Pharmacogn. Res.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcogres.com | www.phcog.net

ORIGINAL ARTICLE

Ilex paraguariensis Promotes Orofacial Pain Relief After Formalin Injection: Involvement of Noradrenergic Pathway

Eudislaine Fonseca de Carvalho, Simone Kobe de Oliveira[1], Viviane Koepp Nardi, Tathiana Carla Gelinski, Marcelo Carlos Bortoluzzi[2], Marcelo Maraschin[1], Geisson Marcos Nardi

Laboratory of Pharmacology, Area of Biological and Health Science, University of the West of Santa Catarina, Joaçaba, [1]Department of Plant Science, Plant Morphogenesis and Biochemistry Laboratory, Federal University of Santa Catarina, Florianópolis, SC, [2]Department of Oral Medicine, State University of Ponta Grossa, Ponta Grossa, PR, Brazil

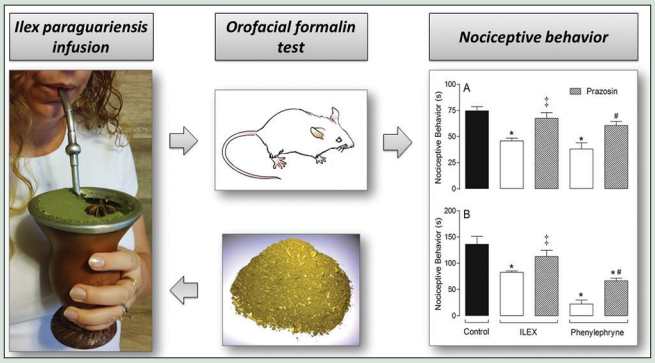
ABSTRACT

Background: Drinking mate or *chimarrão*, a hot infusion of *Ilex paraguariensis* (ILEX) leaves, is a common habit in Southern South America that has a social and almost ritualistic role. It has been used as a stimulant beverage in South America and analgesic in regions of Argentina for treatment of headache and others painful inflammatory conditions such as arthritis and rheumatism. **Objective:** The aim of this study was to evaluate the pharmacological activity of *I. paraguariensis* infusion (ILEX) on orofacial nociception model induced by formalin, and study its mechanism of action. **Materials and Methods:** The analgesic effect of ILEX was assessed through writhing test, paw formalin test, paw edema induced by carrageenan, and orofacial pain induced by formalin. To study the action mechanism of ILEX, opioidergic, dopaminergic, nitrergic, and adrenergic pathways were investigated. **Results:** The high-performance liquid chromatography analysis of ILEX infusion revealed caffeine and theobromine. The treatment with ILEX reduced the number of writhing. However, it was effective neither in the formalin paw test nor in the paw edema induced by carrageenan. Different from formalin paw test, ILEX was able to reduce the orofacial reactivity to formalin in 31.8% (70.4 ± 2.5 s; first phase), and 20% (127.3 ± 18.9 s; second phase). The analgesic effect of ILEX results from the modulation of noradrenergic pathways since prazosin (α_1-adrenoceptor antagonist, 0.15 mg/kg; intraperitoneal) reversed the analgesic effect of ILEX. **Conclusions:** The present report demonstrates that analgesic effect of ILEX in orofacial formalin test is due mainly to modulation of noradrenergic pathways.

Key words: Caffeine, *Ilex paraguariensis,* mate, noradrenergic pathways, orofacial pain

SUMMARY

- *Ilex paraguariensis* (ILEX) has been used as a stimulant beverage in South America and analgesic in regions of Argentina for the treatment of headache and others painful inflammatory conditions such arthritis and rheumatism.
- The aim of this study was to evaluate the pharmacological activity of ILEX on orofacial nociception model induced by formalin, and study its mechanism of action.
- ILEX reduced the number of writhing and orofacial reactivity to formalin in mice. However, it was effective neither in the formalin paw test nor in the paw edema induced by carrageenan.
- The analgesic effect of ILEX results from the modulation of noradrenergic pathways.



Abbreviation Used: ILEX: Infusion of *Ilex paraguariensis*, NSAIDs: Nonsteroidal anti-inflammatory drugs, L-NOARG: L-NG-nitro-arginine, UV: Ultraviolet, i.p.: Intraperitoneal, NOS: Nitric Oxide Synthase, ANOVA: Analysis of variance, S.E.M.: Standard error of mean, HPLC: High-performance liquid chromatography, NO: Nitric Oxide, v.o.: Oral route, DCQ: dicaffeoylquinic acid, BMS: Burning mouth syndrome, PBS: Phosphate-buffered saline.

Correspondence:
Dr. Geisson Marcos Nardi,
Laboratory of Pharmacology, Life Sciences Branch, University of the West of Santa Catarina, Campus II, Getúlio Vargas Street 2125, Joaçaba, SC, ZIP Code: 89600-000, Brazil.
E-mail: geisson.nardi@unoesc.edu.br
DOI: 10.4103/0974-8490.178643

Access this article online
Website: www.phcogres.com
Quick Response Code:

INTRODUCTION

Drinking mate or *chimarrão*, a hot infusion of *Ilex paraguariensis* (ILEX) leaves, is a common habit in Southern South America, including Argentina, Uruguay, Brazil, and Paraguay that has a social and almost ritualistic role. This plant belonging to *Aquifoliaceae* family popularly known as "erva Mate" or "yerba Mate" is used as a tonic stimulant. ILEX shows central nervous system stimulant properties attributed to its methylxanthine alkaloids content such as caffeine and is also known to have compounds with antioxidant properties such as phenolic acids and tannins that are the most abundant compounds in leaves.[1]

I. paraguariensis has been used as a stimulant beverage in South America and analgesic in regions of Argentina for the treatment of

Cite this article as: de Carvalho EF, de Oliveira SK, Nardi VK, Gelinski TC, Bortoluzzi MC, Maraschin M, *et al*. *Ilex paraguariensis* promotes orofacial pain relief after formalin injection: Involvement of noradrenergic pathway. Phcog Res 2016;8:S31-7.

headache and others painful inflammatory conditions such arthritis and rheumatism.[2,3] Some studies have also suggested that part of the effects is due to antioxidant and anti-inflammatory activity, reducing free radicals and inflammatory markers such as tumor necrosis factor-α and interleukin-6, that may interfere with painful and inflammatory conditions, including dental surgical procedures as third molar extraction.[4-6]

The orofacial pain is recognized as pain localized in the tissues of the head, face, neck, and oral cavity structures. Some diseases or clinical situations such as trigeminal neuralgia, headache, dental surgical pain, third molar extraction, and temporomandibular disorders are associated with orofacial pain. In fact, clinical studies have revealed that around 20% of people are affected by this pain. After diagnosis, the mainly treatment involve nonsteroidal anti-inflammatory drugs (NSAIDs), corticosteroids, tricyclic antidepressants, or benzodiazepines. However, in a considerable proportion of patients, these compounds do not adequately relieve pain; remain intractable, even after surgical interventions.[7]

This study is supported in part on observations of Bortoluzzi *et al*. that regular consumption of mate tea reduced pain intensity after third molar surgical removal.[6] Thus, the aim of this study was to evaluate the pharmacological activity of *I. paraguariensis* infusion in orofacial nociception induced by formalin and its possible action mechanism.

MATERIALS AND METHODS

Animals

Adult Swiss mice weighing 25–35 g were maintained in controlled temperature 22°C ± 2°C, light/dark cycle of 12 h, free access to water and food and acclimatized in the pharmacology laboratory 24 h before. The experiments were made between 8:00 A.M. and 06:00 P.M. All procedures were approved by the Institutional Ethics Committee of the University of the West of Santa Catarina under protocol number 019/2010.

Drugs and reagents

Carrageenan type IV, aspirin, indomethacin, sulpiride, apomorphine, L-arginine, NG-nitro-L-arginine (L-NOARG), prazosin, and phenylephrine purchased from Sigma-Aldrich, ST, Missouri, USA. Fentanyl, naloxone, and halothane from Cristália, São Paulo, SP, Brazil. Acetic acid and formaldehyde from Nuclear, São Paulo, SP, Brazil.

Plant material and preparation of *Ilex paraguariensis* infusion

The commercial package of 1 kg of *I. paraguariensis*, batch number 10/2010, was bought in a supermarket located in the city of Joaçaba, Santa Catarina, Brazil in April of 2010, batch number 04/2010. A voucher specimen was collected and deposited at the Barbosa Rodrigues Herbarium (Itajaí, SC, Brazil) under number HBR 55344. By following the previous method for preparing the popular mate drink, 100 g of plant material was a mixture with 1 L of distilled water at 85°C. After 10 min, the mixture was filtered to remove the particulates. The infusion of *I. paraguariensis* (ILEX) was filtrated and dried at 40°C, resulting in a yield of 22.55 g of extract/liter of infusion. ILEX was kept at −20°C until use and dissolved in distilled water before all experiments.

Determination of the dosage of ILEX

One of the objectives of this study was to treat the animals with the same amounts of ILEX that humans ingest. According to Victora *et al*.,[8] the mean daily consumption among "mate" drinkers is an average of 1799 mL (standard deviation = 1244 mL; interquartile range, 850–2418 mL; median, 1608 mL). Based on this study and on the yield of ILEX infusion, we calculated the doses according to Table 1. Each animal received the extract diluted in 0.1 mL of vehicle (distilled water) per 10 g of weight. Control group received the same volume of distilled water. After treatment, each distinct group was subjected to the tests described below.

Quantitative and qualitative assay of total phenolic compounds

Total phenolic compounds were quantified in the aqueous extract as previously described[9] using the Folin-Ciocalteu reagent (Sigma, St. Louis, MO, USA), by means of the absorbance determination (750 nm). The calculation of the total content of phenolic compounds was performed by a standard curve of gallic acid (10–200 μg/mL; r^2 = 0.99). For the high-performance liquid chromatography (HPLC) analysis, aliquots (10 μL per sample) were injected into a liquid chromatograph (Shimadzu LC-10, Tokyo, Japan) equipped with a reverse-phase column (Shim-pack C18, 4.6 mm i.d. × 250 mm long; Shimadzu), thermostated at 40°C, and a ultraviolet (UV)-visible detector (Shimadzu SPD 10 A, λ = 280 nm). An isocratic mobile phase of water: acetic acid: *n*-butanol (350:1:10, v/v/v) mixture was used with a flow rate of 0.8 mL/min.[10] The identification of the compounds of interest was confirmed by chromatography of reference compounds.

Extraction and chromatographic analysis of the xanthine alkaloids

The aqueous infusion (0.43 g) of mate leaves was resuspended in distilled water (5 mL) and incubated for 1 h in 15 mL of dichloromethane. The organic solvent extract was recovered, dried under N_2 flux, and resuspended in dichloromethane (1 mL). Aliquots (10 μL/sample) were injected (three consecutive injections) into a liquid chromatograph (Shimadzu LC-10) equipped with a reverse-phase column (Shim-pack C18, 4.6 mm ID × 250 mm long) thermostatized at 30°C, and a UV-visible detector (Shimadzu SPD 10 A, λ = 272 nm). An isocratic mobile phase of acetonitrile: 0.1% formic acid (15:85) was used with a flow rate at 1.0 mL/min.[11] For purpose of quantitative analysis, a standard calibration curve was obtained by plotting the area of peaks against different concentrations (10.0–100.0 μg/mL; r^2 = 0.99) of caffeine and theobromine (5.0–50.0 μg/mL; r^2 = 0.96) (Sigma, St. Louis, MO, USA).

Writhing test

Writhing was induced by an intraperitoneal (i.p.) injection of acetic acid (0.6%), and writhing movements were evaluated by 30 min of observation immediately after the acetic acid injection. Animals were pretreated orally (v.o.) with ILEX at doses of 262.0–778.0 mg/kg, 1 h before the i.p. injection of formalin. Control animals received vehicle (Distilled

Table 1: Determination of the dose of *Ilex paraguariensis* and the amount of methylxanthines

Groups	Human consume by day (mL/day)	*Dose of ILEX to human with 70 kg (g)	Dose of ILEX to mice (mg/kg)	Total phenols (mg/kg)	Methylxanthines (mg/kg)	
					Theobromine	Caffeine
Light drinker	800	18.40	262.0	51.1±2.7	0.26±0.04	6.4±1.8
Moderate drinker	1600	36.08	515.0	100.5±5.3	0.5±0.06	12.6±3.5
Heavy drinker	2400	54.48	778.0	151.8±8.0	0.76±0.09	19.0±5.3

*The human dose was determined from the yield of 22.5 g of extract obtained from 100 g of ILEX. ILEX: *Ilex paraguariensis*

water; 0.1 mL/10 g) 1 h before the irritant agent. Positive control animals received aspirin (100 mg/kg; v.o.), 30 min before formalin. The results are expressed as mean ± standard error of mean (S.E.M.) of number of writhing and statistical significance was determined by comparing treated groups with the control group.

Paw formalin test

Nociceptive behavior was induced by injection of formalin in the ventral surface of the right hindpaw. Animals were pretreated orally with ILEX at doses of 262.0–778.0 mg/kg, 1 h before the i.p. injection of acetic acid. Control animals received vehicle (Distilled water; 0.1 mL/10 g), 1 h before the irritant agent. Positive control animals received indomethacin (10 mg/kg; v.o.), 30 min before formalin. After pretreatments, the animals received an injection of 20 μL of a 2.5% formalin solution (0.92% formaldehyde) made up in phosphate-buffered saline (PBS). Following the formalin injection, animals were immediately placed in glass cones and the time spent licking/flinching and biting the injected paw was measured with a stopwatch and considered as an indication of nociception (expressed in seconds). That nociceptive behavior was recorded in two phases, the first phase of nociceptive response normally peaks at 0–5 min, and the second phase 15–30 min after the formalin injection. The results are expressed as mean ± S.E.M. of nociceptive behavior in seconds, and statistical significance was determined by comparing treated groups with the control group.

Paw edema induced by carrageenan

Aiming to determine whether the analgesic effect of ILEX was due to an anti-inflammatory effect, we decided to test this hypothesis using the paw edema model. Therefore, different groups of animals were pretreated orally with ILEX at doses of 262.0–778.0 mg/kg and control animals received only distilled water 0.1 mL/10 g. Positive control animals received indomethacin (10 mg/kg; v.o.), 1 h before carrageenan. After pretreatment, the animals were slightly anesthetized with halothane and received a subcutaneous injection of carrageenan 300 μg/paw into the right paw. The contralateral paw received the same volume of sterile PBS and served as a control. The volume of the paw was measured with a plethysmometer (Panlab, Spain) immediately after PBS or carrageenan administration and carried out at different time-points (30, 60, 120, and 240 min after injection of phlogistic agent). Results are expressed as mean ± S.E.M. to the difference of volume in milliliters between the carrageenan and the saline-treated paw. Statistical significance was determined by comparing treated groups with the control group.

Orofacial pain induced by formalin

The procedure used was essentially the same as that previously described by Luccarini *et al*.,[12] with minor modifications. Animals were pretreated orally with ILEX at doses of 262.0–778.0 mg/kg 1 h before injection of formalin for acute experiment. To assess chronic effect of ILEX on orofacial analgesia, half of each dose of the infusion was administered every 12 h, for 15 days. Control animals received vehicle (Distilled water; 0.1 mL/10 g). After acute and chronic pretreatment, the animals received a 10 μL subcutaneous injection using a 27-Gauge needle of 2.5% formalin into the right upper lip, lateral to the nose. Following injection, the animals were immediately placed back in cone glasses measuring the number of seconds that the animals spent rubbing the injected area for 30 min. The recording time was divided into two phases, the first phase of nociceptive response normally peaks at 0–5 min, and the second phase 15–30 min after the formalin injection. The results are expressed as mean ± S.E.M. of nociceptive behavior in seconds, and statistical significance was determined by comparing treated groups with the control group.

Study of action mechanism

We investigated the role played by the opioidergic, dopaminergic, serotoninergic, L-arginine/nitric oxide (NO), and adrenergic pathways in antinociceptive effect of ILEX in orofacial pain induced by formalin. Distinct groups of mice were pretreated with naloxone (an opioid antagonist, 20 mg/kg; i.p.), sulpiride (a D_2-dopaminergic receptor antagonist, 5 mg/kg; i.p.), L-arginine (precursor of NO; 600 mg/kg, i.p.), or prazosin (an α_1-adrenoceptor antagonist, 0.15 mg/kg; i.p.). After 15 min, the animals were treated with ILEX (778.0 mg/kg; v.o.), fentanyl (an opioid agonist, 60 μg/kg; s.c.), apomorphine (a nonselective dopaminergic receptor agonist, 5 mg/kg; i.p.), L-NOARG (75 mg/kg; i.p., a NO synthase [NOS] inhibitor), or phenylephrine (an α_1-adrenoceptor agonist, 10 mg/kg; i.p.). After treatment, the animals received a 10 μL subcutaneous injection of 2.5% formalin into the right upper lip, and nociceptive behavior was evaluated as described above in item 2.10.

Statistical analysis

Statistical differences between groups were made using one-way analysis of variance followed by *post hoc* test "Dunnett's" or "Tukey's" when necessary. $P < 0.05$ was considered indicative of significance.

RESULTS

Analysis of total phenolic compounds in ILEX

As expected, ILEX infusion has a high amount of total phenolic compounds and is expressed in terms of mg/kg of rat weight, shown in Table 1. The HPLC analysis of ILEX infusion revealed a fingerprint with eight compounds (data not shown), in which the major phenolic acid detected was chlorogenic acid. These data are in agreement with the results for the polyphenol content in *I. paraguariensis*.[13]

Analysis of the xanthinic alkaloids compounds in ILEX

The HPLC analysis of ILEX infusion revealed in agreement with previous reports on mate[14,15] that theophylline was not detected, whereas caffeine and theobromine were (data not shown), with the massive majority of caffeine. The quantitative analysis of the alkaloids present in ILEX infusion is expressed in terms of mg/kg of rat weight, shown in Table 1.

Writhing test, paw formalin test, and paw edema induced by carrageenan

Previous treatment of animals with ILEX reduced the writhing response induced by injection of acetic acid in 53.8, 46.5, and 56.9%

Table 2: Effect of *Ilex paraguariensis* infusion on the writhing test, paw formalin test and paw edema in mice

Models	Control	Dose of ILEX (mg/kg)		
		262.0	515.0	778.0
Writhing test				
Writhing	31.8±2.2	14.7±3.4*	17.0±3.4*	13.7±2.1*
Paw formalin test (s)				
1st phase	40.0±4.8	44.2±5.2	40.6±8.9	49.2±2.6
2nd phase	88.0±15.6	90.6±24.1	73.0±21.6	70.8±20.6
Paw edema (10^{-2}) mL (min)				
30	1.7±0.6	2.3±0.6	2.5±0.5	2.2±0.2
60	2.2±0.3	2.3±0.4	3.2±0.3	3.3±0.6
120	2.7±0.8	2.8±0.2	3.3±0.5	3.2±0.5
240	3.2±0.3	3.5±0.6	3.3±0.6	2.7±0.2

n=6-10 animal for experimental group. *P<0.05, different from control group. (ANOVA, Dunnett's test). ANOVA: Analysis of variance; ILEX: *Ilex paraguariensis*

to the doses of 262.0, 515.0, and 778.0 mg/kg, respectively. Similarly, aspirin was also able to reduce the reactivity of animals to acetic acid in 73% (8.6 ± 4.1) [Table 2]. Interestingly, the treatment with ILEX was unable to reduce any phase of the paw formalin test, nor paw edema induced by carrageenan [Table 2]. Unlike, indomethacin was able to reduce both phases of formalin-induced nociception, more effectively in the second phase, as well as paw edema induced by carrageenan, at all times evaluated (data no show).

Orofacial formalin test

Acute administration of ILEX resulted in an inhibition of both the first in and the second phase of orofacial formalin test in all doses used. In the first phase, the nociceptive behavior was reduced in 28.2% (74.2 ± 6.1 s), 30.3% (72.0 ± 3.2 s), and 31.8% (70.4 ± 2.5 s), and second phase in 20% (127.3 ± 18.9 s), 36% (102.2 ± 10.0 s), and 29.9% (111.9 ± 6.2 s) to the doses of 262.0, 515.0, and 778.0 mg/kg, respectively [Figure 1a and b]. Chronic treatment suppresses the response to formalin quite similar to acute treatment. In first phase, nociceptive response to formalin was reduced in 22.6 (69.7 ± 2.3 s), 23 (69.6 ± 2.9 s), and 22.8% (69.5 ± 1.3 s) and in second phase in 25.1 (112.2 ± 17.1 s), 29.3 (105.8 ± 20.2 s), and 28.2% (107.5 ± 12.8 s), respectively to the doses used [Figure 1c and d].

Study of action mechanism

The results presented in Figure 2a and b show that the treatment of mice with naloxone (opioid antagonist), given 15 min earlier, fully prevented the antinociception caused by fentanyl (opioid agonist), when analyzed against both phases of orofacial formalin test. However, under the same conditions, naloxone did not modify the antinociception caused by ILEX in both phases of orofacial formalin test [Figure 2a and b]. The mice treatment with sulpiride (D_2-dopaminergic antagonist), 15 min beforehand, significantly reversed the antinociception caused by apomorphine (a nonselective dopaminergic agonist) but did not change the antinociception caused by ILEX in both phases of the orofacial formalin test [Figure 2c and d]. In the same way, the treatment of animals with L-arginine (precursor of NO) reversed the antinociception caused by L-NOARG (an NOS inhibitor) only in the second phase of orofacial formalin test. The L-NOARG was not able to produce antinociception in the first phase of formalin orofacial test. Nevertheless, L-arginine did not modify the antinociception caused by ILEX in both phases of the test [Figure 2e and f]. Differently from the study of other systems, when we studied the noradrenergic pathways, the treatment of animals with prazosin (α_1-adrenoceptor antagonist.) reversed the antinociception caused by ILEX in both phases of orofacial formalin test. Under the same conditions, prazosin treatment significantly antagonized the antinociceptive action of phenylephrine (α_1-adrenoceptor agonist) [Figure 3].

DISCUSSION

The mainly findings reported here indicate that treatment with infusion of *I. paraguariensis* was able to relieve the orofacial pain induced by formalin in mice. Moreover, part of its mechanism of action involves modulation of noradrenergic pathways.

Third molar removal is a common procedure frequently associated with pain, of moderate or severe intensity. This intervention involves trauma to soft and bony tissues and can result in considerable pain, for this reason, it has become the most frequently used model in acute pain trials. Several studies have shown that natural products have been demonstrated antinociceptive activity in experimental models of orofacial nociception.[16,17] Recently, a clinical trial conducted by Bortoluzzi *et al.*[6] demonstrated that regular consumption of "mate," especially when consumed daily, causes a reduction of pain intensity associated with third molar extraction. The mechanism proposed by the authors is that the analgesic effect of *I. paraguariensis* results from the

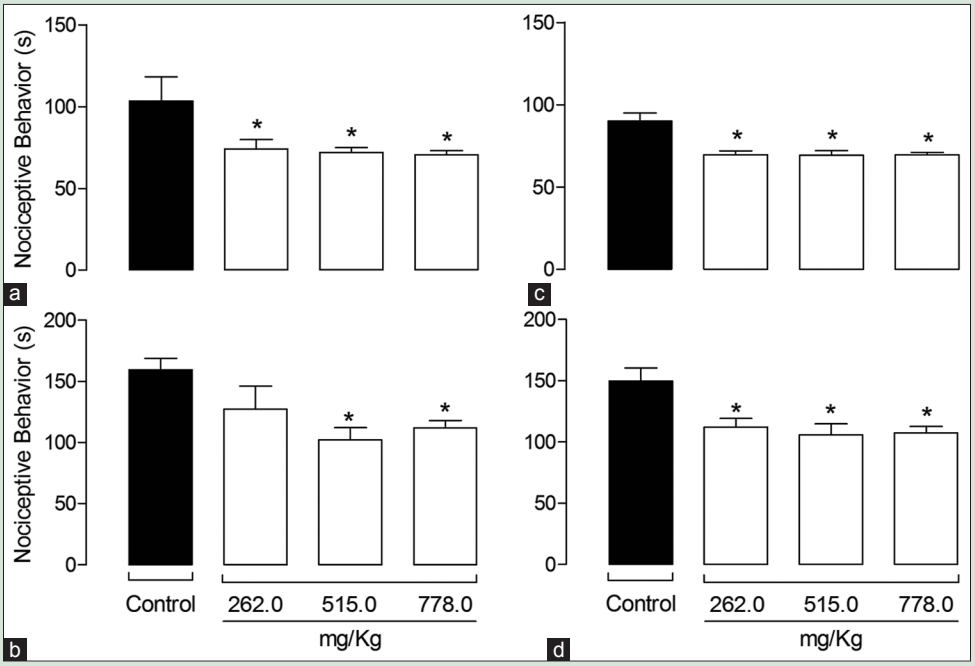


Figure 1: Orofacial antinociceptive effect of ILEX. (a and b) Represent the acute experiment where animals were treated with doses of 262.0–778.0 mg/kg of infusion, p.o. (c and d) Represent the chronic experiment, in which mice received the same doses, twice a day, for 15 days. In both cases, 1 h after last treatment, 20 µl of formalin 2.5% was injected into the right upper lip, and the evaluation of nociceptive behavior was performed in the first phase (a and c) and in second phase (b and d). Mean ± standard error of the mean of 6–9 animals/group. *$P < 0.05$ (analysis of variance, Dunnett's test)

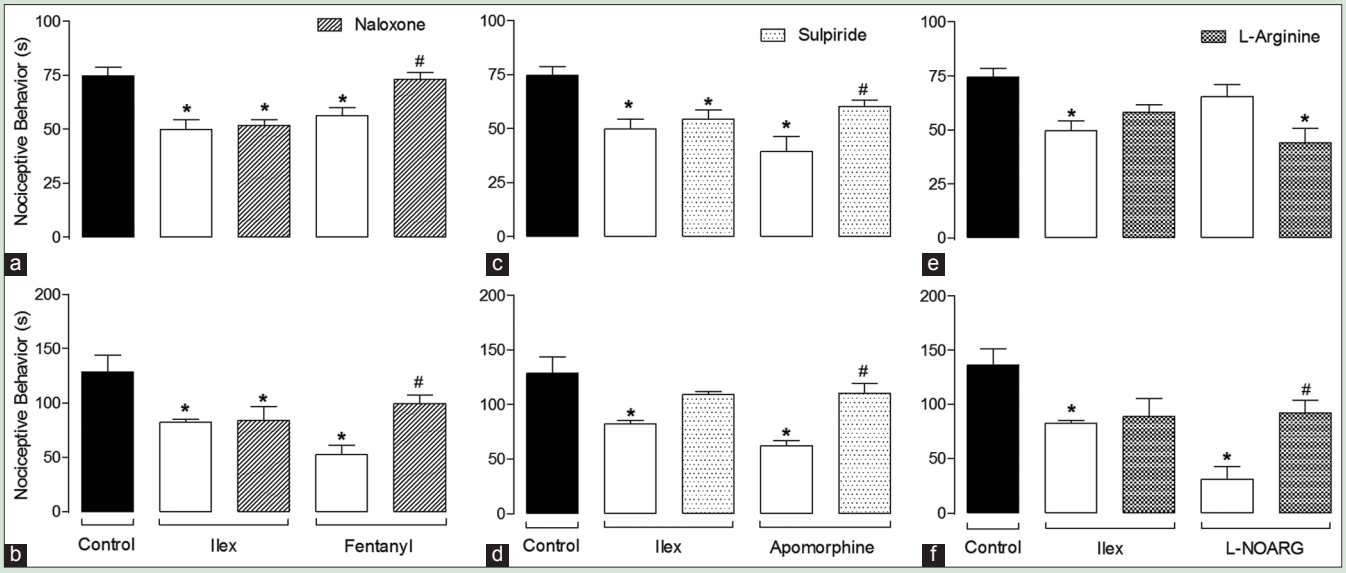


Figure 2: Influence of antagonists on the orofacial antinociception induced by ILEX. Animals were treated with naloxone (20 mg/kg; intraperitoneal a and b), sulpiride (5 mg/kg; intraperitoneal c and d), and L-arginine (600 mg/kg; intraperitoneal e and f), 15 min before the administration of ILEX (778.0 mg/kg; p.o.) or fentanyl (60 μg/kg, s.c.), apomorphine (5 mg/kg, intraperitoneal), and L-NG-nitro-arginine (75 mg/kg, intraperitoneal). Nociceptive behavior in first (a, c and e) and the second phase (b, d, and f) was performed. The mean ± standard error of mean of 6–9 animals. **P* < 0.05, different from control. #*P* < 0.05, different from fentanyl open bar (analysis of variance, Bonferroni's test)

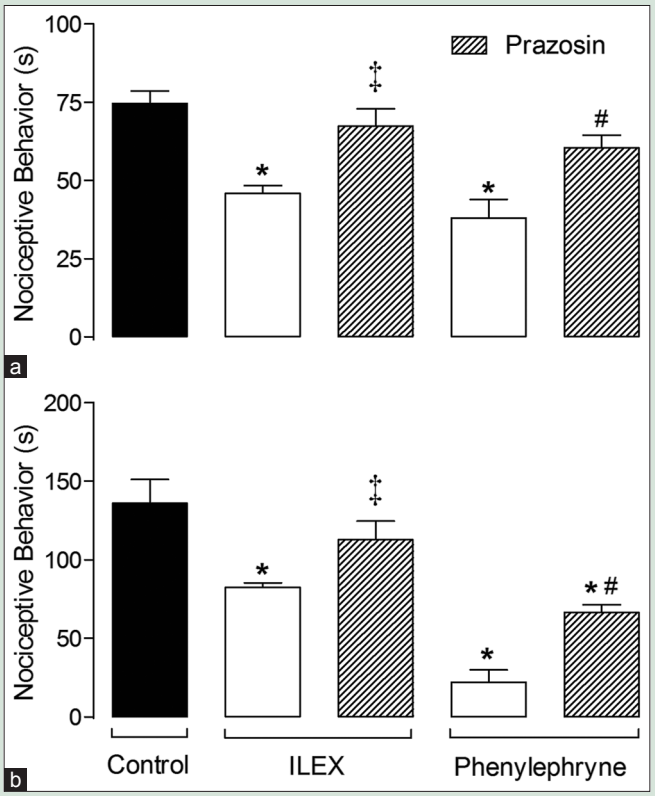


Figure 3: Influence of prazosin on the orofacial antinociception induced by ILEX. Animals were pretreated with prazosin (0.15 mg/kg; intraperitoneal), 15 min before the administration of ILEX (778.0 mg/kg; p.o.) or phenylephrine (10 mg/kg, intraperitoneal). Formalin 2.5 % (20 μL) was injected 1 h after last treatment and evaluation of nociceptive behavior in first (a) and in the second phase (b) was performed. Mean ± standard error of mean of 6–8 animals/group. **P* < 0.05, different from control. #*P* < 0.05, different from phenylephrine open bar. ‡*P* < 0.05, different from ILEX open bar (analysis of variance, Bonferroni's test)

modulation of inflammatory mediators. For this reason, we evaluated the effect of *I. paraguariensis* infusion in classical models of inflammation and pain. One of our concerns was to ensure that doses used in this trial were very close to those of human consumption [Table 1], and doses were determined according to Victora *et al.*[8]

The writhing test is a model of visceral pain-sensitive centrally acting analgesics and NSAIDs. Although not a specific test is helpful to carry out pharmacological screening. The treatment with ILEX reduced the number of writhes indicating an analgesic effect. However, in our experimental conditions, the ILEX did not reverse paw edema in mice. This model is sensitive to NSAIDs, thus reducing orofacial pain does not seem to be a result of anti-inflammatory activity of the compounds present in the plant.

Some of the pharmacological properties attributed *I. paraguariensis* mate have been related to the high content of phenolic acid compounds such as chlorogenic acids, 4, 5 DCQ, and others.[18-20] Evaluation of the pharmacological properties of chlorogenic acid pointed out strong anti-inflammatory and antinociceptive activities, but it failed to display antinociceptive activity when tested by the tail-flick test indicating that its analgesic activity is not due its anti-inflammatory action, but modulation of central pain pathways.[21]

Interestingly, ILEX did not induce analgesia in any phases of formalin paw test [Table 2] but was effective in reducing the orofacial pain [Figure 1]. There is an important difference between nociceptive responses derived from facial stimulation when compared with those using peripheral stimuli. The orofacial region is innervated by the trigeminal nerve, which has purely sensory fibers. While other regions, such as the sciatic nerve, exhibit a mixture of sensory and motor fibers. According to Dodd and Kelly,[22] these anatomical differences are responsible for the different effectiveness of drugs used in orofacial pain, compared to those who promote analgesia in the sciatic neuropathic pain. Indeed, doses of morphine that cause complete analgesia in models of peripheral pain does not present the same results in models of orofacial pain.[23] Therefore, we suggest that the ability of ILEX to induce orofacial analgesia, but not plantar analgesia, may be the result of anatomical differences described above.

After verifying the effectiveness of ILEX to reduce orofacial pain, we studied the effect of specific antagonists upon several pain systems

processing to establish the mechanism of action. The systemic administration of opioids such as morphine, dose-dependently inhibits formalin-induced orofacial pain in mice. Furthermore, systemic opioids could produce antinociception in trigeminal and spinal animal pain models and this effect is blocked by opioid antagonists.[12] Our results showed that naloxone reversed the antinociception induced by fentanyl. However, the opioid antagonist did not interfere in analgesic effect of ILEX.

Dopamine plays an important role in nociception control in several models of chronic and acute pain. In burning mouth syndrome (BMS), a chronic orofacial pain state, an increased D_2 receptor availability in the putamen is demonstrated.[24,25] Stuginski-Barbosa *et al.*[26] using pramipexole, a nonergotic dopaminergic agonist, related a complete improvement of the painful symptoms associated with BMS. However, in our experiments, the previously treatment with sulpiride did not reverse the analgesic effect of ILEX, on the other hand, reversed the analgesic effect of apomorphine. Therefore, the dopaminergic system is not involved in analgesic effects of ILEX extract.

NO may play a pronociceptive role in the inflammatory pain of orofacial region. Several studies have indicated a correlation between NO production and the generation and/or maintenance of chronic pain including temporomandibular joint disorders. NO firstly contributes to vessel homeostasis and causes vasodilation and increasing blood flow. NOS inhibitor NG-nitro-L-arginine methyl ester significantly reduces the hyperalgesia in formalin-induced orofacial pain.[27,28] In our experimental conditions, the inhibition of NO production with L-NOARG reduce the second phase of pain and this effect was reversed by the administration of NO formation substrate L-arginine. However, L-arginine was unable to reverse the effect of ILEX, demonstrating that the analgesic effect does not involve the modulation of nitrergic pathway.

We also investigated the possible involvement of descending inhibitory noradrenergic pathway in the antinociceptive effect of ILEX. The pretreatment of animals with prazosin, an α_1-adrenoreceptor antagonist, reversed the antinociceptive effect induced by phenylephrine. Similar to these results, prazosin reversed the antinociceptive effect promoted by oral administration of ILEX in the orofacial formalin test. This result discloses a participation of α_1-adrenergic receptor in the antinociceptive effect of ILEX. Studies on the analgesic action of antidepressants suggest that α_1-adrenergic receptors are involved in formalin-induced pain. Furthermore, the densities of these receptors change in different brain areas involved in pain after formalin injection.[29,30]

Caffeine and theobromine are prominent xanthines involved with biological activities of *I. paraguariensis*. Of these, caffeine was found in the highest concentration. Caffeine is present in different analgesic formulations commonly found on the market, usually associated with dipyrone, acetaminophen, or acetylsalicylic acid, and clinical studies initially indicated that caffeine was just an adjuvant.[31,32] However, further analysis knocked down this theory, and several reports have revealed that caffeine activates descending noradrenergic pathways originated in the locus coeruleus producing antinociception. The action mechanism proposed to antinociceptive effect of caffeine involve firing of neurons in the locus coeruleus and enhancing the turnover of noradrenaline in a number of brain regions innervated by the locus coeruleus.[33-35] However, the analgesic effect of caffeine is not derived from direct action on noradrenergic neurons, but rather, by its effect on the blockade of a tonic inhibition of neurons in the locus coeruleus by adenosine,[36,37] as well as blockade of inhibitory adenosine receptors on central noradrenergic nerve terminals, since the caffeine is described as an antagonist of both adenosine A_1 and A_2 receptors.[38]

CONCLUSIONS

The results of the present report demonstrate the analgesic effect of ILEX in the orofacial formalin test. Moreover, in our experimental conditions, we suggested that part of the effect of *I. paraguariensis* infusion is due to the modulation of noradrenergic pathways as a result of the presence of methylxanthines such as caffeine. More studies will be necessary to elucidate the complete mechanism of orofacial pain relief induced by ILEX.

Financial support and sponsorship

This work was supported by grants from FAPESC/CNPq (Edital Universal 2012, PRONEM 2012 and Jovem Pesquisador, 2011) and UNOESC.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Heck CI, de Mejia EG. Yerba mate tea (*Ilex paraguariensis*): A comprehensive review on chemistry, health implications, and technological considerations. J Food Sci 2007;72:R138-51.
2. Crovetto RM. Plants used in popular medicine at the Corrientes North-western area (Argentina Republic). Miscelânea 1981;69:7-139.
3. Rondina RD, Bandoni AL, Coussio JD. Argentine Medicinal Species with Potential Analgesic Activity. Dominguezia 2008;24:47-69.
4. Schinella GR, Troiani G, Dávila V, de Buschiazzo PM, Tournier HA. Antioxidant effects of an aqueous extract of *Ilex paraguariensis*. Biochem Biophys Res Commun 2000;269:357-60.
5. Arçari DP, Bartchewsky W Jr., dos Santos TW, Oliveira KA, DeOliveira CC, Gotardo ÉM, *et al.* Anti-inflammatory effects of yerba maté extract (*Ilex paraguariensis*) ameliorate insulin resistance in mice with high fat diet-induced obesity. Mol Cell Endocrinol 2011;335:110-5.
6. Bortoluzzi MC, Guollo A, Capella DL. Pain levels after third molar surgical removal: An evaluation of predictive variables. J Contemp Dent Pract 2011;12:239-44.
7. Krzyzanowska A, Avendaño C. Behavioral testing in rodent models of orofacial neuropathic and inflammatory pain. Brain Behav 2012;2:678-97.
8. Victora CG, Muñoz N, Horta BL, Ramos EO. Patterns of maté drinking in a Brazilian city. Cancer Res 1990;50:7112-5.
9. Swain T, Hillis WE. The phenolic constituents of *Prunus domestica*. The quantitative analysis of phenolic constituents. J Sci Food Agric 1959;10:63-8.
10. Vieira MA, Maraschin M, Pagliosa CM, Podestá R, de Simas KN, Rockenbach II, *et al.* Phenolic acids and methylxanthines composition and antioxidant properties of mate (*Ilex paraguariensis*) residue. J Food Sci 2010;75:C280-5.
11. Robb CS, Geldart SE, Seelenbinder JA, Brown PR. Analysis of green tea constituents by HPLC-FTIR. J Liq Chromatogr Relat Technol 2002;25:787-801.
12. Luccarini P, Childeric A, Gaydier AM, Voisin D, Dallel R. The orofacial formalin test in the mouse: A behavioral model for studying physiology and modulation of trigeminal nociception. J Pain 2006;7:908-14.
13. Cardozo EL Jr., Ferrarese-Filho O, Filho LC, Ferrarese ML, Donaduzzi CM, Sturion JA. Methylxanthines and phenolic compounds in mate (*Ilex paraguariensis* St. Hil.) progenies grown in Brazil. J Food Compost Anal 2002;20:553-8.
14. Reginatto FH, Athayde ML, Gosmann G, Schenkel P. Methylxanthines accumulation in *Ilex* species – Caffeine and the obromine in erva-mate (*Ilex paraguariensis*) and other *Ilex* species. J Braz Chem Soc 1999;10:443-6.
15. Saldaña MD, Zetzl C, Mohamed RS, Brunner G. Extraction of methylxanthines from guaraná seeds, maté leaves, and cocoa beans using supercritical carbon dioxide and ethanol. J Agric Food Chem 2002;50:4820-6.
16. Mittal N, Joshi R, Hota D, Chakrabarti A. Evaluation of antihyperalgesic effect of curcumin on formalin-induced orofacial pain in rat. Phytother Res 2009;23:507-12.
17. Bonjardim LR, Silva AM, Oliveira MG, Guimarães AG, Antoniolli AR, Santana MF, *et al. Sida cordifolia* leaf extract reduces the orofacial nociceptive response in mice. Phytother Res 2011;25:1236-41.
18. Mazzafera P. Caffeine, theobromine and theophyline distribution in *Ilex paraguariensis*. Braz J Plant Physiol.1994;6:149-51.
19. Filip R, López P, Giberti G, Coussio J, Ferraro G. Phenolic compounds in seven South American *Ilex* species. Fitoterapia 2001;72:774-8.

20. Gugliucci A, Bastos DH. Chlorogenic acid protects paraoxonase 1 activity in high density lipoprotein from inactivation caused by physiological concentrations of hypochlorite. Fitoterapia 2009;80:138-42.
21. Yonathan M, Asres K, Assefa A, Bucar F. *In vivo* anti-inflammatory and anti-nociceptive activities of *Cheilanthes farinosa*. J Ethnopharmacol 2006;108:462-70.
22. Dodd J, Kelly JP. Trigeminal system. In: Kandel ER, Schwartz JH, Jessell TM, editors. Principles of Neural Science. New York: Elsevier; 1995. p. 701-10.
23. Idänpään-Heikkilä JJ, Guilbaud G. Pharmacological studies on a rat model of trigeminal neuropathic pain: Baclofen, but not carbamazepine, morphine or tricyclic antidepressants, attenuates the allodynia-like behaviour. Pain 1999;79:281-90.
24. Zarrindast MR, Nassiri-Rad S, Pazouki M. Effects of dopaminergic agents on antinociception in formalin test. Gen Pharmacol 1999;32:517-22.
25. Hagelberg N, Forssell H, Aalto S, Rinne JO, Scheinin H, Taiminen T, *et al.* Altered dopamine D2 receptor binding in atypical facial pain. Pain 2003;106:43-8.
26. Stuginski-Barbosa J, Rodrigues GG, Bigal ME, Speciali JG. Burning mouth syndrome responsive to pramipexol. J Headache Pain 2008;9:43-5.
27. Jung HS, Jeon HB, Jeon IS, Lee BJ, Yoo HW, Ahn DK, *et al.* Preventing extracellular diffusion of trigeminal nitric oxide enhances formalin-induced orofacial pain. Korean J Physiol Pharmacol 2009;13:379-83.
28. Fan W, Huang F, Wu Z, Zhu X, Li D, He H. The role of nitric oxide in orofacial pain. Nitric Oxide 2012;26:32-7.
29. Yokogawa F, Kiuchi Y, Ishikawa Y, Otsuka N, Masuda Y, Oguchi K, *et al.* An investigation of monoamine receptors involved in antinociceptive effects of antidepressants. Anesth Analg 2002;95:163-8.
30. Nalepa I, Vetulani J, Borghi V, Kowalska M, Przewlocka B, Roman A, *et al.* Changes induced by formalin pain in central alpha1-adrenoceptor density are modulated by adenosine receptor agonists. J Neural Transm (Vienna) 2010;117:549-58.
31. Zhang WY. A benefit-risk assessment of caffeine as an analgesic adjuvant. Drug Saf 2001;24:1127-42.
32. Sawynok J. Methylxanthines and pain. In: Methylxanthines, Handbook of Experimental Pharmacology. Berlin: Springer; 2011. p. 311-29.
33. Corrodi H, Fuxe K, Jonsson G. Effects of caffeine on central monoamine neurons. J Pharm Pharmacol 1972;24:155-8.
34. Grant SJ, Redmond DE Jr. Methylxanthine activation of noradrenergic unit activity and reversal by clonidine. Eur J Pharmacol 1982;85:105-9.
35. Hadfield MG, Milio C. Caffeine and regional brain monoamine utilization in mice. Life Sci 1989;45:2637-44.
36. Shefner SA, Chiu TH. Adenosine inhibits locus coeruleus neurons: An intracellular study in a rat brain slice preparation. Brain Res 1986;366:364-8.
37. Regenold JT, Illes P. Inhibitory adenosine A1-receptors on rat locus coeruleus neurones. An intracellular electrophysiological study. Naunyn Schmiedebergs Arch Pharmacol 1990;341:225-31.
38. Müller CE, Jacobson KA. Recent developments in adenosine receptor ligands and their potential as novel drugs. Biochim Biophys Acta 2011;1808:1290-308.

Geisson Marcos Nardi

ABOUT AUTHOR

Geisson Marcos Nardi, PhD in Pharmacology. Professor of Pharmacology at School of Medicine, University of the West of Santa Catarina (Unoesc). Contribute as professor at Unoesc Postgraduate Program of Bioscience and Health, and Program of Science and Biotecnology. It has experience in pharmacology of natural products and cardiovascular pharmacology.