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Original Article

Phytochemical Screening and Antiinflammatory Activity of the Extract from the Leaves of *Desmodium molliculum* (Kunth) DC (Fabaceae) in Rats with Acute Inflammation

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ABSTRACT

Inflammation and pain are the initial response mechanisms to environmental aggression on the human body. The traditional use of plants such as *Desmodium Molliculum* (Kunth) DC, among the Peruvian population for the treatment of inflammatory diseases, has occurred since ancient times. The objective of this research was to determine the presence of secondary metabolites and evaluate the anti-inflammatory activity of *Desmodium molliculum* (EDM) leaves in rats with acute inflammation induced using carrageenan. The phytochemical profile was performed for the main secondary metabolites with biological activity. Subsequently, 25 rats were divided into 5 groups and treated as follows: Group I and II: Physiological Saline Solution (PSS) by oral administration. Group III: Sodium Diclofenac (25 mg/kg body weight) by intraperitoneal administration. Group IV and V: EDM at 250 mg/kg bw and 500 mg/kg bw by oral administration, respectively; 30 minutes after administration, acute inflammation was induced in Groups II, III, IV, and V using the subplantar edema technique with 1% w/v carrageenan. The volume displaced by the hind paw was evaluated in all 5 groups using a digital plethysmometer every 60 minutes for 5 hours. The results were obtained from the displaced volume (Mean ± SD), with the most representative values obtained at 240 minutes, where EDM at 250 mg/kg (0.57 ± 0.07 ml) bw and 500 mg/kg bw (0.578 ± 0.051 ml) showed significant anti-inflammatory activity (ANOVA $p<0.05$). We concluded that *Desmodium Molliculum* has anti-inflammatory activity at doses of 250 mg/kg bw and 500 mg/kg bw.

Key words: Acute inflammation, Carrageenan, *Desmodium*, Dog's Paw, Edema Subplantar, Fabaceae.

INTRODUCTION

Peruvian traditional medicine has used plant resources from pre-Columbian cultures to the present day, and the Desmodium genus is no exception.[1] It has been used in traditional medicine for its diverse phytotherapeutic benefits,[2] such as its antirheumatic,[3] balsamic,[4] diaphoretic,[5] asthmatic crisis treatment,[6] menstrual pain relief[7] and its anti-inflammatory properties.[5,6] *Desmodium molliculum* (Kunth) DC, also known as "dog's paw" or "ma nayupa", is a perennial herbaceous species belonging to the Fabaceae family, native to Peru and Latin America, which grows predominantly in the Andean region and reaches a height of approximately 50 cm.[8,9] It has been traditionally used as an anti-inflammatory and analgesic.[10] It is known that this plant has several active compounds such as: tryptamine,[10] steroids,[11] flavonoids,[2] isoflavonoids,[10] saponins,[12] triterpenoids,[10,11] anthocyanins, polyphenols,[12] tannins, terpenes,[11,13] unsaturated fatty acids[13] and traces of alkaloids that are probably responsible for its therapeutic action.[11,14] Inflammation is described as a natural physiological process of defense of the body against aggressions from the external environment, which results in a series of signs and symptoms, such as flushing (redness), heat, edema, and pain (known as the 4 cardinal points of inflammation), and sometimes, the loss of functionality.[15]

In acute inflammation, there is an immediate reaction of the body against the pathogen, where cells of the immune system such as phagocytes try to destroy it by secreting mediator substances on endothelial cells, which increases vascular permeability so that leukocytes migrate to the pathogen. inflammatory focus and subsequently engulf the pathogen.[16] The most common causes of inflammation are infections or medical conditions (for example, dermatitis), injuries (for example, cuts), and autoimmune diseases (for example, psoriasis).[17] The pharmacological group of non-steroidal anti-inflammatory drugs (NSAIDs) is used as first-line therapy for pain and inflammation. Its mechanism of action is based on the inhibition of cyclooxygenase (COX) 1 and 2; these are the enzymes responsible for producing various types of prostaglandins (PGs), which contribute to pain signaling and inflammation.[18] However, the adverse effects of these drugs make it necessary to search for new options in the treatment of pain and inflammation.[19] The present research aims to investigate the anti-inflammatory properties attributed to *Desmodium molliculum* leaves in a model of acute inflammation induced with carrageenan.

MATERIALS AND METHODS

Collection of plant material

The sample consisted of packets of *Desmodium molliculum* plant leaves, which were packaged by Natura Express® (RSDS 004-2000-SA) and obtained from health food stores in Trujillo, Peru in May 2022.

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Preparation of extracts

The leaves selected were washed under tap water followed by washing with distilled water to remove the surface debris. The plant sample (350 g) was macerated in 4.2 liters of 70% v/v ethanol for 5 days. After maceration, the extract was filtered and dried under reduced pressure using a rotary evaporator. The resulting dried extract was stored in a light-protected flask at -4°C until needed and was reconstituted in physiological saline solution (0.9% sodium chloride) prior to oral administration.

Phytochemical screening

The phytochemical screening was performed using the Lock method.[20] Ten milliliter aliquots of the extract were taken and placed in three capsules until the solvent evaporated. The extract was then replaced with solvents of increasing polarity, including dichloromethane, ethanol, and water. Identification was determined by staining and/or precipitation reactions. Steroids and/or triterpenes, flavonoids, phenols, cardiotonic glycosides, alkaloids, saponins, and tannins were identified.

Drug and chemical used

Diclofenac sodium 25 mg/ml (Genfar ®); λ- Carrageenan (Sigma Aldrich) were found in May 2022.

Experimental animals

In this study, 25 healthy adult male Sprague Dawley rats, weighing 200-250 g and obtained from the National Institute of Health (INS) in Lima, Peru, were used for *in vivo* evaluation. The animals were maintained under standard conditions of 12 hours of light and 12 hours of darkness at an ambient temperature of 22 ± 2°C, with 65 ± 5% humidity. The rats were fed standard laboratory chow and given access to tap water ad libitum before the experiment. Animals are weighed, randomized into groups (n=5), and kept for one week to acclimatize to the laboratory conditions. The study was approved by the ethics committee of the School of Medicine of the Universidad Privada Antenor Orrego, Peru.

Antiinflammatory activity: carrageenan-induced paw oedema

The method used was like that described by Winter and Morris.[21,22] A total of 25 rats were divided into five groups of five animals each. Group I (Vehicle control) was treated orally with a physiological saline solution (0.9% sodium chloride) without inducing inflammation with carrageenan. Group II (Carrageenan control) served as a control and received a suspension of 0.1% carrageenan in physiological saline. Group III (Standard Drug) was treated intraperitoneally with diclofenac sodium at a dose of 25 mg/kg body weight as a standard drug. Groups IV and V (Experimental Groups) received hydroalcoholic extracts of *Desmodium Molliculum* leaves at doses of 250 mg and 500 mg/kg body weight, respectively. Acute edema was induced in the left hind paw of the rats by sub-plantar injection of 0.1 ml of freshly prepared (1% w/v) carrageenan suspension in physiological saline 30 minutes after drug or extract administration. The paw volume was measured at 0, 60, 120, 180, 240, and 300 minutes after the carrageenan injection using a digital plethysmometer (Ugo Basile® M-37140), and the mean volume displaced in the paw was calculated. The percentage inhibition of paw edema was calculated as:

Percentage inhibition of paw oedema = (1–Vt/Vc) × 100

Where Vc represents the increase in paw volume (mean swelling) of the control group of rats at a given time, and Vt represents the swelling of rats treated with *Desmodium Molliculum* leaf extracts at the same time.

RESULTS AND DISCUSSION

Desmodium molliculum (Synonyms: *Desmodium mexicanum*, *Heteroloma lanatum*, *Hedysarum molliculum*, *Meibomia mollicula*) commonly known as "dog's paw" or "manayupa" is a plant that grows between 500-3500m.s.n.m, is a creeping herb belonging to the order Fabales, family Fabaceae and genus Desmodium.[8,23] It is a plant native to the Peruvian Andes; however, it is distributed from Mexico to South America.[24] It grows in warm and temperate climates, with rainfall between 500 and 1000 mm, with temperatures between 12 °C and 30°C, and with atmospheric humidity between 70 and 90%.[8] The extracts used in the research of *Desmodium molliculum* are aqueous, methanolic and ethanolic, the results show that the most active extracts were the ethanolic ones, followed by the aqueous extracts obtained by decoction.[11,25]

Phytochemical screening

The results of the qualitative chemical analysis of EDM are shown in Table 1.

Phytochemical studies in different species of the genus Desmodium have characterized mainly metabolites such as flavonoids and alkaloids, followed by steroids, phenolic compounds, tannins and saponins.[26-28]

Preliminary qualitative phytochemical screening documented that *Desmodium molliculum* leaf extracts show the presence of several bioactive compounds, such as steroids, phenols, flavonoids, saponins, and tannins (Table 1). These compounds are believed to be responsible for the anti-inflammatory effect. Similar studies in *Desmodium molliculum* species identified the presence of tannins, triterpene steroids,[11] flavonoids (flavones, flavonols such as vitexin and isoflavones (5-o-methylgenistein and genistein), steroidal saponins;[26,29] phenolic compound, alkaloids, and carbohydrates.[25]

Anti-inflammatory activity has been previously described for the genus Desmodium using the carrageenan-induced paw edema model, the reduction of inflammation was dose-dependent, showing anti-inflammatory activity between 14.6% and 51.0% after 3 hours of carrageenan solution administration.[30] The acute inflammatory response measured in displaced volume according to the paw edema model of untreated animals and those treated with EDM, and diclofenac is shown in Table 2.

Carrageenan is a sulfated polysaccharide from marine algae (Rhodophyceae) commonly used as an acute inflammation inducer.[31] Several *in vitro* studies have revealed that CGN is able to induce inflammation, triggering innate immune pathways of inflammation, involving the canonical and noncanonical pathways of NF-kB activation with a central role in transcriptional activation of the IL8 gene.[32,33] Inflammation caused by carrageenan occurs in two phases: the

Table 1: The phytochemical profile of hydroalcoholic extract of *Desmodium molliculum* leaves (EDM).

Secondary Metabolites	Assays	Results
Esteroids or triterpenoids	Liebermann Burchard	(+)
Flavonoids	Shinoda	(+)
Phenols	Ferric Chloride	(+)
Saponins	Foaming	(+)
Tannins	Gelatin	(+)
Alkaloids	Dragendorff, Mayer and Bertrand	--
Cardiac Glycosides	Baljet	–

Note: Positive: (+) Negative: –
(Reproduction size at column width)

Table 2: Acute inflammatory response in experimental groups using the carrageenan-induced paw edema model.

Group	Displaced Volume (ml)/time (min)				
	Time 1 (basal)	Time 2 (60 min)	Time 3 (120 min)	Time 4 (180 min)	Time 5 (240 min)
G1 (Vehicle control)	0,672±0.06	0,574±0,068	0,57±0,045	0,548±0,073	0,544±0,065
G2 (Carrageenan)	0,866±0.03	1,79±0,025	1,848±0,028	1,964±0,086	1,806±0,053
G3 (Diclofenac)	0,822±0,024	1,180±0,025	1,302±0,026	1,022±0,038	0,69±0,045
G4 (D. *Molliculum* 250mg/kg bw)	0,850±0,027	1,368±0,031	1,508±0,018	1,338±0,040	1,12±0,035
G5 (D. *Molliculum* 500mg/kg bw)	0,796±0,022	1,266±0,034	1,492±0,033	1,3±0,067	1,002±0,063

Note: ANOVA test; Post-HOC Tuckey: (p-value ≤ 0.05; (p-value ≤ 0.01)
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Table 3: Percentage inhibition (%) of carrageenan-induced paw edema.

Group	Displaced Volume (ml)/time (min)				
	Time 1 (*) (basal)	Time 2 (*) (60 min)	Time 3 (**) (120 min)	Time 4 (*) (180 min)	Time 5 (*) (240 min)
G3 (Diclofenac)	5,079±0,596	34,071±1,543	29,535±1,634	47,948±0,776	63,105±1,303
G4 (*D. Molliculum* 250mg/kg bw)	1,854±0,651	23,581±0,872	18,387±1,316	31,832±1,529	37,938±2,729
G5 (*D. Molliculum* 500mg/kg bw)	1,854±0,651	29,278±1,333	20,226±1,315	34,116±1,685	44,561±1,874

Note: ANOVA test; Post-HOC Tuckey: (*p-value ≤ 0.01; **p-value ≤ 0.05)

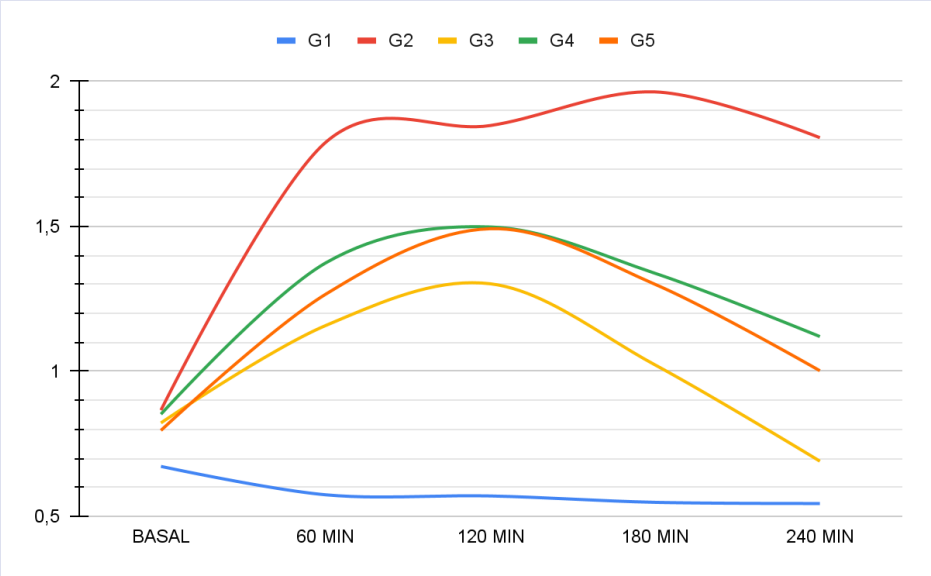

Figure 1: Measurement of volume displacement (mL) over time (minutes) in the carrageenan-induced paw model

Note: G1 (Vehicle control); G2 (Carrageenan), G3 (Diclofenac); G4 (D. *Molliculum* 250mg/kg bw); G4 (D. *Molliculum* 250mg/kg bw); G5 (D. *Molliculum* 500mg/kg bw).

first phase is mediated by the release of histamine and serotonin, while the second phase is triggered by the release of proteases, bradykinins, lysosomes and prostaglandins.[35] Some studies have reported that in the second phase of edema the anti-inflammatory response is more effective.[34,36]

The results of the paw volume measured before the carrageenan administration and at the basal, 1st, 2nd, 3rd, and 4th time of the administration, are shown in Figure 1. According to the results, it was seen that both doses of *D. molliculum* administration significantly reduced edema formation compared to the Carrageenan group ($p < 0.01$).

The percentage inhibition of inflammation (anti-inflammatory activity) obtained in the treated and untreated groups are shown in Table 3. It can be observed that at time 03 (120 minutes), the anti-inflammatory activity of the standard drug and the extracts shows values with a lower difference ($p \leq 0.05$).

This is associated with the beginning of the second phase of inflammation induced by carrageenan. Subsequently, the anti-inflammatory percentages between the groups show greater differences. However, the activity on inflammation of *D. molliculum* extracts continues to increase until time 5 (240 minutes).

CONCLUSION

Phytochemical screening of the hydroalcoholic extract of *D. molliculum* leaves detected steroids, flavonoids, phenolic compounds, saponins, and tannins. The volumes displaced by the paw edema model of the *D.*

molliculum 500mg/kg bw group at time 04 (180 minutes) and 05 (240 minutes) were 1.3±0.067 ml and 1.002±0.063ml, respectively, being the most representative of the anti-inflammatory activity in this plant. According to these results, the hydroalcoholic extract of *D. molliculum* leaves can be used as a potential source of new anti-inflammatory drugs.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest in this study.

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