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Original Article

Toxicity and antinociceptive effects of *Hamelia patens*

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ABSTRACT

Many medicinal herbs are used in folk medicine without taking into account their toxicity. *Hamelia patens* Jacq. (Rubiaceae), a Mexican endemic species, is used for the empirical treatment of pain. The aim of this work was to evaluate the toxicity and antinociceptive effects of ethanolic extracts of *H. patens* leaves. The toxicity of *H. patens* leaves (500–5000 mg/kg) was evaluated in acute (14 days) and subacute (28 days) assays. In the subacute assay, a blood analysis (both hematology and chemistry) was carried out. The antinociceptive effects of *H. patens* leaves (50–200 mg/kg) were evaluated using thermal-induced nociception (hot plate) and the chemical-induced nociceptive tests (acid acetic and formalin). In the acute toxicity test, the LD_{50} estimated for *H. patens* leaves was 2964 mg/kg *i.p.* and >5000 mg/kg *p.o.*, whereas in the subacute test HPE did not affect hematological or biochemical parameters. In chemical-induced nociception models, *H. patens* (100 and 200 mg/kg *p.o.*) showed antinociceptive effects with similar activity than 100 mg/kg naproxen. In the hot plate test, HPE at 100 mg/kg (17%) and 200 mg/kg (25%) showed moderate antinociceptive effects. HPE could be a good source of antinociceptive agents because of its good activity and low toxicity.

Introduction

Worldwide, there is an increasing interest for using medicinal plants for the prevention and treatment of many diseases. The general population perceives herbal medicines as safe because of their natural origin, their efficacy and their long use in traditional medicine. Nevertheless, many plant extracts are used in folk medicine without taking into account their toxicity aspect.

Hamelia patens Jacq., (Rubiaceae), commonly known as "bayetilla," "trompetilla," "coralillo," or "hierba coral, is a bush widely distributed in tropical areas of the American continent. *H. patens* has shown antidiarrheal (Pérez et al., 1996), anti-inflammatory (Sosa et al., 2002), cytotoxic (Taylor et al., 2013), wound healing (Gomez-Beloz et al., 2003), antifungal (Abubacker et al., 2013), antibacterial (Camporese et al., 2003) and vasorelaxant (Reyes-Chilpa et al., 2004) properties. The chemical constituents isolated from this plant are pteropodine, isopteropodine, isomaruquine, maruquine, narirutin, palmirine, rosmarinic acid, rumberine, (−)-hameline, tetrahydroalstonine, aricine, uncarine F, stigmast-4-ene-3, 6-dione and 5,7,2′,5′-tetrahydroxyflavanone 7-rutiroside (Aquino et al., 1990; Ahmad et al., 2012; Paniagua-Vega et al., 2012).

In traditional medicine, *H. patens* is used as diuretic and for the empirical treatment of pain, inflammation, rheumatism, diabetes, wound healing, gastritis, stomach ache, snake and scorpion bites, fever and others (Coe and Anderson, 1999; Leonti et al., 2001; Andrade-Cetto, 2009; Ahmad et al., 2012). Nevertheless, the toxicity and antinociceptive effects of *H. patens* remain to be studied.

In Mexican traditional medicine there are many plants that remain to be studied in terms of their chemical, biological and pharmacological properties. As part of our validation of pharmacological effects in Mexican medicinal plants, this study presents that *H. patens* shows low toxicity and exerts antinociceptive effects.

Materials and methods

Reagents

Naproxen sodium was obtained from Tripharma (Distrito Federal, Mexico). N,*O*-bis (trimethylsilyl) trifluoroacetamide (BSTFA) was acquired from Sigma Aldrich (St Louis, MO, USA). Buprenorphine (BNP) was from Schering Plough Mexico (Distrito Federal,

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Mexico). Ketamine and xylazine were obtained from Pisa Agropecuaria (Atitalaquia, Hidalgo, Mexico).

Plant material

Hamelia patens Jacq., Rubiaceae, was collected in the municipality of Axtla de Terrazas, located at 21° 26′ north latitude and 98° 52′ west longitude in the southeastern portion of the state of San Luis Potosi, Mexico. A voucher specimen (HPHP-SP-102) was deposited at Centro de Investigación de la reserva de la biosfera de Mapimi.

Preparation of ethanol extract of H. patens leaves (HPE)

Powdered dry leaves of *H. patens* (20 g) were extracted with ethanol using a Soxtherm apparatus (Soxtherm automatic, Gerhadt, Germany) for 4 h. The extract was filtered by an acrodisk nylon membrane 0.45 mm pore size (Gelman Sciences, Northampton, UK). The solvent was evaporated to dryness in a vaccum rotator evaporator below 60 °C. The residue was protected from light.

Sample preparation for gas chromatography–mass spectrometry (GC–MS) analysis

Approximately 10 mg of HPE was transferred to polyethylene tube and dissolved with 2 ml isooctane. For silylation, 100 μl of BSTFA was added to the samples and incubated at 100 °C for 10 min in a CEM Discover microwave equipment at 150 W, 290 psi.

GC–MS analysis

Analysis of the HPE was performed on gas chromatograph 6890 (Agilent Technology, Santa Clara, CA, USA) with a selective mass detector 5973. DB-5HT column (15 m × 0.25 mm ID, 0.10 μm film thickness) was used for the analysis. The operating conditions of the column were as follows: oven temperature programmed from 100 °C (3 min) to 320 °C at 15 °C/min, and 2 min hold. The injector temperature was maintained at 320 °C and the volume of injected sample was 1 μl. The MS ran in electron impact at 71 eV and Mass spectral data were acquired in the scan mode in the *m*/*z* range 33–800. The identification of compounds was performed by comparing their mass spectra with data from NIST 11 (National Institute of Standards and Technology, USA), WILEY 09.

Animals

Balb/c or C57BL/6 male mice weighing 25–30 g or Wistar male rats weighing 150–200 g, from the Universidad Autónoma de San Luis Potosi animal facility, were housed in isolated cages at 24 °C under a light–dark cycle of 12:12. The animals were supplied with food and water *ad libitum*. The experiments were carried out according to Official Mexican Norm NOM 062-ZOO-1999 (technical specifications for the production, care and use of laboratory animals). The research also followed the Guidelines on Ethical Standards for Investigations of Experimental Pain in Animals (Zimmerman, 1983).

Acute toxicity test

Six weeks old C57BL/6 mice were used. The acute toxicity test of the HPE, *i.p.* or *p.o.* administration, and the selection of doses was performed according to the method described by Lorke (1983). Mice ($n = 5$ per group) received HPE at doses of 500, 1000. 2500, 3700 and 5000 mg/kg and the control group received saline solution. Mice were observed daily during 14 days for mortality, behavioral changes and other toxic signs. Body weight of mice was recorded at the beginning and end of the experiment. The dose leading to 50% death (LD_{50}) in mice was calculated by regression analysis (percent death *vs.* log dose).

Subacute toxicity test

Eight week old Wistar rats ($n = 10$ per group) were used. HPE was administered orally at doses of 500, 1000, 2500, 3700 and 5000 mg/kg daily during 28 days. The control group consisted of rats administered with saline solution. Body weight of rats was recorded throughout the study. At the end of the experiment, rats were fasted overnight and anesthetized with a single intraperitoneal application of ketamine (50 mg/kg) and xylazine (12 mg/kg), for blood collection by cardiac puncture.

Blood analysis

Blood samples were collected into heparinized and non-heparinized centrifuge tubes. A blood analysis (both hematology and chemistry) was carried out. The heparinized blood was used for a hematological study which included the estimation of white blood cells (WBC) and differential leukocyte counts, platelet, hematocrit and hemoglobin (Hb). The non-heparinized blood was allowed to coagulate before being centrifuged and the serum was separated. Hematological analysis was performed using an automatic hematological analyzer (XT-1800i, Sysmex, Mundelein, IL, USA). The parameters of blood samples were: red blood cells (RBC), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), neutrophils, lymphocytes, monocytes, eosinophils and basophils. Biochemical analysis of serum samples was performed using an automatic chemistry analyzer (Vitros 250, Ortho Clinical Diagnostics, Rochester, NY, USA). The biochemical parameters measured were: glucose, creatinine, blood urea nitrogen (BUN), alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), glucose, creatinine and glycated hemoglobin (HbA1c).

Antinociceptive assays

Hot plate

For antinociceptive tests, Balb/c mice ($n = 8$ per group) were used. The hot plate test was conducted on a thermostatically controlled heated metal plate at a temperature of 55 ± 1 °C, according to the method of Turner (1965). The time (in s) that elapsed between placing the mouse on the hot plate and the manifestation of signs of acute discomfort such as licking of the hind paw or jumping in an attempt to escape from the heat was taken as the reaction time or latency. Mice exhibiting latency time between 10 and 20 s were chosen. The latency time was determined at 60, 120 and 240 min after the application of the following test samples ($n = 8$ each group): vehicle (saline solution), BNP (1 mg/kg *i.p.*) or HPE (50, 100 and 200 mg/kg *p.o.*). A cut off time of 45 s was allowed to avoid thermal injury to the paws. The antinociceptive response was calculated according to the following formula:

$$\% \text{ Antinociceptive response} = \frac{\text{Reaction time} - \text{basal latency}}{\text{Cut off latency} - \text{basal latency}} \times 100$$

Formalin

The formalin test was carried out as described by Hunskaar and Hole (1987). One hour prior to formalin injection, mice ($n = 8$ per group) orally received 100 mg/kg NPX, HPE at doses of 50, 100 and 200 mg/kg and saline solution (the vehicle group). Mice were

Table 1
Effect of HPE on hematological parameters on subacute toxicity test in rats.

Parameter	Control	HPE mg/kg				
		500	1000	2500	3700	5000
RBC (10^6/ml)	9.62 ± 0.54	9.97 ± 0.22	9.11 ± 0.92	9.45 ± 0.27	8.82 ± 0.20	8.84 ± 0.14
Hb (g/dl)	17.93 ± 0.93	17.47 ± 0.47	16.17 ± 1.17	16.47 ± 0.38	15.40 ± 0.30	15.43 ± 0.38
HCT (%)	48.13 ± 2.73	49.00 ± 1.45	48.53 ± 4.37	47.80 ± 0.60	45.17 ± 0.87	44.13 ± 1.0
MCV (fL)	50.03 ± 0.67	49.13 ± 0.59	53.30 ± 0.70	50.57 ± 0.95	51.23 ± 0.31	49.93 ± 1.31
MCH (pg)	17.93 ± 0.32	17.50 ± 0.26	17.87 ± 0.29	17.43 ± 0.12	17.47 ± 0.23	17.50 ± 0.46
MCHC (g/dl)	35.80 ± 0.20	35.63 ± 0.25	33.47 ± 0.59	34.43 ± 0.42	34.10 ± 0.26	35.0 ± 0.10
Platelets (10^3/μl)	1074 ± 50.48	977.7 ± 27.06	1022.3 ± 81.8	870.3 ± 21.2	847.6 ± 93.4	757.8 ± 84.4
WBC (10^6)	2.61 ± 0.49	2.65 ± 0.80	5.39 ± 0.24*	3.22 ± 1.89	2.05 ± 0.58	2.04 ± 0.21
Neutrophils (%)	19.67 ± 4.50	23.23 ± 5.06	15.00 ± 2.77	14.03 ± 4.37	33.83 ± 5.25*	25.53 ± 2.58
Lymphocytes (%)	72.93 ± 2.92	71.60 ± 6.01	78.90 ± 6.60	72.50 ± 15.23	63.30 ± 6.60	70.37 ± 3.74
Monocytes (%)	2.70 ± 0.35	4.37 ± 1.99	3.23 ± 2.67	3.03 ± 2.35	1.87 ± 1.15	3.50 ± 1.18
Eosinophils (%)	1.13 ± 0.21	0.97 ± 0.74	1.53 ± 0.43	1.17 ± 0.50	0.43 ± 0.40	0.47 ± 0.06
Basophils (%)	0.23 ± 0.11	0.23 ± 0.11	0.33 ± 0.13	0.17 ± 0.15	0.57 ± 0.17	0.23 ± 0.13

RBC, red blood cells; Hb, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; WBC, white blood cells.
* Statistically different from the control group (ANOVA followed by Tukey, $p \leq 0.05$).

injected with 30 μl of 1% formalin (in 0.9% saline) into the subplantar space of the right hind paw, and individually placed into glass *cylinders*. The duration of paw licking was recorded at 0–15 min (first phase) and 15–45 min (second phase) after formalin injection.

Acetic acid-induced writhing

The acetic acid method was carried out as described by Koster et al. (1959). One hour prior to acetic acid injection, mice ($n = 8$ per group) orally received 100 mg/kg NPX, HPE at doses of 50, 100 and 200 mg/kg and saline solution (the vehicle group). Each group was administered 10 ml/kg body weight (*i.p.*) of an aqueous solution of acetic acid (1.0%). The mice were individually placed into glass cylinders and observed for the number of abdominal constrictions and stretching, counted over a period of 0–30 min.

Statistical analysis

All experimental values are expressed as the mean ± the standard deviation of at least two independent experiments. Statistically significant differences from the vehicle group were identified by Student's *t*-test or ANOVA with *post hoc* Tukey test for paired data. The level of $p \leq 0.05$ was used to determine statistical significance. All calculations were performed using the Graph Pad Prism V.3 software system (GraphPad Software, San Diego, CA, USA).

Results

Chemical composition of HPE

The chromatogram of HPE using GC–MS showed the presence of several components (Fig. 1A), including polyphenol (catechin), hydroxycinnamic acid (caffeic acid), cyclohexane carboxylic acids (shikimic acid and quinic acid), diterpene (phytol), phytosterol (β-sitosterol), triterpenes (squalene, oleanolic acid, ursolic acid), tocopherols and alkaloid (mitraphylline) (Fig. 1B). The most abundant component was ursolic acid (40%) (Fig. 1B).

Acute toxicity

The LD_{50} estimated by the Lorke method (1983) was 2964 mg/kg *i.p.* and >5000 mg/kg *p.o.* At doses of 2500 mg/kg (*i.p.* or *p.o.*) or higher, neurological deficit was observed in the mice only in the first 4 h after treatment. The symptoms included immobility and sedation. HPE tested at any concentration, administered orally or intraperitoneally, lacked of toxic effects in body weight of mice. An autopsy at the end of the experimental period revealed no apparent changes in organs.

Subacute toxicity

Hematological parameters

HPE at dose of 1000 mg/kg increased significantly ($p \leq 0.05$) the WBC count. Similarly, 3700 mg/kg HPE caused a significant ($p \leq 0.05$) increase in the neutrophil count. Other hematological parameters were not changed by the application of HPE (Table 1).

Biochemical parameters

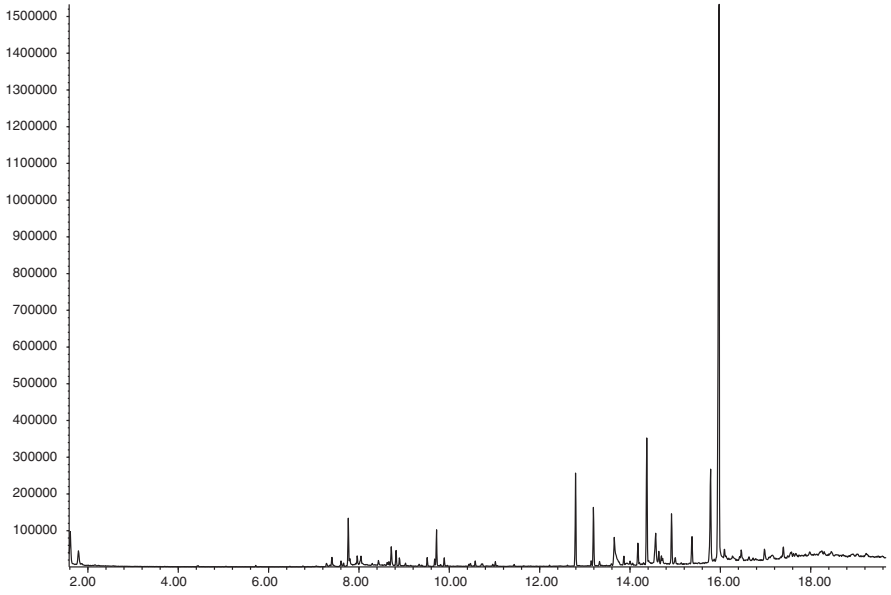
The data indicated that BUN decreased ($p \leq 0.05$) in rats treated with 5000 mg/kg HPE. The levels of AST decreased ($p \leq 0.05$) in rats treated with 3700 mg/kg HPE, whereas the levels of ALP increased ($p \leq 0.05$) in the group of 2500 mg/kg HPE. Other biochemical parameters were not altered by the administration of HPE (Table 2).

Table 2
Effect of HPE on biochemical parameters on subacute toxicity test in rats.

	Control	HPE (mg/kg)				
		500	1000	2500	3700	5000
Glucose (mg/dl)	132.67 ± 11.93	110 ± 17.69	171.67 ± 43.36	128.33 ± 42.71	104.67 ± 12.22	155.57 ± 82.97
HbA1c (%)	3.2 ± 0.20	3.17 ± 0.06	3.50 ± 0.00	3.40 ± 0.00	3.26 ± 0.00	3.36 ± 0.00
BUN (mg/dl)	63.20 ± 5.11	54.20 ± 4.53	54.23 ± 7.25	58.37 ± 3.55	65.20 ± 9.43	36.67 ± 4.35*
Creatinine (mg/dl)	0.27 ± 0.11	0.23 ± 0.06	0.40 ± 0.00	0.33 ± 0.06	0.40 ± 0.00	0.60 ± 0.35
AST (U/l)	103.00 ± 31.18	114.33 ± 28.59	90.43 ± 7.02	147.67 ± 18.56	124.00 ± 15.62	103.00 ± 17.28
ALT (U/l)	116.33 ± 53.25	83.00 ± 13.0	47.00 ± 16.64	87.67 ± 17.74	20.33 ± 2.89*	30.33 ± 1.53
ALP (U/l)	137.77 ± 34.27	144.67 ± 19.50	188.67 ± 29.67	229.00 ± 29.72*	150.23 ± 31.37	118.67 ± 19.01

BUN, blood urea nitrogen; ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; HbA1c, glycated hemoglobin.
* Statistically different from the control group (ANOVA followed by Tukey, $p \leq 0.05$).

A



B

Constituents	Retention time	%
glycerol	1.61	2.25
malic acid	4.44	0.11
shikimic acid	7.40	0.63
hexadecanoic acid	8.71	1.16
caffeic acid	9.33	0.17
phytol	9.51	0.48
α-linolenic acid	9.72	1.66
octadecanoic acid	9.89	0.47
dehydroabietic acid	10.57	0.40
bis (3-tert-butyl-2-hydroxy-5-methylphenyl) methane	10.74	0.29
squalene	12.79	4.52
catechin	13.19	3.02
γ-tocopherol	13.59	0.25
mitraphylline	13.65	4.02
α-tocopherol	14.17	1.29
campesterol	14.57	2.67
β-sitosterol	14.92	2.92
1-triacontanol	15.00	0.59
oleanolic acid	15.78	6.58
ursolic acid	15.97	40.59

Fig. 1. GC–MS chromatogram (A) and chemical constituents (B) of ethanol extract of *H. patens* leaves (HPE).

HPE exerts antinociceptive effects

HPE showed good antinociceptive activity in two of the three *in vivo* models. In the hot plate test at 60 min of treatment, HPE showed the highest antinociceptive effects by 6% (50 mg/kg), 17% (100 mg/kg) and 25% (200 mg/kg), in comparison to the vehicle group (Fig. 2A). After 120 min, the antinociceptive effect of HPE decreased. On the contrary, the highest antinociceptive effects (70%) of BNP (1 mg/kg) were shown at 120 min of treatment. These effects remained up to the end of the experiment (Fig. 2A).

In the formalin test at phase 1 and phase 2, HPE decreased significantly ($p \leq 0.05$) the licking time, compared to the vehicle group (Fig. 2B). In the phase 1, HPE exerted antinociceptive effects by 19%

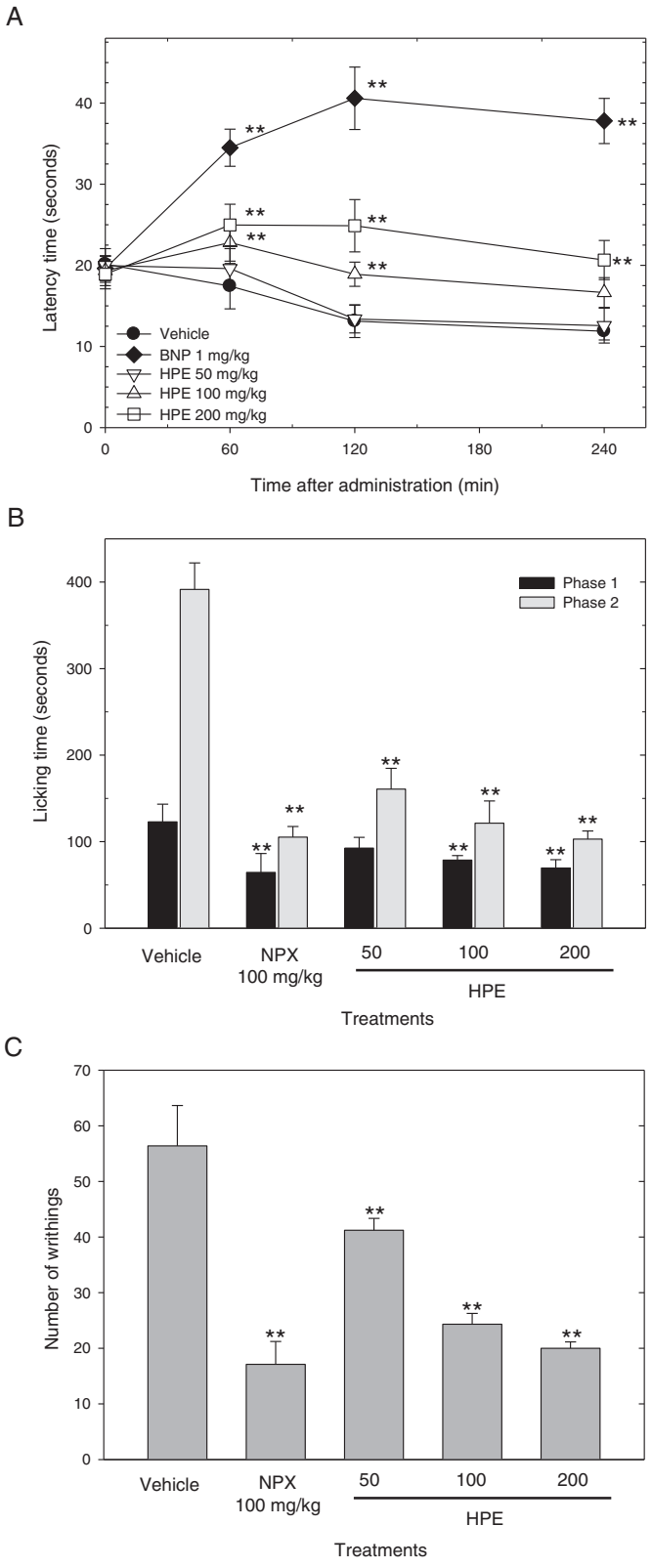


Fig. 2. HPE exerts antinociceptive effects. The antinociceptive effects of HPE (50–200 mg/kg) were evaluated using thermal-induced nociception hot plate (A), and the chemical-induced nociceptive tests formalin (B) and acid acetic (C). The positive control BNP (1 mg/kg) was used for the hot plate test, whereas the positive control NPX (100 mg/kg) was used for the formalin and acid acetic tests. Results represent the mean ± standard deviation (SD). ** denotes $p \leq 0.05$, compared to the vehicle group.

(50 mg/kg), 30% (100 mg/kg) and 39% (200 mg/kg), respectively, whereas in the phase 2 the antinociceptive effects of HPE were 60% (50 mg/kg), 69% (100 mg/kg) and 75% (200 mg/kg) (Fig. 2B). The antinociceptive effects of HPE were comparable with those found by the positive control 100 mg/kg NPX, which showed antinociceptive effects by 48% (phase 1) and 74% (phase 2) (Fig. 2B).

In the acid acetic test, HPE decreased significantly ($p \leq 0.05$) the number of writhings in a dose dependent manner, compared to the vehicle group (Fig. 2C). The maximum percentage inhibition of acetic acid-induced abdominal constrictions by HPE was found at 100 mg/kg (57%) and 200 mg/kg (65%). This effect was comparable to 100 mg/kg NPX (70%) (Fig. 2C).

Discussion

Hamelia patens Jacq., (Rubiaceae), is used in traditional medicine for the empirical treatment of pain (Ahmad et al., 2012). Nevertheless, the antinociceptive effects of *H. patens* as well as its toxicity remain to be studied. Toxicity tests are commonly used in the preliminary studies of medicinal plants to detect pharmacological activities (Malone and Robichaud, 1962). In this study, the toxicity of *H. patens* in acute and subacute assays was recorded.

In our knowledge, this is the first report that indicates the chemical composition of ethanol extract from *H. patens* leaves using GC–MS. The compounds found in HPE had not been previously reported.

In the acute toxicity test, if no mortality is observed at the dose of 5.0 g/kg, the drug tested can be considered as not toxic (Hayes, 1989). The results indicated that HPE orally administered could be considered as non-toxic. Nevertheless, HPE administered intraperitoneally could be considered as moderate toxic. The reduction in body weight is a simple and sensitive index of toxicity. The data indicated that there were no changes of body weight in mice treated with HPE.

Several hematological and biochemical parameters were estimated in the subacute toxicity test in rats to evaluate the safe use of *H. patens*. The results indicated that HPE did not induce anemia or disturbances in the erythrocytes or in the hemoglobin production. Although there was an increase in WBC (1000 mg/kg HPE) and neutrophils (3700 mg/kg HPE), the values are in the physiological limits described for the species (Harkness and Wagner, 1993). The increase of neutrophils may be the result of the administration of HPE as a physiological response against foreign compounds, which might be a normal response from rat organism. In rats treated with HPE there were no changes in the other hematologic parameters. These findings suggest that *H. patens* did not induce hematologic alterations.

The levels of serum glucose and glycated hemoglobin were also evaluated. These two parameters were not significantly changed in all the groups. Therefore, HPE does not affect the carbohydrate metabolism.

Renal function was evaluated by means of urea and creatinine serum levels. Renal damage is considered when both urea and creatinine are increased concomitantly (Satyanarayana et al., 2001). The level of urea decreased in the rats treated with 5000 mg/kg HPE. However, the values are in the physiological limits of rat specie (Harkness and Wagner, 1993).

Hepatic function is commonly monitored by the evaluation of the serum level of transaminases ALT, AST and ALP. Although there was an increase of ALP in HPE at 2500 mg/kg, it has been described that a five-fold higher increase in ALP level is considered as moderate or acute hepatic toxicity. Increases lower than five-fold are considered as no relevant (Wingard et al., 2000). Therefore, treatment with HPE does not alter hepatic function in rats.

Together, the results indicate that *H. patens* could be considered as a non-toxic plant. Nevertheless, other studies including the mutagenic, genotoxic and carcinogenic effects of HPE should be performed. In addition, a subchronic toxicity test should be conducted to establish the adverse effects of a repeated response to *H. patens*.

Nociception is a mechanism by which noxious stimuli are transmitted to the central nervous system (CNS). The nociceptors are pain-sensitive neurons located in the skin, vessels, muscles, fascia, joints and viscera (Calixto et al., 2000). In spite the use of many analgesics, it is needed to find new antinociceptive drugs with higher or similar efficacy and potency than current analgesics but with less toxic effects. Three models were used in this study to evaluate the antinociceptive actions of *H. patens*. One model of thermal-induced nociception (hot plate) and two models of chemical-induced nociception (acid acetic and formalin). The doses of HPE used to evaluate its antinociceptive effects were selected based on the following aspects: (1) their lack of toxic effects as evaluated in acute and subacute toxicity tests, (2) on preliminary studies carried out in our laboratory and (3) to analyze the antinociceptive activity of HPE (50–200 mg/kg) compared to NPX (100 mg/kg).

The hot-plate test is a predominantly a spinal reflex that evaluates centrally acting antinociceptive agents (Turner, 1965). The results suggest that HPE might not induce central antinociceptive effects because HPE increased moderately the latency time in this model.

The formalin test is considered a model of persistent pain produced in two phases. The first phase (0–15 min) is characterized by neurogenic pain and the second phase (15–45 min) is characterized by inflammatory pain (Hunskaar and Hole, 1987). In this model, centrally acting drugs inhibited both phases, while peripheral acting drugs inhibited mainly the second phase. HPE decreased the pain mainly in the second phase.

Acetic acid is an inducer of abdominal contractions and twisting of abdominal muscles by the increase in the level of pro-inflammatory agents (interleukin-1β, tumor necrosis factor-α and interleukin-8) in the peripheral tissue fluid (Ikeda et al., 2001). This pain model is used for evaluating peripherally acting antinociceptive agents (Ikeda et al., 2001). In this study, HPE at the doses of 50, 100 and 200 mg/kg significantly reduced the number of writhing episodes in mice, indicating the inhibition of acetic acid-induced visceral nociception. These results suggest that HPE might exert peripheral antinociceptive effects. In the chemical-induced nociceptive tests, *H. patens* showed antinociceptive effects with similar activity than 100 mg/kg NPX.

Ursolic acid, the most abundant component (40%) found in HPE, has been shown to exert antinociceptive effects in different models (Tapondjou et al., 2003; González-Trujano et al., 2012; Rodrigues et al., 2012). The good antinociceptive effects of HPE could be due the presence of ursolic acid. Nevertheless, further studies are necessary to evaluate the electrophysiological, anatomical and molecular mechanisms by which *H. patens* exerts its antinociceptive effects. The results here presented validate the traditional use of *H. patens* for the treatment of pain.

Conclusions

HPE shows low toxicity in acute and subacute tests. *H. patens* exerts antinociceptive effects with similar activity than 100 mg/kg NPX in chemical-induced nociception models. Therefore, this plant could be a good source of antinociceptive agents because of its good activity and low toxicity. In addition, it is interesting to evaluate the antinociceptive effect of *H. patens* constituents.

Author contribution

AJAC, SBR and JRZM carried out the pharmacological studies. AJAC drafted the manuscript. AHM participated in the design of the study. MMGC performed the phytochemical studies. CCA conceived the study, and participated in its design and coordination and helped draft the manuscript. All authors read and approved the final manuscript.

Conflict of interest

The authors declare no conflicts of interest.

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