

**ATIVIDADES EDEMATOGÊNICA E ANTINOCICEPTIVA DA LECTINA ISOLADA DA FRAÇÃO ALBUMINA DE *Acacia farnesiana* (EDEMATOGENIC AND ANTINOCICEPTIVE ACTIVITIES OF THE LECTIN ISOLATED FROM THE ALBUMIN FRACTION OF *Acacia farnesiana*)**

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**ABSTRACT**

This study aims to evaluate the activity of AFAL in the models of paw edema and chemical nociception. AFAL was injected s.c. or i.v before s.c. injection of carrageenan or dextran in rats to evaluate its pro- or anti-edematogenic effect. The antinociceptive activity was investigated by AFAL i.v. administration in mice in the writhing and formalin tests. Results were expressed as Mean  $\pm$  SEM and considered significant for  $p < 0.05$  (ANOVA). AFAL (0.1 and 1 mg/kg; s.c.) induced paw edema in 30 min, remained constant until the 3<sup>rd</sup> h, increasing paw volume by 2.9x and 3.4x, respectively, compared to saline. However, the lectin did not change the edema induced by carrageenan, dextran or formalin. AFAL reduced the number of writhes by 53% (0.1 mg/kg) 60% (1 mg/kg) and 74% (10 mg/kg), but had no effect at any phases of the formalin test. In conclusion, AFAL presents pro-inflammatory and antinociceptive activity.

**KEYWORDS:** *Acacia farnesiana*, lectin, inflammation, nociception

**INTRODUCTION**

Leguminous lectins are proteins that recognize and reversibly bind to carbohydrates. Studies had demonstrated their pro-inflammatory (Alencar et al., 2003; Assreuy et al., 2009) or anti-inflammatory (Assreuy et al., 1997; Alencar et al., 1999) activities, depending on the route of administration employed. The antinociceptive activity was also described (Napimoga et al., 2007; Pinto et al., 2013).

*Acacia farnesiana* lectin (AFAL) is a chitin-binding protein that possesses bacteriostatic effect (Santi-Gadelha et al., 2008; Santi-Gadelha, 2012) and inhibits neutrophil migration in the peritonitis model (Abrantes et al., 2013). This study aims to evaluate the activity of AFAL in the models of paw edema and chemical nociception.

**METHODS**

**Lectin and Animals**

The lectin was isolated from the albumin fraction of *Acacia farnesiana* (voucher at Herbarium Prisco Bezerra, UFC - EAC 20273) by ion-exchange (DEAE-Sephacel) and chitin affinity

chromatography (Santi-Gadelha et al., 2008).

Male Wistar rats (150-200g) and Swiss mice (25-30 g) were used in protocols approved by the Animal Care and Use Committee of UECE (N° 10130208-8/40).

**Experimental Protocols**

Rat paw edema was measured by hydroplethysmometry before (zero time) and after (0.5, 1, 2, 3, 4 h) subcutaneous (s.c.) intraplantar injection of inflammatory stimuli. Results were expressed as the variation in paw volume (ml) and area under the time-course curve (AUC) (Landucci et al., 1995). The pro-inflammatory activity was assessed via s.c. injection of AFAL (0.01 - 1 mg/kg) as stimuli. The anti-inflammatory activity was assessed via intravenous (i.v.) injection of AFAL (1 mg/kg) 30 min before stimuli (carrageenan or dextran - 300  $\mu$ g/paw). Control groups received saline (s.c. or i.v.).

Chemical nociception was evaluated in the Writhing and Formalin tests in mice. Acetic acid 0.8% (v/v; 0.1 mL/10 g body weight) was injected intraperitoneally (i.p.) and the number of abdominal writhes was counted from 10 to 30 min post-injection (Koster et al., 1959). Formalin (20  $\mu$ l; 2.5% v/v) was injected intraplantar and the time that animals spent licking its paws was recorded at first (0-5 min) and second (15-30 min) phases (Hunskar et al., 1987). After 4 h animals were sacrificed and the paws were cut and weighed.

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AFAL (0.1 - 10 mg/Kg; i.v.), indomethacin (5 mg/kg; i.p.), morphine (5 mg/kg; s.c.) or saline (i.v.) was injected 30 min before tests.

Results are presented as mean  $\pm$  S.E.M. and analyzed by One-way ANOVA, followed by Bonferroni test. Values of  $p < 0.05$  were considered significant.

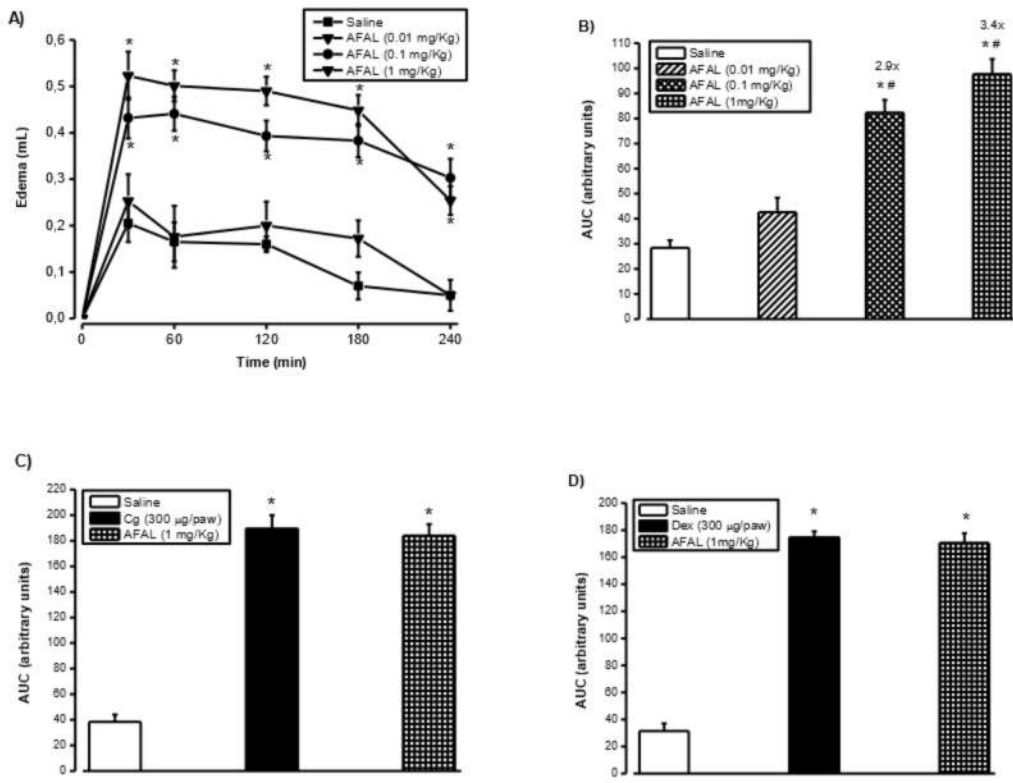
## RESULTS AND DISCUSSION

AFAL (0.1 and 1 mg/Kg) induced paw edema, that was initiated at 30 min, remained constant until the 3<sup>rd</sup> h and decreased at the 4<sup>th</sup> h, characterizing an acute inflammation (Fig. 1A). The increase in paw volume by AFAL at 0.1 mg/kg was by 2.9x ( $81.92 \pm 4.93$  AUC) and at 1 mg/kg by 3.4x ( $97.41 \pm 6.87$  AUC), compared to saline ( $28.71 \pm 3.63$  AUC) (Fig. 1B). In this line studies had demonstrated the capacity of plant lectins, by local administration, to activate cells of the immune system by different mechanisms (Assreuy et al., 2009; Silva et al., 2011). However, AFAL injected i.v. did not alter the edema induced by carrageenan ( $189.61 \pm 10.8$  vs. AFAL:  $184.49 \pm 5.74$  AUC) or dextran ( $169.79 \pm 8.59$  vs. AFAL:  $174.45 \pm 3.22$  AUC) (Fig. 1C, D). This data is apparently in conflict with the demonstration of the AFAL inhibitory effect in the neutrophil migration elicited by carrageenan in the peritonitis model (Abrantes et al., 2013). It is important to highlight that the paw edema model allows mainly the evaluation of vascular events (vasodilatation and permeability), while the peritonitis model is more appropriate to access cellular events of inflammation (DiRosa, 1971).

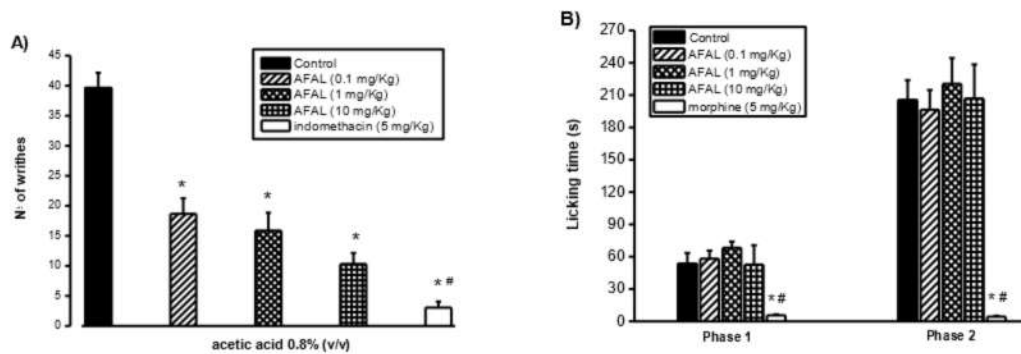
The number of writhes elicited by acetic acid ( $39.62 \pm 2.51$ ) was reduced by AFAL at: 0.1 mg/kg ( $18.67 \pm 2.57$ ) by 53%; 1 mg/kg ( $15.85 \pm 3.03$ ) by 60% and 10 mg/kg ( $10.29 \pm 1.86$ ) by 74% (Fig. 2A), accordingly to other leguminous lectins (Pinto et al., 2013). The writhing test has been useful to evaluate antinociceptive natural products (Labuz et al., 2007), since the acetic acid intraperitoneal irritation triggers central and peripheral inflammatory components of pain (Ribeiro et al., 2000; Ikeda et al., 2001). This result is in accordance with the inhibition of peritonitis by AFAL (Abrantes et al., 2013). On the other hand, the licking time evaluated in the formalin test was unaltered by AFAL (Fig 2B). It could be possible that in the protocols used in the present investigation AFAL did not modify the functional changes in neurons in peripheral (first phase - neurogenic) or central (second phase - inflammatory) nervous system induced by formalin. In addition the paw edema induced by formalin ( $0.14 \pm 0.016$ g) was not reduced by AFAL at 0.1 mg/kg ( $0.13 \pm 0.014$ g), 1 mg/kg ( $0.14 \pm 0.01$ g) or 10 mg/kg ( $0.12 \pm 0.01$ g) corroborating the lack of activity in the model of paw edema.

## CONCLUSIONS

In summary, the present data demonstrate that AFAL has pro-inflammatory and antinociceptive activities, being useful as a tool for better understanding the underlying mechanisms of pain and inflammation.



**FIGURE 1.** AFAL presents edematogenic effect but does not inhibit the edema elicited by carrageenan or dextran. AFAL was injected s.c. (A and B); or i.v. before s.c. injection of carrageenan (Cg) (C) or dextran (Dex) (D). Mean  $\pm$  S.E.M. (n=6). \*p<0.05 compared to saline; # p<0.05 compared to AFAL (0.01mg/kg).



**FIGURE 2.** AFAL antinociceptive effect. AFAL was injected i.v. 30 min before acetic acid (0.6%; i.p.) (A) or formalin (2,5%; s.c.) (B). N° of writhes after acetic acid (10 - 30 min) and licking time (s) after formalin in phase 1 (0-5 min) and phase 2 (15-30 min) were recorded. Mean  $\pm$  S.E.M. (n=8). \*p<0.05 compared to control; #p<0.05 compared to AFAL.

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