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ARTIGO ORIGINAL

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Cicatrização de feridas e efeitos anti-inflamatórios do extrato etanólico de Tropaeolum pentaphyllum em camundongos.

Wound healing and anti-inflammatory effects of the ethanolic extract of Tropaeolum pentaphyllum in mice.

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Palavras-chave: carragenina; inflamação; pleurisia; edema de pata; Tropaeolum pentaphyllum.

Key-words: carrageenan; inflammation; pleurisy; paw oedema; Tropaeolum pentaphyllum.

Resumo

A planta *Tropaeolum pentaphyllum* (Tropaeolaceae), é conhecida e nominada popularmente em certas regiões brasileiras como *crem, batata-crem capuchinha, flor-de-sangue*. Suas raízes são utilizadas na medicina popular da região sul do Brasil, como anti-inflamatórias. No entanto, relatos sobre a atividade anti-inflamatória desta planta são escassos na literatura. Os objetivos deste trabalho foram avaliar o potencial biológico do extrato etanólico das raízes de *T. pentaphyllum* (EETP) em modelos experimentais de inflamação tais como: cicatrização de feridas excisionais, edema de pata e pleurisia em camundongos. O tratamento com EETP reduziu de maneira mais efetiva o tempo necessário para a cicatrização das lesões excisionais, quando comparado ao grupo tratado com iruxol. No teste de edema da pata induzido pela injeção de carragenina, a administração de EETP reduziu essa condição fisiopatológica de maneira dose dependente. Apenas o tratamento com a maior dose de EETP (500mg/kg) foi capaz de reduzir significativamente os eventos fisiopatológicos observados durante a pleurisia. Este estudo sugere que os tubérculos da *T. pentaphyllum* podem dar origem a um promissor fitoterápico anti-inflamatório.

Abstract

Tropaeolum pentaphyllum (Tropaeolaceae) is known popularly in certain regions of Brazil as *crem capuchinha flower blood*. Its roots are used in folk medicine of southern Brazil, as anti-inflammatory. However, reports of anti-inflammatory activity of this plant are scarce in the literature. The objectives of this study were to evaluate the biological potential of the ethanol extract of the roots of *T. pentaphyllum* (EETP) in experimental models of inflammation such as healing of excisional wounds, paw edema and pleurisy in mice. The treatment with EETP reduced more effectively the time required for the healing of excisional lesions when compared to the group treated with iruxol. In the paw edema test induced by injection of carrageenan, the EETP administration reduced this pathophysiological condition (dose-dependent manner). Only treatment with the highest dose of EETP (500mg/kg) was able to significantly reduce the pathophysiological events observed during pleurisy. This study suggests that the tubers of *T. pentaphyllum* may be a promising anti-inflammatory herbal.

1. Introduction

The empirical use of medicinal plants has been corroborated by scientific literature in recent decades and the scientific studies on these medicinal plants enabled the development of new pharmacological agents (Rio, 2001, Cruz-Silva et al., 2015).

The research for new molecules, has taken a slightly different route based in discovery of new compounds (Gurib-Fakim, 2006). The plants belonging to family Tropaeolaceae are used in popular medicine for the treatment of several diseases, including inflammatory processes (Zanetti et al., 2004).

The inflammatory process or inflammation is a pathophysiological response to a tissue injury which may be physical origin, chemical and/or biological (Martins, 2010) with the purpose of eradicating the aggressor or agent such as micro-organisms, burns, physical trauma or tumor cells (Pioneiro, 2010) and promotes tissues repair. On the other hand, chronic inflammation is recognized as the cause or a consequence of a number of several human diseases, such as asthma, heart diseases, diabetes and cancer, among other pathophysiologies. Any event that promotes cell injury is intimately involved with the induction of the inflammatory response and contributes to the pathogenesis of chronic inflammatory diseases (Martins, 2010).

Inflammation is a crucial biological process which can contribute to a better understanding of the key mechanisms and development of different pathophysiology (Chung et al., 2011). During the inflammatory process, leukocytes from the circulating blood are attracted to the site where the tissue injury occurred and they are stimulated by inflammatory mediators. The presence of these cells, especially neutrophils, indicates the beginning of inflammation, characterized by the acute phase (Bach et al., 2002). This series of events related to cell regeneration and subsequent recovery of the injured site active resistance to the cell by the immune system. This activation induces innate immunity in healthy tissue whenever it is injured again (Sloane et al., 2010).

In some pathophysiological conditions, the inflammatory response can be quantitatively and/or qualitatively excessive. On this way, clinical interventions are needed to decrease this response by the administration of anti-inflammatory medications such as non-steroidal anti-inflammatory and glucocorticoids (Simons et al., 1979). Although being widely used in clinical practice, they are associated with a spectrum of toxic effects, because the nonselective cyclooxygenase inhibition (Silverstein et al., 2000). The discovery of new anti-inflammatory compounds from natural origin provided the clinical use of these substances because they are less adverse effects compared to anti-inflammatory drugs reference (Foglio et al., 2006).

T. pentaphyllum (Tropaeolaceae) is a brazilian plant popularly known as crem capuchinha flower blood, is commonly found in the Santa Catarina state (Fabbri and Valla, 2011). The crem tubers are used locally as antiscorbutic, anti-inflammatory and depurative. However scientific studies about Tropaeolaceae family and their biological effects are scarce in the literature (Pioneiro, 2010). The most studied specie from this family is T. majus being commonly used for the treatment of cardiovascular disorders, urinary infections, asthma, inflammatory process and constipation (Zanetti et al., 2004).

Previous studies from our laboratory have shown biological activity of T. majus in different experiments. Thus, these preliminary findings stimulate our research group to study the possible effects of T. majus on the inflammatory response in different experimental models.

2. Material and Methods

2.1. Preparation of ethanolic extract of Tropaeolum pentaphyllum (EETP)

Tubers of T. pentaphyllum were collected in the region of Videira, Santa Catarina state. The plant identification was performed by the Botanical Museum of Curitiba, Paraná state, where a voucher specimen was deposited (MBM 331903). The extract was prepared by Maria Elida Alves Stefanello (Chemistry Department, PH.D.) Tubers were dried at 40°C and then ground. The biological material was extracted at room temperature with ethanol 95%, followed by extraction with methanol-water 50%. The evaporation of the solvents provided the hydroalcoholic extract and ethanol, and the extracts were tested for evaluation suggested.

2.2.1. Drugs and plant extract

The reference drug used in the experiments were: carrageenan Lambda type IV (Sigma Chemical Co., St. Louis, USA), dexamethasone (Lab) and EETP. All drugs used were dissolved in saline solution.

2.2.2. Animals

The experiments were performed in Swiss mice (25-35g), housed at 22±2°C, under a 12-h light/12-h dark cycle and with access to food and water ad libitum. The animals were acclimatized to the laboratory for at least 1h 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 (Asuzu et al., 2015).

2.2.3. Measurement of paw oedema

Experimental animals were pretreated with the EETP at doses of 100, 300 e 500mg/kg or vehicle (1% of tween 80 in saline, 0.9%) by oral route (p.o.) or subcutaneously (dexamethasone 1.0mg/kg, s.c, positive control), 1 h prior to the induction of the oedema. The animals received a 50 µl s.c. injection of vehicle (saline, 0.9%) containing carrageenan (300 µg/paw) into the right hindpaw. In the contralateral paw only vehicle was used as a control. The thickness of the paw oedema was measured using a digital micrometer before the induction of the oedema and at different time points after the injection of the phlogistic agent (0.5, 1, 2 and 4h).

2.2.4. Wound induction, animal grouping and drug administration

The back of the mice were shaved and a 6mm full thickness open excision wound was made by removing a patch of skin under ketamine (100mg/kg) and xilazine (10mg/kg) by intraperitoneal route were administered for anesthesia (Suguna et al., 1993). A total of 18 animals were divided into three groups: The control group (n=6) that received only vehicle (50µL of unbuffered physiological saline, once daily, for a period of 11 days), EETP group (n=6) that received 50µL of the EETP (30mg/mL) incorporated in natrosol gel, applied topically, for a period of 11 days and commercial colagenase group (n=6) that received 50µL of iruxol topical application for a period of 11 days. The wound closure is expressed as mm.

2.2.5. Pleural cell migration and protein exudation

The animals received a pretreatment p.o. with EETP at doses of (100, 300 e 500mg/kg) or vehicle, 1h before the carrageenan induction of inflammation or dexamethasone s.c. (1.0mg/kg, positive control), and naive (saline-treated 0.9% negative control), administered orally by gavage, in different groups of mice. Pleurisy was induced by the intrapleural injection of carrageenan 1% (100 µl), as previously described (Zanusso-Junior et al., 2011). The carrageenan was diluted in saline buffer. An adapted needle was inserted into the right side of the thoracic cavity of the animals to enable intrathoracic administration of carrageenan. Control mice received the same volume (100 µL) of sterile pyrogenfree saline. After 4h, the animals were killed and the thoracic cavity was washed with 1mL of phosphate-buffered saline (PBS). The exudate volume was measured, and an aliquot of 20 µL was diluted in Turk solution (1:20) used to determine the total number of leukocytes in a Neubauer chamber. For a differential counting of leukocytes, the remaining fluid was centrifuged at 3200 rpm for 20 min, and the cells were resuspended. The protein exudation was evaluated directly from the lavage by Bradford's reaction, using the commercially available Bradford kit (Bioagency, São Paulo, Brazil). The total and differential cell counts were performed under light microscopy and the results are measured as the number of cells/ml of pleural fluid.

2.2.6. Statistical analysis

The results are presented as the mean ± SEM which are calculated as geometric means accompanied by their respective 95% confidence limits. The statistical significance among groups was assessed by means one-way (ANOVA) followed by Student-Newman-Keuls. P values less than 0.05 were considered statistically not significant.

3. Results

3.1. Effects of EETP on wound healing

The groups treated with iruxol and treated groups EETP had a shorter time to occurrence of the healing of excisional wounds compared to the control group. However EETP treatment was more effective than treatment with iruxol (Figure 1).

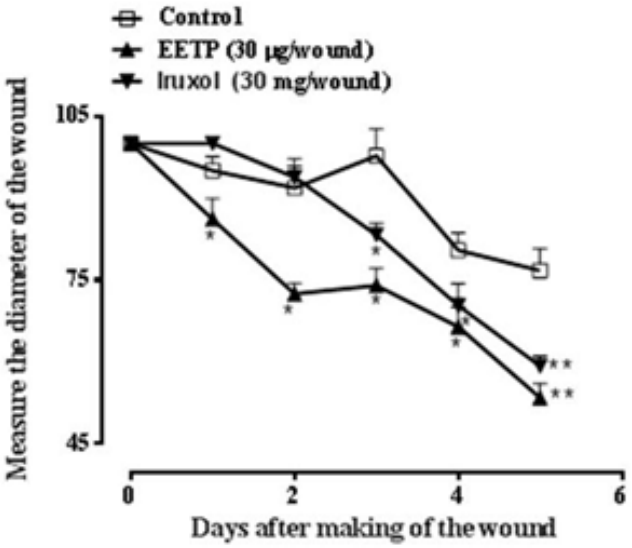

Figure 1- Effects of local administration of ethanolic extract from T. pentaphyllum (30g/injury), on wound healing in mouse. Each point show the mean of 4-6 animals and vertical lines show the SEM. Asterisks represent the statistical difference when compared with control values (gel natrosol basis) *P < 0.05, **P < 0.01 one-way ANOVA followed by Student-Newman-Keuls.

3.3.2. Effects of EETP on carrageenan-induced paw oedema

Treatment with EETP orally was effective in reducing the paw edema induced by carrageenan, a time-dependent manner. However, their effect persists for up to 2h after the occurrence of its biological effect (Figure 2) and only occurs with administration of high doses of extract (Figure 2a) compared to respective control groups. After 2 h from carrageenan injection, the inhibition of 49 ± 5 % was observed for dose of 300 mg/kg while 47 ± 4 % was verified for group treated with 500 mg/kg (Figure 2).

3.3.3. Effects of EETP on pleural cell migration and protein exudation

The pleural injection of carrageenan increased the leukocyte migration and protein extravazation (Figure 3A and 3B). The oral administration of EETP (500 mg/kg) inhibits leukocyte migration and protein exudation in the experimental model of pleurisy. The inhibition for EETP (500 mg/kg) were 75 ± 4 % for leukocyte migration and 59 ± 3 % for protein extravazation. The same event was observed with the subcutaneous administration of the dexamethasone highest dose orally compared to respective control groups (Figures 3). The inhibition for dexamethasone were 88 ± 6 % for leukocyte migration and 77 ± 6% for protein extravazation.

4. Discussion

The inflammatory process is a defense response of the body against injury and for the most part is a self-limiting process. Then when this response becomes chronic, deleterious effects on the body can arise due to hyperactivation of the immune system (Martins, 2010, Pioneiro, 2010).

Local response in the inflammatory process initiates with tissue damage and triggers various biochemical events including vasodilation, increased vascular permeability and

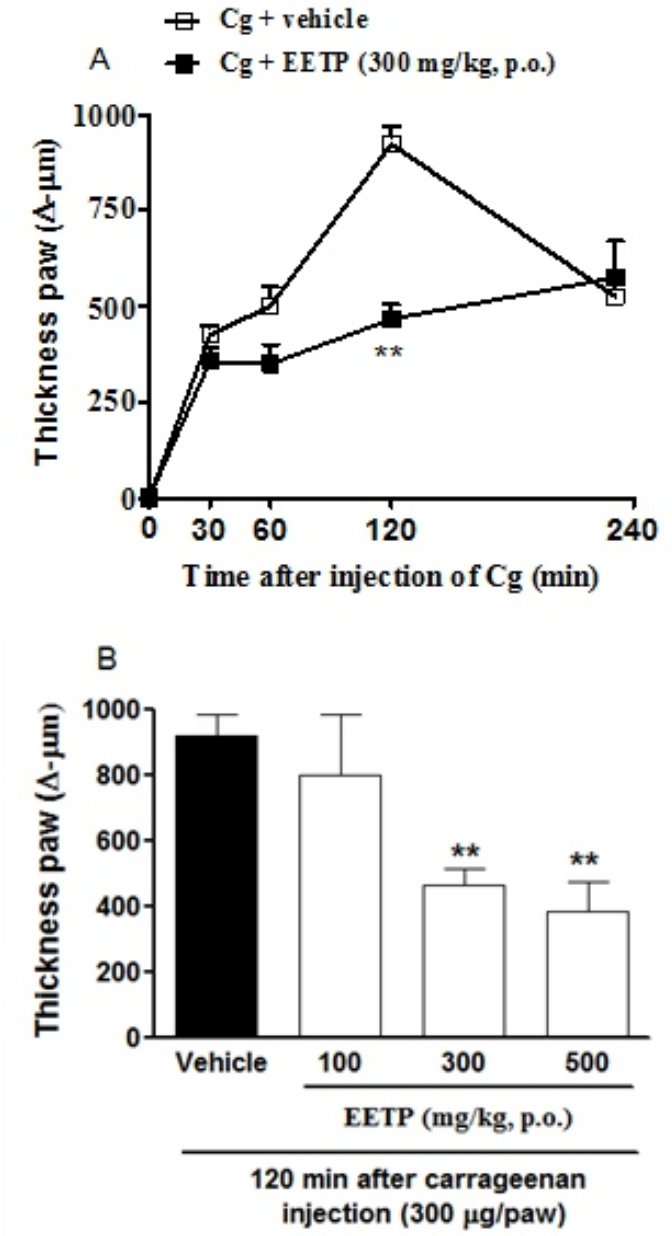

Figure 2- Effects of oral administration of ethanolic extract from T. pentaphyllum (300mg/kg) on different time points after carrageenan injection (A) and with differents doses (100, 300 and 500mg/kg) on thickness paw 120 min after carrageenan injection. Each point and bar show the mean of animals and vertical lines show the SEM. Asterisks represent the statistical difference when compared with respective control group. **P < 0.01 and**P < 0.01 one-way ANOVA followed by Student-Newman-Keuls.

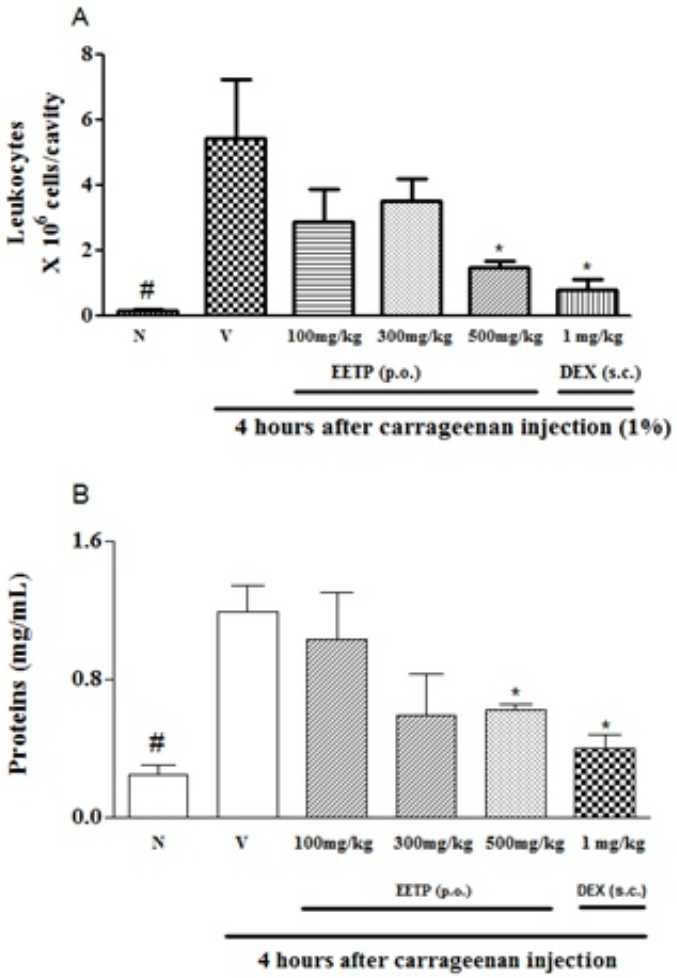

Figure 3- Effects of oral administration of ethanolic extract from T. pentaphyllum (100, 300 and 500mg/kg) on the total leukocytes (A) and protein extravazation induced by carrageenan in the pleural cavity of mice during pleurisy experiments. Vertical lines showed in the histograms represent the SEM. Asterisks represent the statistical difference when compared with respective control group. *P < 0.05 one-way ANOVA followed by Student-Newman-Keuls. # represent the statistical difference when compared the control group with naive. #P < 0.05 one-way ANOVA followed by Student-Newman-Keuls.

increased blood flow implying the oedema formation. This complex cascade of physiological events promotes protection to tissues, restricting the damage at the site of infection or injury, but it may have deleterious effects when so exacerbated.

Vasodilation and plasma extravasation is a characteristic response of paw oedema induced by carrageenan, which is a widely used model to assess the anti-inflammatory acute effect.

Carrageenan-induced inflammatory response is characterized by an early phase (1-2h) where there is the release of inflammatory mediators as histamine, serotonin and bradykinin, followed by a late stage (3-4 h) with the release of prostaglandins (Zanusso-Junior et al., 2011).

In the present study, the oral treatment of mice with EETP, were able to reduce the paw oedema, leukocyte migration and protein extravasation induced by carrageenan.

The results obtained in the wound healing studies have shown that EETP is more effective than the reference drug for this pathophysiological condition.

Our results also suggest that the efficacy of EETP against signs and symptoms associated with the inflammatory response in different experimental models is plausible. However, this therapeutic efficacy is dose-dependent. The reduction of paw oedema induced by carrageenan, is observed with administration of higher doses (300 and 500mg orally) of EETP. The inhibition of leukocyte migration and the inhibition of protein exudation in the pleurisy model are observed only after oral administration of 500mg EETP.

Based on these experimental results, we suggests that EETP presents biological potential to be utilized in clinical therapy as alternatives to the reference drugs currently available and reinforces the idea that the empirical medicinal knowledge is an indicator to discovery of new natural compounds biologically active against different pathophysiology.

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