Anais da Academia Brasileira de Ciências
ISSN: 0001-3765
aabc@abc.org.br
Academia Brasileira de Ciências
Brasil

LIMA, JOSÉLIA A.; COSTA, THIAGO W.R.; SILVA, LEANDRO L.; MIRANDA, ANA LUÍSA P.; PINTO, ANGELO C.

Antinociceptive and anti-inflammatory effects of a Geissospermum vellosii stem bark fraction

Anais da Academia Brasileira de Ciências, vol. 88, núm. 1, marzo, 2016, pp. 237-248
Academia Brasileira de Ciências
Rio de Janeiro, Brasil

Anais da Academia Brasileira de Ciências (2016) 88(1): 237-248
(Annals of the Brazilian Academy of Sciences)
Printed version ISSN 0001-3765 / Online version ISSN 1678-2690
http://dx.doi.org/10.1590/0001-3765201520140374
www.scielo.br/aabc

Antinociceptive and anti-inflammatory effects of a *Geissospermum vellosii* stem bark fraction

JOSÉLIA A. LIMA[1,2], THIAGO W.R. COSTA[2,3], LEANDRO L. SILVA[4], ANA LUÍSA P. MIRANDA[4] and ANGELO C. PINTO[1]

[1]Departamento de Química Orgânica, Instituto de Química, Universidade Federal do Rio de Janeiro, Av. Athos da Silveira Ramos, 149, 21941-909 Rio de Janeiro, RJ, Brasil
[2]Escola de Ciências da Saúde, Universidade do Grande Rio, Rua Professor José de Souza Herdy, 1160, 25071-202 Duque de Caxias, RJ, Brasil
[3]Escola Técnica Estadual de Santa Cruz, Fundação de Apoio à Escola Técnica, Largo do Bodegão, 46, 23550-050 Rio de Janeiro, Brasil
[4]LEFEx, Departamento de Biotecnologia Farmacêutica, Faculdade de Farmácia, Universidade Federal do Rio de Janeiro, Av. Carlos Chagas Filho, 373, 21941-902 Rio de Janeiro, RJ, Brasil

Manuscript received on July 16, 2014; accepted for publication on May 13, 2015

ABSTRACT

Geissospermum vellosii (Pao pereira) is a Brazilian tree whose stem barks are rich in indole alkaloids that present intense anticholinesterase activity. The present study evaluated the effects of a stem bark fraction (PPAC fraction) and ethanolic extract (EE) of Pao pereira in classic murine models of inflammation and pain. The EE and PPAC fraction, both at a dose of 30 mg/kg, significantly reduced mice abdominal constriction induced by acetic acid by 34.8% and 47.5%, respectively. In the formalin test, EE (30 mg/kg) and PPAC fraction (30 and 60 mg/kg) inhibited only the second phase, by 82.8%, 84.9% and 100%, respectively. Compared with indomethacin, similar doses of EE or PPAC fraction were approximately twice as effective in causing antinociception. PPAC fraction was not effective in the hot plate test but reduced the inflammatory response at the second (50.6%) and third (57.8%) hours of rat paw edema induced by carrageenan. Antihyperalgesic activity was observed within 30 min with a peak at 2 h (60.1%). These results demonstrate that compounds in PPAC fraction have anti-inflammatory and antinociceptive activity by a mechanism apparently unrelated to the opioid system. Regardless of similar responses to indomethacin, the effects of PPAC fraction are mainly attributed to acetylcholine actions.

Key words: *Geissospermum vellosii*, Pao pereira, antinociception, inflammation, cholinergic anti-inflammatory pathway.

INTRODUCTION

Geissospermum vellosii (Pao pereira) stem bark fraction (PPAC fraction) is rich in indole alkaloid substances and presents an intense anticholinesterase activity as previously characterized by our group (Lima et al. 2009). We demonstrated *in vitro* that PPAC fraction is more selective in inhibiting butyrylcholinesterase (BChE) than acetylcholinesterase (AChE). We found *in vivo* that mice that were treated with PPAC fraction reversed the scopolamine-induced amnesia with no peripheral or central cholinergic side effects (Lima et al. 2009). The main alkaloid with

Correspondence to: Josélia Alencar Lima
E-mail: joselialima@terra.com.br

anticholinesterase activity in PPAC was isolated and identified as geissospermine (Lima et al. 2009). In addition, geissospermine, the main constituent of the fraction, binds strongly to human AChE (Araújo et al. 2011).

Recently, an immunomodulatory circuit termed the "cholinergic anti-inflammatory pathway" was reported as a mechanism for neural inhibition of inflammation (Tracey 2002, Blalock 2002) and interfaces the brain with the immune system (Pavlov et al. 2003, Rosas-Ballina and Tracey 2009). Immune cells, such as macrophages, possess a complete cholinergic system consisting of acetylcholine (ACh), muscarinic and nicotinic receptors (mAChR and nAChR, respectively), choline acetyl-transferase and AChE (Kawashima and Fujii 2003, Ley et al. 2010, Chernyavsky et al. 2010). Stimulation of the vagus nerve in animals that were subjected to carrageenan-induced paw swelling significantly attenuated the development of edema formation and inhibited the local production of cytokines in the zone of inflammation (Borovikova et al. 2000a). Furthermore, the vagus nerve stimulation of mice in a model of subcutaneous inflammation caused by injecting carrageenan into an air pouch significantly inhibited the recruitment of polymorphonuclear leukocytes to the inflammatory zone (Saeed et al. 2005). As the vagus nerve does not signal directly to either the paw or the subcutaneous tissues, it is likely that the reduction in inflammation that was observed following vagus nerve stimulation in these models is attributable to the downregulation of cytokine production by the reticuloendothelial system and the redirection of leukocyte trafficking away from the periphery (Huston et al. 2006). ACh is a major parasympathetic neurotransmitter and inhibits the lipopolysaccharide (LPS)-induced production of pro-inflammatory cytokines, including interleukin–1 (IL-1), tumoral necrosis factor (TNF) from macrophages (Tracey 2002) and microglia (Shytle et al. 2004), by a mechanism that depends on the α7 nicotinic ACh receptor (α7nAChR), which inhibits nuclear factor kappa B (NF-kB) nuclear translocation and suppresses cytokines (Tracey 2007, Kamal et al. 2009). Vagus nerve stimulation or the administration of α7nAChR agonists inhibits not only TNF but also IL-1, IL-6, IL-8 and high mobility group box 1 (HMGB1) (Wang et al. 2004). In addition, nicotine inhibits resident peritoneal macrophage activation *ex vivo*, attenuating pro-inflammatory cytokine release through the α7nAChR-mediated activation of the JAK/STAT pathway (De Jonge et al. 2005). The cholinergic stimulation of α7nAChR on mouse macrophages down-regulates the production of proinflammatory cytokines in a lethal endotoxemia model (Borovikova et al. 2000b, Wang et al. 2003, Tracey 2007).

Considering all of the stated anti-inflammatory roles that are mediated by the cholinergic system (Borovikova et al. 2000b, Tracey 2002, 2007, Wang et al. 2003, 2004, Shytle et al. 2004, Kamal et al. 2009) and the previously described PPAC fraction anticholinesterase activity (Lima et al. 2009), the aim of this work was to examine the effects of PPAC fraction and the ethanolic extract (EE) of the *Geissospermum vellosii* in classic murine models of inflammation and pain. Therapies that bring together anticholinesterase and pro-cognitive properties associated with anti-inflammatory actions might represent new insight for the treatment of pathologies involving neuroinflammation and memory loss, such as Alzheimer disease (AD).

MATERIALS AND METHODS

Preparation of Ethanol Extract and Fractions

Stem barks of *Geissospermum vellosii* were dried at room temperature (30 - 35 °C). The dried material was ground, macerated six times with 95% ethanol (2 days each, for 12 days) and dried by evaporating the ethanol extracts (2 days) under reduced pressure. The yield of the ethanol extracts from stem bark

was 3.6% of the dried materials. Water was added to the dried material, and the mixture was defatted with hexane (1:5). The defatted aqueous extract was extracted with chloroform ($CHCl_3$) at different pH values (3.0 - 12.0). The fractions were eluted in thin-layer chromatography and revealed with Dragendorff´s reagent. Fractions at pH values of 5.0, 7.0, 9.0 and 12.0 presented orange colored points, characteristic of alkaloids. As we previously demonstrated (Lima et al. 2009), after evaluation of the fractions for anticholinesterase activity, the fraction at pH 7, which we named "PPAC", was chosen for pharmacological studies.

Animals

Adult Wistar rats (120 – 220 g) and Swiss albino mice (18 – 25 g) of both sexes were obtained from the Faculty of Pharmacy-UFRJ-Brazil breeding unit. The experimental procedures were in compliance with the recommendations of the Animal Care and Use Committee of Universidade Federal do Rio de Janeiro (CEUA-UFRJ protocol FARMACIA04). All of the animals were maintained under standardized conditions, only with water *ad libitum* for 12 hours before the experiment.

Drugs and Chemicals

Acetic acid, Tween 80, ethanol and formaldehyde were purchased from Merck, and indomethacin and carrageenan were purchased from Sigma Chemical. PPAC fraction and EE were prepared as a fine suspension in EtOH/Tween 80/H_2O (1:1:8, v/v/v; vehicle).

Antinociceptive Activity

Acetic acid-induced abdominal constriction in mice

The antinociceptive activity was determined *in vivo* using the abdominal constriction test induced by 0.6% acetic acid in 0.9% sodium chloride solution (0.1 ml/10 g; *i.p.*) as previously described (Collier et al. 1968). PPAC fraction and EE were administered orally (30 mg/kg; 0.1 ml/20 g) as a fine suspension one hour before acetic acid injection. Ten minutes after acetic acid injection, the number of constrictions *per* animal was recorded for 20 minutes. Control animals received an equal volume of vehicle or indomethacin (10 mg/kg) and were used as positive controls. The antinociceptive activity was reported as the percentage of inhibition of constriction compared to that of the vehicle control group.

Formalin-induced pain in mice

The formalin-induced pain test was performed as described by Hunskaar and Hole (1987). All of the groups received treatment 60 min before formalin injection, PPAC fraction (10, 30 and 60 mg/kg), EE (30 mg/kg), control (equal volume of vehicle) and indomethacin (35.8 mg/kg), which was used as a positive control. Animals were injected subplantarly with 20 µl of 2.5% formalin in 0.9% sodium chloride solution in the left hind paw. The time that mice spent licking or biting the injected paw or leg was recorded. Two distinct periods of intensive licking activity were identified and scored separately unless otherwise stated. The first period (neurogenic phase) was recorded 0-5 min after formalin injection, and the second period (inflammatory phase) was recorded 15-30 min after injection.

Hot plate test

The central analgesic activity was investigated using the hot plate test as previously described (Tejwani et al. 1992). In these experiments, the hot plate apparatus (Ugo Basile, Model-DS 37) was maintained at 56 ± 1 °C. Each mouse received two trials on the hot plate, separated by a 30-min interval from one another. The first trial familiarized the animal with the test procedure, and the second trial served as the control reaction time (first sign of paw licking or jumping) for the animal. Mice were pre-

selected, and those showing a reaction time greater than 10 s were not used. The reaction time for each mouse was determined on the hot plate surface at 30, 60, 90 and 120 min after intraperitoneal administration of the vehicle or PPAC fraction (30 mg/kg), and the time between placement and the first sign of paw licking or jumping was recorded as latency. A cut-off time of 30 s was established to avoid possible skin injury.

Carrageenan-Induced Rat Paw Edema and Hyperalgesia

The antiedematogenic and antihyperalgesic activities were measured using the carrageenan-induced rat paw edema test as previously described (Ferreira 1979) and the hyperalgesia on hot plate as previously described (Lavich et al. 2005). PPAC fraction (30 mg/kg) and an equal volume of vehicle (control) were administered intraperitoneally. After 60 min, animals were injected subplantarly with 1% carrageenan in saline or sterile saline (0.9% NaCl) into the right and left hind paws, respectively (0.1 ml/paw). Then, edema and thermal hyperalgesia were quantified.

Edema - Paw volumes were measured using a glass plethysmometer that was coupled to a peristaltic pump at each hour until three hours after the subplantar injection. Edema was calculated as the volume difference between carrageenan and saline-treated paw. The anti-inflammatory activity was expressed as the percentage inhibition of the edema compared to that of the vehicle control group.

Hyperalgesia - Carrageenan-evoked hyperalgesia was quantified as a measure of the nociceptive response to a thermal stimulus (51 ± 1 °C) that originated from a hot plate apparatus (Ugo Basile, Model-DS 37). Each animal received two trials on the hot plate, separated by a 30-min interval from one another. The first trial familiarized the animal with the test procedure, and the second trial served as the control reaction time (abrupt withdrawal of the right hind paw) for the animal. The withdrawal latency of the right hind paw was determined 0, 30, 60, 120 and 180 min after carrageenan injection. Hypernociception to heat is defined as a decrease in the withdrawal latency and is calculated as follows: Δ paw withdrawal latency (s) = (right paw withdrawal latency at time 0) – (right paw withdrawal latency at the others times). The cut-off time was 20 s to avoid possible skin injury.

Statistical Analysis

Results are expressed as the mean ± SEM of 7-10 animals per group. The data were statistically analyzed by a one-way or two-way ANOVA Bonferroni post-test for a significance level of *p<0.05 or ***p<0.001. When appropriated, the ID_{50} value (i.e., the dose that reduces response by 50%) was determined by non-linear regression using GraphPad Prism software.

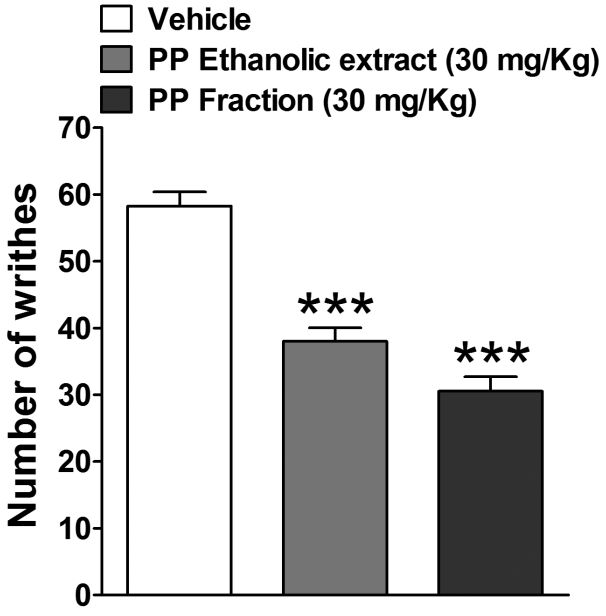

Figure 1 - Effect of EE (30 mg/kg) and PPAC fraction (30 mg/kg) on abdominal constriction induced by 0.6% acetic acid (0.1 ml/10 g*; i.p.*) in mice after oral administration. Each column represents the mean ± SEM. of 8–11 animals. ***p<0.001 in a one-way ANOVA Bonferroni post-test.

RESULTS

ANTINOCICEPTIVE ACTIVITY

Acetic acid-induced abdominal constrictions in mice

As shown in Figure 1, EE and PPAC fraction, both at a dose of 30 mg/kg (*po*), significantly reduce abdominal constrictions induced by acetic acid by 34.8 and 47.5% ($p<0.001$), respectively. The inhibition is comparable to that of indomethacin (10 mg/kg; *po*), a standard non steroidal anti-inflammatory drug (NSAID) that was used as a positive control, whose inhibition of the acetic acid-induced abdominal constrictions in mice was 44.5% (data from Lima et al. 2005).

Formalin-induced pain in mice

The analgesic activity of EE (30 mg/kg, *i.p.)* and PPAC fraction (10, 30, 60 mg/kg, *i.p.*) was evaluated using indomethacin (35.8 mg/kg*, i.p.)* as a positive control. As shown in Figure 2, EE, different doses of PPAC fraction or indomethacin did not exert an effect on the first phase (0-5 min), whereas in the second phase (15-30 min), the paw-licking time was significantly ($p<0.001$) inhibited by EE (82.8%), PPAC fraction at 30 mg/kg (84.9%) and 60 mg/kg (100%), and indomethacin (49.0%). These two phases correspond to neurogenic and inflammatory pain. Compared to indomethacin, similar doses (30 mg/kg) of EE or PPAC fraction were approximately twice as active in causing antinociception. PPAC

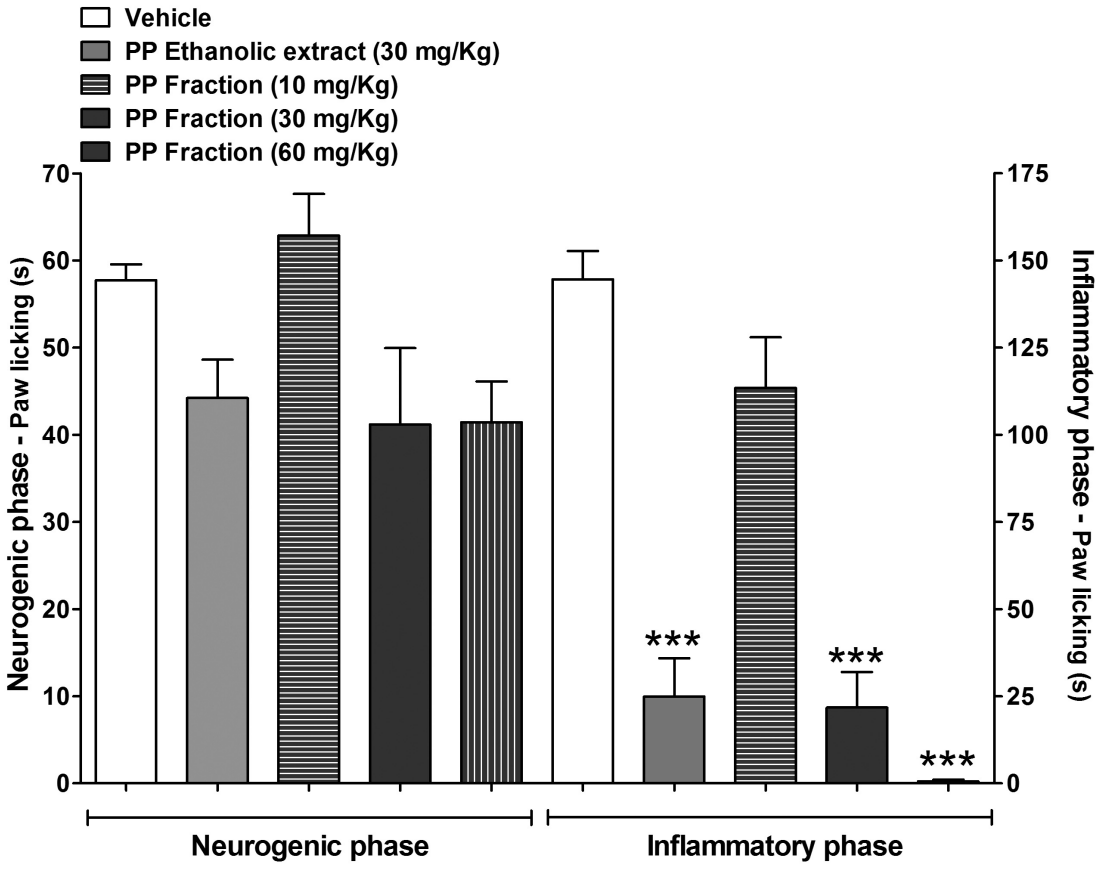

Figure 2 - Effect of PPAC fraction (10, 30 and 60 mg/kg) and EE (30 mg/kg) on formalin-induced pain in mice after *i.p.* administration. Each column represents the mean ± SEM. of 6–11 animals. ***$p<0.001$ in a one-way ANOVA Bonferroni post-test.

fraction caused dose-dependent inhibition of the second phase, with no statistically significant effect at 10 mg/kg, presenting an antinociceptive ED50 of 15.9 mg/kg.

Hot plate test

PPAC fraction (30 mg/kg, *i.p.*) did not affect the latency time response in any of the times compared to that of the vehicle group (data not shown).

CARRAGEENAN-INDUCED RAT PAW EDEMA AND HYPERALGESIA

In the carrageenan-induced paw edema, there was a gradual increase in paw volume (edema) in the control group throughout the entire experiment (3 hours). PPAC fraction at a dose of 100 mg/kg, *i.p.*, was able to inhibit the inflammatory response 2 and 3 hours post carrageenan injection. The percentage of inhibition was, respectively, 50.6 and 57.8% ($p<0.001$) (Figure 3). The simultaneous evaluation of PPAC fraction (100 mg/kg, *i.p.)* in the carrageenan-induced hyperalgesia showed that this fraction ($p<0.001$) significantly increased the withdrawal latency in all of the times (30, 60, 120 and 180 min) after carrageenan injection in more than 40%, reaching a maximum inhibition (60.1%) at 120 min (Figure 4). Indomethacin (10 mg/kg) inhibited both edema and hyperalgesia by 70%, showing an ID50 of 0.96 mg/kg at the 3rd hour under our experimental conditions (data from Lima et al. 2005, Viegas Jr et al. 2008). No gastric irritations were observed.

DISCUSSION

The potent anticholinesterase activity of PPAC fraction and its effects on memory tests in mice were previously evaluated by our group (Lima et al. 2009). In a previous work, we demonstrated that PPAC fraction inhibits rat brain and *Electrophorus electricus* (eel) AChE, as well as BChE from horse serum, in a concentration-dependent manner, being more potent against BChE than AChE. We also demonstrated that PPAC fraction can reverse scopolamine-induced amnesia in passive avoidance

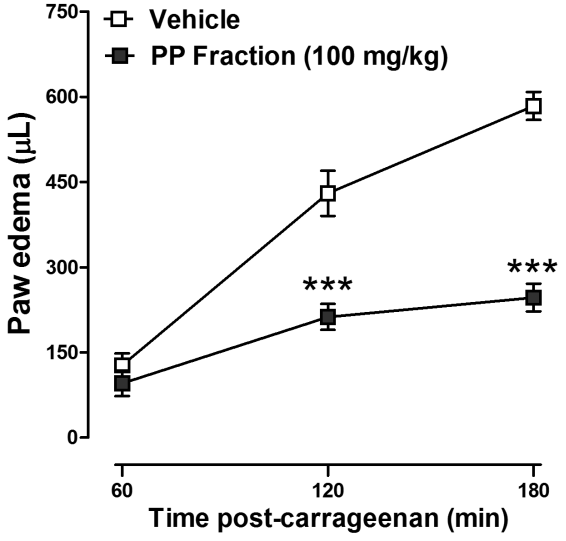

Figure 3 - Effect of PPAC fraction (100 mg/kg; po) on carrageenan-induced rat paw edema. Each point represents the mean ± SEM of 7–10 animals. ***p<0.001 in a two-way ANOVA Bonferroni post-test.

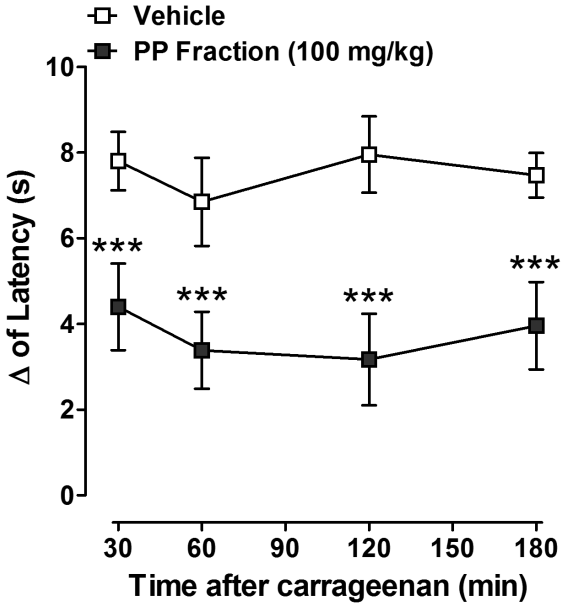

Figure 4 - Effect of PPAC fraction (100 mg/kg; po) on carrageenan-induced rat hyperalgesia. Each point represents the mean ± SEM of 7–10 animals. ***p<0.001 in a two-way ANOVA Bonferroni post-test.

and Morris water maze tests at 30 mg/kg, *i.p.*, with no noticeable peripheral or central cholinergic side effects up to a dose of 200 mg/kg, when mice showed convulsions affecting the whole body followed by death. Considering the recent findings demonstrating the prominent anti-inflammatory functions of the cholinergic signaling (Borovikova et al. 2000b, Tracey et al. 2001, Tracey 2002, Blalock 2002, Pavlov et al. 2003, Rosas-Ballina and Tracey 2009), we decided to examine the indole alkaloid-enriched fraction (PPAC fraction) and EE of *Geissospermum vellosii* stem bark in classical models of pain and inflammation *in vivo* and compare them with those of indomethacin, a non-steroidal anti-inflammatory drug. In this paper, we demonstrate that EE and PPAC fraction significantly inhibited the abdominal constriction induced by acetic acid in mice. Acetic acid causes an increase in the prostaglandin (PGE2 and PGF2α) levels in the peritoneal fluid, which in part act on peritoneal receptors to induce inflammatory pain and increase capillary permeability (Deraedt et al. 1980, Amico-Roxas et al. 1984). The test did not indicate if this potential resulted from central and/or peripheral actions. For clarification, a formalin test (Hunskaar and Hole 1987) was conducted. This test indicated that EE and PPAC fraction present peripheral analgesic properties (inhibition of inflammatory pain corresponding to phase 2 of the formalin test) without apparent central analgesic effects (neurogenic phase or phase 1 of the formalin test). The early phase, named non-inflammatory pain, is a result of the direct stimulation of nociceptors and reflects centrally mediated pain; the late phase, named inflammatory pain, is caused by local inflammation with a release of inflammatory and hyperalgesic mediators (Hunskaar and Hole 1987), such as serotonin, histamine, bradykinin and prostaglandins (Tjolsen et al. 1992). To confirm that *G. vellosii* had no central analgesic actions, the hot plate test, which is commonly used to assess narcotic analgesia (Asongalem et al. 2004), was conducted. PPAC fraction did not show antinociceptive effects in this test. Therefore, it is assumed that *G. vellosii* has no central analgesic effect that would contribute to its peripheral analgesic effect. This observation, together with the antinociceptive effect that was observed in the writhing test and the lack of activity in the first phase of the formalin test, suggests that the antinociceptive activity of PPAC fraction may be unrelated to the activation of the opioid system and reinforces the anti-inflammatory property of *G. vellosii*.

To demonstrate the anti-inflammatory property of *G. vellosii*, the effects of the PPAC fraction on the carrageenan-induced paw edema and hyperalgesia in rat were evaluated. PPAC fraction presented an antiedematogenic effect acting on the second phase of inflammatory process, inhibiting the edema in the 2nd and 3rd (57.8%) hours. Antihyperalgesic activity was observed within 30 min with a peak at 2 hours (60.1%). Indomethacin, a well-known prostaglandin inhibitor, inhibited both edema and hyperalgesia by 70%. Edema formation due to carrageenan in the rat paw is a biphasic event (Vinegar et al. 1969). Initial phase is attributed to the release of histamine and serotonin (Crunkhon and Meacock 1971). The second phase of edema is due to the release of prostaglandins, proteases and lysosome (Vinegar et al. 1969, Crunkhon and Meacock 1971) and is sensitive to most clinically effective anti-inflammatory drugs (Vinegar et al. 1969, DiRosa et al. 1971). The edema that is produced at the peak (3rd hour) is thought to be due to the release of kinin-like substances, especially bradykinin (Van Arman et al. 1965, Crunkhon and Meacock 1971). The hyperalgesic changes that are evoked by carrageenan are clearly sensitive to the cyclooxygenase (COX) inhibitor indomethacin (Lavich et al. 2005).

The results demonstrate that *Geissospermum vellosii* stem barks present an anti-inflammatory and antinociceptive activity, similar to that observed to

indomethacin, through a mechanism that seems to be unrelated to the opioid system. All together, results suggest prostanoids, such as prostaglandins (PGs), as facilitating agents of the inflammatory process. Regardless of similar responses, the mechanism of action of PPAC fraction differs from that of indomethacin, as the actions are mainly assigned to ACh, the major vagus nerve neurotransmitter. It has long been known that ACh (Pedigo et al. 1975), other direct cholinergic agonists (Yaksh et al. 1985, Hendershot and Forsaith 1959, Wang et al. 2005) and anticholinesterase agents (ChEIs) (Ireson 1970, Yaksh et al. 1985, 1995, Cozanitis et al. 1983, Gurun et al. 1997) induce antinociception in laboratory animals. Systemic administration of ChEIs, which cross the blood-brain barrier, produces analgesia and enhances analgesia from opiates (Eisenach 1999). Galanthamine, an indole alkaloid that is used to treat AD, had its analgesic effects evaluated and compared to those of physostigmine and morphine in the hot plate test in rats and in mice writhing test (Cozanitis et al. 1983). In the hot plate test, naloxone, an antagonist of opioid receptors, partially blocked the analgesic effect of galantamine but not that of physostigmine. Both ChEIs produced analgesia in abdominal writhing test in mice. According to Metys et al. (1969), physostigmine produces an analgesia that is mediated through mAChRs. Honda et al. (2000) reported the involvement of M3 mAChRs of the spinal cord in formalin-induced nociception in mice. The muscarinic antagonist atropine and the M3 mAChRs antagonist 4-DAMP were able to inhibit the second phase response of formalin at low doses (0.1–20 ng, *i.t.*), whereas the M1 mAChRs antagonist pirenzepine only inhibited the second-phase response at a high dose (1000 ng, *i.t.*). In addition, M2 mAChRs antagonist AF-DX116 had no effect. Wang et al. (2005) used the hot plate and formalin tests to evaluate the antinociception of choline, an α7 nAChRs agonist, and showed that choline can reduce acute or inflammatory nociceptive behavior through α7 nAChRs on CNS and periphery after *i.c.v*. and *i.v.* treatment, respectively. In formalin test, choline did have an effect in the second phase. M1 mAChRs in sheep (Bouaziz et al. 1995) and rats (Naguib and Yaksh 1997) and M3 mAChRs have been suggested to be involved mainly in spinal antinociception (Hwang et al. 1999). nAChRs are extensively distributed in central nervous system (CNS) and peripheral sites. Although most reports focus on the central sites of the analgesic action of nicotinic agonists (Damaj et al. 1998, Khan et al. 1994, Sahley and Berntson 1979), α-7 nAChRs were found in peripheral sites, such as ganglion neurons of the dorsal root (Boyd et al. 1991, Hu and Li 1997, Genzen et al. 2001).

Indomethacin prevents the synthesis of PGs through the inhibition of the catalytic activity of the COX family, COX1 and COX2, with greater selectivity for COX1. Both of these isoenzymes are expressed in the CNS (Kaufmann et al. 1997), and experimental induction of peripheral inflammation is associated with an increase in the expression of COX2 in the spinal cord (Beiche et al. 1996). COX2 expression was also detected in neuroinflammatory disorders, such as AD, and its induction is attributed to Aβ deposition (Rojo et al. 2008). AD is characterized by forebrain cholinergic neuron loss and a progressive decline in ACh. In AD, Aβ can attract and activate microglia, leading to an aggregation of cells around these Aβ deposits in the brain (Tuppo and Arias 2005), culminating in the secretion of pro-inflammatory mediators, including IL-1β, TNF-α, IL-6 and COX2 expression, among others (Heneka and O'Banion 2007, Rojo et al. 2008). Additionally, AChE, an enzyme that selectively catalyzes the hydrolysis of ACh within cholinergic synapses in the brain, promotes the generation of amyloid aggregates by accelerating the assembly of the Aβ peptide into Aβ fibrils (Inestrosa et al. 1996, Bartolini et al. 2003), forming highly toxic AChE–amyloid-β peptide complexes, whose neurotoxicity is higher

than that induced by the Aβ peptide alone, both *in vitro* and *in vivo* (Alvarez et al. 1998). Therefore, to treat AD, it is necessary restore cholinergic transmission by increasing ACh levels, stopping the neuronal inflammatory process, and protecting and augmenting the activity of the surviving cholinergic neurons. The most promising currently available drugs for the palliative treatment of AD are the acetylcholinesterase inhibitors (AChEI), such as galantamine, rivastigmine and donepezil (Giacobini 2000), and the n-methyl d-aspartate (NMDA) receptor antagonist memantine.

The inhibition of AChE activity reduces Aβ deposition and indirectly reduces Aβ-enhanced COX2 expression (Giacobini 2000, Greig et al. 2005). Recent evidence indicates a direct role of AChEIs in the inhibition of the release of inflammatory mediators from specialized cells. Galantamine, for example, attenuated the release of cytokines from activated murine microglia (Giunta et al. 2004). In mice, the peripheral administration of AChEIs almost completely blocked activated microglia cytokine production in the hippocampus and blood (Pollak et al. 2005). Physostigmine and neostigmine conferred similar protection against septic shock induced by cecal ligation and puncture together with a down-regulation of NF-kB and a reduction of the circulating levels of pro-inflammatory cytokines TNF-α, IL-1β and IL-6 (Hofer et al. 2008). Donepezil exerts its anti-inflammatory effects through the inhibition of the production of IL-1β, IL-6, IL-18 and monocyte chemoattractant protein-1 (MCP-1) (Hwang et al. 2010). In humans, donepezil treatment of AD patients for 1 month led to an attenuation of the release of cytokines from peripheral monocytes (Reale et al. 2005). All of the cholinesterase enzyme inhibitors (ChEIs) that are currently licensed for AD inhibit AChE and, to a varying extent, BChE, which is a second ChE in the brain (Lahiri et al. 2003).

In contrast to the drugs that are currently used to treat AD, PPAC fraction is approximately twice as selective for BChE than AChE (Lima et al. 2009). BChE has an important function in constitutive hydrolysis of acetylcholine in the central nervous system, where it could play a more extensive role in normal cholinergic transmission (Mesulam et al. 2002). Within the human nervous system, AChE predominates over BChE. However, whereas AChE is localized mainly in neurons, BChE is associated with glial cells as well as neurons (Das 2007, Darvesh and Hopkins 2003, Darvesh et al. 2003). BChE efficiently catalyzes the hydrolysis of endogenous substances, including choline esters (Giacobini 2004). In AD, AChE activity can decrease during disease progression, while BChE activity increases (Arendt et al. 1992, Atack et al. 1987). The ratio between BChE and AChE can change from 0.6 in normal brain to as high as 11 in cortical areas that are affected by this disease (Greig et al. 2002).

In conclusion, we demonstrate that the indole alkaloid-enriched PPAC fraction presented significant analgesic and anti-inflammatory activities *in vivo*. The mechanisms that are involved might be related to the anticholinesterase activity, which would enhance the anti-inflammatory cholinergic pathway. Once PPAC fraction presents slightly more selectivity to BChE, it can improve cognition and potentially control neuro-inflammation and could be useful for treating moderate to severe phases of AD.

ACKNOWLEDGMENTS

We thank Professor Josiane S. Neves for helpful comments and suggestions. This study was supported by grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

RESUMO

O *Geissospermum vellosii* (Pau pereira) é uma árvore brasileira, cujas cascas do caule são ricas em alcalóides indólicos que apresentam intensa atividade

anticolinesterásica. Este estudo avaliou os efeitos de uma fração da casca (fração PPAC) e do extrato etanólico (EE) do Pau pereira em modelos clássicos de inflamação e dor em murinos. O EE e a fração PPAC, ambos na dose de 30 mg/kg, reduziram significativamente a constrição abdominal induzida por ácido acético em camundongos em 34,8% e 47,5% respectivamente. No teste da formalina, o EE (30 mg/kg) e a fração PPAC (30 e 60 mg/kg) inibiram somente a segunda fase em 82,8%; 84,9% e 100%, respectivamente. Comparado com a indometacina, doses similares do EE ou da fração PPAC foram cerca de duas vezes mais eficazes em causar antinocicepção. A fração PPAC não foi eficaz no teste da placa quente, enquanto reduziu a resposta inflamatória na segunda (50,6%) e na terceira (57,8%) horas do edema de pata de rato induzido por carragenina. A atividade anti-hiperalgésica foi observada depois de 30 min com um pico em 2 h (60,1%). Esses resultados mostrararam que os compostos presentes na fração PPAC têm atividade anti-inflamatória e antinociceptiva por um mecanismo aparentemente não relacionado ao sistema opióide. A despeito das respostas similares às observadas para a indometacina, os efeitos da fração PPAC são atribuídos principalmente às ações da acetilcolina.

Palavras-chave: *Geissospermum vellosii*, Pau pereira, antinocicepção, inflamação, via anti-inflamatória colinérgica.

REFERENCES

ALVAREZ A ET AL. 1998. Stable complexes involving acetylcholinesterase and amyloid-β peptide change the biochemical properties of the enzyme and increase the neurotoxicity of Alzheimer's fibrils. J Neurosci 18: 3213-3223.

AMICO-ROXAS M, CARUSO A, TROMBADORE S, SCIFO R AND SCAPAGNINI U. 1984. Gangliosides antinociceptive effects in rodents. Arch Int Pharmacodyn Ther 272: 103-117.

ARAÚJO JQ, LIMA JA, PINTO AC, DE ALENCASTRO RB AND ALBUQUERQUE MG. 2011. Docking of the alkaloid geissospermine into acetylcholinesterase: a natural scaffold targeting the treatment of Alzheimer's disease. J Mol Model 17: 1401-1412.

ARENDT T, BRÜCKNER MK, LANGE M AND BIGL V. 1992. Changes in acetylcholinesterase and butyrylcholinesterase in Alzheimer's disease resemble embryonic development--a study of molecular forms. Neurochem Int 21: 381-396.

ASONGALEM EA, FOYET HS, EKOBO S, DIMO T AND KAMTCHOUING P. 2004. Antiinflammatory, lack of central analgesia and antipyretic properties of *Acanthus montanus* (Ness) T. Anderson. J Ethnopharmacol 95: 63-68.

ATACK JR, PERRY EK, BONHAM JR AND PERRY RH. 1987. Molecular forms of acetylcholinesterase and butyrylcholinesterase in human plasma and cerebrospinal fluid. J Neurochem 48: 1845-1850.

BARTOLINI M, BERTUCCI C, CAVRINI V AND ANDRISANO V. 2003. Beta-amyloid aggregation induced by human acetylcholinesterase: inhibition studies. Biochem Pharmacol 65: 407-416.

BEICHE F, SCHEUERER S, BRUNE K, GEISSLINGER G AND GOPPELT-STRUEBE M. 1996. Up-regulation of cyclooxygenase-2 mRNA in the rat spinal cord following peripheral inflammation. FEBS Lett 390: 165-169.

BLALOCK JE. 2002. Harnessing a neural-immune circuit to control inflammation and shock. J Exp Med 195: F25-28.

BLASKO I, VEERHUIS R, STAMPFER-KOUNTCHEV M, SAURWEIN-TEISSL M, EIKELENBOOM P AND GRUBECK-LOEBENSTEIN B. 2000. Costimulatory effects of interferon-gamma and interleukin-1beta or tumor necrosis factor alpha on the synthesis of Abeta1-40 and Abeta1-42 by human astrocytes. Neurobiol Dis 7: 682-689.

BOROVIKOVA LV, IVANOVA S, NARDI D, ZHANG M, YANG H, OMBRELLINO M AND TRACEY KJ. 2000a. Role of vagus nerve signaling in CNI-1493-mediated suppression of acute inflammation. Auton Neurosci 85: 141-147.

BOROVIKOVA LV, IVANOVA S, ZHANG M, YANG H, BOTCHKINA GI, WATKINS LR, WANG W, ABUMRAD N, EATON JW AND TRACEY KJ. 2000b. Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. Nature 405: 458-461.

BOUAZIZ H, TONG C AND EISENACH JC. 1995. Postoperative analgesia from intrathecal neostigmine in sheep. Anesth Analg 80: 1140-1144.

BOYD RT, JACOB MH, MCEACHERN AE, CARON S AND BERG DK. 1991. Nicotinic acetylcholine receptor mRNA in dorsal root ganglion neurons. J Neurobiol 22: 1-14.

CHERNYAVSKY AI, ARREDONDO J, SKOK M AND GRANDO SA. 2010. Auto/paracrine control of inflammatory cytokines by acetylcholine in macrophage-like U937 cells through nicotinic receptors. Int Immunopharmacol 10: 308-315.

COLLIER HOJ, DINNEEN LC, CHRISTINE A, JOHNSON CA AND SCHNEIDER C. 1968. The abdominal constriction response and its suppression by analgesic drugs in the mouse. Br J Pharmac Chemother 32: 295-310.

COZANITIS DA, FRIEDMANN T AND FÜRST S. 1983. Study of the analgesic effects of galanthamine, a cholinesterase inhibitor. Arch Int Pharmacodyn 266: 229-238.

CRUNKHON P AND MEACOCK SER. 1971. Mediators of the inflammation induced in the rat paw by carrageenan. Br J Pharmacol 42: 392-402.

DAMAJ MI, FEI-YIN M, GLASSCO W, GLENNON RA AND MARTIN BR. 1998. Antinociceptive responses to nicotinic acetylcholine receptor ligands after systemic and

intrathecal administration in mice. J Pharmacol Exp Ther 284: 1058-1065.

DARVESH S AND HOPKINS DA. 2003. Differential distribution of butyrylcholinesterase and acetylcholinesterase in the human thalamus. J Comp Neurol 463: 25-43.

DARVESH S, HOPKINS DA AND GEULA C. 2003. Neurobiology of butyrylcholinesterase. Nat Rev Neurosci 4: 131-138.

DAS UN. 2007. Acetylcholinesterase and butyrylcholinesterase as possible markers of low-grade systemic inflammation. Med Sci Monit 13: RA214-221.

DE JONGE WJ ET AL. 2005. Stimulation of the vagus nerve attenuates macrophage activation by activating the Jak2-STAT3 signaling pathway. Nat Immunol 6: 844-851.

DERAEDT R, JOUQUEY S, DELEVALLEE F AND FLAHAUT M. 1980. Release of prostaglandin E and F in an algogenic reaction and its inhibition. Eur J Pharmacol 61: 17-24.

DI ROSA M, GIROUD JP AND WILLOUGHBY DA. 1971. Studies on the mediators of the acute inflammatory response induced in rats in different sites by carragenan and turpentine. J Pathol 104: 15-29.

EISENACH JC. 1999. Muscarinic-mediated analgesia. Life Sci 64: 549-554.

FERREIRA SH. 1979. A new method for measuring variations of rat paw volume. J Pharm Pharmacol 31: 648.

GENZEN JR, VAN CLEVE W AND MCGEHEE DS. 2001. Dorsal root ganglion neurons express multiple nicotinic acetylcholine receptor subtypes. J Neurophysiol 86: 1773-1782.

GIACOBINI E. 2000. Cholinesterase inhibitors stabilize Alzheimer's disease. Ann NY Acad Sci 920: 321-327.

GIACOBINI E. 2004. Cholinesterase inhibitors: new roles and therapeutic alternatives. Pharmacol Res 50: 433-440.

GIUNTA B, EHRHART J, TOWNSEND K, SUN N, VENDRAME M, SHYTLE D, TAN J AND FERNANDEZ F. 2004. Galantamine and nicotine have a synergistic effect on inhibition of microglial activation induced by HIV-1 gp120. Brain Res Bull 64: 165-170.

GREIG NH, LAHIRI DK AND GIACOBINI E. 2005. Editorial: advances in Alzheimer therapy: something old, something new, something borrowed, something blue. Curr Alzheimer Res 2: 275-279.

GREIG NH, LAHIRI DK AND SAMBAMURTI K. 2002. Butyrylcholinesterase: an important new target in Alzheimer's disease therapy. Int Psychogeriatr 14(Suppl. 1): 77-91.

GURUN MS, LEINBACH R, MOORE L, LEE CS, OWEN MD AND EISENACH JC. 1997. Studies on safety of glucose and paraben-containing neostigmine for intrathecal administration. Anesth Analg 85: 317-323.

HENDERSHOT LC AND FORSAITH J. 1959. Antagonism of the frequency of phenylquinone-induced writhing in the mouse by weak analgesics and non analgesics. J Pharmacol Exp Ther 125: 237-240.

HENEKA MT AND O'BANION MK. 2007. Inflammatory processes in Alzheimer´s disease. J Neuroimmunol 184: 69-91.

HOFER S ET AL. 2008. Pharmacologic cholinesterase inhibition improves survival in experimental sepsis. Crit Care Med 26: 404-408.

HONDA K, HARADA A, TAKANO Y AND KAMIYA H. 2000. Involvement of M3 muscarinic receptors of the spinal Cord in formalin-induced nociception in mice. Brain Res 859: 38-44.

HU HZ AND LI ZW. 1997. Modulation of nicotinic ACh-, GABAA- and 5-HT3-receptor functions by external H-7, a protein kinase inhibitor, in rat sensory neurones. Br J Pharmacol 122: 1195-1201.

HUNSKAAR S AND HOLE K. 1987. The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. Pain 30: 103-114.

HUSTON JM ET AL. 2006. Splenectomy inactivates the cholinergic antiinflammatory pathway during lethal endotoxemia and polymicrobial sepsis. J Exp Med 203: 1623-1628.

HWANG J, HWANG H, LEE HW AND SUK K. 2010. Microglia signaling as a target of donepezil. Neuropharmacology 58: 1122-1129.

HWANG JH, HWANG KS, LEEM JK, PARK PH, HAN SM AND LEE DM. 1999. The antiallodynic effects of intrathecal cholinesterase inhibitors in a rat model of neuropathic pain. Anesthesiol 90: 492-499.

INESTROSA NC, ALVAREZ A, PÉREZ C, MORENO D, VICENTE M, LINKER C, CASANUEVA OI, SOTO C AND GARRIDO J. 1996. Acetylcholinesterase accelerates assembly of amyloid-β peptides into Alzheimer's fibrils: possible role of the peripheral site of the enzyme. Neuron 16: 881-891.

IRESON JD. 1970. A comparison of the antinociceptive actions of cholinomimetic and morphine-like drugs. Br J Pharmacol 40: 92-101.

KAMAL MA, GREIG NH AND REALE M. 2009. Anti-Inflammatory Properties of Acetylcholinesterase Inhibitors Administred in Alzheimer's Disease. Anti-Inflam Anti-Allergy Agents Med Chem 8: 85-100.

KAUFMANN WE, ANDREASSON KI, ISAKSON PC AND WORLEY PF. 1997. Cyclooxygenases and the central nervous system. Prostaglandins 54: 601-624.

KAWASHIMA K AND FUJII T. 2003. The lymphocytic cholinergic system and its biological function. Life Sci 72: 2101-2109.

KHAN I, YAKSH TL AND TAYLOR P. 1994. Ligand specificity of nicotinic acetylcholine receptors in rat spinal cord: Studies with nicotine and cytisine. J Pharmacol Exp Ther 270: 159-166.

LAHIRI D, FARLOW M, SAMBAMURTI K, GREIG N, GIACOBINI E AND SCHNEIDER L. 2003. A critical analysis of new molecular targets and strategies for drug developments in Alzheimer's disease. Curr Drug Targets 4: 97-112.

LAVICH TR, CORDEIRO RS, SILVA PM AND MARTINS MA. 2005. A novel hot-plate test sensitive to hyperalgesic stimuli and non-opioid analgesics. Braz J Med Biol Res 38: 445-451.

LEY S, WEIGERT A AND BRÜNE B. 2010. Neuromediators in inflammation— a macrophage/nerveconnection. Immunobiol 215: 674-684.

LIMA JA, COSTA RS, EPIFÂNIO RA, CASTRO NG, ROCHA MS AND PINTO AC. 2009. Geissospermum vellosii stembark: anticholinesterase activity and improvement of scopolamine-induced memory deficits. Pharmacol Biochem Behav 92: 508-513.

LIMA JA, OLIVEIRA AS, MIRANDA ALP, REZENDE CM AND PINTO AC. 2005. Anti-inflammatory and antinociceptive activities of an acid fraction of the seeds of *Carpotroche brasiliensis* (Raddi) (Flacourtiaceae). Braz J Med Biol Res 38: 1095-1103.

MESULAM MM, GUILLOZET A, SHAW P, LEVEY A, DUYSEN EG AND LOCKRIDGE O. 2002. Acetylcholinesterase knockouts establish central cholinergic pathways and can use butyrylcholinesterase to hydrolyze acetylcholine. Neuroscience 110: 627-639.

METYS J, WAGNER N, METYSOVA J AND HERZ A. 1969. Studies on the central antinociceptive action of cholinomimetics agents. Int J neuropharmac 8: 413-425.

NAGUIB M AND YAKSH TL. 1997. Characterization of muscarinic receptor subtypes that mediate antinociception in the rat spinal cord. Anesth Analg 85: 847-853.

PAVLOV VA, WANG H, CZURA CJ, FRIEDMAN SG AND TRACEY KJ. 2003. The cholinergic anti-inflammatory pathway: A missing link in neuroimmunomodulation. Mol Med 9: 125-134.

PEDIGO NW, DEWEY WL AND HARRIS LS. 1975. Determination and characterization of the antinociceptive activity of intraventricularly administered acetylcholine in mice. J Pharmacol Exp Ther 193: 845-852.

POLLAK Y, GILBOA A, BEN-MENACHEM O, BEN-HUR T, SOREQ H AND YIRMIYA R. 2005. Acetylcholinesterase inhibitors reduce brain and blood interleukin-1β production. Ann Neurol 57: 741-745.

REALE M, IARLORI C, GAMBI F, LUCCI I, SALVATORE M AND GAMBI D. 2005. Acetylcholinesterase inhibitors effects on oncostatin-M, interleukin-1β and interleukin-6 release from lymphocytes of Alzheimer's disease patients. Exp Gerontol 40: 165-171.

ROJO LE, FERNÁNDEZ JA, MACCIONI AA, JIMENEZ JM AND MACCIONI RB. 2008. Neuroinflammation: Implications for the pathogenesis and molecular diagnosis of Alzheimer´s disease. Arch Med Res 39: 1-16.

ROSAS-BALLINA M AND TRACEY KJ. 2009. Cholinergic control of inflammation (Review). J Intern Med 265: 663-679.

SAEED RW, VARMA S, PENG-NEMEROFF T, SHERRY B, BALAKHANEH D, HUSTON J, TRACEY KJ, AL-ABED Y AND METZ CN. 2005. Cholinergic stimulation blocks endothelial cell activation and leukocyte recruitment during inflammation. J Exp Med 201: 1113-1123.

SAHLEY TL AND BERNTSON GG. 1979. Antinociceptive effects of central and systemic administrations of nicotine in the rat. Psychopharmacology (Berl) 65: 279-283.

SHYTLE RD, MORI T, TOWNSEND K, VENDRAME M, SUN M, ZENG J, EHRHART J, SILVER AA, SANBERG PR AND TAN J. 2004. Cholinergic modulation of microglia activation by alpha 7 nicotinic receptors. J Neurochem 89: 337-343.

TEJWANI GA, RATTAN AK AND MCDONALD JS. 1992. Role of spinal opioid receptors in the antinociceptive interactions between intrathecal morphine and bupivacaine. Anesth Analg 74: 726-734.

TJOLSEN A, BERGE OG, HUNSKAAR S, ROSLAND JH AND HOLE K. 1992. The formalin test: an evaluation of the method. Pain 51: 5-17.

TRACEY KJ. 2002 The inflammatory reflex. Nature 420: 853-859.

TRACEY KJ. 2007. Physiology and immunology of the cholinergic antiinflammatory pathway. J Clin Invest 117: 289-296.

TRACEY KJ, CZURA CJ AND IVANOVA S. 2001. Mind over immunity. FASEB J 15: 1575-1576.

TUPPO EE AND ARIAS HR. 2005. The role of inflammation in Alzheimer's disease. Int J Biochem Cell Biol 37: 289-305.

VAN ARMAN CG, BEGANY AJ, MILLER LM AND PLESS HH. 1965. Some details of the inflammations caused by yeast and carrageenan. J Pharmacol Exp Ther 150: 328-334.

VANEGAS H AND SCHAIBLE HG. 2001. Prostaglandins and cyclooxygenases [correction of cycloxygenases] in the spinal cord. Prog Neurobiol 64: 327-363.

VIEGAS JR C, ALEXANDRE-MOREIRA MS, FRAGA CA, BARREIRO EJ, BOLZANI VS AND DE MIRANDA AL. 2008. Antinociceptive profile of 2,3,6-trisubstituted piperidine alkaloids: 3-*O*-acetyl-spectaline and semi-synthetic derivatives of (-)-spectaline. Chem Pharm Bull 56: 407-412.

VINEGAR R, SCHREIBER W AND HUGO R. 1969. Biphasic development of carrageenin edema in rats. J Pharmacol Exp Ther 166: 96-103.

WANG H ET AL. 2003. Nicotinic acetylcholine receptor alpha7 subunit is an essential regulator of inflammation. Nature 421: 384-388.

WANG H ET AL. 2004. Cholinergic agonists inhibit HMGB1 release and improve survival in experimental sepsis. Nat Med 10: 1216-1221.

WANG Y, SU DM, WANG RH, LIU Y AND WANG H. 2005. Antinociceptive effects of choline against acute and inflammatory pain. Neuroscience 132: 49-56.

YAKSH TL, DIRKSEN R AND HARTY GJ. 1985. Antinociceptive effect of intrathecally injected cholinomimetics drugs in the rat and cat. Eur J Pharmacol 117: 81-88.

YAKSH TL, GRAFE MR, MALKMUS S, RATHBUN ML AND EISENACH JC. 1995. Studies on the safety of chronically administered intrathecal neostigmine methylsulfate in rats and dogs. Anesthesiology 82: 412-427.