Available online at www.sciencedirect.com
ScienceDirect
Journal of Ethnopharmacology 114 (2007) 218–226
Journal of ETHNO-PHARMACOLOGY
www.elsevier.com/locate/jethpharm

Pharmacological analysis of paregoric elixir and its constituents: *In vitro* and *in vivo* studies

Edinéia Lemos Andrade [a], Juliano Ferreira [a,b], Adair R.S. Santos [a,c], João B. Calixto [a,*]

[a] *Department of Pharmacology, Federal University of Santa Catarina, Florianópolis, SC, Brazil*
[b] *Department of Chemistry, Federal University of Santa Maria, Santa Maria, RS, Brazil*
[c] *Department of Physiology, Federal University of Santa Catarina, Florianópolis, SC, Brazil*

Received 28 February 2007; received in revised form 26 July 2007; accepted 3 August 2007
Available online 10 August 2007

Abstract

Paregoric elixir is a phytomedicinal product which is used widely as an analgesic, antispasmodic and antidiarrheal agent. Here, we investigated the pharmacological actions and some of the mechanisms of action of paregoric elixir and compared its action with some of its components, the alkaloids morphine and papaverine. The paregoric elixir given orally to mice did not present relevant toxic effects, even when administered in doses up to 2000-fold higher than those used clinically. However, it showed an antinociceptive action that was more potent, but less efficacious, than morphine. In contrast to morphine, its effect was not dose-dependent and not reversed by the non-selective opioid antagonist naloxone. Moreover, paregoric elixir produced tolerance, but did not cause cross-tolerance, with the antinociceptive actions of morphine. When assessed in the gastrointestinal motility *in vivo*, paregoric elixir elicited graduated reduction of gastrointestinal transit. Finally, like morphine and papaverine, paregoric elixir concentration-dependently inhibited electrically-induced contraction of the guinea pig isolated ileum. *In vivo* and *in vitro* gastrointestinal actions of paregoric elixir were not reversed by naloxone. Collectively, the present findings lead us to suggest that the pharmacological actions produced by paregoric elixir are probably due to a synergic action of its constituents.

Keywords: Paregoric elixir; Antinociception; Gastrointestinal transit; Toxicity

1. Introduction

Opium (sumo in Greek) is a dried extract obtained by evaporation and heating of the latex from incision of the capsules of non-ripe *Papaver somniferum* (Papaveracea), known popularly as oriental poppy (Trease and Evans, 1978). The actions of opium have been known for thousands of years. The first reports on the use of opium were from Sumerians who lived in Mesopotamia between 5000 and 4000 B.C. (for review: Calixto et al., 2000, 2001). The tincture of opium or laudanum used currently was first introduced by Paracelsus (1490–1540), and Le Mort created the camphorated tincture of opium known as paregoric elixir in 1702 (Trease and Evans, 1978; Woods, 1958).

The isolation of the alkaloid morphine from *P. somniferum* is attributed to Friedrich Sertürner in 1805, this substance being the first alkaloid obtained and identified in pure form shape (Rocha and Silva, 1973; Brownstein, 1993; Benyhe, 1994). Besides morphine, opium contains some 30 alkaloids, which can be combined with the organic acid meconic acid (Robbers et al., 1997). Despite the high number of alkaloids, only some of them such as morphine, codeine and papaverine seem to be relevant for the pharmacological action of *P. somniferum* and are actually used in clinical practice (Resine and Pasternak, 1996). These alkaloids are found in different concentrations in opium and are divided into two chemical classes, the phenanthrenes and the benzylisoquinolines.

The phenanthrenes alkaloids morphine and codeine exert several biological effects that are mediated by interaction with G-protein-coupled receptors known as opioids, mainly of the μ subtype. The coupling of these receptors to G_i protein inhibits the activity of the cyclase adenylate and voltage-dependent Ca^{2+} channel mechanisms, besides stimulating the efflux of K^+ ions (Dhawan et al., 1996). These receptors are activated by several

* Corresponding author at: Department of Pharmacology, Federal University of Santa Catarina, CCB, Campus Universitário Trindade, 88049-900 Florianópolis, SC, Brazil. Tel.: +55 483721 9491; fax: +55 483232 9139.
E-mail addresses: calixto@farmaco.ufsc.br, calixto3@terra.com.br (J.B. Calixto).

0378-8741/$ – see front matter © 2007 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.jep.2007.08.004

endogenous ligands such as encephalins, dinorphins, endorphins and endomorphins.

The opioid receptors are widely distributed in the central and peripheral nervous systems, and following activation they are responsible for the therapeutic effects of the opioids. These include analgesic, anticough and antidiarrheal effects that can be inhibited by non-selective antagonist receptors opiods, such as naloxone. Furthermore, a great amount of evidence reveals that morphine and codeine cause important side effects such as euphoria, constipation, tolerance and probably addiction that complicate their clinical usage (Furlan et al., 2006).

Differently of the morphine and codeine, the papaverine, a benzylisoquinoline alkaloid which is also present in opium, is practically devoid of side effects in the central nervous system since it is pharmacologically and chemically distinct from the phenantrene alkaloids morphine and codeine. Among the principal actions described for papaverine is its ability to relax the smooth musculature (Kaneda et al., 1998).

Even after the isolation of morphine and other constituents present in the opium, preparations containing opium extract are still used nowadays, including the opium tincture or laudanum and the camphorated tincture of opium known as paregoric elixir. These preparations are employed mainly for the treatment of gastrointestinal disturbances, and present advantages in relation to isolated morphine (Krantz and Carr, 1961; Osol, 1980). Besides morphine, the opium extract contains papaverine that presents antispasmodic action. The paregoric elixir is a pharmaceutical preparation described in British and American pharmacopeias that contains about 0.05% morphine and 0.005% papaverine, besides camphor, essence of aniseed, benzoic acid, alcohol and other alkaloids present in the poppy (Krantz and Carr, 1961; Osol, 1980).

Although it has been known for over 300 years and is present in the main pharmacopeias, paregoric elixir has not been scientifically evaluated to examine its pharmacological and possible toxicological effects. The aim of the present study was therefore to investigate, through *in vitro* and *in vivo* assays, the pharmacological actions, some aspects about its toxicology, and possible side effects of paregoric elixir. We also compared the effects of paregoric elixir with the main alkaloids present in opium, namely morphine and papaverine, with the purpose of analysing the participation of these alkaloids in the paregoric elixir's action.

2. Materials and methods

2.1. Animals

Adult male and female Swiss mice weighing 25–35 g and guinea pigs weighing 300–500 g of both sexes were used throughout the experiments. All animals were housed in a room maintained at a constant temperature of 22 ± 2 °C under a 12 h light/12 h dark cycle at 60–80% humidity with food and water available *ad libitum*. Animals were acclimatized to the laboratory for at least 1 h before the tests. All procedures were approved by our Institutional Ethics Committee (process numbers 262/CEUA and 23080.035334/2003-16/UFSC) and were in accordance with the National Institutes of Health Animal Care Guidelines (NIH Publications No. 80-23).

2.2. The fingerprint of paregoric elixir established by HPLC

The HPLC system consisted of an Waters Model 2695 Diode Array Detector, a Waters Model 2695 Pump solvent delivery, an Atlantis-d C_{18} (100 mm × 2.1 mm; particle size 5 μm) reversed-phase column. The column was maintained at 30 °C.

Separations were achieved on linear gradient of aqueous formic acid 0.1%:methanol (0 min, 100:0; 10 min, 100:0; 10 min, 70:30; 10 min, 60:40; 10 min, 40:60; 10 min, 100:0; 10 min, 100:0) at a flow rate of 0.3 ml/min, injection volume of 10 μl and UV detection at 254 nm.

The fingerprint analysis was carried out by the comparison with some standards compounds, namely: morphine (500 μg/ml), codeine (400 μg/ml) and papaverine (500 μg/ml). Before the injection, samples of paregoric elixir have been diluted to 10% (v/v) with formic acid 0.1%.

2.3. Acute toxicity

To investigate the possible toxic effects of paregoric elixir, increasing doses (5.3–1600 μg morphine/kg, p.o.) of the product were administered in an attempt to determine the lethal dose 50 (LD_{50}). Another group of animals was pre-treated with morphine (4.4–44.0 mg/kg, p.o.), paregoric elixir (0.53–10.6 μg morphine/kg, p.o.) or saline (10 ml/kg, p.o.) in order to assess events characteristic of toxicity, such as abdominal constriction, pilo-erection, paralysis, tremor, convulsion or Straub's signal. The animals were kept in plastic boxes and observed for 24 h after treatment.

2.4. Open-field test

To assess the possible effects of paregoric elixir on the animals' spontaneous motor activity and defecation, they were evaluated in the open-field paradigm, according to the method characterized by Montgomery (1955). Mice were placed individually in an acrylic box (30 cm × 15 cm × 30 cm) with the floor divided into nine squares. The number of squares crossed with the four paws and the number of fecal boluses was registered during a period of 5 min. The animals were treated with paregoric elixir (0.53–10.6 μg morphine/kg, p.o.), morphine (4.4–44.0 mg/kg, p.o.) or saline (1 ml/kg, p.o.) 1 h before the experiments.

2.5. Antinociceptive activity

To evaluate the possible antinociceptive effect of the paregoric elixir, the formalin model of nociception was used. The procedure employed was as described previously (Hunskaar et al., 1985, 1986; Corrêa and Calixto, 1993). Briefly, 20 μl of 2.5% formalin solution (0.92% formaldehyde), made up in phosphate buffered saline (137 mM NaCl, 2.7 mM KCl and 10 mM phosphate buffer), was injected intraplantarly under the surface of the right hindpaw. This model provided evidence of two phases

of painful sensitivity: an immediate early phase lasting for 5 min (pain of neurogenic origin) and a late phase, lasting from 15 to 30 min after the injection of formalin (pain of inflammatory origin) (Hunskaar and Hole, 1987). The mice were treated by oral route with paregoric elixir (0.53–530 μg morphine/kg), morphine (0.088–44.0 mg/kg) (used as positive control) or saline (10 ml/kg) 1 h before formalin injection. After intraplantar injection of formalin, the animals were placed immediately in a glass cylinder 20 cm in diameter, and the time spent licking and biting the injected paw was considered as indicative of nociception.

To investigate the role of the opioid system in the antinociceptive effect of paregoric elixir, animals were pre-treated with naloxone (a non-selective opioid receptor antagonist, 1 mg/kg, i.p.) 15 min prior to paregoric elixir (10.6 μg morphine/kg, p.o.) or morphine (8.8 mg/kg, p.o.) administration. One hour after treatment, the nociception was assessed in the formalin model of pain.

Next, we also verified whether the repeated administration of paregoric elixir, similar to morphine, produced tolerance to its antinociceptive effect or cross-tolerance with morphine. To do this, mice were treated twice a day for 4 consecutives days with morphine (8.8 mg/kg, p.o.), paregoric elixir (10.6 μg morphine/kg, p.o.) or saline (10 ml/kg, p.o.). The animals received two orally administered doses of each drug or saline per day at 9:00 am and 5:00 pm for the first 3 days and a single injection at 8:00 am on day 4. One hour after the last treatment, animals were pre-treated with saline (10 ml/kg, p.o.), morphine (8.8 mg/kg, p.o.) or paregoric elixir (10.6 μg morphine/kg, p.o.), and the antinociceptive effect was assessed 1 h later in the formalin test, as described above.

2.6. Gastrointestinal transit (GIT)

This test was conducted with the objective of assessing whether or not paregoric elixir, like morphine, could cause inhibition of the gastrointestinal motility. The experiments were carried out as described previously (Santos et al., 1999). The mice were treated with paregoric elixir (0.53–53.0 μg morphine/kg, p.o), morphine (0.88–44.0 mg/kg, p.o.), papaverine (1.06 μg/kg, p.o.) or vehicle (1 ml/kg, p.o.) 1 h before receiving a standard charcoal meal (0.3 ml, p.o.). The animals were sacrificed 20 min after administration of the charcoal, and the total extension of gut and the distance that the meal had travelled were measured. Data was expressed as the percentage of gut that the charcoal meal had travelled.

To investigate the role of the opioid system in the gastrointestinal transit effect of paregoric elixir, animals were pre-treated with naloxone (1 mg/kg, i.p.) 15 min prior to paregoric elixir (10.6 μg morphine/kg, p.o.). One hour after treatment, the gastrointestinal motility was analysed.

2.7. Electrical field stimulation-induced guinea pig ileum contraction

In order to assess the antispasmodic actions of paregoric elixir, morphine and papaverine, electrical stimulation of the isolated guinea pig ileum was used. Guinea pigs of both sexes were anaesthetized with choral hydrate (0.7%) and killed by cervical dislocation. A 10–30 cm portion of ileum from the ileocecal junction was excised rapidly and flushed gently with warm Krebs' solution (composition: NaCl, 118 mM; KCl, 4.8 mM; $CaCl_2$, 2.5 mM; $MgSO_4$, 1.2 mM; KH_2PO_4, 0.9 mM; $NaHCO_3$, 25 mM; glucose, 11 mM; pH 7.4) to remove contents and adhering adipose tissue. Whole segments (1–3 cm long, six to eight segments per animal) were set up for recording of isometric contractions along their longitudinal axis in a jacketed organ bath containing 5 ml of gassed (5% CO_2 and 95% O_2) Krebs' solution maintained at 37 °C, under a basal tension of 1 g. Isometric contractions were measured by means of TRI-201 force displacement transducers and recorded on a polygraph (Letica Scientific Instruments, Barcelona, Spain).

After at least 30 min of equilibration, with renewals of the nutrient solution every 10 min, preparations were submitted to field stimulation with rectangular 1 ms pulses of supramaximal voltage (ca. 50–70 V), delivered at 0.1 Hz via platinum electrodes (Guimarães and Rae, 1992; Santos et al., 1999). Once the twitch contractions evoked by field stimulation had attained a steady level, a single non-cumulative concentration–response curve was plotted for paregoric elixir (50–1500 ng/ml), morphine (3.75–1125 ng/ml) or papaverine (9–90.000 ng/ml).

2.8. Drugs

Paregoric elixir was provided by Laboratório Catarinense (Joinville, SC, Brazil). The following drugs were used: formalin, morphine hydrochloride, codeine phosphate (Merck AG, Darmstadt, Germany), chloral hydrate, papaverine (Sigma Chemical Co., St. Louis, MO, USA) and naloxone (Research Biochemicals International, Natick, MA, USA). All tested drugs were diluted in saline. The dose ranges of paregoric elixir were selected taking into account the recommended dose for humans: 50 drops three times a day (containing 0.05% morphine). Thus, the use solution was obtained dissolving 50 times 150 drops (5.3 ml) of elixir (which corresponded to 2.65 mg of morphine) in saline. The resultant dose was 530 μg of morphine/kg. The dose of papaverine employed in gastrointestinal motility test was ten times lesser in comparison to dose of morphine, because the opium contains 10% of morphine and only 1% papaverine.

3. Statistical analysis

All values are presented as mean ± S.E.M. of inhibitions obtained for each individual experiment. The ID_{50} or IC_{50} values (i.e., dose or concentration of paregoric elixir, papaverine or morphine necessary to reduce responses by 50% relative to the control value, respectively) are presented as geometric means accompanied by their respective 95% confidence limits. The ID_{50} and IC_{50} values were estimated using linear regression for individual experiments with the GraphPad Prism software. Statistical comparison of data was performed by means of analysis of variance (ANOVA) followed by Dunnett's or Newmann–Keuls' test. *P*-values less than 0.05 were considered significant. For the toxicity tests, data was analysed by means of the χ^2-nonparametric test.

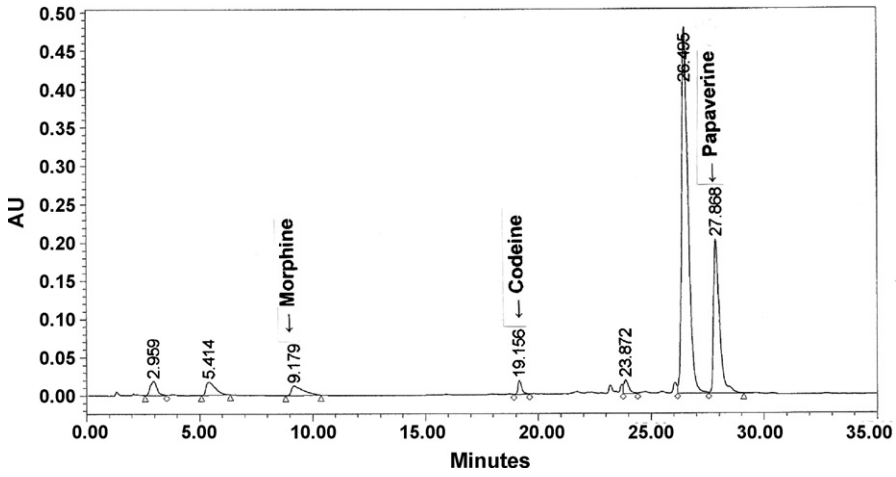

Fig. 1. The fingerprint for the herbal drug paregoric elixir obtained by HPLC showing several compounds, being three compounds identified as morphine (retention time = 9.179 min), codeine (retention time = 19.156 min) and papaverine (retention time = 27.868 min).

4. Results

4.1. Fingerprint of paregoric elixir

The fingerprint of paregoric elixir was obtained by HPLC (Fig. 1). From the chromatogram of the mixture, three component peaks could be detected: morphine (retention time = 9.179 min), codeine (retention time = 19.156 min) and papaverine (retention time = 27.868 min).

4.2. Evaluation of paregoric elixir's acute toxicity

The acute treatment of mice with paregoric elixir (5.3–1600 μg morphine/kg, p.o.) did not cause the death of any animals at the tested doses (data not shown). Moreover, paregoric elixir (0.53–10.6 μg morphine/kg) did not produce any significant alterations in behaviour response which were characteristic of toxicity, such as abdominal constriction, paralysis, tremor, convulsion or Straub's signal (data not shown). In addition, we did not observe abdominal constriction, convulsion, tremor or paralysis in animals treated with several doses of morphine (4.4–44.0 mg/kg, p.o.) (data not shown). However, we were able to detect Straub's signal, in animals treated with morphine (χ^2-test, d.f. 9.903, 3; $P = 0.019$) (Fig. 2A), but not in animals treated with paregoric elixir. Moreover, the higher dose of paregoric elixir (10.6 μg morphine/kg) or morphine (44.0 mg/kg) produced pilo-erection in 30% and 10% of treated animals, respectively (Fig. 2B). However, this response did not reach any statistical significance, either for paregoric elixir (χ^2-test, d.f. 2.654, 1; $P = 0.103$) or for morphine (χ^2-test, d.f. 0.9545, 1; $P = 0.328$) in comparison to the saline-treated group.

4.3. Evaluation of spontaneous movement in the open-field test

The results presented in Fig. 2C show that paregoric elixir (0.53–10.6 μg morphine/kg, p.o.) and morphine (4.4–44.0 mg/kg, p.o.) caused dose-dependent increase in the motor activity of mice when assessed in the open-field test. Interestingly, paregoric elixir, like morphine, significantly reduced the number of fecal boluses in relation to the control group (Fig. 2D).

4.4. Evaluation of antinociceptive effect

The results presented in Fig. 3A and B show that paregoric elixir, administered by oral route (0.53–10.6 μg morphine/kg, p.o.), caused partial but significant inhibition of both phases of formalin-induced nociception. Furthermore, paregoric elixir was more effective in inhibiting the first than the second phase of nociception, with maximal inhibitions of $61.9 \pm 3.1\%$ and $41.3 \pm 8.9\%$, respectively. However, its antinociceptive effect was not proportional to the doses used.

On the other hand, the oral administration of morphine (0.08–44.0 mg/kg) dose-dependently inhibited both phases of formalin-induced nociception (Fig. 3C and D). The calculated mean DI_{50} values for these effects were 24.4 (19.9–29.8) and 16.4 (10.9–24.7) mg/kg, with inhibitions of $85 \pm 4\%$ and 100% for the first and second phases of the formalin test, respectively. Interestingly, morphine-induced antinociception (8.8 mg/kg, p.o.) in both phases of the formalin test was completely reverted by the opioid antagonist naloxone (1 mg/kg, i.p.). In marked contrast, the antinociception caused by paregoric elixir (10.6 μg morphine/kg, p.o.) was not significantly changed by naloxone (Fig. 4A and B).

The pre-treatment of animals (p.o., twice a day for 4 consecutives days) with morphine (8.8 mg/kg) or with paregoric elixir (10.6 μg morphine/kg) produced tolerance to its own antinociceptive effects in comparison to animals pre-treated with saline and analysed using the formalin test in mice. However, no cross-tolerance between morphine and paregoric elixir was detected, i.e., morphine (8.8 mg/kg) continued to induce antinociception in paregoric elixir pre-treated animals and paregoric elixir (10.6 μg morphine/kg) also continued to induce antinociception in morphine pre-treated animals (Fig. 4C and D).

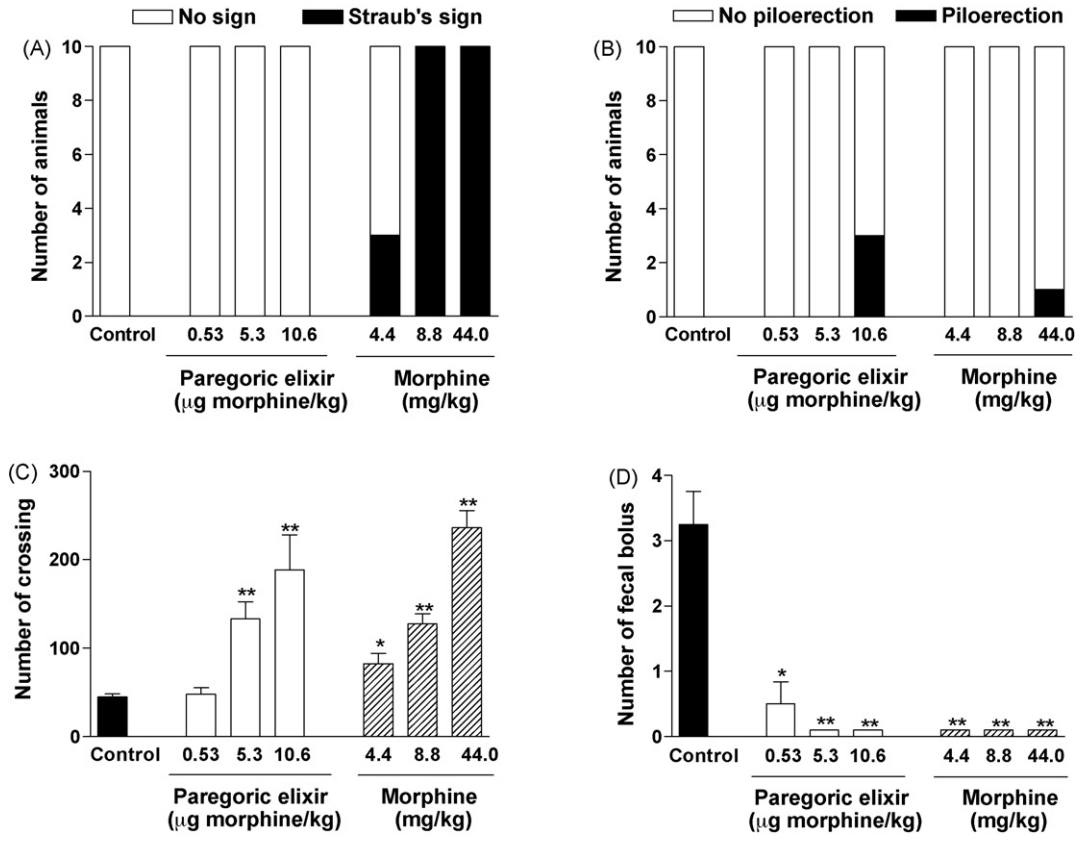

Fig. 2. Effect of treatment with paregoric elixir (0.53–10.6 μg morphine/kg, p.o.) or morphine (4.4–44.0 mg/kg, p.o.) on the behaviour of mice, indicating possible toxic effects such as Straub's signal (A) and pilo-erection (B), on the number of crossings (C) and fecal boluses (D) in the open field. Each column represents the mean ± S.E.M. of six to eight mice. The asterisks denote the significance levels, $*P<0.05$, $**P<0.01$, compared with control groups treated with vehicle.

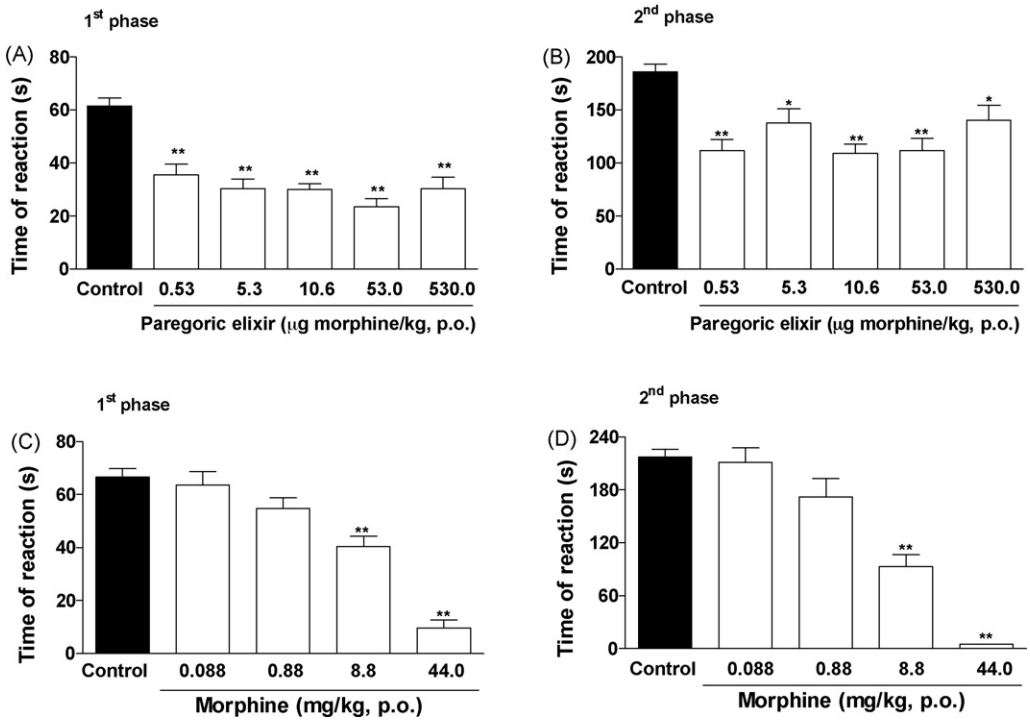

Fig. 3. Effect of treatment with paregoric elixir (0.53–530 μg morphine/kg, p.o.; A and B), morphine (0.088–44 mg/kg, p.o.; C and D) on formalin-induced nociception in mice. Each column represents the mean ± S.E.M. of six to eight mice. The asterisks denote the significance levels, $*P<0.05$; $**P<0.01$, compared to control group.

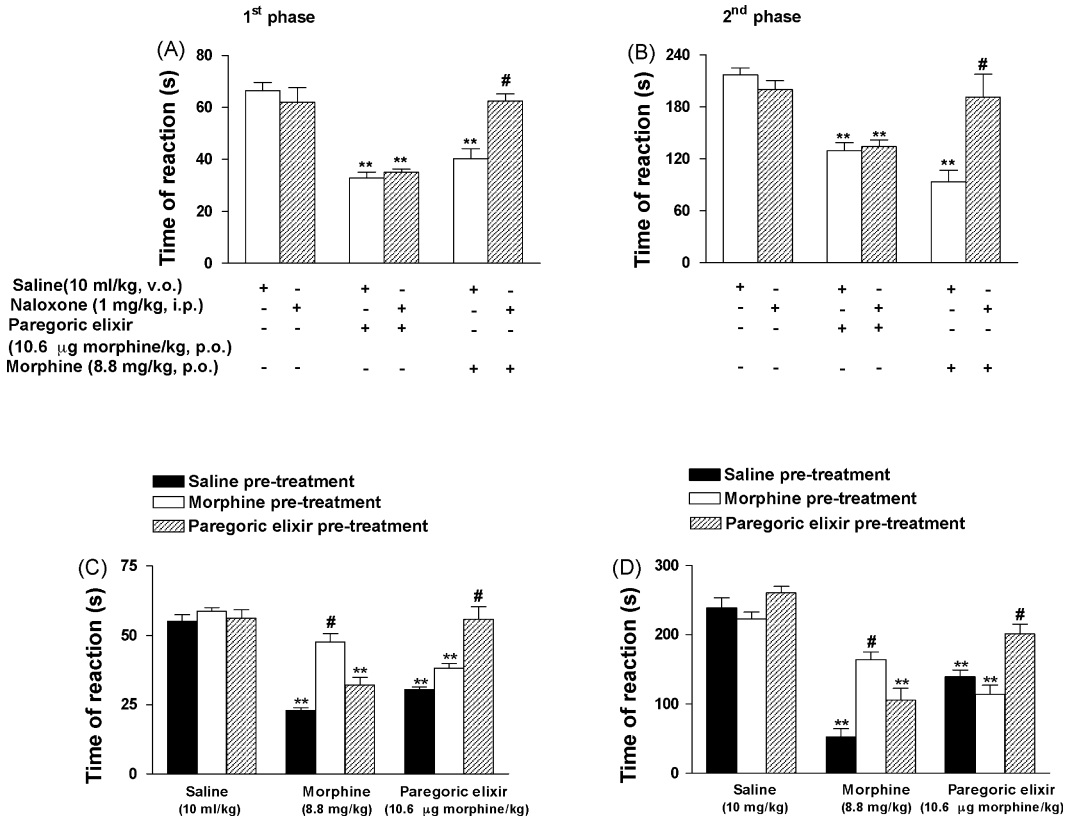

Fig. 4. Effect of naloxone (1 mg/kg, i.p.) on the antinociceptive effect caused by paregoric elixir (10.6 μg morphine/kg, p.o.) or morphine (8.8 mg/kg, p.o.) (A and B) on formalin-induced nociception in mice. Cross-tolerance effect of the treatment with paregoric elixir (10.6 μg morphine/kg, p.o.), morphine (8.8 mg/kg, p.o.) or vehicle (10 ml/kg, p.o.) twice a day for 4 days on the antinociceptive profile caused by paregoric elixir (10.6 μg morphine/kg, p.o.) or morphine (8.8 mg/kg, p.o.) on formalin-induced nociception in mice (C and D). Each column represents the mean ± S.E.M. of six to eight mice. $^{*}P < 0.05$; $^{**}P < 0.01$, compared to control group pre-treated with vehicle; $^{\#}P < 0.01$ compared to group treated with vehicle plus morphine or paregoric elixir.

4.5. Evaluation of gastrointestinal motility

The results in Fig. 5 show that similar to morphine, paregoric elixir caused dose-dependent inhibition of the gastrointestinal motility. However, a dose of morphine which was about 80 times higher than that of paregoric elixir was necessary for inhibition of gastrointestinal motility. Similarly, the treatment with papaverine (1.06 μg/kg, p.o.) significantly reduced the gastrointestinal motility in mice (Fig. 5A). In addition, the inhibition of the gastrointestinal motility produced by paregoric elixir was not affected by pre-treatment of animals with the non-selective opioid antagonist naloxone (1 mg/kg, i.p.) (Fig. 5B). The same treatment with naloxone reversed totally the gastrointestinal inhibitory effect caused by morphine (44.0 mg/kg, p.o.) (result not shown).

4.6. Evaluation of antispasmodic effect in vitro

The electrical field stimulation of guinea pig ileum-induced contractions that were abolished by tetrodotoxin 1 μM (result

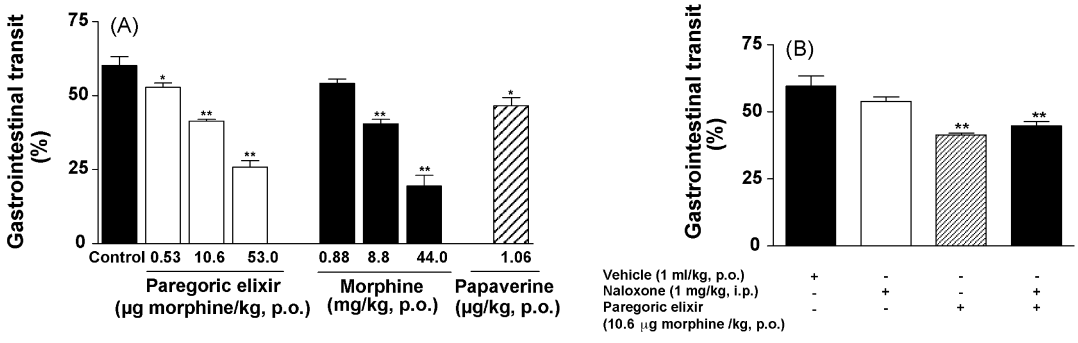

Fig. 5. Effect of treatment with paregoric elixir (0.53–53.0 μg morphine/kg, p.o.), morphine (0.88–44.0 mg/kg, p.o.) or papaverine (1.06 μg/kg, p.o.) (A) on gastrointestinal transit in mice. Effect of treatment with naloxone (1 mg/kg, i.p.) on the inhibition of gastrointestinal transit induced by paregoric elixir (10.6 μg morphine/kg) (B). Each column represents the mean ± S.E.M. of six to eight mice. The asterisks denote the significance levels, $^{*}P < 0.05$, $^{**}P < 0.01$, compared to control group treated with vehicle.

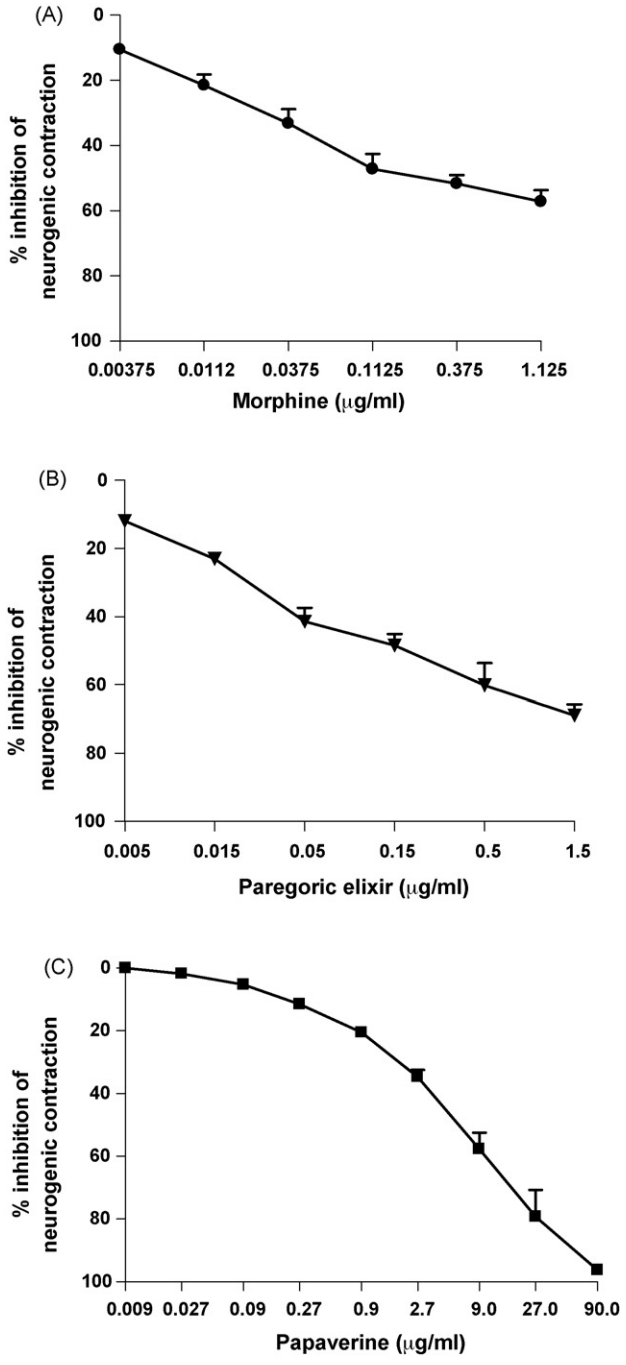

Fig. 6. Inhibitory effect of morphine (0.00375–1.125 μg/ml, A), paregoric elixir (0.005–1.5 μg/ml, B) or papaverin (0.009–90 μg/ml, C) on the contractile response induced by electrical field stimulation (continuous stimulation with square pulses of 1 ms duration, delivered at 0.1 Hz supramaximal voltage) in the isolated guinea pig ileum. Each point represents the mean ± S.E.M. of five to eight experiments.

not shown). The results presented in Fig. 6A show that non-cumulative concentrations of morphine (3.75–1125 ng/ml) induced a concentration-dependent inhibition of field electric stimulation contraction in guinea pig isolated ileum, with a mean IC_{50} value of 64.8 (33.4–122.2) ng/ml and maximal inhibition of 57 ± 3.5%. Similarly, paregoric elixir (50–1500 ng/ml) inhibited, in a concentration-dependent manner, the contractions evoked by field electric stimulation in ileum with a mean IC_{50} value of 32.9 (19.4–5.8) ng/ml and maximal inhibition of 69 ± 3.3% (Fig. 6B). Likewise, papaverine (9–90.000 ng/ml) also caused concentration-dependent inhibition of contractions evoked by field electric stimulation in guinea pig ileum, with the mean IC_{50} value of 6800 (4100–11.600) ng/ml and maximal inhibition of 96 ± 1.5% (Fig. 6C).

5. Discussion

Although paregoric elixir has been used for therapeutic purposes for more than 300 years, to our knowledge, no pharmacological studies on this product have been conducted. The results of the present study show for the first time that even when tested at higher doses in mice, paregoric elixir did not exhibit any evidence of toxic events. In addition, we detected Straub's signal in all animals treated with morphine, but not in those treated with paregoric elixir. These results in relation to paregoric elixir are somewhat unexpected, as paregoric elixir is standardized as the morphine alkaloid, and the initial belief is that this product should produce the same side effects as morphine. However, the yield of morphine in paregoric elixir is very low (0.05%), and besides morphine, the opium extract also contains other constituents such as codeine and papaverine (see Section 1 and also confirmed in the fingerprint of Fig. 1).

The experiments designed to evaluate the antinociceptive effect of paregoric elixir also furnished further support for the pharmacological action of this product, since only partial antinociception was observed, despite the use of higher doses of paregoric elixir. These results further suggest that constituents others than morphine, present in opium, must be responsible for this action. Another piece of evidence that supports this concept were the results showing that in contrast to that observed for morphine, the antinociceptive action of paregoric elixir was not dose-related and was not reversed by the non-selective opioid antagonist naloxone. This suggests that the antinociceptive actions of the product paregoric elixir are probably due to a synergic action of several components present in opium, mainly the alkaloids morphine and codeine. In fact, previous findings have shown that codeine is about 9–12 times less potent that morphine in causing antinociception, depending on the animal model used (Le Bars et al., 2001). Reinforcing this idea, codeine inhibits the two phases of formalin test, in doses five times bigger that those necessary for inhibition by morphine (Hunskaar and Hole, 1987).

Another interesting new aspect that emerged from the present study was the fact that prolonged treatment with paregoric elixir (twice a day for 4 consecutive days), in contrast to morphine, did not produce tolerance to it, nor did it cause cross-tolerance with the antinociceptive actions of morphine. These results reinforce our previous concept that the antinociceptive effects of paregoric elixir are unlikely to be associated directly with the activation of the μ opioid receptors that are sensitive to morphine.

Considering that one of the main indications of paregoric elixir is associated with the treatment of intestinal and menstrual colic, we investigated further the actions of this product, as well as some of its constituents (the alkaloids morphine and

papaverine), on gastrointestinal motility both *in vivo* and *in vitro*. The results show that paregoric elixir caused dose-dependent reduction of gastrointestinal transit *in vivo*. Notably, we observed essentially the same effect with morphine, but only when higher doses were used.

Further *in vivo* evidence supporting a non-opioid mechanism for paregoric elixir in inhibiting the gastrointestinal motility was the demonstration that the inhibitory action of paregoric elixir was not significantly reversed by previous treatment with the non-selective antagonist opioid naloxone. However, using the same doses and schedule of treatment, naloxone was capable of significantly reverting the reduction of the gastrointestinal transit caused by morphine, indicating that this alkaloid, but not the other active constituents present in the product paregoric elixir, are probably responsible for the reduction of the gastrointestinal transit, most likely acting via activation of μ opioids receptors.

To establish whether the reduction of gastrointestinal transit caused by paregoric elixir could be due to the presence of the alkaloid papaverine, a separate series of animals received papaverine at a dose of 1.06 μg/kg, calculated on the basis of the amount present in paregoric elixir. The results show that although papaverine was less potent than morphine when it was assessed in *in vitro* tests, it was more potent than morphine in reducing the gastrointestinal motility *in vivo*. Therefore, a great part of the inhibitory effect on the gastrointestinal motility caused by paregoric elixir is probably due to the presence of the alkaloid papaverine, known for its strong antispasmodic actions (Calixto et al., 1984; Kaneda et al., 1998). Furthermore, the above-mentioned results could also explain the complete absence of the reversion of the inhibitory effect of the paregoric elixir on gastrointestinal transit after treatment, with naloxone. It is important to mention that papaverine, one of the principal constituents responsible for the antispasmodic action of paregoric elixir, appears to cause relaxation in smooth muscle by multiple mechanisms, mainly via intracellular accumulation of cAMP and/or cGMP, by inhibiting phosphodiesterase, effects on Ca^{2+} movement and inhibition of mitochondrial respiration, but in ileal smooth muscle would be due to the last mechanism (Kaneda et al., 1998, 2005).

6. Conclusion

Collectively, the *in vivo* and *in vitro* results presented in the current study show that the phytomedicine paregoric elixir that is standardized as 0.05% in the alkaloid morphine, dosed orally to mice, is a safe product, being able to inhibit both phases of formalin-induced nociception as well as gastrointestinal transit in mice. Furthermore, like morphine and papaverine, paregoric elixir also concentration-dependently inhibits field electric stimulation contraction in the guinea pig isolated ileum. In marked contrast to morphine, none of the pharmacological effects caused by paregoric elixir were significantly reversed by naloxone, suggesting an opioid independent-mediated mechanism. Finally, the present results strongly suggest that the studied pharmacological actions of paregoric elixir are probably mediated by the synergistic action of its constituents, mainly papaverine, morphine and codeine.

Acknowledgements

This work was supported by grants from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), the Programa de Apoio aos Núcleos de Excelência (PRONEX) (Brazil), and Laboratório Catarinense (Brazil). E.L.A. is a post-graduate student in pharmacology receiving a grant from CNPq.

References

Benyhe, S., 1994. Morphine: new aspects in the study of an ancient compound. Life Science 55, 969–979.

Brownstein, M.J., 1993. A brief history of opiates, opioid peptides, and opioid receptors 90. In: Proceedings of the National Academy of Sciences of the United States of America, pp. 5391–5393.

Calixto, J.B., Yunes, R.A., Neto, A.S., Valle, R.M., Rae, G.A., 1984. Antispasmodic effects of an alkaloid extracted from *Phyllanthus sellowianus*: a comparative study with papaverine. Brazilian Journal of Medical and Biological Research 17, 313–321.

Calixto, J.B., Beirith, A., Ferreira, J., Santos, A.R., Cechinel, V., Yunes, R.A., 2000. Naturally occurring antinociceptive substances from plants. Phytotherapy Research 14, 401–418.

Calixto, J.B., Scheidt, C., Otuki, M., Santos, A.R., 2001. Biological activity of plant extracts: novel analgesic drugs. Expert Opinion on Emerging Drugs 6, 261–279.

Corrêa, C.R., Calixto, J.B., 1993. Evidence for participation of B_1 and B_2 kinin receptors in formalin-induced nociceptive response in mouse. British Journal of Pharmacology 110, 193–198.

Dhawan, B.N., Cesselin, F., Raghubir, R., 1996. International union of pharmacology. XII. Classification of opioide receptors. Pharmacology Reviews 48, 567–591.

Furlan, A.D., Sandoval, J.A., Mailis-Gagnon, A., Tunks, E., 2006. Adverse effects of oral morphine: an evidence-based report. Canadian Medical Association Journal 174, 1589–1594.

Guimarães, C.L., Rae, G.A., 1992. Dual effects of endothelins-1, -2 and -3 on guinea pig field-stimulated ileum: possible mediation by two receptors coupled to pertussis toxin-insensitive mechanisms. Journal of Pharmacology and Experimental Therapeutics 261, 1253–1259.

Hunskaar, S., Hole, K., 1987. The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. Pain 30, 103–114.

Hunskaar, S., Fasmer, O.B., Hole, K., 1985. Formalin test in mice, a useful technique for evaluating mild analgesia. Journal of Neuroscience Methods 14, 69–76.

Hunskaar, S., Berge, O.G., Hole, K., 1986. Dissociation between antinociceptive and anti-inflammatory effects of acetylsalicylic acid and indomethacin in the formalin test. Pain 25, 125–132.

Kaneda, T., Shimizu, K., Nakajyo, S., Urakawa, N., 1998. The difference in the inhibitory mechanisms of papaverine on vascular and intestinal smooth muscles. European Journal of Pharmacology 355, 149–157.

Kaneda, T., Takeuchi, Y., Matsui, H., Shimizu, K., Urakawa, N., Nakajyo, S., 2005. Inhibitory mechanism of papaverine on carbachol-induced contraction in bovine trachea. Journal of Pharmacological Sciences 98, 275–282.

Krantz, J.C., Carr, C.J., 1961. The Pharmacological Principles of Medical Practice. Williams & Wilkins, Baltimore, pp. 541–588.

Le Bars, D., Gozariu, M., Cadden, S.W., 2001. Animal models of nociception. Pharmacological Reviews 53, 597–652.

Montgomery, K.C., 1955. The relation between fear induced by novel stimulation and exploratory behavior. Journal of Comparative and Physiological Psychology 48, 254–260.

Osol, A., 1980. Remington's Pharmaceutical Sciences. Mack Publishing, Easton, pp. 1044–1047.

Resine, T., Pasternak, T.G., 1996. Opioid analgesics and antagonists. In: Hardman, J.G., Limbird, L.E. (Eds.), Goodman & Gilman: The Pharmacological Basis of Therapeutics. McGraw-Hill, New York, pp. 521–555.

Robbers, J.E., Speedie, M.K., Tyler, V.E., 1997. Farmacognosia e Farmacobiotecnologia. Premier, São Paulo, pp. 163–208.

Rocha, Silva, M., 1973. Fundamentos da farmacologia e suas aplicações à terapêutica. Edart, São Paulo.

Santos, A.R., Miguel, O.G., Yunes, R.A., Calixto, J.B., 1999. Antinociceptive properties of the new alkaloid, cis-8,10-di-*N*-propyllobelidiol hydrochloride dihydrate isolated from *Siphocampylus verticillatus*: evidence for the mechanism of action. Journal of Pharmacology and Experimental Therapeutics 289, 417–426.

Trease, G.E., Evans, W.C., 1978. Pharmacognosy. Baillière Tindall, London, pp. 569–576.

Woods, L.A., 1958. Narcotic analgesics I: opium alkaloids. In: Drill, V.A. (Ed.), Pharmacology in Medicine. McGraw-Hill, New York, pp. 215–228.