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Pharmacology, Biochemistry and Behavior 83 (2006) 342–348
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
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Antinociceptive activity of 3-*O*-β-D-glucopyranosyl-23, 24-dihydrocucurbitacin F from *Hintonia standleyana* (Rubiaceae)☆

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Received 18 November 2005; received in revised form 13 February 2006; accepted 18 February 2006

Abstract

Oral administration of a MeOH–CH_2Cl_2 (1:1) extract of the stem bark of *Hintonia standleyana* (HSE) produced a dose-dependent antinociceptive effect when tested in mice using the writhing (150–750 mg/kg) and the hot-plate (150–600 mg/kg) models. From the active extract 3-*O*-β-D-glucopyranosyl-23,24-dihydrocucurbitacin F (GDHCF), 5-*O*-[β-D-apiofuranosyl-(1→6)-β-D-glucopyranosyl]-7-methoxy-3′,4′-dihydroxy-4-phenylcoumarin (AG4-PC) and desoxycordifolinic acid (DCA) were isolated. GDHCF (10–100 mg/kg, p.o.) significantly reduced acetic acid-induced abdominal contortions and increased the hot-plate latency in comparison to vehicle-treated mice. Metamizol (50–100 mg/kg) and morphine (2.5–5 mg/kg) were used as positive controls, respectively. GDHCF-induced antinociception was partially blocked by naloxone (1 mg/kg, i.p.), L-NAME (150 mg/kg, i.p.) and glibenclamide (10 mg/kg, i.p.) suggesting that its pharmacological effect could be due to the activation of the nitric oxide pathway, followed by the opening of the ATP-sensitive K^+ channels, as well as an activation of the opioid receptors.
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Keywords: *Hintonia standleyana*; Rubiaceae; Cucurbitacins; Antinociception; 3-*O*-β-D-glucopyranosyl-23,24-dihydrocucurbitacin F; Desoxycordifolinic acid; Inflammatory pain

1. Introduction

Hintonia standleyana Bullock (Rubiaceae), known in Mexico with the popular names of "copalchi", "quina amarilla" and "falsa quina" (false quina), is used to treat diabetes and complains associated with malaria (González-Chevez et al., 2000). According to Bullock (Bullock, 1935), *H. standleyana* is distinguished from the related species *Hintonia latiflora* by the small, almost smooth fruit, which is not lenticellate as that of *H. latiflora*, and by the absence of bracteoles. In a previous investigation, it was demonstrated that an extract (100 mg/kg) of the stem bark of *H. standleyana* did cause a significant decrease in blood glucose levels, in both normal and streptozotozin (STZ)-diabetic rats when compared with vehicle-treated groups (Guerrero-Analco et al., 2005). 3-*O*-β-D-glucopyranosyl-23, 24-dihydrocucurbitacin F (GDHCF), 5-*O*-β-D-glucopyranosyl-7-methoxy-3′,4′-dihydroxy-4-phenylcoumarin and 5-*O*-[β-D-apiofuranosyl-(1→6)-β-D-glucopyranosyl]-7-methoxy-3′,4′-dihydroxy-4-phenylcoumarin (AG4-PC) were isolated from the active extract (Guerrero-Analco et al., 2005). GDHCF and AG4-PC did not decrease blood glucose levels in normal rats. However, in two different long term subacute experiments, using animals with a developing diabetes condition and with STZ-induced diabetes, both compounds at daily doses of 10 mg/kg (developing diabetes condition) or 30 mg/kg (STZ-induced diabetes condition) provoked a significant antihyperglycemic activity. Furthermore, AG4-PC restored near to normal the blood glucose levels in STZ-induced diabetic rats. In all cases, the groups treated with the active principles and the extract showed less body weight lost than the glibenclamide-treated and diabetic control groups.

The use of *H. standleyana* for treating the symptoms of malaria (fevers, pains and chilling) in folk medicine suggested the presence of compounds with analgesic/and or anti-inflammatory properties; in order to corroborate this hypothesis the present investigation was undertaken to establish the

☆ This work was taken in part from the PhD thesis of J.A. Guerrero-Analco.
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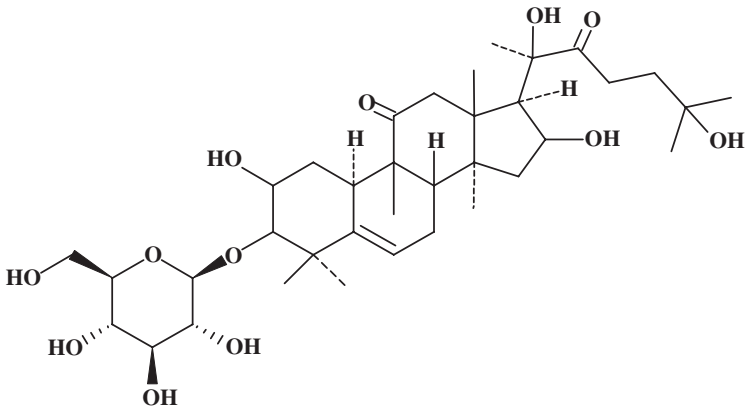
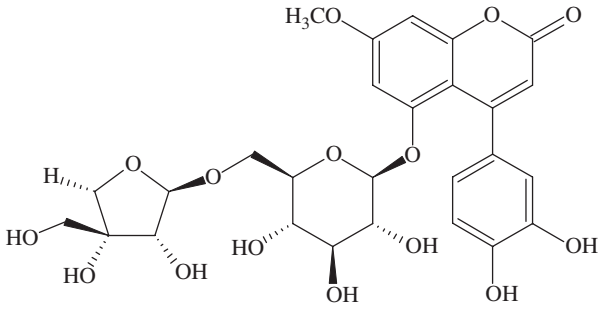
doi:10.1016/j.pbb.2006.02.020

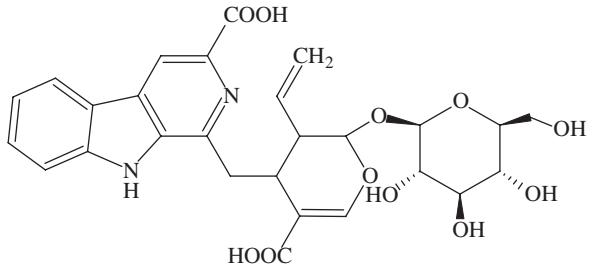
antinociceptive effect of an extract and constituents of the plant. Two well known animal models were used in this study to accomplish such endeavor, the writhing and the hot-plate tests (Woolfe and MacDonald, 1944; Eddy and Leimbach, 1953; Collier et al., 1968; Chiang and Zhuo, 1989; Le Bars et al., 2001). The methods were selected because of their usefulness to investigate peripheral and central mediated effects, respectively. In addition, this work was carried out to complete the pharmacological profile of *H. standleyana* in order to integrate a scientific monograph on this plant; such monograph, alike to those published by the American Herbal Pharmacopoeia, the European Scientific Cooperative of Phytotherapy and the World Health Organization for several medicinal plants, would permit better assessment of this very Mexican plant. Herein, we report the analgesic effect of a crude extract and some metabolites isolated from the plant namely, GDHCF, AG4-PC as well as desoxycordifolinic acid (DCA) (Fig. 1).



3-O- β-D-glucopyranosyl-23,24-dihydrocucurbitacin F



5-*O*-[β-D-apiofuranosyl-(1→6)-β-D-glucopyranosyl]-7-methoxy-3',4'-dihydroxy-4-phenylcoumarin



desoxycordifolinic acid

Fig. 1. Chemical structures of 3-*O*-β-D-glucopyranosyl-23,24-dihydrocucurbitacin F (GDHCF), 5-*O*-[β-D-apiofuranosyl-(1→6)-β-D-glucopyranosyl]-7-methoxy-3′,4′-dihydroxy-4-phenylcoumarin (AG4-PC), desoxycordifolinic acid (DCA) obtained from the extract of *Hintonia standleyana*.

2. Methods

2.1. Plant material

The stem bark of *H. standleyana* Bullock was collected in Atenango del Rio, Guerrero (Mexico) in January 2003. A voucher specimen (P. Hersch No 824) was deposited at the ASFM-INAH Herbarium, Cuernavaca, Mexico.

2.2. General procedures

Melting points were determined on a Fisher-Johns apparatus and are uncorrected. IR spectra were recorded as KBr pellets or films on a Perkin-Elmer 59913 spectrophotometer. Optical rotations were registered on a Perkin-Elmer 241 digital polarimeter. NMR spectra were recorded on a Varian Unity Plus-500 spectrometer in CD_3OD, either at 500 MHz (^1H) or 125 (^{13}C) MHz, using tetramethylsilane (TMS) as an internal standard. EIMS were obtained on a JMS-AX505HA mass spectrometer. Positive FABMS data were obtained in a JEOL SX 102 mass spectrometer using a NBA matrix. HPLC was carried out with a Waters instrument equipped with Waters 996 UV photodiode array detector (900) set at 208–215 nm, using a Symmetry® C-18 5μ column (4.6 mm i.d. × 250 mm) at a flow rate of 0.4 mL/min. Control of the equipment, data acquisition, processing, and management of chromatographic information were performed by the Millennium 2000 software program (Waters). The analyses were achieved using CH_3CN–H_2O (8:2) as mobile phase. TLC analyses were performed on silica gel 60 F_{254} plates (Merck), and visualization of plates was carried out using ceric sulfate (10%) or *N,N′*-dimethyl-aminebenzaldehyde (10% in EtOH) reagents.

2.3. Extraction and isolation of compounds GDHCF, AG4-PC and DCA

Dried and shredded stem bark (900 g) was macerated with CH_2Cl_2–MeOH (1:1) (5 L × 3) during 6 days at room temperature. The combined extracts were evaporated in vacuo to yield 200 g of a brown residue. The dried extract was chromatographed in a glass column packed with silica gel (2 kg) eluting with hexane-EtOAc (1:1→0:1) and EtOAc–MeOH (1:0→0:1) to yield ten primary fractions (FH1–FH10). Fraction FH4 (8.5 g), eluted with EtOAc–MeOH (9:1), was further chromatographed over a silica gel column using CH_2Cl_2–EtOAc–MeOH (47.5:47.5:5) as a mobile phase to give ten fractions (FH4I–FH4X). From fraction FH4V spontaneously crystallized 300 mg of pure GDHCF (Fig. 1), mp = 199–204 °C, identical to an authentic sample isolated from *H. latiflora* (Mata et al., 1990). After elimination of the solvent from the mother liquors of fraction FH4V, 3.6 g of a white crystal containing mainly GDHCF was obtained. HPLC analysis of this material revealed that GDHCF [R_t (retention time) = 9.0 min] represented ~75% of fraction FH4V. Thus, the total yield of GDHCF was ~0.3% of the dry plant material. Further column chromatography over silica gel (190 g) of FH8 (10 g), eluted with CH_2Cl_2–MeOH (7:3), using CH_2Cl_2–MeOH (9.5:0.5→0:1) as mobile phase yielded seven secondary fractions, FH8I–FH8VII. From fraction FH8IV eluted with CH_2Cl_2–MeOH (7:3), crystallized 1.2 g of AG4-PC (Fig. 1), mp > 300 °C (Guerrero-Analco et al., 2005). From primary fraction X (30 g), eluted with CH_2Cl_2–MeOH (1:1), was purified by column chromatography on silica gel (300 g) using a gradient of CH_2Cl_2–MeOH (9:1→1:1) to yield thirteen secondary fractions (FHX-I–FHX-XIII). Fraction XII eluted with CH_2Cl_2–MeOH (1:1), yielded 300 mg of DCA (Fig. 1), mp > 300 °C.

2.4. Identification of GDHCF, AG4-PC and DCA

All the spectra of GDHCF and AG4-PC were identical to those of authentic samples (Guerrero-Analco et al., 2005). DCA was characterized by comparison of its spectral data with those previously described (Adeoye and Waigh, 1983): Glassy white solid; $[\alpha]_D$: −80.5° (*c* 0.02, MeOH); UV λ_{max}(methanol) 236, 262 and 268 nm; IR (KBr): ν_{max} = 3412, 1690, 1624, 1593, 1560,1389 1324, 1250, 1080 cm^{-1} [^{13}C]-NMR ($CDOD_3$, 125 MHz); δ_C 137.1 (C-2), 146.8 (C-3), 142.9 (C-5), 115.4 (C-6), 130.9 (C-7), 122.9 (C-8), 123.0 (C-9), 121.6 (C-10), 130.2 (C-11), 113.1 (C-12), 143.3 (C-13), 35.5 (C-14), 39.6 (C-15), 116.5 (C-16), 150.4 (C-17), 118.6 (C-18), 135.4 (C-19), 46.2 (C-20), 97.6 (C-21), 172.7 (C-22), 173.3 (C-23), 101.1 (C-1′), 74.7 (C-2′), 78.0 (C-3′), 72.0 (C-4′), 78.6 (C-5′) and 63.3 (C-6′); [^1H]-NMR ($CDOD_3$, 500 MHz) δ_H 8.75 (1H, s, H-6), 8.23 (1H, brd, *J* = 8.0, H-9), 7.32 (1H, ddd, *J* = 8.0, 8.0, 1.0, H-10), 7.61 (1H, ddd, *J* = 8.0, 8.0, 1.0, H-11), 7.65 (1H, ddd, *J* = 8.0, 1.0, 1.0, H-12), 3.46 (1H, dd, *J* = 14.5, H-14a), 3.20(1H, brd, *J* = 14.5, H-14b), 3.56 (1H, dd, *J* = 8.5, 6.0, H-15), 7.42 (1H, s, H-17), 4.28 (1H, brd, *J* = 10.5, H-18a), 4.81 (1H, brd, *J* = 18.5, H-18b), 5.70 (1H, ddd, *J* = 18.5, 10.5, 8.0, H-19), 2.58 (1H, ddd, *J* = 8.5, 8.5, 5.5, H-20), 5.72 (1H, d, *J* = 8.5, H-21), 4.82 (1H, d, *J* = 7.5, H-1′), 3.18 (1H, dd, *J* = 8.0, 9.0, H-2′), 3.42 (1H, dd, *J* = 9.0, 9.0, H-3′), 3.21 (1H, dd, *J* = 10.0, 9.0, H-4′), 3.45 (1H, m, H-5′), 4.09 (1H, dd, *J* = 12.0, 2.0, H-6′a) and 3.72 (1H, dd, *J* = 12.0, 7.5, H-6′b); HRFABMS: *m/z* = 557.5271 $[M+H]^+$ (calcd. for $C_{27}H_{29}N_2O_{11}$: 557.518); FABMS: *m/z* = 579 [M +Na], 557 [M+H], 513 [M-CO_2], 468 [M-2CO_2]), 415 [M-180 + K], 399 [M-180+Na].

2.5. HPLC analysis of an infusion of the stem bark of H. standleyana

Dried and shredded stem bark (20 g) was treated with boiling water (250 mL) during 30 min. The resulting extract was analyzed by HPLC using the same conditions above indicated; the resulting analytical chromatogram revealed the presence of three major organic compounds: AG4-PC (R = 20.0 min), GDHCF (R_t = 8.0 min) and DCA (R_t = 8.5 min) in a ratio of 3:1:1 approximately. The peaks were identified by coelution with authentic samples.

2.6. Drugs

HSE, GDHCF, AG4-PC and DCA were isolated from *H. standleyana*. N^G-L-nitro-arginine methyl ester (L-NAME),

glibenclamide, and naloxone were purchased from Sigma (St. Louis, MO, USA). The extract, GDHCF, AG4-PC and DCA were suspended in 0.2% Tween-80. Morphine was purchased from Laboratorios Pisa (Mexico City). Metamizol was a gift of Aventis Pharma (Mexico City). L-NAME, naloxone, morphine and metamizol were dissolved in saline solution. Glibenclamide was dissolved in a saline solution of dimethylsulfoxide (DMSO 2%).

2.7. Animals

Experiments were performed on male mice ICR (body weight range, 25–30 g), from Centro UNAM/Harlan (Harlan Mexico, S.A. de C.V). All experiments followed the Guidelines on Ethical Standards for Investigation of Experimental Pain in Animals (Zimmerman, 1983) and Mexican Official Norm for Animal Care and Handing (NOM-062-ZOO-1999). Efforts were made to minimize animal suffering and to reduce the number of animals used. Mice were only used one time. Mice were housed in a climate-and light-controlled room with a 12-h light/dark cycle. Twelve hours before experiments, food was withheld, but animals had free access to drinking water. The CH_2Cl_2–MeOH (1:1) extract of *H. standleyana* and isolated compounds were suspended in vehicle (Tween-80, 2% in saline solution). At the end of the experiment, animals were euthanized. No side effects were observed in any of the studied groups of animals.

2.8. Measurement of antinociceptive activity

2.8.1. Acetic acid-induced writhing

The acetic acid-induced writhing test was performed in mice as previously described (Koster et al., 1959). The CH_2Cl_2–MeOH (1:1) extract of *H. standleyana* (100–750 mg/kg in 0.2% Tween-80) or isolated compounds (GDHCF: 10–100 mg/kg; AG4-PC: 100 mg/kg and DCA: 100 mg/kg) were administered 30 min before intraperitoneal (i.p.) injection of 0.6% acetic acid (10 ml/kg). Control animals received a similar volume of vehicle (0.2% Tween-80) or the positive control (metamizol, 50–100 mg/kg, p.o.). Animals were then placed in an observation box, and the abdominal constrictions were counted cumulatively over a period of 30 min. Antinociceptive activity was expressed as the reduction in the number of abdominal constrictions, i.e., the differences between control animals and animals pretreated with the extract or pure compounds.

2.8.2. Hot-plate test

The conventional hot-plate apparatus (Ugo Basile, Italy) was also used to measure the nociceptive response (Woolfe and MacDonald, 1944; Eddy and Leimbach, 1953). Mice were placed into an acrylic cylinder on the heated surface (55.5 ±0.2 °C), and the time between placement and shaking or licking of the paws, or jumping was recorder as the response latency. The extract (150–600 mg/kg) and all substances (GDHCF: 10–100 mg/kg; AG4-PC: 100 mg/kg and DCA: 100 mg/kg) were administered 30 min before beginning the experiment. Mice were observed before and at 30, 60, 90 and 120 min after drugs administration. A cutoff of 30 s was used to avoid injury. Morphine (2.5–5 mg/kg, p.o.) was used as positive control.

2.9. Analysis of possible mechanism of action of GDHCF

The possible mechanisms by which GDHCF caused antinociception in the hot-plate test were investigated pre-treating (−15 min) mice with L-NAME (150 mg/kg, i.p. in saline solution), glibenclamide (10 mg/kg, i.p. in DMSO 2%) or naloxone (1 mg/kg, i.p. in saline solution); then, GDHCF (100 mg/kg, p.o.) was administered and the antinociceptive effect recorded as described above.

2.10. Acute toxicity study in mice

Mice were treated with doses of 10, 100, 1000, 1600, 2900 and 5000 mg/kg of the crude extract. The animals were kept under close observation over a period of 14 days as described elsewhere (Lorke, 1983). Restlessness, respiratory distress, convulsions, diarrhea, motor activity, posture and reflexes were qualitatively determined. In addition, internal organs (stomach, heart, lung, liver, kidneys, etc.) were removed and examined under dissecting microscope to detect internal lesions. Finally, the weight of the animals was monitored throughout the experiments.

2.11. Statistical analysis

All experimental results are given as the mean±S.E.M. of the data obtained in 6 animals per group. Curves were constructed plotting the number of writhes or latency as a function of time. The area under the latency against time curves (AUC), an expression of the duration and intensity of the effect, was calculated by the trapezoidal rule. One-way analysis of variance (ANOVA), followed by Tukey's test was used to compare differences between treatments. Differences were considered to reach statistical significance when $P<0.05$.

3. Results and discussion

In order to asses any potential toxic effects of *H. standleyana*, mice were treated orally with increasing doses (10–5000 mg/kg) of its crude extract (Lorke, 1983). Treated mice did not present behavioral alterations during the experiment. In addition, no lesions or bleedings were observed in the organs removed. Since no death or damage was observed throughout the experiment, the LD_{50} of the extract must be higher than 5000 mg/kg. The lack of toxicity of the extract in mice, the long term use of *H. standleyana* in Mexican folk medicine as well as our previous enduring experiments on diabetic rats are good indicators of the safety of the herbal preparations made up with the plant.

Oral administration of an extract *H. standleyana* significantly reduced ($P<0.05$) the number of abdominal constrictions in a dose-dependent manner when tested in the writhing model (Fig. 2A). The effect was similar to that of metamizol, a

standard analgesic drug used as a positive control (Abbott and Hellemans, 2000). Moreover, the extract was also able to significantly increase ($P<0.05$) latency to thermal stimuli in the hot-plate test (Fig. 2B) but in this case the pharmacological action was lower than that of morphine (positive control). Altogether, these results supported that *H. standleyana* reduce inflammatory and nociceptive pain in mice.

In order to determine the analgesic principles of the plant, the effect of some compounds isolated from the active extract was determined in both nociceptive models. The tested substances included GDHCF, AG4-PC and DCA. Compounds AG4-PC and DCA were not active in both pain models. On the other hand, oral administration of GDHCF reduced acetic acid-induced abdominal contractions (Fig. 3A) in a dose-related fashion. The effect produced by GDHCF at 100 mg/kg was comparable to that of metamizol (100 mg/kg) (Fig. 3B). The level of activity displayed by GDHCF in this model could be related with the ability of some triterpenoids to inhibit the synthesis of prostaglandin $E_{2]}$ (Peters et al., 1999). Further work is in progress to evaluate this possibility.

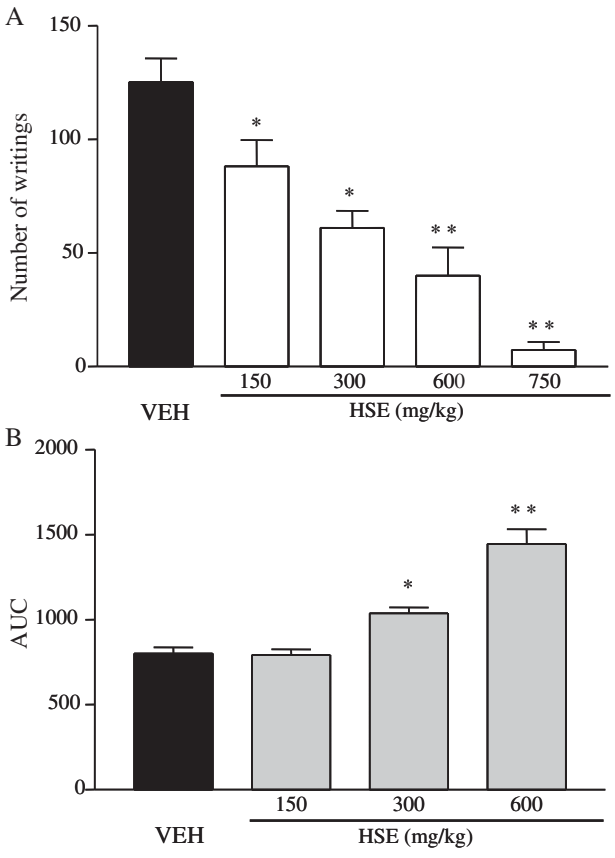


Fig. 2. Antinociceptive effect of the CH_2Cl_2–MeOH extract of *Hintonia standleyana* (HSE) in mice submitted to the writhing (panel A) and hot-plate (panel B) tests. The extract was administered orally 30 min before the tests. Number of writhes was counted over a 30-min period following the injection of 0.6% acetic acid. Thermal latency was assessed during 2 h. Data in the writhing test are the total number of writhes in 30 min, whereas that data in the hot-plate test are the area under the latency against time curve (AUC). In both cases bars are the means of six mice ± S.E.M. *Significantly different from vehicle group ($P<0.05$) and **significantly different from the vehicle (VEH) group ($P<0.01$), as determined by analysis of variance followed by the Tukey's test.

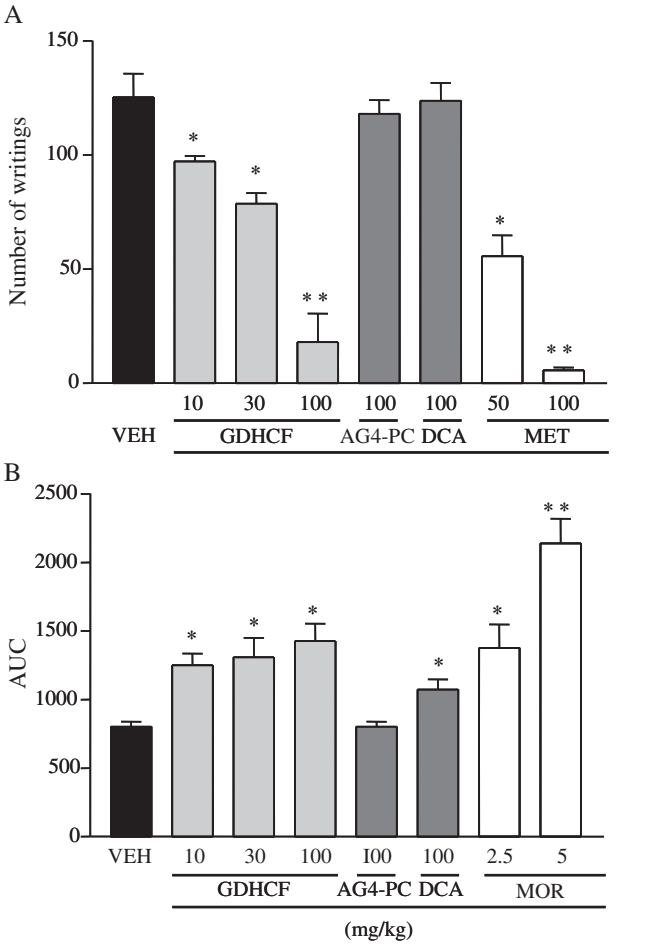


Fig. 3. Antinociceptive effect of 3-*O*-β-D-glucopyranosyl-23 24-dihydrocucurbitacin F (GDHCF), 5-*O*-[β-D-apiofuranosyl-(1→6)-β-D-glucopyranosyl]-7-methoxy-3′,4′-dihydroxy-4-phenylcoumarin (AG4-PC) and desoxycordifolinic acid (DCA) in mice submitted to the writhing (panel A) and hot-plate (panel B) tests. Drugs were administered orally 30 min before the tests. Metamizol (MET, used as positive control in the writhing test) and morphine (MOR, used as positive control in the hot-plate test) were given 15 min before the noxious stimulation. Number of writhes was counted over a 30-min period following the injection of 0.6% acetic acid. Thermal latency was assessed during 2 h. Data in the writhing test are the total number of writhes in 30 min, whereas that data in the hot-plate test are the area under the latency against time curve (AUC). In both cases bars are the means of six mice ± S.E.M. *Significantly different from vehicle (VEH) group ($P<0.05$) and **significantly different from the vehicle group ($P<0.01$), as determined by analysis of variance followed by the Tukey's test.

In the hot-plate model, GDHCF (100 mg/kg) increased the pain latency (Fig. 3B) but its action was lower than that provoked by morphine (5 mg/kg). Thus, the results clearly reveal that GDHCF is able to produce antinociception in central and peripheral pain models in mice.

HPLC analysis of the infusion of the plant revealed the presence of three major organic compounds in a ratio ~3:1:1. The compounds were identified by coelution with authentic samples as AG4-PC, DCA and GDHCF, respectively. AG4-PC and DCA were not active in both assays performed in this investigation therefore the major antinociceptive active principle of the plant is GDHCF. Considering the total yield of GDHCF in the crude extract, the doses used for testing seems to

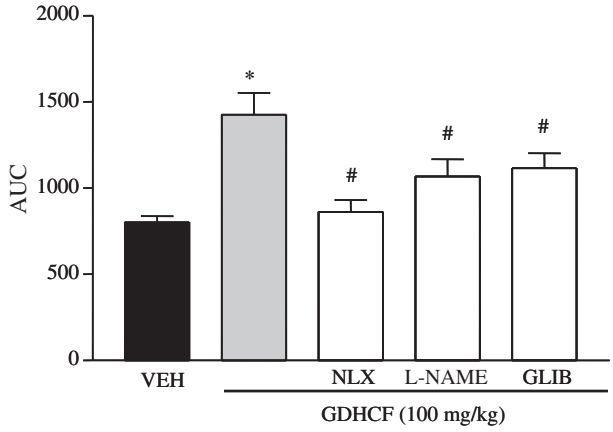


Fig. 4. Effect of naloxone (NLX), N^G-L-nitro-arginine methyl ester (L-NAME), and glibenclamide (GLIB) on the antinociceptive effect produced by 3-*O*-β-D-glucopyranosyl-23 24-dihydrocucurbitacin F (GDHCF) in the hot-plate test. Mice were pretreated (−15 min) with either L-NAME (10 mg/kg, i.p.), glibenclamide (10 mg/kg, i.p.) or naloxone (1 mg/kg, i.p.) before 3-*O*-β-D-glucopyranosyl-23 24-dihydrocucurbitacin F (GDHCF) (100 mg/kg) administration and 30 min after the thermal latency was assessed. Data are expressed as the area under the latency response to 55.5 °C against time curve (AUC). Each point indicates the mean of six mice ± S.E.M. *Significantly different from vehicle (VEH) group ($P<0.05$) and #significantly different from the 3-*O*-β-D-glucopyranosyl-23 24-dihydrocucurbitacin F (GDHCF) group ($P<0.05$), as determined by analysis of variance followed by the Tukey's test.

be in agreement with the amount detected of this compound in the traditional preparation (i.e. infusion).

To our knowledge this is the first report on the antinociceptive effect of cucurbitacin-type of compounds, which then represent new leads for the development of analgesic drugs. Furthermore, 23,24-dihydrocucurbitacins did not exhibited the cytotoxic properties of the related analogs possessing a 23,24 double bond (Rahman et al., 1973; Fang et al., 1984; Mata, 1993).

Next, to asses the possible mode of action of GDHCF, its antinociceptive action was determined in mice pretreated with L-NAME, glibenclamide or naloxone, using the hot-plate model. According to the results shown in Fig. 4, the effect of GDHCF (100 mg/kg) was blocked by the nitric oxide synthase inhibitor L-NAME, suggesting that its action involves the activation of the nitric oxide-cyclic GMP pathway at peripheral and/or central levels (Duarte et al., 1990; Duarte and Ferreira, 1992; Tonussi and Ferreira, 1994; Lorenzetti and Ferreira, 1996; Granados-Soto et al., 1995; 1997; Nozaki-Taguchi and Yamamoto, 1998; Islas-Cadena et al., 1999).

The analgesic activity of GDHCF was also blocked by glibenclamide, an ATP-sensitive K^+ channel blocker. It has been reported that glibenclamide specifically blocks ATP-sensitive K^+ channels, with no effect on Ca^{2+}- or voltage-dependent K^+ channels (Amoroso et al., 1990; Davies et al., 1991; Edwards and Weston, 1993). Therefore, our data suggest that opening of ATP-sensitive K^+ channels might be related with the analgesic action of this cucurbitacin.

It is likely that GDHCF could have a mechanism of action similar to diclofenac, metamizol, ketorolac, sodium nitroprusside and morphine all of which activate the nitric oxide-cyclic GMP-K^+ channel pathway. (Carrier et al., 1997; Rodrigues and Duarte, 2000; Soares et al., 2000; Lázaro-Ibáñez et al., 2001; Alves and Duarte, 2002; Ortiz et al., 2002; Alves et al., 2004).

Finally, the opiate receptor antagonist naloxone (1 mg/kg) significantly reversed GDHCF-induced antinociception, suggesting an activation of opioid receptors and/or an increment of endogenous opioids might be involved in the antinociceptive effect of GDHCF, as well (Bjorkman et al., 1990).

The information generated in this study indicates that *H. standleyana* and one of its major metabolite, namely GDHCF, have antinociceptive effect in the writhing and hot-plate tests in mice. The antinociceptive mode of action of this compound seems to be due to the activation of the nitric oxide pathway, followed by the opening of the ATP-sensitive K^+ channels, as well as an activation of the opioid receptors. The results also tend to support the popular use of the species in folk medicine for the treatment of painful complaints.

From the chemotaxonomic point of view this work represents the first report of alkaloids in the genus *Hintonia*. The presence of this type of compounds in this genus was questioned for several years because the antipaludic alkaloid quinine could not be found in the related species *H. latiflora*. The involvement of the alkaloid DCA in the claimed antipaludic effect of this plant remains an open question.

Acknowledgements

This research was supported by a CONACYT grant (CO1-018) and DGAPA IN212005. Myrna Déciga-Campos is a DGAPA-UNAM postdoctoral fellow. We are indebt to Dr. Vinicio Granados-Soto for valuable discussion and suggestions. We also are grateful to Dr. Jorge E. Torres-López for facilitating the hot plate equipment and to Isabel Rivero, Laura Acevedo, Guadalupe Ángeles and Gabriela Castañeda for technical assistance.

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