol 1990; 186: 289–293
20 *Granados-Soto V, Flores-Murrieta F, Castañeda-Hernández G, López-Muñoz FJ.* Evidence for the involvement of nitric oxide in the antinociceptive effect of ketorolac. Eur J Pharmacol 1995; 277: 281–284
21 *Granados-Soto V, Rufino MO, Lopes LDG, Ferreira SH.* Evidence for the involvement of the nitric oxide – cGMP pathway in the antinociception of morphine in the formalin test. Eur J Pharmacol 1997; 340: 177–180
22 *Amoroso S, Schmid-Antomarchi H, Fosset M, Lazdunski M.* Glucose, sulfonylureas, and neurotransmitter release: role of ATP-sensitive K^+ channels. Science 1990; 247: 852–854
23 *Davies NW, Pettit AI, Agarwal R, Standen NB.* The flickery block of ATP-dependent potassium channels of skeletal muscle by internal 4-aminopyridine. Pflugers Arch 1991; 419: 25–31
24 *Edwards G, Weston AH.* Induction of a glibenclamide-sensitive K-current by modification of a delayed rectifier channel in rat portal vein in insulinoma cells. Br J Pharmacol 1993; 110: 1280–1281
25 *Alves D, Duarte I.* Involvement of ATP-sensitive-K(+) channel in the peripheral antinociceptive effect induced by dipyrone. Eur J Pharmacol 2002; 444: 47–52
26 *Alves DP, Tatsuo MA, Leite R, Duarte ID.* Diclofenac-induced peripheral antinociception is associated with ATP-sensitive K^+ channels activation. Life Sci 2004; 74: 2577–2591
27 *Soares AC, Leite R, Tatsuo MAKF, Duarte IDG.* Activation of ATP-sensitive K^+ channels: mechanism of peripheral antinociceptive action of the nitric oxide donor, sodium nitroprusside. Eur J Pharmacol 2000; 400: 67–71
28 *Bermúdez-Ocaña DY, Ambriz-Tututi M, Pérez-Severiano F, Granados-Soto V.* Pharmacological evidence for the participation of NO-cyclic GMP-PKG-K^+ channel pathway in the antiallodynic action of resveratrol. Pharmacol Biochem Behav 2006; 84: 535–542
29 *Ortíz MI, Medina-Tato DA, Sarmiento-Heredia D, Palma-Martínez J, Granados-Soto V.* Possible activation of the NO-cyclic GMP-protein kinase G–K^+ channels pathway by gabapentin on the formalin test. Pharmacol Biochem Behav 2006; 83: 420–427
30 *Hernández-Pacheco A, Araiza-Saldaña CI, Granados-Soto V, Mixcoatl-Zecuatl T.* Possible participation of the nitric oxide-cyclic GMP-protein kinase G–K^+ channels pathway in the peripheral antinociception of melatonin. Eur J Pharmacol 2008; 596: 70–76

This is a copy of the author's personal reprint