Research Article

Activity of the hydroethanolic extract of Buddleja brasiliensis Jacq. ex Spreng. Fam. on acute pain induced in mice -

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Citation this article: Almanca CCJ, Marinho BG. Activity of the Hydroethanolic Extract of Buddleja brasiliensis Jacq. Ex Spreng. Fam an Acute Pain Induced in Mice. Int J Pain Relief. 2017;1(1): 001-007.

ABSTRACT

Buddleja brasiliensis is a species found in Southern Brazil, being popularly used in the treatment of inflammatory, respiratory and rheumatic diseases. From the genus Buddleja, several types of chemical compounds have been isolated, including flavonoids and other shikimate-derived compounds. The aim of this study was to study the antinociceptive activity of the hydroethanolic extract of *Buddleja brasiliensis* in acute pain models induced in mice. Male Swiss mice (20-22 g) were used in models of acute pain (Acetic acid-induced abdominal writhing, formalin, tail flick and hot plate tests) and in model for evaluation of spontaneous motor performance (open field test). Furthermore, we evaluate the possible action mechanism of *B. brasiliensis* using naloxone, L-NAME, glibenclamide, atropine, mecamylamine and ondansetron in tail flick and hot plate tests. The hydroethanolic extract from the *B. brasiliensis* was administered orally at doses of 50, 100 and 300 mg/ kg. The chemical analyzes performed in the phytochemical screening revealed the presence of flavonoids, triterpenes, phenylethanoid glycosides and phenolic acids. The extract showed antinociception properties in models of acute pain induced by chemical and thermal stimuli and the prior administration of naloxone reduced the antinociceptive effect of *B. brasiliensis.* The results showed that the hydroethanolic extract of *B. brasiliensis* produced central antinociceptive effect, suggesting the participation of the opioid system. The extract did not develop symptoms of toxicity.

Keywords: *Buddleja brasiliensis*; Antinociception; Mice; Medicinal plants

INTRODUCTION

The pain correspond a subjective response that the organism presents when it is submitted to some potentially harmful stimulus. It is a protective function, representing in many cases, the only symptom for the diagnosis of various diseases, but when it is persistent, it provokes negative emotional reactions, becoming debilitating and causing suffering, and causing damage in social and economic relations. Pain should therefore be rapidly and effectively controlled and treated [1].

Pharmacological control of pain basically involves two strategies. The first is the use of drugs that directly block the mechanism of conduction of pain, resulting in antinociception; these drugs are represented by opioids, such as morphine, codeine, fentanyl and tramadol, which directly abolish nociceptive transmission by binding to opioid receptors [2]. The second strategy involves the use of drugs that inhibit the sensitization of nociceptors and, therefore, the development of hypernociception; this is the main mechanism of action of drugs that inhibit the cyclooxygenase, non-steroidal anti-inflammatory drugs (NSAIDs) [3].

Phytotherapy is the basis of modern pharmacology; it is also the oldest medical system in the world. The World Health Organization (WHO) defines a medicinal plant as "any plant species, cultivated or not, used for therapeutic purposes" [4]. Medicinal plants are important for pharmacological research and drug development, not only when their constituents are used directly as therapeutic agents but also as materials for synthesis, or models for pharmacologically active compounds [5].

The genus Buddleja (Scrophulariaceae) consists of about 100 species distributed in the tropics of America, Asia and Africa [6]. Buddleja species have been used in traditional medicine. *Buddleja brasiliensis* is a species found in Southern Brazil, being popularly used in the treatment of inflammatory, respiratory and rheumatic diseases. From the genus Buddleja, several types of chemical compounds have been isolated, including flavonoids and other shikimate-derived compounds, such as the phenylethanoid glycosides typified by verbascoside [7]. The aim of this study was to study the antinociceptive activity of the hydroethanolic extract of *Buddleja brasiliensis* in order to prove its use by the native population and to establish a new alternative in the treatment of algic conditions.

MATERIALS AND METHODS

Animals

Male Swiss mice (20 – 22 g) were obtained from our animal facility. The animals were maintained in a room with a controlled temperature (22 ± 2 °C) and a 12 h light/dark cycle with free access to food and water. Eight hours before each experiment only water was available to animals, to avoid interference of food with absorption of the drug. The experimental protocol was approved by the Ethics Committee on the use of animals of Institute of Biological Sciences and Health of the Federal Rural University of Rio de Janeiro (CEUA-IB – UFRRJ) under number 008/2015.

Plant material

The leaves of *Buddleja brasiliensis*, Scrophulariaceae, were collected in the city of Muniz Freire, ES, Brazil (20º 26' 19'' S e 41º 23' 44'' W) in March 2009. The plant was authenticated by Érika Von Sohsten de Souza Medeiros (Department of Systematic Botany, Botanical Garden of Rio de Janeiro, Brazil) and a voucher specimen was deposited in the Herbarium of the Botanical Garden under the number RB492873.

Preparation of **the plant extract:** The leaves (250 g) were grounded and extracted with 70% hydroethanolic solution for 72 h, then filtered and exposed again to extraction with hydroethanolic solution. To obtain the crude hydroethanolic extract the filtrates were concentrated in a rotary vacuum evaporator. The yield of the extract was about 25% (w/w). The material was stored at -20°C until use.

Phytochemical analysis: The hydroethanolic extract was fractionated by solvent partition dissolving it in ethanol/water (7:3) and the solution was submitted to successive extractions with hexane (n - C_6H_{14}), dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc) and butanol (BuOH) yielding, after distillation of the solvents. The identification of the chemical constituents was performed through spectra analysis by infrared and nuclear magnetic resonance 1H and 13C spectroscopy using uni and bi-dimensional techniques, and compared results with the literature.

Chemicals: The following substances were used: acetic acid (Vetec, Rio de Janeiro, Brazil), formalin (Merck, Darmstadt, Germany), glibenclamide, ondansetron, L-NAME, mecamylamine, atropine and dimethyl sulfoxide (Sigma Aldrich, St. Louis, MO, USA), morphine and naloxone (Cristália, São Paulo, Brazil).

Treatments

Increasing doses of the *B. brasiliensis* were administered orally (50, 100 and 300 mg/ kg – p.o.). Morphine was used as positive controls. The dose of morphine (5.01 [2.47 – 8.68] mg/ kg) and (8.15 [6.24 – 10.44] – p.o. - opioid analgesic drug) were obtained by calculating the ED_{50} (confidence limits) in acetic acid-induced abdominal writhing test and Tail Flick test, respectively, that were performed beforehand. The ED_{50} value for the antinociceptive action was obtained by fitting the data points representing the antinociceptive effect demonstrated in this model by nonlinear regression using Graph Pad Prism software version 6.0 (San Diego, California, USA) (data not shown).

Different groups were treated orally with saline solution, vehicle, morphine or the *B. brasiliensis* (50, 100 and 300 mg/ kg) for evaluation in the Acetic acid-induced abdominal writhing, formalin, tail- flick, hot-plate and open field tests. Distilled water mixed with dimethyl sulfoxide, a solubilising agent, was used as a vehicle (5%). In the control group the animals received saline solution

To evaluate the participation of specific systems on the effect shown by *B. brasiliensis*; Naloxone (5 mg/ kg), N_{ω}-Nitro-L-arginine methyl ester hydrochloride (3 mg/ kg), Ondansetron (0.5 mg/ kg), Glibenclamide (1 mg/ kg), Atropine (5 mg/ kg) and Mecamylamine (1 mg/ kg) were administered intraperitoneally (i.p.) 15 min before the oral administration of 300 mg/ kg *B. brasiliensis* in the tail-flick and hot-plate tests.

Acetic acid-induced abdominal writhing test: Model used to screen of the antinociceptive activity [8]. It was performed by the intra peritoneal administration of acetic acid (1.2%). The count of the total number of writhes was started immediately after the acetic acid injection and remained for 30 minutes. The pattern for counting of abdominal contortions was the appearance of strong abdominal contractions, followed by trunk twisting and extension of the hind limbs and contact of the abdomen with the floor of the recipient.

Formalin test: This model was performed to discriminate between activity on inflammatory or Non-inflammatory pain. Formalin-induced behaviour was accomplished as previously described by [9]. In this test, 0.02 ml of formalin solution (2.5% v/v) was administered into the hind paw of the mice. Subsequently, the time in seconds that the mice remained licking the paw was evaluated. The evaluation was done in 2 phases: the first (neurogenic phase) between 0-5 min after the formalin injection and the second (inflammatory phase) between 15-30min after the injection.

Tail flick test: This model was used to evaluate spinal antinociceptive activity, as previously described by [10]. The mice were placed in an acrylic tube prior to each determination and then placed on equipment to perform the tail-flick test. A light beam was focused approximately 4 cm from the tip of the tail and the tail withdrawal latency was recorded automatically. The light intensity was adjusted for baseline values between 3 - 5 s; this intensity was not changed and the animals that had baseline values outside these limits were excluded from the experiment. Measures of latency time (LT) were made at intervals of 20 min. The first two measures were made before drug administration. The average of these measures was called the 'baseline'. In order to evaluate the contribution of different endogenous systems on the antinociceptive activity of the extract experimental groups that received specific antagonists prior to administration of the extract were used. After determination of baseline latency, mice received the extract, and the reaction

latency was determined in different times after injection. The tail-flick latencies were converted to the percentage of increase over the baseline (IBL) according to the following formula:

$$IBL\ \% = 100 - \frac{LT \times 100}{BL}$$

Hot plate test: The procedure used was as described previously [11]. This model was used to evaluate supra-spinal antinociceptive activity. The animals were placed on a hot plate set at 55 ± 1°C. The reaction time (RT) was recorded when the animals licked their hind paws or jumped. Two measures were taken before the administration of substances and six measures were taken after the application of substances at 30-min intervals. The mean of RTs obtained before the administration of the substances is called the baseline. In order to evaluate the contribution of different endogenous systems on the antinociceptive activity of the extract experimental groups that received specific antagonists prior to administration of the extract were used. The mean RT obtained before administration of substances was considered the baseline (BL). Antinociception was quantified as the percentage increase over the baseline shown in each measurement time, calculated using the following formula:

$$Increase\,in\,baseline\ (\%) = \frac{RT \times 100}{BL} - 100$$

Open-field test: This model was realized to measure the level of spontaneous locomotor activity in mice and was performed according to the method described by [12]. Five days before behavioural testing, each animal was handled daily for a few minutes. The mice were placed individually in the center of the observation chamber (60 minutes after oral administration of substances), in which the floor was divided into 50 squares (5 x 5 cm). The mice explored freely the observation chamber for 5 min. The result is based on the total number of squares covered by the animals.

In vivo toxicological evaluation

An acute toxicity test was performed according to the WHO guidelines [13]. Acute toxicity was determined following the experimental model described previously by [14]. A single oral dose of *B. brasiliensis* (1000 mg/ kg) was administered to a group of ten mice. The animals were placed in an observation chamber and videotaped for a period of 8 hours per day. Behavioural parameters such as: convulsion, hyperactivity, grooming, loss of righting reflex, increased or decreased respiration, and sedation were observed for a period of 7 days. A control group of 10 animals administered with the vehicle was also subjected to the same protocol.

Statistical analysis

All experimental groups consisted of 7–10 animals. The results are presented as the mean ± Standard Error of the Mean (SEM). Statistical significance between the groups was determined using one-way analysis of variance (ANOVA) followed by Bonferroni's test for the acetic acid-induced abdominal writhing, formalin and open field tests. The statistical significance between the groups was determined by two-way analysis of variance (ANOVA) followed by Bonferroni's test for the tail-flick and hot-plate tests. *P* values of less than 0.05, 0.01 and 0.001 were considered to be statistically significant.

RESULTS

Phytochemical analysis

The results of the chemical analyzes performed in the phytochemical screening revealed the presence of flavonoids,

triterpenes, phenylethanoid glycosides and phenolic acids in the hydroethanolic leaf extract of *B. brasiliensis*.

Acetic acid-induced writhing tests

Oral administration of *B. brasiliensis* inhibited writhing by approximately 41% (34.2 + 2.2 writhes) and 58% (24.2 + 3.4 writhes) at doses of 100 and 300 mg/ kg, respectively. The control group showed 57.8 + 5.2 writhes, [F (5,30) = 14.11; *p* < 0.001]. The Morphine (5.01 mg/ kg) inhibited the number of writhes by approximately 50% compared with the control group (Figure 1A).

Formalin test

Pre-treatment with the *B. brasiliensis* significantly reduced the time that the mice spent licking their injected paws after formalin injection. In the first phase, the inhibitory effect was observed only with the highest dose (300 mg/ kg), while in the second phase was observed at all doses (Figure 1B).

In the first phase, the control group showed 53.2 + 6.3s (seconds), and the *B. brasiliensis* showed 65% (18.4 + 3.0s) inhibition at dose of 300 mg/ kg. In the second phase, doses of 50, 100 and 300 mg/ kg presented 33% (116.0 + 3.9s), 38% (109.2 + 3.6s) and 85% (26.4 + 4.0s) inhibition, respectively (Figure 1B), and control group showed (175.2 + 15.0s), [F(11,60) = 59.91; *p* < 0.001]. Morphine (5.01 mg/ kg) inhibited the number of licks by approximately 50% compared with the control group in both the phases.

Tail-flick test

The administration of *B. brasiliensis* at dose of 300 mg/ kg increased significantly the latency time from the measurement time of 20 min to the measurement time of 120 min, while the morphine increased significantly the latency time from the measurement time of 60 min to the measurement time of 120 min, when compared to control group (Figure 2A), [F (5,180) = 7.81; *p* < 0.001].

The figures 2B and 2C show the results of administration of *B. brasiliensis* in association with increasing doses of Naloxone, Atropine, Mecamylamine, Glibenclamide, L-NAME and ondansetron to determine the lowest dose capable of producing a change in the antinociceptive activity of the compound.

Only naloxone reduced the antinociceptive effect of *B. brasiliensis* in 74%; while prior administration of mecamylamine, atropine, ondansetron, glibenclamide and L-NAME were not able to reduce the effect of *B. Brasiliensis*. Similar results to those obtained using the control group were observed when the antagonists were administered alone (Figure 2B and 2C).

Hot-Plate test

The administration of *B. brasiliensis* at doses of 50, 100 and 300 mg/ kg increased significantly the latency time from the measurement time of 150 min to the measurement time of 180 min, from the measurement time of 90 min to the measurement time of 180 min and from the measurement time of 90 min to the measurement time of 180 min, respectively, while the morphine increased significantly the latency time from the measurement time of 30 min to the measurement time of 120 min, when compared to control group (Figure 3A), [F(5,180) = 4.68; *p* < 0.001].

Figures 3B and 3C show the results of administration of *B. brasiliensis* in association with increasing doses of Naloxone, Atropine, Mecamylamine, Glibenclamide, L-NAME and ondansetron, and
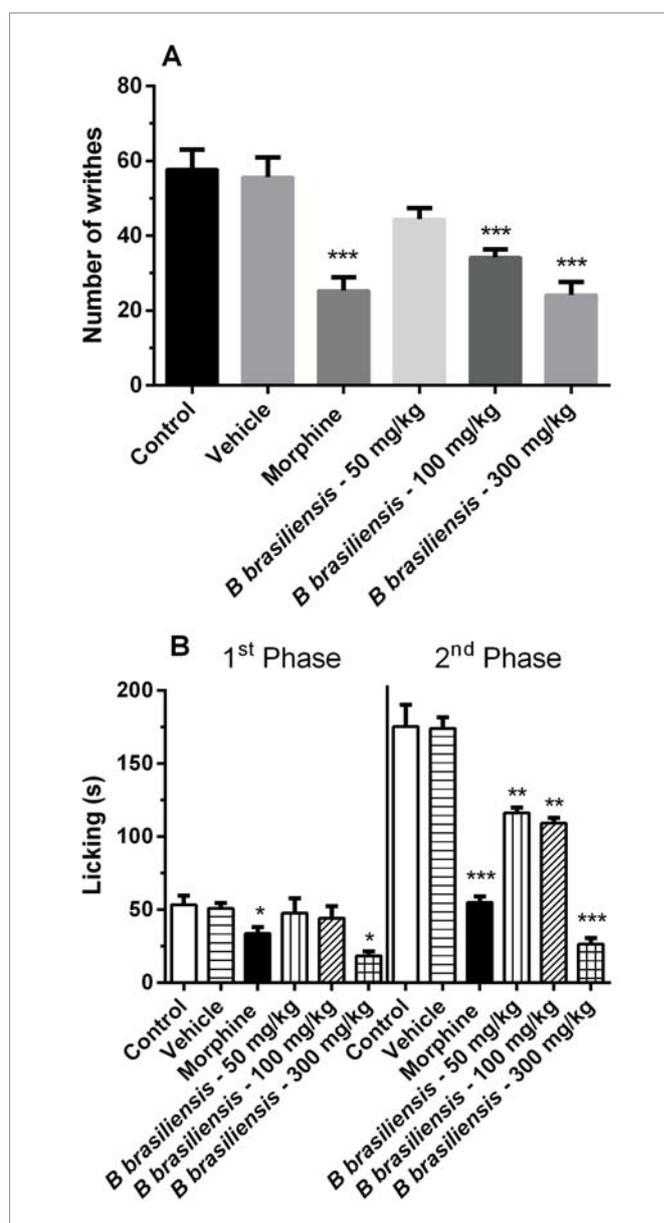
Figure 1: The effects of orally administered hydroethanolic extract of Buddleja brasiliensis (B. brasil) on acetic acid-induced writhing (A) and formalin (B) tests. The mice were pre-treated with saline solution, vehicle, morphine (5.01 mg/ kg), or B. brasiliensis (50, 100 and 300 mg/ kg) 60 min before intraperitoneal injection of acetic acid and intraplantar injection of formalin. The results are expressed as the mean ± SEM (n = 7-10). The statistical significance was calculated by One-way ANOVA followed by Bonferroni's test. * p < 0.05, ** p < 0.01 and *** p < 0.001 when comparing the B. brasiliensis-, morphine- and vehicle-treated groups with the control group.

again only the naloxone reduced the antinociceptive effect of *B. brasiliensis* in 63%. Similar results to those obtained using the control group were observed when the antagonists were administered alone (Figure 3B and 3C).

Open Field test

In the open-field test, the *B. brasiliensis* had not significant effect on locomotor activity compared with the control group at the dose of 300 mg/ kg or another dose tested (data not shown), while the morphine significantly decreased locomotor activity (Figure 4), [F(3,20) = 0.58; *p* < 0.05].

In vivo toxicological evaluation

The *B. brasiliensis* was evaluated for acute toxicity in mice. No intoxication symptoms (convulsion, hyperactivity, grooming, loss of righting reflex, respiratory rate change and sedation) were observed in the animals. The *B. brasiliensis* was not toxic after oral administration (1000 mg/ kg; $LD_{50} > 1000$ mg/ kg).
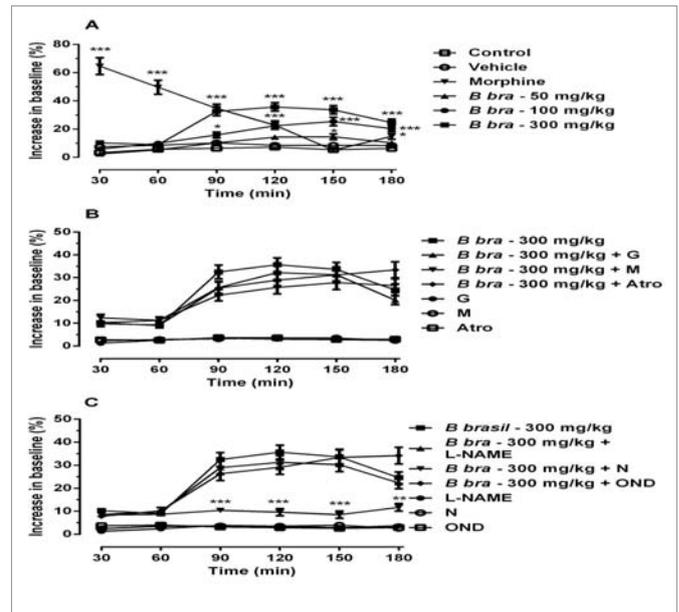
DISCUSSION

The results of the present study demonstrated, that oral administration of the hydro alcoholic extract of *Buddleja brasiliensis* showed a potent dose-dependent inhibition of the nociceptive behavioral response in animal models of pain without causing sedation or motor impairment. This antinociceptive effect may be mediated, at least in part, by the activation of opioid system.

The intraperitoneal injection of acetic acid is a test used to search new analgesic drugs that act in peripheral, spinal and supraspinal levels. The nociceptive response of this test is produced by release cytokines such as TNF-α and IL-1β from macrophages and mast cells present in the abdominal cavity, and also involves the release and biosynthesis of prostaglandins [15,16]. The *B. brasiliensis* was able to reduce the abdominal writhing number induced by acetic acid with higher doses.

The formalin test is a classic acute pain animal model for study of drugs with analgesic and anti-inflammatory effects [17]. Injection of
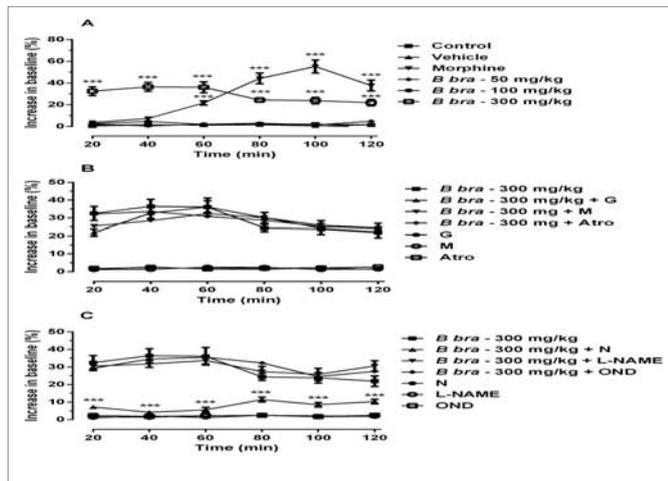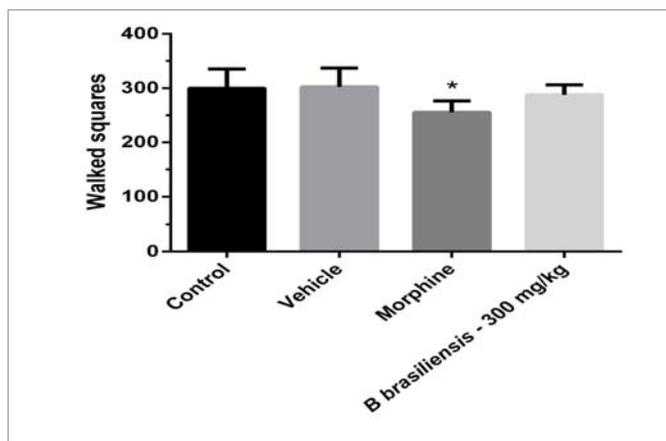

Figure 2: Effects of orally administered hydroethanolic extract of Buddleja brasiliensis (B bra) on the tail-flick test. In A, the mice were treated with saline solution, vehicle, and morphine (8.15 mg/ kg), or B. brasiliensis (50, 100 and 300 mg/ kg). In B, The mice were pre-treated intraperitoneally with Glibenclamide (G – 1 mg/ kg), Mecamylamine (M - 1 mg/ kg) and Atropine (Atro - 5 mg/kg) 15 min before the administration of B. Brasiliensis and Glibenclamide, Mecamylamine, Atropine isolated. In C, The mice were pre-treated intraperitoneally with Naloxone (N – 5 mg/ kg), Nω-Nitro-L-arginine methyl ester hydrochloride (L-NAME - 3 mg/kg) and Ondansetron (OND – 0, 5 mg/kg) 15 min before the administration of B. Brasiliensis and Naloxone, L-NAME, Ondansetron isolated. The dose of B. brasiliensis used was 300 mg/kg. The results are expressed as the mean ± SEM (n = 7 – 10). The statistical significance was calculated by two-way ANOVA followed by Bonferroni's test. In A, * p < 0.05, ** p < 0.01, and *** p < 0.001 when comparing B. brasiliensis -, vehicle and morphine-treated groups with the control group. In B, * P < 0.05, ** p < 0.01, and *** p < 0.001 when comparing Glibenclamide-, Mecamylamine-, Atropine-, B. brasiliensis + Glibenclamide-, B. brasiliensis + Mecamylamine-, B. brasiliensis + Atropine- groups with the B. brasiliensis - group. In C, * P < 0.05, ** p < 0.01, and *** p < 0.001 when comparing Naloxone-, L-NAME-, Ondansetron-, B. brasiliensis + Naloxone-, B. brasiliensis + L-NAME-, B. brasiliensis + Ondansetron- groups with the B. brasiliensis – group.

Figure 3: Effects of orally administered hydroethanolic extract of Buddleja brasiliensis (B bra) on the hot-plate test. In A, the mice were treated with saline solution, vehicle, and morphine (8.15 mg/kg), or B. brasiliensis (50, 100 and 300 mg/ kg). In B, The mice were pre-treated intraperitoneally with Glibenclamide (G – 1 mg/ kg), Mecamylamine (M - 1 mg/ kg) and Atropine (Atro - 5 mg/ kg) 15 min before the administration of B. Brasiliensis and Glibenclamide, Mecamylamine, Atropine isolated. In C, The mice were pretreated intraperitoneally with Naloxone (N – 5 mg/ kg), Nω-Nitro-L-arginine methyl ester hydrochloride (L-NAME - 3 mg/ kg) and Ondansetron (OND – 0, 5 mg/ kg) 15 min before the administration of B. Brasiliensis and Naloxone, L-NAME, Ondansetron isolated. The dose of B. brasiliensis used was 300 mg/ kg. The results are expressed as the mean ± SEM (n = 7–10). The statistical significance was calculated by two-way ANOVA followed by Bonferroni's test. In A, * p < 0.05, ** p < 0.01, and *** p < 0.001 when comparing B. brasiliensis -, vehicle and morphine-treated groups with the control group. In B, * p < 0.05, ** p < 0.01, and *** p < 0.001 when comparing Glibenclamide-, Mecamylamine-, Atropine-, B. brasiliensis + Glibenclamide-, B. brasiliensis + Mecamylamine-, B. brasiliensis + Atropine- groups with the B. brasiliensis - group. In C, * p < 0.05, ** p < 0.01, and *** p < 0.001 when comparing Naloxone-, L-NAME-, Ondansetron-, B. brasiliensis + Naloxone-, B. brasiliensis + L-NAME-, B. brasiliensis + Ondansetron- groups with the B. brasiliensis – group.

formalin into the hind paw induces a biphasic pain response; the first phase is neurogenic (early phase) and results from direct activation of primary afferent sensory neurons, predominantly by C-fiber activation, while the second phase is inflammatory (late phase) and appears to be dependent on the combination of an inflammatory reaction in the peripheral tissue and central sensitization in the dorsal horn of the spinal cord [17]. Thus, the early phase can be suppressed by the administration of centrally acting analgesics, such as morphine, while the late phase responds to various drugs with established clinical analgesic, such steroidal and non-steroidal anti-inflammatory [17]. The effect observed with the *B. brasiliensis* suggested that the antinociceptive action would be related to the central mechanisms. Similarly, morphine, presented the same response profile, inhibiting the two phases.

Nociceptive information is processed and integrated peripherally with the spinal and supraspinal levels to the central nervous system. Tail-flick test is a spinally integrated nociceptive reflex, while hot-plate test is a complex response which is supraspinally integrated. The *B. brasiliensis* showed effect in both models, confirming its central antinociceptive activity shown in the formalin test [18].

Figure 4: Effects of orally administered hydroethanolic extract of Buddleja brasiliensis on open field test. The mice were treated with B. brasiliensis (50, 100 and 300 mg/ kg), morphine (8.15 mg/ kg), vehicle and saline solution, 60 minutes later the animals were placed in the observation chamber. The results are expressed as the mean ± SEM (n = 7-10). The statistical significance was calculated by One-way ANOVA followed by Bonferroni's test. * p < 0.05, ** p < 0.01 and *** p < 0.001 when comparing the B. brasiliensis-, vehicle- and morphine-treated groups with the control group.

It has been shown that both cholinergic receptor agonists and acetyl cholinesterase inhibitors produce analgesia after spinal administration in rats and humans [19]. The importance of the cholinergic system in the modulation of pain is illustrated by the fact of ACh spinal release is increased as a consequence of the noradrenergic bulbospinal descending inhibition in response to noxious stimuli [20]. It has been shown that high frequency transcutaneous electric nerve stimulation in rats can inhibit hyperalgesia and this effect is dependent of cholinergic mechanisms [21].

The nitrergic pathway has an important role in the modulation of nociception [22]. The involvement of NO in nociception is dual; studies show that administration of NO donors inhibits PGE2-induced hypernociception and that this effect is reversed with the use of L-NAME, a nitric oxide synthase inhibitor, responsible for NO production [23]. However, other studies have shown the participation of the nitrergic pathway in the analgesic effect of opioids [24].

Modulation of nociception by 5-HT at the spinal level is a complex process, because it may inhibit and/or facilitate nociceptive transmission depending of the type of nociceptive stimuli and, most importantly, the nature of the 5-HT receptors expressed centrally [25]. There is evidence that the intrathecal administration of exogenous 5-HT reverses tactile allodynia in spinal nerve ligated in rats [26]. The antiallodynic effect of 5-HT has been attributed to the activation of 5-HT1/5 receptors [27], while 5-HT2/7 receptors have been associated with pro-nociception [28].

Based on this, the previous administration of N_ω-Nitro-L-arginine methyl ester hydrochloride (non-selective nitric oxide synthase inhibitor), Atropine (non-selective muscarinic antagonist), mecamylamine (non-selective nicotinic antagonist), Ondansetron (5-HT$_3$ serotoninergic antagonist), and Glibenclamide (selective ATP-sensitive K$^+$ channel blocker) did not change the analgesic effect of *B. brasiliensis*. Depending of the dose and route of administration used, these antagonists could induce alterations in the nociceptive response when used alone [29-31], but at the doses used in this paper, it was not observed.

The opioid hypothesis was investigated by attempting to reverse analgesia, through prior administration of naloxone (opioid antagonist). In this regard, our results show that preadministration of naloxone was able to reverse the antinociception of *B. brasiliensis*, clearly demonstrating an important involvement of the extract in the opioid system activation to produce analgesia.

The open field test was used to exclude the possibility that the antinociceptive action of extract could be related to nonspecific disturbances in the locomotor activity of the animals. We observed that with effective antinociceptive dose, the hydroethanolic extract of *B. brasiliensis* did not alter the spontaneous motor performance of mice.

In this study four experimental models of acute pain were used to assess the analgesic property of the hydroethanolic extract of *Buddleja brasiliensis* leaves. This study has demonstrated the antinociceptive activity of *B. brasiliensis* in the models of chemical nociception induced by acetic acid and formalin, as well as in models of nociception induced by thermal stimuli (tail flick and hot plate tests). The tail-flick and hot-plate tests revealed central activity, while the formalin test investigated both central and peripheral effects. Our result suggests the antinociceptive effect of *B. brasiliensis* at the spinal and supraspinal levels using tail-flick and hot-plate tests. According to these results, we can infer that the antinociceptive effect of *B. brasiliensis* seemed to be mediated by activation of the opioid system. The absence of adverse effects and intoxication was observed in our study by the lack of symptoms of intoxication in the animals that received the *B. brasiliensis* orally. More studies are needed for a better understanding of its mechanism.

ACKNOWLEDGMENT

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