Journal of Ethnopharmacology 125 (2009) 163–169

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jethpharm

Evidence for a role of 5-HT$_{1A}$ receptor on antinociceptive action from *Geissospermum vellosii*

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ARTICLE INFO

Article history:
Received 15 October 2008
Received in revised form 8 May 2009
Accepted 24 May 2009
Available online 2 June 2009

Keywords:
Geissospermum vellosii
Alkaloids
Formalin
Acetic acid
Mice
Serotonergic system

ABSTRACT

Ethnopharmacological relevance: Geissospermum vellosii is a tree widely found throughout the Amazonic forest and frequently used by the native population for painful disorders.
Aim of the study: The present study examined the antinociceptive effects of *Geissospermum vellosii* in behavioral models of nociception.
Materials, methods and results: Oral administration of crude extract of *Geissospermum vellosii* or its dichloromethane fraction (1–100 mg/kg) inhibited formalin-induced inflammatory nociception and acetic acid-induced visceral nociception. The antinociceptive effect of *Geissospermum vellosii* was unrelated with motor dysfunctions. Furthermore, the alkaloid 12-metoxy-1-methyl-aspidospermidine (0.001–1 mg/kg), isolated from the dichloromethane fraction, also produced antinociception. The antinociception caused by the dichloromethane fraction was significantly attenuated by pre-treatment of mice with p-chlorophenylalanine methyl ester (PCPA, an inhibitor of serotonin synthesis, 100 mg/kg once a day for 4 consecutive days) and WAY-100635 (a 5-HT$_{1A}$ receptor antagonist, 0.3 mg/kg). In contrast, dichloromethane fraction antinociception was not affected by pre-treatment of animals with ketanserin (a 5-HT$_2$ receptor antagonist, 0.3 mg/kg) or ondansetron (a 5-HT$_3$ receptor antagonist, 0.5 mg/kg).
Conclusions: Together, these results indicate that *Geissospermum vellosii* produces antinociception through an interaction with 5-HT$_{1A}$ receptors. Furthermore, the alkaloid 12-metoxy-1-methyl-aspidospermidine contributes to the antinociceptive properties reported for *Geissospermum vellosii*.

1. Introduction

The *Geissospermum vellosii* or *Geissospermum leave*, as is scientifically known, is a medium-sized tree, widely found throughout the Amazonic forest. This plant belongs to the family Apocynaceae and is popularly known as "Pau-Pereira" (Manske and Holmes, 1952). The bark of this tree is frequently used by the native population therapeutically for malaria, liver pain, fever, stomach disorders, constipation and as sexual stimulant (Muñoz et al., 2000; Dos Santos, 2007). Like other plants of the family Apocynaceae, *Geissospermum vellosii* has several classes of compounds, particularly alkaloids. Indeed, it was shown that the bark of *Geissospermum vellosii* presents the alkaloids geissospermine, geissosquizine, geissoschizoline and flavopereirine (Hughes and Rapoport, 1958; Dos Santos, 2007; Almeida et al., 2007).

According to Costa et al. (2006), a geissospermine-rich fraction originated from bark's stem extract of the plant is able to reduce amnesia induced by scopolamine in animal models of inhibitory avoidance and in the water maze. Recently, it was showed that the compounds flavopereirine, geissoschizoline and geissospermine reduce the uptake of serotonin by synaptosomes from rat hippocampus (Lima-Landman et al., 2006).

It is well known that serotonin (5-HT) pathways within the CNS arise from a series of nuclei situated in the midline of the brain stem, the raphe nuclei, which represent the richest source of neuronal 5-HT synthesized in the mammalian brain (Fields et al., 1991; Millan, 2002). In addition, several studies have shown that the bulb

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doi:10.1016/j.jep.2009.05.026

spinal serotonin system may suppress incoming noxious input to the spinal cord and inhibit pain transmission (Basbaum and Fields, 1984; Alhaider et al., 1991; Millan, 1995). Moreover, the multiple 5-HT receptor types within the spinal cord appear to fulfill different roles in the control of nociception, reflecting their contrasting patterns of coupling to intracellular transduction mechanisms (Millan, 1995; Bardin et al., 2000). The activities of 5-HT receptors are complex and sometimes even contrasting, and can depend on: (1) the receptor subtype being activated, (2) the relative contributions of presynaptic versus postsynaptic actions of receptors, (3) the nociceptive paradigm in terms of quality and intensity of stimulus (Sawynok and Reid, 1996; Millan, 2002), and (4) the dose-related effect, which can be pro- or antinociceptive, of agonists and antagonists of serotonergic receptor subtypes (Hylden and Wilcox, 1983). Several pieces of evidence point to 5-HT_{1A}, 5-HT_2 and 5-HT_3 receptors modulating nociceptive transmission, as activation of these receptors in the spinal cord produces antinociception in the formalin test and other models of pain (Bardin et al., 2000; Sasaki et al., 2001; Millan, 2002).

Taking into account the biological activities of *Geissospermum vellosii* and its possible involvement with the serotonergic systems, it is surprising that no pharmacological study has been carried out on the possible antinociceptive effects of the *Geissospermum vellosii* up to now. Here, we have therefore examined the possible antinociceptive action of the crude extract and fractions obtained from *Geissospermum vellosii* in chemical models of nociception in mice. Attempts have been made to further investigate the possible involvement of the serotonergic systems in the antinociceptive action of *Geissospermum vellosii* extract. In addition, we also analyzed the possible antinociceptive effect of the alkaloid 12-metoxy-1-methyl-aspidospermidine isolated from this plant.

2. Material and methods

2.1. Animals

Experiments were conducted using male Swiss mice (20–30 g), housed at 22 ± 2 °C under a 12-h light/12-h dark cycle (lights on at 06:00) and with access to food and water *ad libitum*. Animals were acclimatized to the laboratory for at least 1 h before testing and were used only once throughout the experiments. The experiments were performed after approval of the protocol by the Institutional Ethics Committee and were carried out in accordance with the current guidelines for the care of laboratory animals and the ethical guidelines for investigations of experimental pain in conscious animals (Zimmermann, 1983). The numbers of animals and intensities of noxious stimuli used were the minimum necessary to demonstrate the consistent effects of the drug treatments.

2.2. Preparation of the crude extract, fraction and isolated compound from Geissospermum vellosii

The *Geissospermum vellosii* (Apocynaceae) was collected in the northeast of Brazil in November 2002. It was identified by comparison with exsiccate (number 36060) from *Geissospermum vellosii* deposited at the Botanical Garden from Curitiba, PR, Brazil. Ground bark of *Geissospermum vellosii* (3800 g) was air-dried, powdered and submitted to Soxhlet extraction in absolute ethanol until exhaustion. The crude extract was filtered and submitted to evaporation under reduced pressure until reduction to 1/5 of its initial volume. The crude extract (590 g) was submitted to partition liquid–liquid in Soxhlet with dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc) and buthanol (n-BuOH) resulting in CH_2Cl_2 (76.24 g), EtOAc (19.61 g), n-BuOH (10.93 g) fractions, and remaining aqueous fraction (90.95 g). The CH_2Cl_2 fraction (66.75 g) was submitted to chromatography through an appropriate column using changeable phases (hexane, dichloromethane and methanol) with a rate flow of 1 mL/min. This column isolated the alkaloid identified through RMN ^{13}C and ^{1}H as 12-metoxy-1-methyl-aspidospermidine (324 mg, Fig. 1).

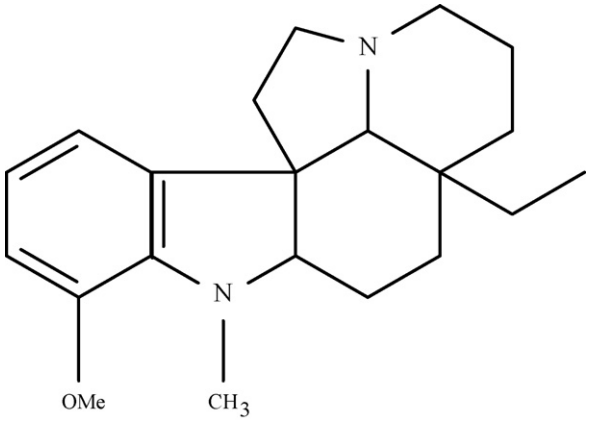

Fig. 1. Molecular structure of alkaloid 12-metoxy-1-methyl-aspidospermidine.

2.3. Abdominal constriction response caused by intraperitoneal injection of acetic acid

The abdominal constrictions were induced according to procedures previously described and resulted in contraction of the abdomen together with a stretching of the hind limbs in response to an intraperitoneal injection of acetic acid (0.6%) at the time of the test (Santos et al., 1999). First, the mice were pre-treated by oral route with the crude extract, CH_2Cl_2, EtOAc, n-BuOH or aqueous fractions (1–300 mg/kg) 60 min before testing. Control animals received the same volume of vehicle (10 ml/kg, p.o.). In another set of experiments, mice were pre-treated with alkaloid (0.001–1 mg/kg) by p.o. routes, 60 min before the irritant injection. Control animals received a similar volume of vehicle (10 ml/kg). After the challenge, the mice were individually placed into glass cylinders of 20 cm diameter, and the abdominal constrictions were counted cumulatively over a period of 30 min. Antinociceptive activity was expressed as the reduction in the number of abdominal constrictions, i.e. the difference between control animals (mice pre-treated with vehicle) and animals pre-treated with drugs.

2.4. Formalin-induced nociception

The procedure used was essentially the same as that previously described (Santos and Calixto, 1997; Santos et al., 1999). Animals received 20 μl of a 2.5% formalin solution (0.92% formaldehyde) made up in saline, injected i.pl. into the ventral surface of the right hindpaw. Animals were observed from 0 to 5 min (neurogenic phase) and from 15 to 30 min (inflammatory phase) and the time spent licking the injected paw was recorded with a chronometer and considered as indicative of nociception. Animals received *Geissospermum vellosii* crude extract (1–100 mg/kg, p.o.) or the dichloromethane fraction (1–100 mg/kg, p.o.) 60 min beforehand. Control animals received vehicle (10 ml/kg, p.o.). After i.pl. injection of formalin, the animals were immediately placed in a glass cylinder 20 cm in diameter, and the time spent licking the injected paw was recorded.

2.5. Measurement of motor performance and locomotor activity

In order to evaluate the possible non-specific muscle relaxant or sedative effects of *Geissospermum vellosii* crude extract or

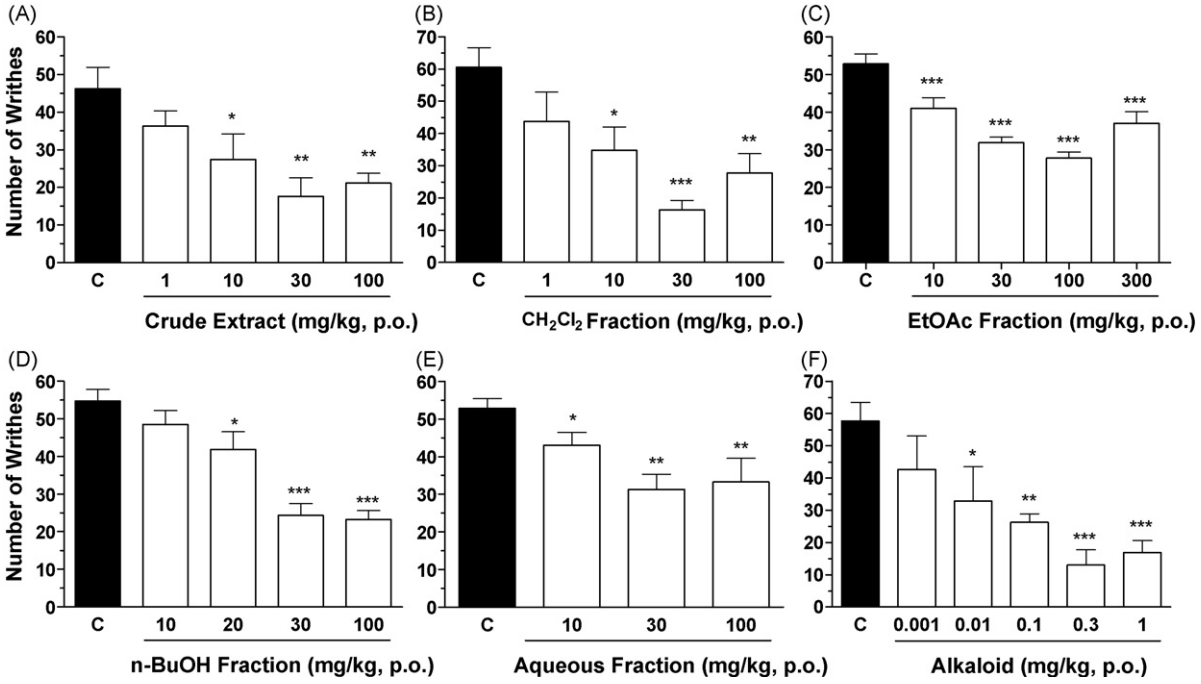

Fig. 2. Effects of crude extract (A), dichloromethane (CH_2Cl_2) fraction (B), ethyl acetate (EtOAc) fraction (C), n-butanol (n-BuOH) fraction (D), aqueous fraction (E) and alkaloid 12-metoxy-1-methyl-aspidospermidine (F) obtained from *Geissospermum vellosii* administered orally against acetic acid-induced visceral pain in mice. Each column represents the mean ± S.E.M. of 10–11 animals. Control value (C) indicates the animals treated with vehicle and the asterisks denote the significance levels, when compared with control groups (one-way ANOVA followed by Student–Newman–Keuls' test) $^{*}P < 0.05$; $^{**}P < 0.01$.

dichloromethane fraction, mice were submitted to the rota-rod task (Godoy et al., 2004) and open-field test (Rodrigues et al., 2002). The rota-rod apparatus consists of a bar with a diameter of 3.7 cm, subdivided into four compartments by 25-cm diameter disks. Twenty-four hours before the experiments, all animals were trained on the rota-rod until they could remain in the apparatus for 60 s without falling. On the day of experiment, the animals were treated with *Geissospermum vellosii* crude extract or the dichloromethane fraction (10–100 mg/kg, p.o.) and 60 min after were tested in the rota-rod test. The latency to fall and number of falls were recorded during a 240-s interval.

2.6. Analysis of possible involvement of the serotonergic systems in the antinociceptive action of Geissospermum vellosii

In order to investigate the participation of the serotonergic system in the antinociceptive action of *Geissospermum vellosii* in the acetic acid test, mice were pre-treated with WAY-100635 (0.3 mg/kg, s.c., a selective 5-HT_1 receptor antagonist), ketanserin (0.3 mg/kg, i.p., a selective 5-HT_{2A} receptor antagonist), ondansetron (0.5 mg/kg, i.p., a 5-HT_3 receptor antagonist) or vehicle (10 ml/kg, i.p.). After 20 min, they received the dichloromethane fraction (30 mg/kg, p.o.) or vehicle injection and 60 min later, the acetic acid (Takeshita and Yamaguchi, 1995; Bhargava and Saha, 2001; Duman et al., 2004). Another group of animals was pre-treated with vehicle and after 20 min they received dichloromethane or vehicle, 60 min before acetic acid injection.

In a separate series of experiments, in order to investigate the possible contribution of the endogenous serotonin in the antinociceptive action of *Geissospermum vellosii* in the acetic acid test, animals were pre-treated with p-chlorophenylalanine methyl ester (PCPA, 100 mg/kg, i.p., an inhibitor of serotonin synthesis) or with vehicle, once a day, for 4 consecutive days. Then, animals received an injection of the dichloromethane fraction (30 mg/kg, p.o.) or vehicle 20 min after the last PCPA or vehicle injection and were tested in the acetic acid test 60 min later (Santos et al., 1999).

Table 1
Inhibition by crude extract, fractions and isolated compounds from *Geissospermum vellossii* on acetic acid-induced abdominal constriction.

Drugs	ID_{50}[a]	MI (%)[b]
Crude extract (mg/kg)	26.3 (6.8–101.4)	62 ± 11
Dichloromethane (CH_2Cl_2) fraction (mg/kg)	9.8 (2.9–32.8)	73 ± 5
n-Butanol (n-BuOH) fraction (mg/kg)	53.1 (32.5–86.5)	57 ± 4
Ethyl acetate (EtOAc) fraction (mg/kg)	ND	48 ± 3
Aqueous fraction (mg/kg)	ND	41 ± 8
12-Metoxy-1-methyl-aspidospermidine (μg/kg)	27.4 (4.8–155.3)	77 ± 8
Aspirin (mg/kg)[c]	108.7 (92.7–126.6)	82 ± 5

Mice (N = 10–12 per group) were treated with alkaloid, extract or fractions 60 min (orally) before acetic acid administration.
[a] 95% confidence limits.
[b] Maximal inhibition (MI); ND not determined.
[c] Data from De Campos et al. (1997).

2.7. Drugs

The following substances were used: acetic acid and formaldehyde (Merck, Darmstadt, Germany); p-chlorophenylalanine methyl ester hydrochloride (PCPA), N-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-N-(2-pyridynyl) cyclohexanecarboxamide (WAY-100635, Sigma Chemical Co., St. Louis, USA); ketanserin tartarate (Tocris Cookson Inc., Ellisville, USA); ondansetron hydrochloride (Cristália, São Paulo, Brazil). Drugs were dissolved only in saline, with the exception of *Geissospermum vellosii* that was dissolved in saline and tween (80%). The final concentration of tween did not exceed 10% and did not cause any "per se" effect.

2.8. Statistical analysis

The results are presented as mean ± S.E.M., except the ID_{50} values (i.e., the dose of *Geissospermum vellosii* crude extract, fraction

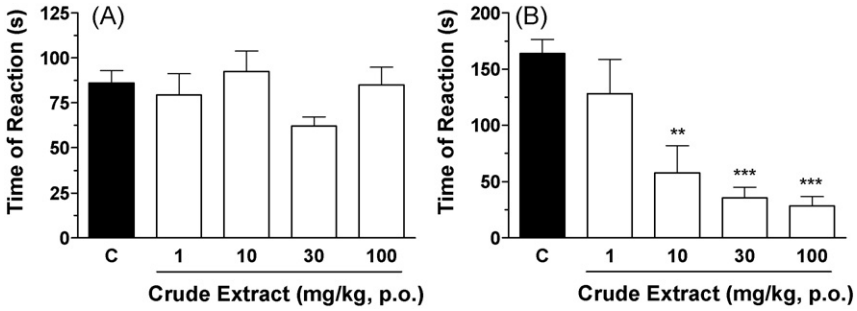

Fig. 3. Effect of crude extract (1–100 mg/kg) obtained from *Geissospermum vellosii* administered orally against formalin-induced licking (neurogenic phase, panel A, and inflammatory phase, panel B) in mice. Each column represents the mean ± S.E.M. of 10–11 animals. Control values (C) indicate the animals injected with vehicle and the asterisks denote the significance levels, when compared with control groups (one-way ANOVA followed by Student–Newman–Keuls test) $^{**}P < 0.01$.

or alkaloid reducing the nociceptive response by 50% relative to the control value), which are reported as geometric means accompanied by their respective 95% confidence limits. The ID_{50} value was determined by nonlinear regression from individual experiments using Graph-Pad software (GraphPad software, San Diego, CA, USA). The statistical significance of differences between groups was detected by ANOVA followed by Student–Newman–Keuls' test. *P*-Values less than 0.05 ($P < 0.05$) were considered as indicative of significance.

3. Results

3.1. Abdominal constriction response caused by intraperitoneal injection of acetic acid

Oral administration of animals with crude extract and fractions (CH_2Cl_2, EtOAc, n-BuOH and aqueous fractions), in the dose of 10–300 mg/kg, given 60 min beforehand, did not produce any irritation by itself (result not shown), but caused a significant reduction of the number of abdominal constrictions induced by acetic acid in mice in relation to control group (Fig. 2A–E). In addition, the results depicted in Fig. 2(A, B and D) show that the crude extract, CH_2Cl_2 and n-BuOH fractions of *Geissospermum vellosii* (1–100 mg/kg, p.o.) produced dose-related inhibition of acetic acid-induced abdominal constrictions in mice, with ID_{50} values (and their respective 95% confidence limits) of 26.3 (6.8–101.4) mg/kg, 9.8 (2.9–32.8) mg/kg and 53.1 (32.5–86.5) mg/kg and maximal inhibition of 62 ± 11%, 73 ± 5% and 57 ± 4% at the dose of 30 mg/kg (Table 1 and Fig. 2A, B and D). Moreover, the oral treatment with the aqueous and EtOAc fractions (10–300 mg/kg) caused partial inhibition of the acetic acid-induced abdominal constrictions in mice, with maximal inhibition of 41 ± 8% and 48 ± 3% at the dose of 30 and 100 mg/kg, respectively (Table 1 and Fig. 2C and E). At the ID_{50} level, CH_2Cl_2 fraction was approximately 2.7–11.1-fold more potent than crude extract, n-BuOH fraction and well-known anti-inflammatory and analgesic drug aspirin (Table 1). Considering that CH_2Cl_2 was the most potent fraction, it was selected for the phytochemical analysis to determine its active constituents.

Interestingly, the results depicted in Fig. 2F show that the alkaloid isolated from *Geissospermum vellosii* (0.001–1 mg/kg, orally) produced dose-related inhibition of acetic acid-induced abdominal constrictions with ID_{50} value of 27.4 (4.8–155.3) μg/kg and the peak of inhibition observed was 77 ± 8% (Fig. 2F). Moreover, the alkaloid was 358-fold more potent than the CH_2Cl_2 fraction (original fraction). In addition, the alkaloid was 3967-fold more potent than well-known anti-inflammatory and analgesic drug (aspirin) (Table 1) when analyzed in the acetic acid test.

3.2. Formalin-induced nociception

The results presented in Fig. 3(A and B) show that crude extract of *Geissospermum vellosii* (1–100 mg/kg, p.o.) also produced dose-related inhibition only in the inflammatory phase of formalin-induced nociception, with ID_{50} value of 2.1 (0.6–7.0) mg/kg and the inhibition of 79 ± 5% at the dose of 100 mg/kg. Moreover, the CH_2Cl_2 fraction in the same doses resulted in a graded and significant inhibition of both neurogenic and inflammatory phases of formalin-induced nociception. The ID_{50} values and the inhibition for the neurogenic and inflammatory phases were 22.6 (7.1–71.4) mg/kg and 4.4 (1.9–9.9) mg/kg and 63 ± 5% and 84 ± 9%, respectively (Fig. 4A and B). However, De Campos et al. (1997) demonstrated that the standard drug aspirin, given orally (100–600 mg/kg), caused significant inhibition against the inflammatory phase (but not the neurogenic phase) of the formalin-induced nociception. The calculated mean ID_{50} value 282.0 (243.0–328.0) mg/kg and the inhibition of 89 ± 5% at the dose of 600 mg/kg. Moreover, the CH_2Cl_2 fraction and crude extract of *Geissospermum vellosii* were 64- and 134-fold

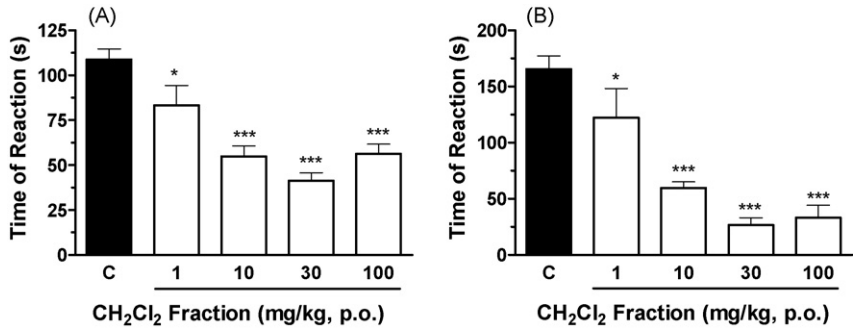

Fig. 4. Effect of dichloromethane fraction (1–100 mg/kg) obtained from *Geissospermum vellosii* administered orally against formalin-induced licking (neurogenic phase, panel A, and inflammatory phase, panel B) in mice. Each column represents the mean ± S.E.M. of 10–12 animals. Control values (C) indicate the animals injected with vehicle and the asterisks denote the significance levels, when compared with control groups (one-way ANOVA followed by Student–Newman–Keuls test) $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

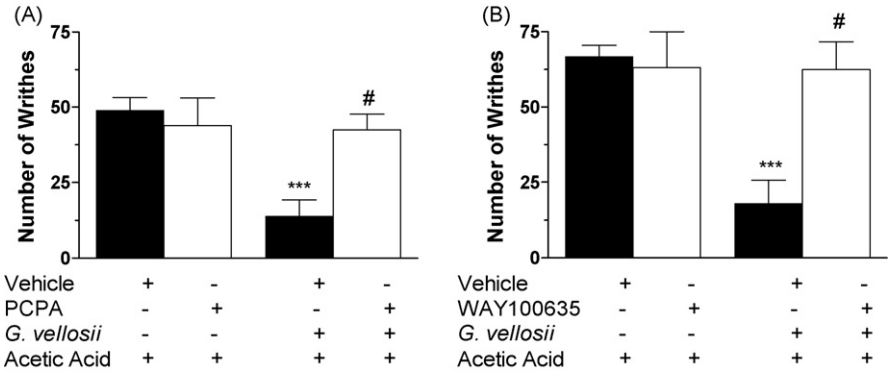

Fig. 5. Effect of pre-treatment of animals with PCPA (100 mg/kg, 4 consecutive days, panel (A) or WAY100635 (3 mg/kg, panel B) on the antinociceptive profile of dichloromethane fraction (30 mg/kg, p.o.) obtained from *Geissospermum vellosii* administered orally against acetic acid-induced visceral pain in mice. Each column represents the mean ± S.E.M. of 10–12 animals. The symbols report the significance level ***$P < 0.01$ compared with control group (animals injected with the vehicle alone) and #$P < 0.001$ compared with *Geissospermum vellosii* treatment (one-way ANOVA followed by Student–Newman–Keuls test).

more potent than the well-known anti-inflammatory and analgesic drug (aspirin) when analyzed in the inflammatory phase of formalin-induced nociception, respectively.

3.3. Analysis of serotonergic system on action of Geissospermum vellosii

The results depicted in Fig. 5A show that the previous treatment of mice with PCPA (100 mg/kg, i.p., for 4 consecutive days) also produced significant inhibition of the antinociception caused by the CH_2Cl_2 fraction (30 mg/kg, p.o.) against acetic acid-induced pain. In addition, treatment of mice with WAY-100635 (0.3 mg/kg, s.c.), but not with ketanserine (0.3 mg/kg, i.p.) or ondansetron (0.5 mg/kg, i.p.), significantly reversed the antinociception caused by CH_2Cl_2 fraction (30 mg/kg, p.o.) against acetic acid-induced nociception (Fig. 5B and results not shown).

3.4. Analysis of possible interaction of Geissospermum vellosii with locomotor activity

The treatment of animals with the crude extract or the CH_2Cl_2 fraction of *Geissospermum vellosii* in high dose (100 mg/kg, p.o.), but not in low doses (10 or 30 mg/kg, p.o.), caused significant reduction of the locomotor activity in the open-field test when compared with animals that received vehicle (control group) (Table 2). However, the same treatment of animals with the crude extract or the CH_2Cl_2 fraction of *Geissospermum vellosii* (10–100 mg/kg, p.o.) did not affect the motor performance in the rota-rod task (Table 2).

Table 2
Effect of crude extract or dichloromethane fraction obtained from *Geissospermum vellossii* on number of crossings in open-field test and on the fall latency or number of falls in rota-rod test in mice.

Treatment	Open-field test	Rota-rod test	
	Crossing	Fall latency	Number of falls
Vehicle (10 ml/kg)	56 ± 5	141 ± 46	0.5 ± 0.2
Crude extract (10 mg/kg)	54 ± 8	89 ± 37	0.8 ± 0.1
Crude extract (30 mg/kg)	80 ± 2	141 ± 45	0.5 ± 0.2
Crude extract (100 mg/kg)	38 ± 3*	163 ± 49	0.3 ± 0.2
Dichloromethane (10 mg/kg)	57 ± 9	203 ± 37	0.2 ± 0.2
Dichloromethane (30 mg/kg)	50 ± 3	125 ± 51	0.5 ± 0.2
Dichloromethane (100 mg/kg)	35 ± 4*	135 ± 47	1.2 ± 0.6

Mice were treated with extract or fractions 60 min (p.o.) before open-field or rota-rod tests. Data represents the mean ± S.E.M. of six animals. The symbols report the significance level.

* $P < 0.05$ compared with control group (animals injected with the vehicle alone) (one-way ANOVA followed by Student–Newman–Keuls test).

4. Discussion

This study showed for the first time that the crude extract, fractions and compounds isolated from *Geissospermum vellosii* displayed antinociceptive effect against visceral pain evoked by intraperitoneal injection of acetic acid in mice. Furthermore, the crude extract of *Geissospermum vellosii* or its CH_2Cl_2 fraction also inhibited both the neurogenic (early phase) and inflammatory (late phase) pain responses caused by formalin injection in mice. Here, we observed that the crude extract and CH_2Cl_2 fraction of *Geissospermum vellosii* did not alter motor performance and locomotor activity in the doses that caused significant antinociception.

A considerable number of studies have suggested that extracts or active principles obtained from *Geissospermum vellosii* have been used in various countries as popular medicine for the treatment of a variety of illnesses, such as malaria, liver pain, fever, stomach disorders, constipation and as sexual stimulant (Muñoz et al., 2000; Dos Santos, 2007). However, in spite of the fact that the extract from *Geissospermum vellosii* has been suggested to produce analgesic effect, the putative antinociceptive activities of the extract or active principles obtained from the *Geissospermum vellosii* as well as its mechanisms of action have not yet been demonstrated.

The results reported here indicate that oral administration of the crude extract, CH_2Cl_2 and n-BuOH, but not EtOAc and aqueous, fractions obtained from *Geissospermum vellosii* inhibited, in a dose-dependent manner, the nociceptive response elicited by acetic acid. At the ID_{50} level, CH_2Cl_2 fraction was approximately 2.7–11.1-fold more potent than the crude extract and n-BuOH fraction and well-known anti-inflammatory and analgesic drug aspirin in inhibiting acetic acid-induced visceral pain. To our knowledge this is the first report of this kind in the literature. Furthermore, the chemical studies carried out on this CH_2Cl_2 fraction allowed us to isolate the alkaloid 12-metoxy-1-methyl-aspidospermidine in *Geissospermum vellosii*, which seems to be responsible, for the antinociceptive properties reported for the CH_2Cl_2 fraction. Moreover, at the ID_{50} level, this alkaloid was 358- and 3967-fold more potent than CH_2Cl_2 fraction and well-known anti-inflammatory and analgesic drug (aspirin) when analyzed in the acetic acid test. In addition, acetic acid-induced writhing reaction in mice, described as a typical model of inflammatory pain, has long been used as a screening tool for the assessment of analgesic or anti-inflammatory properties of new agents (Vinegar et al., 1979; Tjølsen and Hole, 1997).

Also of interest are the results showing that the CH_2Cl_2 fraction, but not the crude extract, obtained of *Geissospermum vellosii* is largely effective in preventing both the neurogenic (early phase)

and inflammatory (late phase) pain responses caused by formalin injection in mice. Furthermore, it has been shown that injection of formalin in rodents produces a significant increase in spinal levels of different mediators, such as excitatory amino acids, PGE_2, nitric oxide, tachykinin, kinins, among other peptides (Tjølsen et al., 1992; Malmberg and Yaksh, 1995; Santos and Calixto, 1997; Santos et al., 1998). Likewise, these data suggest that the CH_2Cl_2 fraction, differently from the crude extract, contains active principles that are effective in reducing both the neurogenic and inflammatory pain induced by formalin.

As mentioned in Section 1, the serotonergic system plays a very important role in the control of pain. In addition, several clinical and preclinical studies have found that antidepressant drugs are able to produce marked analgesia in humans and animals (Korzeniewska-Rybicka and Plaznik, 1998; Millan, 2002; Rojas-Corrales et al., 2003). Recently, it has been shown that some alkaloids isolated from *Geissospermum vellosii* reduced the serotonin uptake by rat synaptossomes *in vitro* (Lima-Landman et al., 2006). Together, these findings strongly suggest that the serotonergic system could indeed be involved in the antinociceptive effects of the CH_2Cl_2 fraction from *Geissospermum vellosii* in mice. In fact, the results of the present study show that CH_2Cl_2 fraction produces antinociception that appears to be mediated by the serotonergic system, especially through the stimulation of 5-HT_1 receptors. This assertion is supported by the demonstration that (1) depletion of endogenous serotonin with the tryptophan hydroxylase inhibitor PCPA, at a dose known to decrease the cortical content of serotonin and to significantly reverse the morphine antinociception, largely antagonized the antinociceptive action of the CH_2Cl_2 fraction (Santos et al., 1999; Mendes et al., 2000; Dailly et al., 2006); (2) selective antagonists of 5-HT_{1A} receptors, namely WAY-100635, consistently reversed the antinociception caused by systemic administration of the CH_2Cl_2 fraction when analyzed against acetic acid-induced pain; in marked contrast, selective antagonists of 5-HT_{2A} and 5-HT_3 receptors, namely ketanserin and ondansetron, respectively, do not appear to account, to any large extent, for the antinociceptive action of the CH_2Cl_2 fraction. Furthermore, several studies have demonstrated the involvement of 5-HT_{1A} receptors in the mechanism of action of many classes of antidepressant drugs, including tricyclics, SSRIs (selective serotonin reuptake inhibitors) and MAOi (monoamine oxidase inhibitors) (Hensler, 2002). Thus, our data clearly indicate that in the model of visceral pain, the antinociceptive action of *Geissospermum vellosii* CH_2Cl_2 fraction was reduced by a selective 5-HT_{1A} receptor antagonist, which suggests that 5-HT_{1A} receptor stimulation by *Geissospermum vellosii* CH_2Cl_2 fraction produces analgesic-like effect.

Recently, it was reported that the alkaloid geissospermine isolated from *Geissospermum vellosii* acts as relaxing skeletal muscle drug by acting in nicotinic receptors (Tanae et al., 2006). Moreover, geissosquizine isolated from *Geissospermum vellosii* significantly reduces the locomotor activity in the open-field test, apparently mediated by central dopaminergic system (Shimada et al., 1999). To determine whether the decrease of nociceptive behaviors could be due to a neuromuscular blockade caused by treatment with *Geissospermum vellosii* and to avoid misinterpretation of our antinociceptive results, the open-field and rota-rod tests were carried out. As indicated, animals showed changes in the open-field test only with higher tested dose of *Geissospermum vellosii* crude extract and CH_2Cl_2 fraction, and the same was not observed in the rota-rod test. These results suggest that the decrease on number of crossings may be occurring for some other reason rather than neuromuscular blockade, since neuromuscular blocker reduces motor performance not only in the open-field test, but also in the rodarod test (Moreira et al., 2000; De Souza et al., 2001). Moreover, since the maximal antinociceptive effect of *Geissospermum vellosii* crude extract and CH_2Cl_2 fraction was obtained in the dose of 30 mg/kg, it may be suggested that the antinociceptive action of *Geissospermum vellosii* is unrelated to motor dysfunctions.

In summary, the results of the present study demonstrate for the first time that the extract and fractions obtained from *Geissospermum vellosii*, especially the crude extract and the dichloromethane fraction, exerts pronounced antinociception against chemical models (acetic acid and formalin) of nociception in mice at a dose that does not interfere with motor performance. In addition, the antinociceptive effect of the dichloromethane fraction involves an interaction with the serotonergic (through 5-HT_{1A}, but not 5-HT_{2A} and 5-HT_3, receptors) system. Furthermore, the alkaloid isolated and identified as 12-metoxy-1-methyl-aspidospermidine contributes to the explanation of the antinociceptive properties reported for the *Geissospermum vellosii*. Finally, the antinociceptive action demonstrated in the present study supports, at least in part, the ethnomedical uses of this plant.

Acknowledgements

This work was supported by grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Programa de Apoio aos Núcleos de Excelência (PRONEX), Fundação de Apoio à Pesquisa Científica Tecnológica do Estado de Santa Catarina (FAPESC) and Financiadora de Estudos e Projetos [FINEP, Rede Instituto Brasileiro de Neurociência (IBN-Net)], Brazil.

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