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Antinociceptive effects of *Psychotria viridis Ruiz & Pav*. on carrageenan-induced hyperalgesia in rats

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ABSTRACT

Psychotria viridis Ruiz & Pav. (*P. viridis*) is a ritual plant used as one of the ingredients in the preparation of the ayahuasca drink, which is consumed by indigenous and religious groups during sacred rituals in countries of South America. Studies have shown that the extract of this plant has therapeutic effects on diseases such as depression, anxiety disorders, and chemical dependencies. The aim of this study is to evaluate the effects of *P. viridis* leaf extracts on nociception. Male Wistar rats (180-200 g) were used in this study. To induce the experimental inflammatory pain, carrageenan (200 µg/paw) was injected into the paw. Nociception was measured using the mechanical paw pressure test. *P. viridis* extracts used include methanolic (PVM), ethyl acetate (PVA) and hexanic (PVH). A surprising variation in the antinociceptive effect of the three extracts evaluated (PVM, PVA e PVH) was observed, a fact that may occur due to the large number of substances contained in them. Based on the dose-response curve, the peak of action and the doses with the greatest antinociceptive effect of the extracts were determined, that is 9.6 mg (time; t = 2.5 h) for PVM; 2 mg (t = 4 h) for PVA; and 4.8 and 19.2 mg (t = 0.5 h and 2.5 h, respectively) for PVH (there were two points with significant differences). In the subsequent stage, the peak action of the extracts in the doses with the greatest antinociceptive effect coincided with the peak action of carrageenan. PVH and PVM were able to partially reverse the nociceptive effect of carrageenan, while PVA showed a promising total reversal of the effect of carrageenan. It is possible to conclude that *P. viridis* has a high analgesic potential and therefore, is promising in the development of new drugs for pain control.

KEYWORDS: ayahuasca, *Psychotria viridis* extracts, antinociception, inflammatory pain, Santo Daime.

INTRODUCTION

Archeological artifacts over 3000 years old found in the Amazon region are related to ritualistic ceremonies with the consumption of entheogen beverages [1]. During the shamanistic ritual, drinks such as ayahuasca were used for the shaman to change his state of consciousness and establish contacts with spiritual entities to obtain prophecies, pathological diagnoses and treatments [2].

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Ayahuasca is a drink that was already known to Amazonian peoples before the pre-Columbian period and is commonly prepared from two plants: *Banisteriopsis caapi* (Spruce ex Griseb.) Morton and *Psychotria viridis Ruiz & Pav*. (*P. viridis*) [1, 3]. In *vegetalismo*, a term used for the practice of shamanism in the Peruvian Amazon, these two plants fall under the concept of professor plant, which are plants capable of generating knowledge [4].

Currently, it is estimated that about 70 indigenous groups use ayahuasca, but consumption is no longer restricted to the forest environment, and is moving towards urban areas [5, 6]. Its consumption is still used in religious ceremonies in Brazil among groups such as "União do Vegetal", "Santo Daime" and "Barquinha" [7-9]. The ethnological importance is so recognized that there is an exception in the Brazilian legislation that allows the consumption of ayahuasca, only during rituals, even containing substances that are prohibited [10, 11].

Studies have shown that ayahuasca has therapeutic effects especially against psychological disorders, including anxiety, depression and chemical dependence [12-15]. These effects are attributed to the constituents of *P. viridis*, mainly to *N*,*N*-dimethyltryptamine (DMT) [16, 17], which can also be found in fungi, marine sponges, tunicates, frogs, legumes and grasses. In addition, there are reports that rats and humans also produce DMT endogenously [18]. This compound is structurally similar to serotonin [19] and is well known for its actions as an agonist of the 5-HT_{2A} receptor [20, 21], since its effects were selectively antagonized by ketanserin [22, 23]. Moreover, DMT also has an affinity for receptors 5-$HT_{1A/1B/1D/2B/2C/6/7}$, whilst for 5-$HT_{1A/2A/2C}$ it has partial agonist activity [24-27]. As the activation of the serotonergic system has a central [28] and peripheral antinociceptive effect promoting analgesic action, especially by 5-$HT_{1B/2A/3}$ receptors [29], possibly the extract of *P. viridis* also has the potential as an analgesic.

The psychoactive effects of *P. viridis* are primordial to rituals. Many substances, such as cannabinoid agonists, have analgesic action associated with the effects of altering the state of consciousness [3, 30]. This activity can help maintain the trance and contribute to the continuation of this state of consciousness during rituals. Thus, the aim of this study is to evaluate the effect of different extracts from the leaves of *P. viridis* in a model of inflammatory pain in rats.

MATERIALS AND METHODS

Animals

All experiments were performed with male Wistar rats (180-200 g) from the Bioterism Center of Federal University of Minas Gerais (CEBIO-ICB/UFMG). The animals were placed in plastic boxes with forage shavings and kept in the trial room for two days before the experiments, for habituation. They were housed in a temperature-controlled room (23 to 25 ºC), on an automatic 12-hour light/dark cycle (07:00 - 19:00). All animal procedures and protocols were approved by the Ethics Committee on the Use of Animals of the Federal University of Minas Gerais (protocol nº 58/2019) and are in accordance with the recommendations for the evaluation of experimental pain in animals [31]. After the experimental procedures, the animals were euthanized.

Plant materials

Leaves of *Psychotria viridis Ruiz & Pav* were collected in the morning, by the Federal Police expert André Dias Cavalcanti, at the planting of the "União do Vegetal" (UDV) nucleus located at Lagoa da Prata municipality (20º 01' 21" south latitude and 45º 32' 37" west longitude), Minas Gerais, Brazil, and the collection process was supervised by an agronomist of the UDV, which guarantee the identity of this species. *P. viridis*, popularly known as "chacrona", cultivated in this region is originally from the State of Rondônia, Brazil, which is located at the center of Amazon region.

The collected *P. viridis* leaves were dried in a forced ventilation oven at 40 °C for two days. The dried leaves of *P. viridis* were powdered in a knife mill. The extracts of the powdered leaves were prepared using direct extraction in a Soxhlet apparatus. Hexane, ethyl, acetate, and methanol were used sequentially with an average extraction time of seven hours for each solvent. After this

stage, each solvent was removed and recovered in a rotary evaporator, at a temperature of 40 ºC with the aim of reducing the pressure of the system and finalizing the process.

Drug administration

Carrageenan λ (200 µg/paw; Sigma, USA) was diluted in sterile physiological saline. The hexanic (PVH; 1.2, 4.8 and 19.2 mg/mL) and ethyl acetate (PVA; 1, 2, 4 and 8 mg/mL) extracts of *P. viridis* were diluted in cremophor 10% and the methanolic extract (PVM; 1.2, 4.8, 9.6 and 38.4 mg/mL) in dimethyl sulfoxide (DMSO) 10%. Carrageenan was injected into the right plantar surface of the paw at a volume of 100 µL per paw and the extracts were orally given (gavage) at a volume of 1 mL/animal.

Measurement of nociceptive threshold

Hyperalgesia was induced using a subcutaneous injection of carrageenan λ (CG; 200 µg/paw) into the plantar surface of the hind paw. Hyperalgesia was measured using the mechanical paw pressure test described by Randall and Selitto [32]. An algesimeter (Ugo Basile, Italy) was used, whereby increasing pressure is applied to the plantar surface of the rat's paw. The weight in grams required to elicit the nociceptive response of paw withdrawal was determined as the nociceptive threshold. A cutoff value of 300 g was used to reduce the possibility of damaging the paw. The nociceptive threshold was measured in the right hind paw and recorded before (baseline nociceptive threshold) and after CG injection. The time course of responses was computed, and the results were expressed as the difference between these two averages (Δ of the nociceptive threshold). The time of injections and time of nociceptive threshold measurements, as well as the doses used, were based on literature data and on the results of pilot experiments. To reduce stress, the rats were habituated to the apparatus 1 day prior to the experiments.

Experimental protocol

In all experiments, the animals were fasted for 6 hours before oral gavage of the extracts. The baseline nociceptive threshold of each animal was first determined before the injection of any substance. Carrageenan was injected into the right hind paw at zero time. For the experiments evaluating temporal curves, the extracts were given, at different doses, by gavage 120 minutes after the CG injection, and the nociceptive threshold was measured at specific times 150 minutes after CG injection. Using the data of the time of the greatest analgesic effect of the extracts in the dose-response curves, new experiments were carried out to coincide these times with the peak effect of the CG (180 minutes) as follows: PVH was orally given 30 and 150 minutes after the CG, PVA 60 min before CG, and PVM 30 minutes after CG; all nociceptive threshold measurements were made 180 minutes after the CG injection.

Statistical analysis

The animals were randomly distributed between the experimental and control groups and the results were expressed as the mean ± standard error of the mean (S.E.M.). Statistical differences between groups were calculated by two-way analysis of variance (ANOVA) followed by the Bonferroni test for the temporal curve evaluation, and t-Student test for the peak action evaluation. Statistical significance was set at $p<0.05$.

RESULTS AND DISCUSSION

The methanolic (PVM), ethyl acetate (PVA) and hexanic (PVH) extracts of *P. viridis* leaves were evaluated separately. The extracts were given orally, at different doses, 150 minutes after the intraplantar injection of carrageenan (200 µg/ paw). An irregular profile was observed for the different extracts (Figures 1A, 2A and 3A). From the temporal development of the dose-response curves, the best dose and time in which there was a greater antinociceptive effect of the various extracts were identified. For the PVM extract, it was possible to observe a greater antinociceptive effect in 270 min with the dose of 9.6 mg (Figure 1A), while for PVA we identified this effect with the time of 240 min for the dose of 2 mg (Figure 2A). For the PVH extract, there were two points with significant differences between the control and treated animals at doses of 4.8 and 19.2 mg, at 30 and 150 min, respectively (Figure 3A).

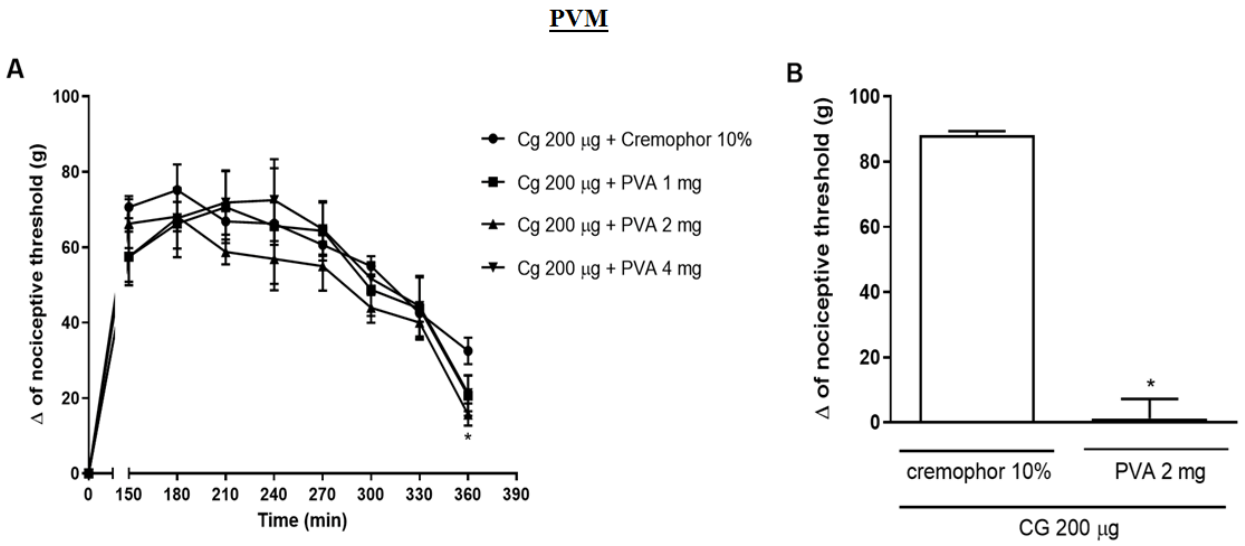

Figure 1. Effect of oral administration of PVM on CG-induced hyperalgesia. Carrageenan (CG; 200 µg/paw) was injected into the right rat paw at time 0 and *P. viridis* methanolic extract (PVM; 1,2, 4,8 and 9,6 mg) or its vehicle (DMSO 1%) was orally given at time 120 minutes for temporal curve evaluation (A) and at time 30 minutes for the peak action evaluation (B). The data are presented as mean ± S.E.M. (n = 4). *Indicates a significant difference compared with CG + DMSO 1% (P<0.05); two-way ANOVA with Bonferroni post test (A) and t-Student test (B).

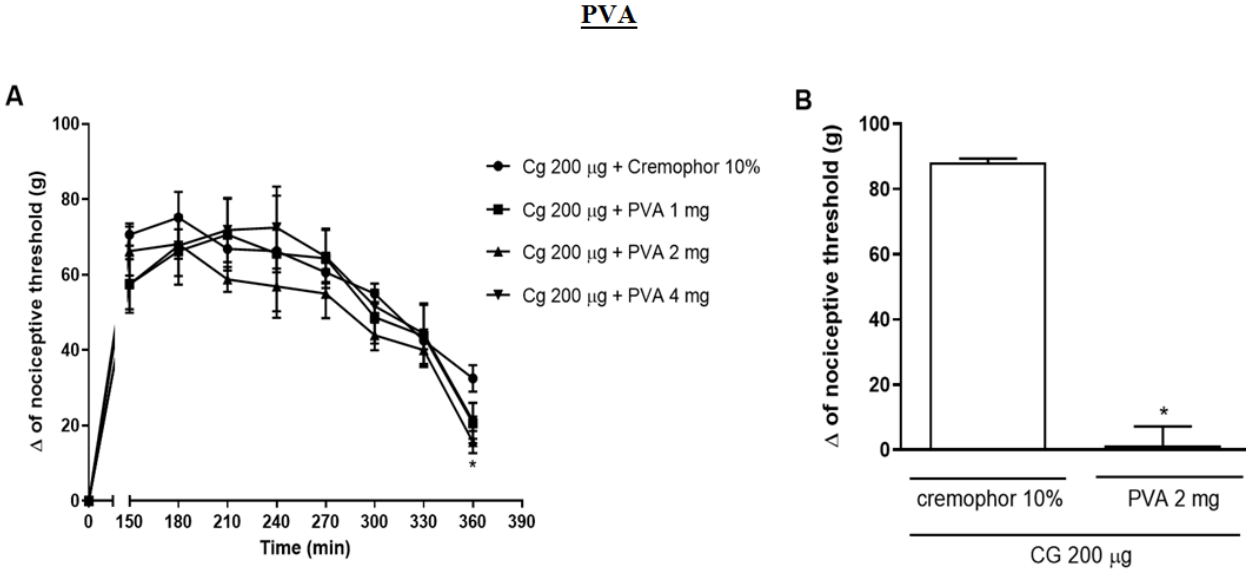

Figure 2. Effect of oral administration of PVA on CG-induced hyperalgesia. Carrageenan (CG; 200 µg/paw) was injected into the right rat paw at time 0 and *P. viridis* ethyl acetate extract (PVA; 1, 2 and 4 mg) or its vehicle (cremophor 10%) was orally given at time 120 minutes for temporal curve evaluation (A) and 60 minutes before CG for the peak action evaluation (B). The data are presented as mean ± S.E.M. (n = 4). *Indicates a significant difference compared with CG + cremophor 10% (P<0.05); two-way ANOVA with Bonferroni post test (A) and t-Student test (B).

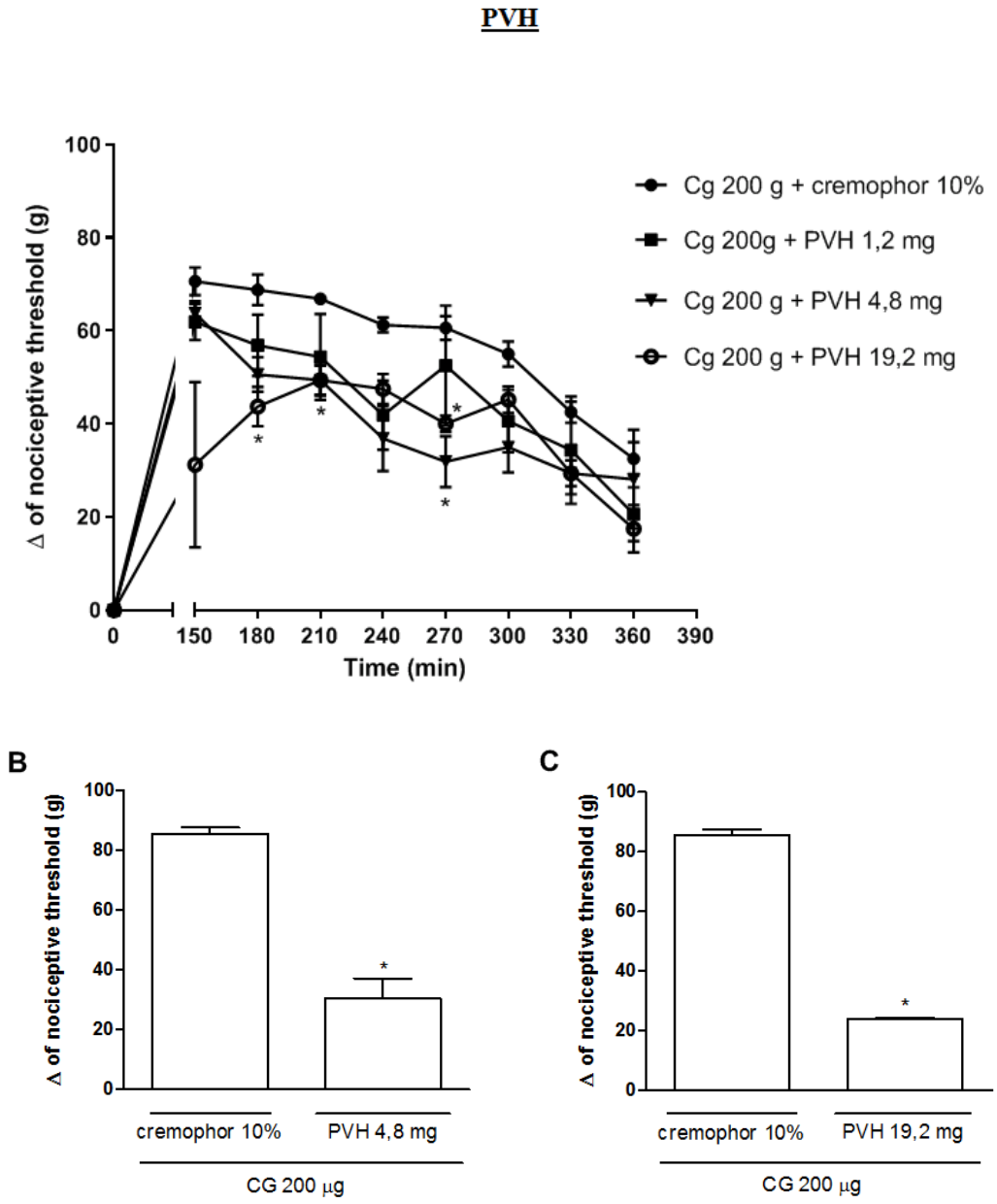

Figure 3. Effect of oral administration of PVH on CG-induced hyperalgesia. Carrageenan (CG; 200 µg/paw) was injected into the right rat paw at time 0 and *P. viridis* hexanic extract (PVH; 1,2, 4,8 and 19,2 mg) or its vehicle (cremophor 10%) was orally given at time 120 minutes for temporal curve evaluation (A) and at time 30 minutes (B) or 150 minutes for the peak action evaluation. The data are presented as mean ± S.E.M. (n = 4). *Indicates a significant difference compared with CG + cremophor 10% ($P<0.05$); two-way ANOVA with Bonferroni post test (A) and t-Student test (B and C).

Plant extracts usually contain several active substances, with different pharmacological effects and pharmacokinetic characteristics [33]. These characteristics of the extracts could explain the disparity observed in the response of the extracts of *P. viridis* tested in this work, since, the presence of 19 different constituents was identified [34] in the *P. viridis* leaf. Such a finding may also indicate that the interaction between the compounds is causing the instability of the curve

or that there is not only one analgesic substance, but several.

From the temporal analysis of the effects of the different extracts, we selected the times when there was a greater antinociceptive effect and the doses when these effects occurred, and the extracts were given in such a way as to coincide with the action peaks of the extracts and carrageenan (180 min). The PVM extract, at the dose of 9.6 mg (Figure 1B), as well as the PVH extract, at the doses of 4.8 mg and 19.2 mg (Figures 3B and 3C, respectively), showed a partial antinociceptive effect, while the PVA extract (Figure 2B) presented a total antinociceptive effect.

Similarly, the administration of plant extracts of the *Psychotria* genus also caused antinociceptive effects. It was showed that extracts obtained from the leaves of *Psychotria colorata* had an antinociceptive effect in the formalin test (first and second phase), writhing test and in the tail-flick test [35]. Another observation made by the same authors was a reversal of this effect in the tail flick-test after pretreatment with naloxone, thereby suggesting the involvement of the opioid system in this event. The alkaloid Hodgkinsine, one of the main constituents of the genus *Psychotria*, showed a dose-dependent antinociceptive effect, that was antagonized by naloxone, in thermal models of nociception and in pain induced by capsaicin [36].

A wide variety of plants are being used by indigenous populations as analgesics [37]. Furthermore, several drugs used commercially today come from plants; for instance, morphine comes from the poppy [38]. Likewise, the compounds present in *P. viridis* extracts have shown promising results for the discovery of new pharmacological tools for pain control. It should be highlighted, however, that further studies are necessary to elucidate the main compounds responsible for the antinociceptive effect of *P. viridis*.

CONCLUSION

With the obtained data it is possible to conclude that the great variety of substances contained in *P. viridis* results in fairly irregular antinociceptive effects over time. However, an important antinociceptive effect was detected, showing that *P. viridis* may contain promising substances for the development of pain control drugs.

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AUTHOR CONTRIBUTIONS

A.C.P., I.D.G.D., and T.R.L.R. conceived and designed the experiments. D.P.D.M and F.C.S.F performed the experiments. I.D.G.D., D.P.D.M., and F.C.S.F. analyzed the data and performed the statistical analysis. M.C.A., S.A.V.F., O.D.H.S., and A.D.C. performed the extraction and purification of the plant. D.P.D.M. and F.C.S.F. wrote the paper. I.D.G.D. provided overall direction to the project and revised the manuscript. All authors reviewed, discussed, and approved the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

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