



Fractions of *Selaginella convoluta* (Arn.) Spring (Selaginellaceae) attenuate the nociceptive behavior events in mice

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(With 10 figures)

Abstract

Selaginella convoluta (Arn.) Spring is a species popularly known as “jericó”, and used in folk medicine as analgesic and anti-inflammatory. This study aimed to investigate in mice the antinociceptive and anti-inflammatory activities of the hexane (Sc-Hex) and chloroform (Sc-CHCl₃) fractions (100, 200 and 400 mg/kg) obtained by partition of crude ethanol extract from *S. convoluta*. The preliminary phytochemical analysis of the fractions was performed. Antinociceptive activity was evaluated by writhing, formalin and hot-plate tests. Anti-inflammatory activity was evaluated using carrageenan-induced pleurisy. The rota-rod test was used to evaluate motor coordination. Preliminary phytochemical screening showed that the Sc-Hex and the Sc-CHCl₃ were positive for the presence of flavonoids, anthracene derivatives, quinones, triterpenes and steroids. Inhibition of writhing was observed for fractions tested. The Sc-Hex at all doses tested was effective in reducing the nociceptive behavior produced by formalin only in the second phase. However, the Sc-CHCl₃ decreased the paw licking time in the first and second phases. In the hot plate no significant effect was observed for any fraction. In the rota-rod test, treated mice did not demonstrate any significant motor performance changes. In the carrageenan-induced pleurisy, Sc-CHCl₃ (200 mg/kg) reduced cell migration to the pleural cavity. These results reveal the antinociceptive properties of *S. convoluta*, which support, in part, its traditional use, since the fractions did not present significant activity in the inflammatory response profile. We further verify that this antinociceptive effect could be by activation of nociceptive peripheral pathway.

Keywords: *Selaginella convoluta*, Selaginellaceae, inflammation, nociception.

Frações de *Selaginella convoluta* (Arn.) Spring (Selaginellaceae) atenuam a resposta nociceptiva em camundongos

Resumo

Selaginella convoluta é uma espécie popularmente conhecida como “jericó”, e usada na medicina popular como analgésica e anti-inflamatória. Este estudo teve como objetivo investigar em camundongos as atividades antinociceptiva e anti-inflamatória das frações hexânica (Sc-Hex) e clorofórmica (Sc-CHCl₃) (100, 200 e 400 mg/kg) obtidas por partição do extrato etanólico bruto de *S. convoluta*. A análise fitoquímica preliminar das frações foi realizada. A atividade antinociceptiva foi avaliada pelos testes de contorções abdominais, formalina e placa quente. A atividade anti-inflamatória foi avaliada usando pleurisia induzida por carragenina. O teste do rota-rod foi utilizado para avaliar a coordenação motora. A triagem fitoquímica preliminar mostrou que Sc-Hex e Sc-CHCl₃ apresentaram reação positiva para a presença de flavonoides, derivados antracênicos, quinonas, triterpenos e esteroides. A inibição das contorções foi observada para as frações testadas. Sc-Hex em todas as doses testadas foi efetivo na redução do comportamento nociceptivo produzido pela formalina somente na segunda fase. No entanto, Sc-CHCl₃ diminuiu o tempo de lambida da pata na primeira e segunda fases. Na placa quente, nenhum efeito significativo foi observado para qualquer fração. No teste do rota-rod os camundongos tratados não demonstraram mudanças significativas na coordenação motora. Na pleurisia induzida por carragenina, Sc-CHCl₃ (200 mg/kg) reduziu a migração celular para a cavidade pleural. Estes resultados revelam a propriedade antinociceptiva de *S. convoluta*, justificando, em parte, seu uso tradicional, uma vez que os extratos não apresentaram atividade significativa no perfil de resposta inflamatória. Verificamos ainda que esse efeito antinociceptivo mostrou-se ligado à ativação da via periférica nociceptiva.

Palavras-chave: *Selaginella convoluta*, Selaginellaceae, inflamação, nocicepção.

1. Introduction

The pain and inflammation are a body defense reaction, in order to eliminate or limit the spread of injurious organisms, toxic chemical substances, and physical injuries from living mammalian tissues (Nahar et al., 2013; Sobota et al., 2000). Narcotic drugs, non-steroidal anti-inflammatory drugs (NSAIDs), steroidal and immunosuppressant drugs have been usually used in the relief of pain and inflammatory diseases by the people of the world for a long time (Lima-Saraiva et al., 2012). Furthermore, these drugs may have various and severe adverse side effects, such as gastric lesions caused by NSAIDs, tolerance and dependence induced by opiates, use of these drugs as anti-inflammatory and analgesic agents have not been successful in all cases (Carvalho et al., 2010; Menichini et al., 2009; Adedapo et al., 2008).

Medicinal plants have been used since ancient times as medicines for the treatment of diseases and still play a key role in world health to be potential source of drugs (Basso et al., 2005; Newman and Cragg, 2016; Jeelani et al., 2017). According to Kumara (2001), plant based drugs in traditional medicine are being paid much attention because of their minimal side effects, cheapness and also the fact that 80% of the world population still rely on them (Kumara, 2001). The chemical diversity of plants has made them one of the main sources for the isolation of bioactive organic compounds (Basso et al., 2005). Brazil is privileged because it ranks first among the richest countries in biodiversity in the world, accounting for 22% of the higher plant species on the planet (Rates, 2001).

The family Selaginellaceae Willk. is a distinctive family including the single genus *Selaginella* which is a nearly worldwide genus of about 700 species (Tryon and Tryon, 1982) to 750 species (Judd et al., 1999), with about 270 of them in America. Members of the Selaginellaceae occurs mostly terrestrial, herbaceous and perennial plants. Vary greatly in size, some small species have stems about 3 cm long, while larger ones have stems 50 cm to approximately 1 m long, under 2 cm tall (Judd et al., 1999).

Pharmacological and phytochemical studies on genus *Selaginella* led to identification of numerous bioactive molecules, including alkaloids, phenols (flavonoids, tannins, saponins), and terpenoids (triterpenes and steroids) (Almeida et al., 2013; Chikmawati et al., 2008), with broad biological activities, including antiviral, antifungal, antibacterial, cytotoxic, anticancer, antioxidant, antiplasmodial, leishmanicidal and anti-inflammatory properties (Lin et al., 1999; Jung et al., 2006; Lee et al., 2009a, b; Chen et al., 2005; Silva et al., 1995; Woo et al., 2006; Subramoniam et al., 2005; Pandey et al., 1993; Kunert et al., 2008; Kim et al., 1998; Gambhir et al., 1987).

Selaginella convoluta is a medicinal plant found in Northeastern Brazil commonly known as “jerico”, “mão-de-sapo” and “mão-fechada” and it is used in folk medicine as antidepressant (Santos et al., 1994; Giorgetti et al., 2007), aphrodisiac, diuretic, against amenorrhea (Agra et al., 2007), cough, bleeding, increases

female fertility (Albuquerque et al., 2007) as well as analgesic and anti-inflammatory (Almeida et al., 2005).

Previous study carried out by our research group demonstrated the antinociceptive effect of ethanolic extract in mice (Sa et al., 2012). In this way, the present work aimed to investigate the antinociceptive and anti-inflammatory effects of the fractions obtained by partition of the crude ethanol extract of *S. convoluta* in experimental models in mice.

2. Material and Methods

2.1. Plant material

Selaginella convoluta was collected in the city of Petrolina (Coordinates: S 09°03'54"; W 40°19'12") State of Pernambuco, Brazil, in March of 2012. A sample was identified by Viseldo Ribeiro de Oliveira, a researcher from Embrapa Semiárido. A voucher specimen (19203) was deposited at the Herbarium Vale do São Francisco (HVASF) of the Federal University of San Francisco Valley (UNIVASF).

2.2. Preparation of plant extracts

The dried and pulverized plant (1935 g) was macerated with ethanol 95% at room temperature for 72 h. The extractive solution was filtered and concentrated under reduced pressure in a rotatory evaporator at 50 °C, producing 146.55 g of crude ethanol extract (Sc-EEB). The Sc-EEB was suspended in a mixture of methanol and water (3:7) and extracted successively with hexane and chloroform in ascending order of polarity to obtain the fractions Sc-Hex (21.26 g) and Sc-CHCl₃ (15.14 g), respectively.

2.3. Phytochemical analysis

The preliminary phytochemical screening, in order to establish the possible chemical nature of the compounds, was carried out with the fractions obtained by partitioning the Sc-EEB. The tests were performed according to the methodology described by Wagner and Bladt (1996) seeking to highlight the main groups of secondary metabolism.

2.4. Animals

Male adult albino Swiss mice (25-35 g) were used in this study. The animals were randomly housed in appropriate cages at 22 ± 2 °C on a 12 h light/dark cycle with free access to food and water. They were used in groups of six animals each, according to the experiments realized. Experiments were performed during the light phase of the cycle. Treatment doses (100, 200 and 400 mg/kg) were chosen based on a previous study (Sá et al., 2012). All tests were carried out by the same visual observer. Experimental protocols and procedures were approved by the Universidade Federal do Vale do São Francisco Animal Care and Use Committee by number 0003/17072012.

2.5. Pharmacological tests

2.5.1. Acetic acid-induced writhing test

This test was done using the method described by Koster et al. (1959). Mice (25-35 g) were divided into nine groups. Acetic acid (0.9% v/v) was administered i.p. in a

volume of 0.1 mL/10 g. Vehicle (saline), morphine (MOR, 10 mg/kg), Indomethacin (INDO, 20 mg/kg) and fractions (100, 200 and 400 mg/kg), were administered i.p. 30 min before acetic acid injection. The number of abdominal constrictions produced in each group for the succeeding 10 min was counted and compared to the response in the control group. Antinociceptive activity was calculated as the percentage inhibition of abdominal constriction.

2.5.2. Formalin test

The method used was similar to the described by Hunskaar and Hole (1987) and Viana et al. (1998). Twenty microliters of 2.5% formalin (in 0.9% saline, subplantar) was injected subcutaneously into the right hind paw of mice. The time (in seconds) spent in licking and biting responses of the injected paw was taken as an indicator of pain response. Responses were measured for 5 min after formalin injection (first phase, neurogenic) and 15-30 min after formalin injection (second phase, inflammatory). Vehicle (saline), morphine (MOR, 10 mg/kg), acetylsalicylic acid (ASA, 150 mg/kg) and fractions (100, 200 and 400 mg/kg), were administered i.p. 60 min before formalin injection. Mice were observed in the chambers with a mirror mounted on three sides to allow view of the paws. Antinociceptive activity was calculated as the percentage inhibition of licking time.

2.5.3. Hot plate test

Mice were divided into eight groups. Mice were pre-selected on the hot plate at 55 ± 0.5 °C. Licks on the rear paws were the parameters of observation. Animals showing a reaction time (latency for licking the hind feet or jumping) greater than 20 s were discarded. The animals were then treated with vehicle (saline, 0.1 mL/10 g), morphine (MOR, 10 mg/kg) and fractions (100, 200 and 400 mg/kg) via i.p. Latency time (in seconds) for each mice was determined on the hot plate during the maximum period of 20 s, at intervals of 30, 60, 90 and 120 min after the administration of the treatment (Almeida et al., 2011).

2.5.4. Carrageenan-induced pleurisy

The animals (n=6 in each group) were pre-treated by intraperitoneal route (i.p.) with fractions (100, 200 and 400 mg/kg) 30 min prior to the induction of pleurisy. Pleurisy was induced by the intrapleural injection (i.t.) of 100 μ L of a 1% (v/v) carrageenan solution. A specially adapted 13 \times 5 syringe was introduced into the right side of the thoracic cavity of mice to inject the carrageenan solution, and an equal volume of sterile saline was injected into the controls. At 4 h after the i.t. injection, the animals were sacrificed in a CO₂ gas chamber and the thoracic cavity was opened and washed with 1 mL PBS containing EDTA (10 mM). These pleural washes were recovered and their volume measured. Pleural wash samples were diluted in Turk fluid (2% acetic acid) for total leukocyte counts using Neubauer chambers. Indomethacin was used as reference drug. Thus, parallel group of animals was pre-treated (30 min before pleurisy induction) with indomethacin (10 mg/kg, i.p.)

and 4 h later the same inflammatory parameters were evaluated (Farias et al., 2011).

2.5.5. Measurement of total protein content

The fluids recovered from the pleural cavity of the animals treated with the extracts Sc-Hex and Sc-CHCl₃ at 100, 200 and 400 mg/kg were centrifuged for 10 min at $1.500 \times g$, and the total protein content was quantified in the supernatant, at 640 nm, using the Folin-Lowry technique (Stauffer, 1975).

2.5.6. Motor coordination test (rota-rod test)

A rota-rod treadmill device (Insight, Brazil) was used for the evaluation of motor coordination. Initially, 24 h before the test, mice capable of remaining on the rota-rod apparatus longer than 180 s (7 rpm) were selected. Thirty minutes after the administration of fractions (100, 200 and 400 mg/kg, i.p.), vehicle (saline, 0.1 mL/10 g; control group) or diazepam (2.5 mg/kg, i.p.), each animal was tested on the rota-rod apparatus at 30 min, 1 and 2 h post-treatment, and the time(s) the mice were able to remain on top of the bar was (were) recorded for up to 180 s.

2.6. Statistical analysis

The data were expressed as mean \pm S.E.M. and the statistical significance were determined using an analysis of variance (ANOVA) followed by Dunnett's test. Values were considered significantly different at $p < 0.05$. All analysis was performed using by GraphPad Prism 5.0 program.

3. Results

Preliminary phytochemical screening showed that the Sc-Hex and the Sc-CHCl₃ were positive for the presence of flavonoids, anthracene derivatives, quinones, triterpenes and steroids.

The intraperitoneal administration of Sc-Hex (100, 200 and 400 mg/kg) showed dose-dependent antinociceptive effect and decreased significantly ($p < 0.05$) the number of writhing movements induced by the i.p. administration of the acetic acid compared with the control group. The percentages of inhibition were 64.93, 77.91 and 99.33%, respectively (Figure 1). The Sc-CHCl₃ produced inhibition of the abdominal writhing response by 90.16, 90.16 and 95.92% (Figure 2). Indomethacin caused a 90.92 and 88.53% reduction in writhing movements for animals treated with Sc-Hex and Sc-CHCl₃, respectively. Morphine abolished all of the nociceptive responses in all treated groups.

The results of the formalin test are shown in Figure 3 and Figure 4. Sc-CHCl₃ showed a significant antinociceptive effect to inhibit the licking time in both phases (neurogenic and inflammatory pain) of the test, but the result was most significant in the second phase. Sc-CHCl₃ (100 mg/kg, i.p.) decreased by 35.46%, the paw licking time in the first phase, as well as 82.06, 74.96 and 92.77%, respectively, in the second phase of the formalin test. However, the Sc-Hex decreased the paw licking time only the second phase by 64.51, 67.80 and 73.90%.

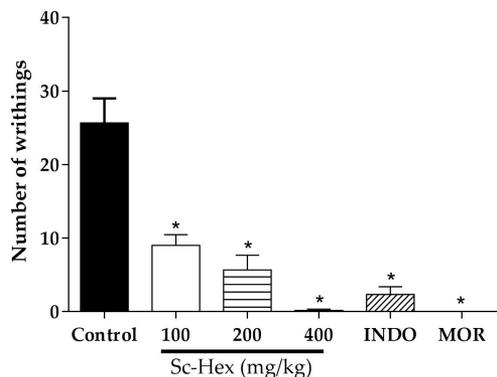


Figure 1. Antinociceptive effect of the hexane fraction from *Selaginella convoluta* (Sc-Hex), indomethacin (INDO, 20 mg/kg) and morphine (MOR, 10 mg/kg) on acetic acid-induced writhing in mice. Values are mean ± S. E.M, n = 6, *p < 0.05 significantly different from control (ANOVA followed by Dunnett's test).

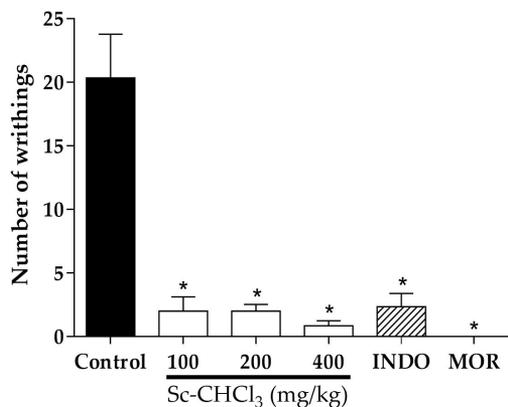


Figure 2. Antinociceptive effect of the chloroform fraction from *Selaginella convoluta* (Sc-CHCl₃), indomethacin (INDO, 20 mg/kg) and morphine (MOR, 10 mg/kg) on acetic acid-induced writhing in mice. Values are mean ± S.E.M, n = 6, *p < 0.05 significantly different from control (ANOVA followed by Dunnett's test).

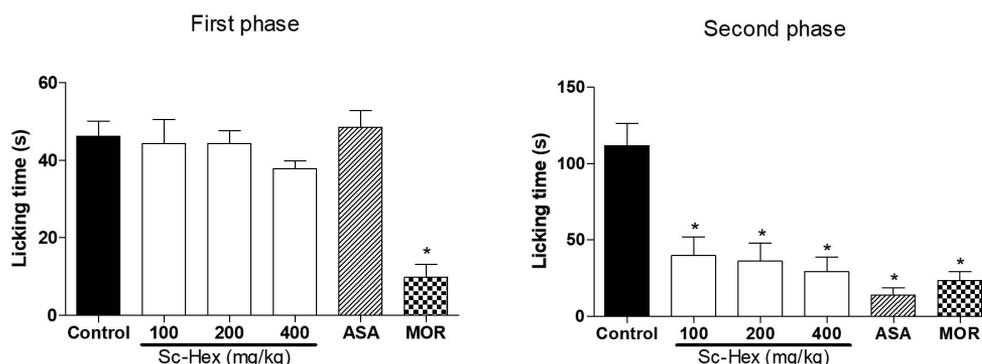


Figure 3. Antinociceptive effect of the hexane fraction of *Selaginella convoluta* (Sc-Hex), acetylsalicylic acid (ASA, 150 mg/kg) and morphine (MOR, 10 mg/kg) on formalin (First and second phase). Values are mean ± S.E.M, n = 6, *p < 0.05 significantly different from control (ANOVA followed by Dunnett's test).

The reference drug acetylsalicylic acid was effective only in the second phase for both fractions (87.63% - Sc-Hex and 88.89% - Sc-CHCl₃). Morphine decreased the licking time during the two phases.

In the hot plate test, no significant effect was observed for any fraction. The effect of morphine (10 mg/kg) was significantly higher. Figure 5 and Figure 6 show these results.

Treatment with the Sc-CHCl₃ only at dose of 200 mg/kg (i.p), caused a significant decrease the volume of the exudates to $1.19 \pm 0.25 \times 10^6$ cells mL/cavity compared with the control group ($6.53 \pm 1.40 \times 10^6$ cells mL/cavity) and in total protein extravasations when it was administrated 30 min before carrageenan (Figure 7 and Figure 8). As expected, the reference drug, indomethacin caused a significant inhibition of volume of the exudates to 0.66 ± 0.14 mL/cavity.

In the rota rod test, the fractions did not impair motor coordination at 0.5, 1 and 2 h post administration. Diazepam (2.5 mg/kg) caused a significant decrease in time that the animals remained on the rota-rod apparatus, compared to the control group (Figure 9 and Figure 10).

4. Discussion

The present study reported the evaluation of antinociceptive and anti-inflammatory effects of *S. convoluta* fractions employing various experimental models. Although the plant is widely used in the folk medicine in the semi-arid region of Brazil, only one report about the antinociceptive activity of crude ethanolic extract is recorded in the literature (Sá et al., 2012). Furthermore, current treatments used to fight pain and inflammation are usually insufficient for having severe side effects and limited effectiveness (Bourinet et al., 2005). Therefore, the continuous search for new molecules that are more effective with reduced side adverse is needed.

The antinociceptive activities of fractions, all given intraperitoneally at the doses of 100, 200 and 400 mg/kg, were evaluated using chemical (acetic acid and formalin) and thermal (hot plate test) stimuli. The writhing test has long been used as a screening tool for assessing the

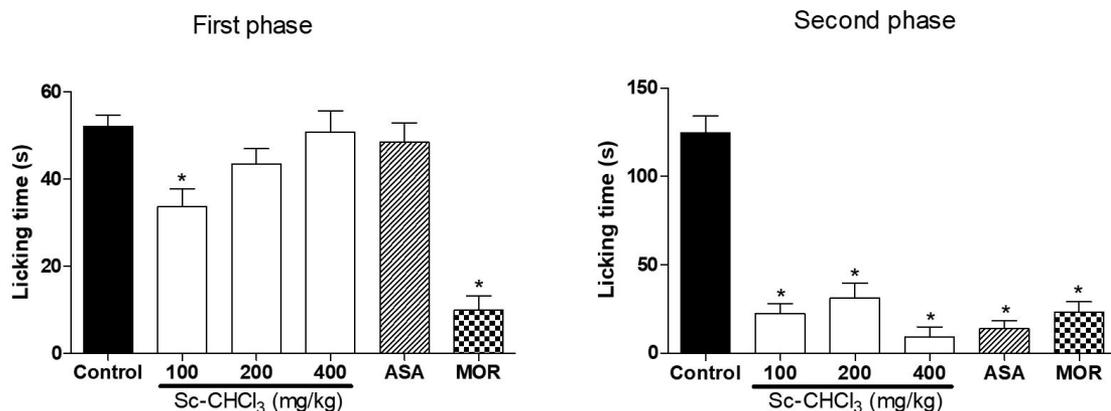


Figure 4. Antinociceptive effect of the chloroform fraction of *Selaginella convoluta* (Sc-CHCl₃), acetylsalicylic acid (ASA, 150 mg/kg) and morphine (MOR, 10 mg/kg) on formalin (First and second phase). Values are mean \pm S.E.M, n = 6, *p < 0.05 significantly different from control (ANOVA followed by Dunnett's test).

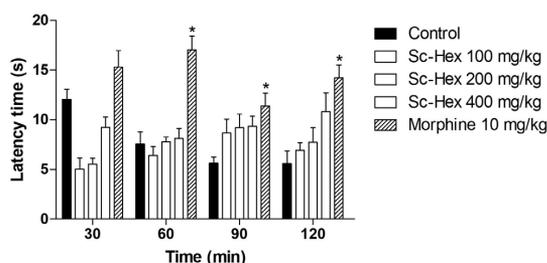


Figure 5. Antinociceptive effect of the hexane fraction of *Selaginella convoluta* (Sc-Hex) and morphine (MOR, 10 mg/kg) on hot plate test. Values are mean \pm S.E.M.; *p < 0.05, significantly different from control; ANOVA followed Dunnett's.

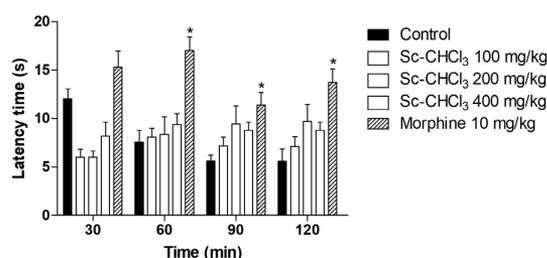


Figure 6. Antinociceptive effect of the chloroform fraction of *Selaginella convoluta* (Sc-CHCl₃) and morphine (MOR, 10 mg/kg) on hot plate test. Values are mean \pm S.E.M.; *p < 0.05, significantly different from control; ANOVA followed Dunnett's.

analgesic properties of new substances (Collier et al., 1968). Intraperitoneal administration of acetic acid induce abdominal writhing and involves the increase in cyclooxygenase (COX), lipoxigenase (LOX), prostaglandins (PGs), histamine, serotonin, bradykinin, substance P, IL-1 β , IL-8, TNF- α in the peritoneal cavity (Farias et al., 2011). These agents

provoke a very stereotyped behavior in the mice which is characterized by abdominal contractions, movements of the body as a whole and twisting of dorso-abdominal muscles. However, it is a non-specific method for evaluation of pain (Silva et al., 2010).

Sc-Hex and Sc-CHCl₃ significantly reduced the acetic acid-induced writhing in mice. The results support the hypothesis that the fractions have antinociceptive effect on the abdominal constrictions. Additionally, different flavonoids have been found to be antinociceptive and anti-inflammatory agents due to their ability to inhibit the arachidonic acid metabolism (Melo et al., 2008; Havsteen, 2002; Aquila et al., 2009). The presence of flavonoids in the fractions of *S. convoluta* may be responsible for the antinociceptive effect (Macêdo et al., 2018).

In order to extend the studies on the antinociceptive and anti-inflammatory effects, we used the model of nociception induced by formalin. The formalin-induced paw linking test is a model comprising two distinct phases. The first phase (neurogenic pain) occurs about 3 min after the injection, and then after a quiescent period, a second phase (inflammatory pain) between 20 and 30 minutes (Clavelou et al., 1995). The first phase results essentially from chemical stimulation of nociceptive afferent fibers, particularly C-fibers, which can be suppressed by opiates like morphine (Amaral et al., 2007). However, the involvement of substance P and bradykinin has also been reported. The second phase (inflammatory pain) is induced due to action of inflammatory mediators such as prostaglandins, serotonin and bradykinin in peripheral tissues and functional changes in the dorsal horn of the spinal cord (Cha et al., 2011; Dalal et al., 1999). The Sc-Hex demonstrated that the number of paw licking was significantly reduced only in second phase (p < 0.05) in a dose-dependent manner. However, the Sc-CHCl₃ showed significant effect in both neurogenic and inflammatory pain phases (p < 0.05).

In this experiment, Sc-CHCl₃ decreased the licking time in both phases, but the effect was more significant

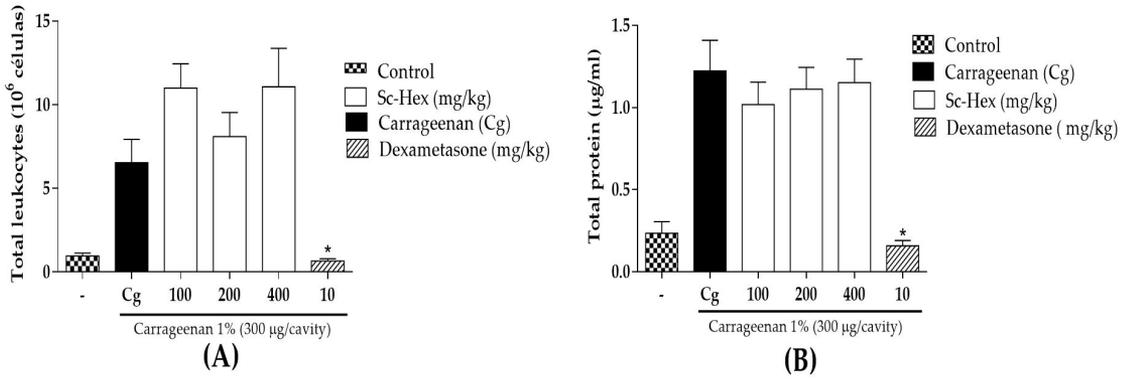


Figure 7. Effect of the hexane fraction of *Selaginella convoluta* (Sc-Hex) and indomethacin (10 mg/kg) on total leukocyte (A) and protein extravasation (B) on Pleurisy induced by carrageenan. Values are mean ± S.E.M.; *p < 0.05, significantly different from control; ANOVA followed Dunnett's.

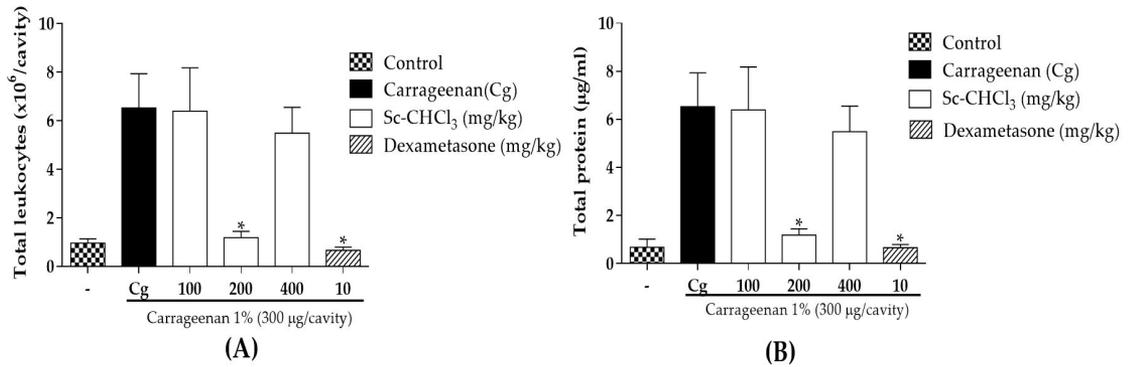


Figure 8. Effect of the chloroform fraction of *Selaginella convoluta* (Sc-CHCl₃) and indomethacin (10 mg/kg) on total leukocyte (A) and protein extravasation (B) on Pleurisy induced by carrageenan. Values are mean ± S.E.M.; *p < 0.05, significantly different from control; ANOVA followed Dunnett's.

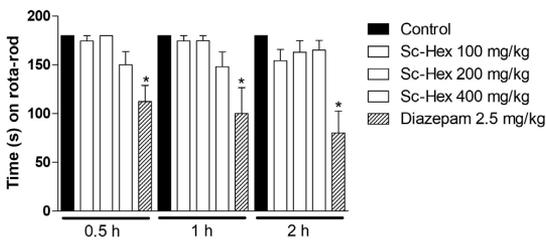


Figure 9. Effect of the hexane fraction of *Selaginella convoluta* (Sc-Hex) and diazepam (2.5 mg/kg) on rota-rod test. Values are mean ± S.E.M.; *p < 0.05, significantly different from control; ANOVA followed Dunnett's.

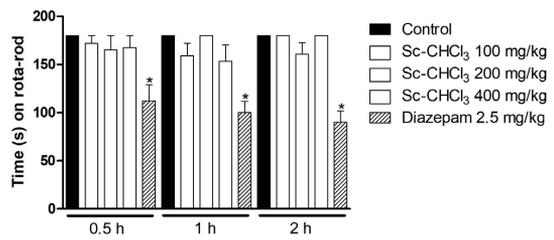


Figure 10. Effect of the chloroformic fraction of *Selaginella convoluta* (Sc-CHCl₃) and diazepam (2.5 mg/kg) on rota-rod test. Values are mean ± S.E.M.; *p < 0.05, significantly different from control; ANOVA followed Dunnett's.

in the second phase. The inhibition in both phases is characteristic of drugs that act centrally, and indicates a possible interaction with opioid receptors. Opioid analgesics seem to be antinociceptive for both phases, although the first is more sensitive to these substances. In contrast, non-steroidal anti-inflammatory drugs such as indomethacin and acetylsalicylic acid seem to suppress only the second phase (Hunnskaar and Hole, 1987).

The results obtained in the acetic acid-induced writhing test and formalin test were similar to those observed by Sá et al. (2012) when evaluating the crude ethanolic extract from *S. convoluta*. They related that the crude ethanolic extract also exhibited reduced writhing and decreased the paw licking time in mice during the first phase of the formalin test as well as during the second phase of the test, but the reduction was most significant in the second phase.

In order to confirm the antinociceptive activity and to investigate the involvement of the central mechanisms of the effects of the fractions, the hot plate test was used. In this test, the intraperitoneal administration of fractions didn't affect mice's reactivity at the thermal stimulus, demonstrating that the antinociceptive effect in the abdominal constriction (writhing) test probably do not involve central mechanisms. In contrast, in the study realized by Sá et al. (2012) treatment at doses of 200 and 400 mg/kg of the crude ethanolic extract increased the latency time in the hot plate test after 60 and 90 minutes, respectively.

To evaluate the anti-inflammatory effect of the fractions, the model of carrageenan-induced pleurisy in mice was used. The pleurisy induced by phlogistic agents is a widely accepted model for the evaluation of the anti-inflammatory effect of compounds from plants.

Inflammation is a protective process that is essential for the preservation of the integrity of the organism in the event of chemical, physical and infectious damage. Often, the inflammatory response to severe lesions erroneously damages normal tissue (Farias et al., 2011).

Recruitment of cells to inflammatory sites is dependent on the release of vasoactive and chemotactic factors that increase the local blood flow and microvascular permeability and promote the migration of leukocytes from the intravascular space into the tissues (Farias et al., 2011). In this study, intrapleural injection of carrageenan induced an acute inflammatory reaction, characterized by marked accumulation of exudates rich in protein and intense migration of polymorphonuclear in the pleural cavities. Treatment of the animals with 200 mg/kg of Sc-CHCl₃ attenuated the number of total leukocytes and total protein concentration in the exudate after carrageenan stimulus (Zhao et al., 2007). However, this result is not sufficient to affirm that the plant has anti-inflammatory activity, because this effect was observed only in one dose of fractions.

Finally, to assess whether extracts produces a loss of motor coordination in animals, the rota-rod test was performed. Motor coordination tests are used in the screening of drugs with possible myorelaxant/neurotoxic effect, since they are tests that are capable to detect the muscle relaxant activity and motor incoordination of agents with pharmacological properties, as is the case with many analgesics (Pultrini et al., 2006; Baggio et al., 2012). The result revealed that the extracts did not produce changes in motor coordination of treated animals.

In conclusion, this study indicates that the hexane and chloroform fractions of *Selaginella convoluta* exhibit antinociceptive activity. Our results support previous claims of its traditional use. The mechanism of action of these extracts showed linked with possible activation of nociceptive peripheral pathway. In addition, we have demonstrated that the extracts from *S. convoluta* have not any anti-inflammatory effect in the pleurisy model induced by carrageenan. Further studies are necessary to identify the biologically active constituents and to define the underlying of molecular mechanisms of the inhibitory effects of these fractions.

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