

The Anti-Inflammatory Effect of *Andira Anthelmia* Lectin In Rats Involves Inhibition of The Prostanoid Pathway, TNF- α And Lectin Domain

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Abstract

Objective: To investigate the effect and mechanisms of *Andira anthelmia* lectin in rat models of acute inflammation.

Material: AAL anti-inflammatory activity was evaluated in Wistar rat models of paw edema, peritonitis and hyperalgesia.

Methods: AAL (0.01-1 mg/kg i.v.) was injected 30 min before stimulation with carrageenan and with initial and late phase inflammatory mediators into the animals paw and peritoneum for evaluation of: cell migration (optical and intravital microscopy), paw edema (plethysmometry and histopathology); hyperalgesia (analgesimetry).

Results: AAL inhibited leukocyte migration induced by carrageenan, mainly neutrophils to the peritoneal fluid, decreasing leukocyte adhesion. In the peritoneal fluid, AAL reduced the gene expression of TNF- α and cyclooxygenase, as well the levels of PGE₂. ALL also inhibited the paw edema induced by carrageenan, mainly the late phase, but also the edema induced by serotonin, histamine, TNF- α , PLA₂ and PGE₂, but not by L-arginine and bradykinin. In this model, AAL inhibited mechanical hypernociception induced by TNF- α , PGE₂, db-cAMP and capsaicin, and the activity of myeloperoxidase in the paw tissues.

Conclusion: AAL presents anti-inflammatory effect in acute models of rat inflammation involving the participation of prostaglandins, TNF- α and lectin domain.

Introduction

The leukocyte adhesion to activated endothelial cells is a sequential, multistep process consisting of tethering, rolling, firm adhesion and transmigration [1] that involves a series of activation mediated by inflammatory agents, such as histamine, cytokines, chemokines and prostaglandins [2, 3].

Recognition between proteins and carbohydrates is critical in many biological processes, such as viral, bacterial and parasitic infections, cell differentiation, cancer and leukocyte adhesion to the vascular endothelium [4]. In this context, there is a growing number of papers using lectins as tools for studying biological processes since lectins are (glyco) proteins capable recognition and binding to carbohydrates in a reversible and highly specific way [5].

Plant lectins are considered a heterogeneous group of proteins regarding its biochemical/physicochemical properties, molecular structure, biding specificity for carbohydrates and biological activities. Studies involving lectins belonging to the Dalbergieae tribe are scarce, being currently approximately 26 purified lectins [6].

The interest in studying the effects of plant lectins has increased because glycoconjugate interactions have been implied in inflammation pathways. Therefore, these proteins are structurally correlated and

differ in the ability of binding to carbohydrates are considered potential tools for application in biotechnology or immunotherapies [7].

Lectins isolated from seeds of leguminous plants belonging to Dalbergieae tribe, genus *Lonchocarpus* (*L. sericeus*, *L. arariensis*, *L. campestris*) have been described to inflammatory processes in animal models via interaction with carbohydrates [6].

The lectin of *Andira anthelmia* (AAL), a native plant of the Brazilian Amazon (Dalbergieae tribe), was isolated by affinity chromatography (sepharose-mannose) and partially characterized. AAL presents glycosylation and double band (molecular weight with approximately 20 kDa and other minor bands of 17, 15, and 13 kDa), possessing molecular weight approximately of 20 kDa and other three minor fragments of 17, 15, and 13 kDa. The AAL hemagglutinating activity was inhibited by D-mannose and D-glucose and was shown to be dependent on divalent cations. AAL presents antinociceptive effect in behavioral tests (writhing and formalin), involving the lectin domain and peripheral mechanisms of inflammatory nociception [8]. However, up to date, there is no report in the scientific literature showing the anti-inflammatory effect of a lectin mannose/glucose-binding belonging to Dalbergieae tribe.

This study aimed to investigate the underlying mechanism of the anti-inflammatory effect of *Andira anthelmia* lectin in rat models of acute inflammation.

Materials And Methods

Drugs and reagents

Ketamine and xylazine were supplied by König S/A (Buenos Aires, province of Buenos Aires, Argentina). α -D-mannose, Carrageenan λ , histamine, serotonin (5-hydroxytryptamine), L-arginine, 1H-[1, 2, 4]oxadiazolo[4,3-a]-quinoxalin-1-one (ODQ), N-nitro-L-arginine methyl ester (L-NAME), hexadecyltrimethylammonium bromide, tetramethylbenzidine (TMB), hydrogen peroxide, phospholipase A₂ (PLA₂), prostaglandin E₂ (PGE₂), tumor necrosis factor alpha (TNF- α), bradykinin, N₆,2'-O-Dibutyryl adenosine 3',5'-cyclic monophosphate sodium salt (db-cAMP) and dexamethasone were obtained from Sigma Chemical Company (St. Louis, Missouri, USA). Brazol kit from LGC Biotecnologia (Cotia - SP, Brazil); DNase and reverse transcriptase SuperScriptTM IV First-Strand Synthesis System from Invitrogen[®] (Carlsbad - California, USA); GelRed from Biotium (Fremont - California, USA); primers Oligo (dT) and dNTP from Thermo Fisher (Waltham - Massachusetts, USA); GoTaq[®] qPCR Master Mix kit from Promega (Madsison - Wisconsin, USA) and primers for β -actin, COX-1, COX-2 and TNF- α from Integrated DNA Technology-IDT (Coralville - Iowa, USA). Kit ELISA PGE₂ was purchased from Abcam[®] (Cambridge, MA-EUA). All other chemicals were of analytical grade.

Lectin isolation and treatment

Andira anthelmia lectin (AAL) was isolated by sepharose–mannose affinity chromatography, presenting binding affinity for mannose and glucose [8]. Animals received AAL (0.01; 0.1; 1 mg/kg) or 0.9% NaCl

(sterile saline) by intravenous (i.v.) route 30 minutes before injection of the inflammatory stimuli and the anti-inflammatory effect of AAL evaluated in models of acute inflammation (peritonitis, paw edema, hypernociception).

Animals

Wistar rats (200–300 g) were maintained in cages (six in each) in a controlled environment (circadian cycle, 25° C, food and water ad libitum). Experimental protocols were previously approved by the Institutional Animal Care and Use Committee of the State University of Ceará (No. 2126961/2015) following the recommendations of the Brazilian College of Animal Experimentation (COBEA), in accordance to NIH guidelines (publication nº 85 – 23, revised 2011).

Peritonitis model

Peritonitis was induced by intraperitoneal (i.p.) injection of carrageenan (500 µg/cavity) 30 min after the treatment with AAL (0.01–1 mg/kg; i.v.). After 4 h, peritoneal fluid was collected in 5 ml of saline (5 IU heparin) for total and differential leukocyte counts [9], dosage of PGE₂ levels and analysis of the relative gene expression of cyclooxygenase enzymes and TNF-α. Leukocyte adhesion and rolling were also evaluated by intravital microscopy 4 h after peritonitis induction. For this, animals were anesthetized (xylazine 5 mg/kg + ketamine 50 mg/kg, i.m.) before exposition of the mesenteric bed for *in situ* microscopic examination. Leukocyte rolling was defined as white blood cells that move at a velocity significantly slower than erythrocytes in a given microvessel. Leukocytes were considered to be adherent to the venular endothelium if they remained stationary for more than 30 s. Rolling and adherent cells were counted for 10 min and expressed as the number of rolled leukocytes/10 min and adherent cells/10 min [10].

Paw edema model

Animals received AAL (1 mg/kg; i.v) 30 min before intraplantar (s.c.) administration of carrageenan (300 µg), serotonin (100 µg/paw), histamine (1 µg/paw), bradykinin (3 nmol/paw), L-arginine (15 nmol/paw), TNF-α (5 ng/paw), PLA₂ (30 µg/paw) or PGE₂ (30 µg/paw). Control animals received saline (s.c.) in the contralateral paw. The edema was measured by hydroplethysmometry (Panlab, LE 7500, Barcelona, Spain) before (zero time) and after (30 min – 4 h) inflammatory stimuli, being expressed as the difference of paw volume displacement (ml) or area under curve-AUC (arbitrary units) [11]. L-NAME (30 mg/kg; s.c.), ODQ (8 µg/kg; s.c.), thalidomide (45 mg/kg; s.c.), dexamethasone (0.5 mg/kg; s.c.) or indomethacin (5 mg/kg; s.c.) were used as reference drugs. Paw tissues were collected at the fourth hour after carrageenan administration for evaluation of myeloperoxidase (MPO) activity and histopathology. The MPO activity was determined by spectrometry (A_{450 nm}) and expressed as MPO U/mg tissue [12]. For histopathology, longitudinal paw segments were fixed overnight in 10% buffered formalin, embedded in paraffin, sliced (5 µm) and stained by HE. Slices were observed by light microscopy coupled to image acquisition systems. The intensity of tissue inflammatory alterations was evaluated according to the following scores: (0) normal tissue - absence of edema and inflammatory cell infiltrate; (1) discrete tissue

changes - mild edema and cellular infiltration; (2) moderate tissue alteration - significant edema and cellular infiltration; (3) intense tissue alteration - wide area of edema and cellular infiltration [13].

Hypernociception model

Animals were individually placed into plexiglas boxes with malleable mesh net floor. A large probe coupled to electronic algometer was used to apply a force on the footpad center in order to cause paw withdrawal reflex from mechanical stimulation. The reduced intensity force required to evoke paw withdrawal is indicative of hypernociception [14]. The paw withdrawal was quantified (g) before (basal value) and up to 4 h thereafter stimuli. The antinociceptive effect of AAL (1 mg/kg i.v.) was evaluated 30 min before s.c. administration of TNF- α (5 ng/paw), PGE₂ (30 μ g/paw), db-cAMP (100 μ g/paw) or capsaicin (1 μ g/paw). Indomethacin (5 mg/kg, s.c., 30 min prior PGE₂) or saline 0.9% were administered as controls.

Measurement of inflammatory mediators: Gene Expression and ELISA

Gene sequences from *Rattus norvegicus* were obtained from GeneBank database. Genes specific primer for COX-1, COX-2 and TNF- α reference gene primer for β -actin gene were obtained using Primer3 (<http://primer3.ut.ee>). All primers were synthesized by IDT with 25 nt length. From resident cells of peritoneal fluid total RNA was extracted using Brazol kit and treated with DNase (Invitrogen). Total RNA was resuspended in RNase-free water and its concentration determined by spectrophotometry (NanoVue) at 260 nm. RNA purity was checked at optical density ratio (OD_{260nm}/OD_{280nm}) between 1.8 and 2.0. RNA quality and integrity were analyzed in 1.2% agarose gel staining with GelRed. The cDNA was synthesized using reverse transcriptase SuperScript™ IV First-Strand Synthesis System (Invitrogen), 1000 ng of total RNA was used and the reaction was performed according to manufacturer's recommendations. At the final, the cDNA concentration was determined by spectrophotometry at 260 nm. qRT-PCR was performed using thermocycler Bioer LineGene 9660. Genes amplification were performed in a reaction including 0.3 μ M of each primer (forward and reverse), 2 μ L cDNA and 10 μ L of GoTaq® qPCR Master Mix kit (Promega) in a total volume reaction of 20 μ L. A initial cycle was performed at 95°C for 10 min, followed by 40 cycles at 95°C for 15 s, and 60°C for 15 s and 60°C for 30 s. Melting curve analysis was performed to evaluated primer dimers and other artifacts. The gene expression was determined using the $2^{-\Delta\Delta CT}$ method [15]. Additionally, the peritoneal fluid was centrifuged (3000 g $\times$ 15 min), and the obtained supernatant was assessed for the content of PGE₂ (pg/ml) by enzyme-linked immunosorbent assay (A_{405nm}).

Statistical Analysis

Statistical differences were determined by analysis of variance (one-way or two-way ANOVA), followed by Bonferroni's test. The results were expressed as mean $\pm$ SEM (n = 8 or n = 3/group for gene expression). Histopathological data was expressed as Median (maximum and minimum) and analyzed by the Mann-Whitney test. p < 0.05 was considered significant.

Results

AAL inhibits leukocyte migration induced by carrageenan

In the peritonitis model, AAL inhibited the number of leukocyte migration induced by carrageenan (5908 ± 490) by 32% (0.01: 3960 ± 353), 48% (0.1: 3460 ± 309) and 78% (1 mg/kg: 2291 ± 241 cells/ml). This inhibition was mainly due to the decrease in neutrophils by 31% (0.01: 2563 ± 240), 47% (0.1: 1967 ± 172) and 78% (1 mg/kg: 803 ± 147) (carrageenan: 3749 ± 374 cells/ml) (Fig. 1a). The inhibitory effect of AAL (1 mg/kg) on leukocyte migration (2825 ± 229) was completely blocked by its association with mannose (5643 ± 742 cells/ml). Mannose *per se* did not induce any effect (6513 ± 243 cells/ml) (Fig. 2b). The intravital microscopy revealed that carrageenan increased the endothelial leukocyte rolling (403.0 ± 13.3) and adhesion (15.1 ± 1.8) compared to saline (107.8 ± 12.42 rolling cells/10 min; 6.8 ± 0.8 adhesion cells/10 min). AAL significantly decreased leukocyte rolling by 37% (259.8 ± 22.7) and adhesion by 50% (7.5 ± 0.6 cells/10 min) (Fig. 1c, d).

AAL inhibits inflammatory parameters induced by carrageenan in the paw edema model

In the paw edema model, AAL at 1 mg/Kg inhibited the paw edema time-course induced by carrageenan from 0–4 h (Fig. 2a). The inhibitory effect was observed in the edema initial phase (0–2 h) by 26% (47 ± 3.7 ASC) compared to carrageenan (6.7 ± 1.8 vs. saline: 18 ± 1.7 AUC) (Fig. 2b), but mainly in the late phase (2–4 h) by 35% (62.6 ± 8.5 AUC) compared to carrageenan (96.2 ± 8.5 vs. saline: 6.3 ± 2 AUC) (Fig. 2c). AAL also reduced the total protein levels by 37% (0.72 ± 0.14 mg/ml vs. carrageenan: 1.15 ± 0.13 vs. saline: 0.59 ± 0.08 mg/ml) (Fig. 2d) and the myeloperoxidase activity by 62% (0.11 ± 0.01 U/mg vs. carrageenan: 0.29 ± 0.02 vs. saline: 0.13 ± 0.01 U/mg) (Fig. 2e).

In the histopathological evaluation, carrageenan induced severe alterations (leucocyte infiltration, edema) in the animal paw-tissues [median score: 3 (+ 3; +2)] (Fig. 3b) compared to saline [score: 0 (+ 1; 0)] (Fig. 3a). AAL treatment reduced these alterations [score: 0 (+ 1; 0)] (Fig. 3c).

AAL inhibits paw edema induced by initial and late-phase inflammatory mediators

In the paw edema model, AAL (1 mg/kg) inhibited by 40% (105.5 ± 25.6 AUC) the paw edema induced by serotonin (177.1 ± 18.3 vs. saline: 45.4 ± 9.1 AUC) and by 32% (93.2 ± 7) that of histamine (137 ± 8.4 vs. saline: 29 ± 1.7 AUC) (Fig. 4a). However, the edema elicited by bradykinin (166.5 ± 19.7 vs. AAL: 136 ± 10.3 vs. saline: 89.4 ± 16.5 AUC) and L-arginine (71 ± 7.8 AUC) was unaltered by AAL (66 ± 8.6 vs. L-NAME: 43.5 ± 4.3 AUC) (Fig. 4b). Animals that received ODQ associated with AAL (98.2 ± 13.3) did not show different responses from those that received both isolated drugs (ODQ: 103.5 ± 10.7 vs. AAL: 93.1 ± 16) compared to carrageenan (150.2 ± 6.7 AUC) (Fig. 4b). Moreover, the edema induced by TNF- α (90.5 ± 10.6), PLA₂ (117.5 ± 4.5) and PGE₂ (102.9 ± 14.9) were notably reduced by AAL by 73 % (24 ± 4), 77% (26 ± 5) and 38% (64.5 ± 5.1 AUC), respectively (Fig. 4c, d). Similar effects were observed the classical

inhibitors of TNF- α (thalidomide: 41 ± 1.5), PLA₂ (dexamethasone: 51 ± 4.8 AUC) and PGE₂ (indomethacin: 54.5 ± 3.5 AUC).

AAL inhibits hypernociception elicited by mediators involved in the TNF- α and prostaglandins pathway

In respect to mechanical hypernociception, TNF, PGE₂, db-cAMP and capsaicin increased animals paw withdrawal in response to mechanical stimulation with electronic analgesometer. At the first hour, AAL (1 mg/kg) recovered (32.9 ± 3.1 vs. saline: 35.1 ± 2) the response elicited by TNF- α (22.2 ± 0.7 g). At the third hour, AAL abolished (31.9 ± 0.9 vs. saline: 37.6 ± 1.8) the hypernociceptive response induced by TNF- α (23.1 ± 3.1 g) (Fig. 5a). The hypernociceptive effect of PGE₂ (41.3 ± 1.7 vs. AAL: 54 ± 1.3 vs. indomethacin: 54 ± 3.9) and db-cAMP (17.8 ± 1.7 vs. AAL: 30.2 ± 1.4 vs. saline: 31.2 ± 0.8 g) was also fully recovered by AAL at the first and third hour, respectively (Fig. 5b, c). AAL also inhibited the hypernociceptive response (50.5 ± 1.9) induced by capsaicin (38.1 ± 3 vs. saline: 57.6 ± 4.6) at the second hour (Fig. 5d).

AAL alters the gene expression of cyclooxygenases and TNF- α ; and the levels of PGE₂

In the qRT-PCR revealed in the carrageenan group significant increase in gene relative expression for COX-1 (3.1 ± 0.01 vs. saline: 1), COX-2 (1 vs. saline 0) and TNF- α (1 vs. saline: 0). AAL reduced the relative expression in 16.3x of COX-1 (0.19 ± 0.01) (Fig. 6a), 13.8x of COX-2 (0.07 ± 0.01) (Fig. 6b) and 3.2x in TNF- α (0.31 ± 0.03) (Fig. 6c). Carrageenan increased the PGE₂ levels in peritoneal fluid (0.23 ± 0.04) and AAL (0.06 ± 0.007 pg/ml) reduced by 73% (Fig. 6d). This effect was completely blocked by the association of lectin with mannose (0.18 ± 0.02 pg/ml).

Discussion

This study demonstrated the modulator effect of the purified *Andira anthelmia* lectin (AAL) on vascular and cellular events of acute inflammation in rats, that was shown to be dependent on the lectin domain. These results corroborate the AAL antinociceptive effect previously in the inflammatory phase of the formalin test [8].

Pre-treatment with AAL showed high efficacy to reduced leukocyte migration induced by carrageenan, a flogistic agent that stimulates resident macrophages to release primary cytokines, such as TNF- α , a powerful chemotactic agent to neutrophils [16]. The differential leukocyte count showed that neutrophils are the principal target cells involved in the anti-inflammatory effect of AAL. Similar results have been observed in rats for the lectins isolated (Dalbergieae tribe) from *Lonchocarpus sericeus* [17, 18] and *L. araripensis* [19], presenting binding affinity for N-acetylglucosamine. In addition, the interference of AAL in neutrophil migration was also confirmed by the reduced MPO activity in the paw, a heme-containing

enzyme that is abundantly expressed in neutrophils. Excessive generation of MPO-derived oxidants has been associated to tissue damage in acute and chronic inflammation [20].

AAL is a pioneer lectin isolated from plants of the Dalbergieae tribe presenting affinity for mannose/glucose, to inhibit leukocyte migration. Intravital microscopy showed the reduction in of leukocyte rolling and adhesion to the endothelium of mesenteric vessels after AAL treatment. Mannose associated to AAL was capable to block the reduction elicited by AAL on leukocyte migration, probably by competitive interactions on selectins–sugar binding sites of endothelial and leukocyte cell membranes, as suggested before [21, 22]. In general, lectins that interact to mannose and glucose residues present anti-inflammatory effect by systemic administration (Assreuy et al., 1997; Pinto et al., 2013) and inflammatory effect by local administration [23, 24].

The edema induced by carrageenan in rodents is biphasic characterized by intense leukocyte influx involving a complex network of inflammatory mediators [25]. The initial phase (0–2 h) is predominantly vascular, triggered by the release of biogenic amines (histamine, bradykinin and serotonin) [26], whereas the second phase (2–4 h) is maintained particularly by neutrophil infiltration with production of prostaglandins, nitric oxide and cytokines [27–28, 25]. AAL inhibited the time-course of the paw edema induced by carrageenan, showing maximal effect in the cell infiltrate phase. Accordingly, the anti-inflammatory effect of leguminous lectins is characterized by the preferential inhibition on cellular events of acute inflammation [21, 29, 30]. Other isolated lectins belonging to the Dalbergieae tribe also showed inhibitory effect on carrageenan-induced paw edema such as that of *L. sericeus* [17–18], *L. araripensis* [30] and *L. campestris* [19].

The cellular nature of AAL anti-inflammatory effect was confirmed either by the paw histopathological analysis and by the evaluation of the edema induced by late phase mediators of carrageenan edema (phospholipase A2, prostaglandin E2, TNF- α). However, we cannot exclude the vascular effect of AAL, since our data also showed the AAL inhibitory response on paw edema elicited by histamine and serotonin, but not by L-arginine, excluding the involvement of the oxide nitric pathway. Histamine and serotonin (5-HT) are mediators released by inflammatory cells that mediate the vascular events of acute inflammation induced by carrageenan in rats [31]. Thus, our data suggest that the anti-edematogenic effect of AAL has the participation of biogenic amines, cytokines (TNF- α) and prostaglandins.

Moreover, AAL inhibited the inflammatory process and hypernociception induced by TNF- α and PGE₂. The enhanced sensitivity by nociceptive stimuli is one of the characteristics of the inflammatory response and results from an increased excitability of primary nociceptive neurons [32]. There is experimental evidence demonstrating that prostaglandins, among other mediators (sympathetic amines, endothelins, cytokines) can sensitize the primary nociceptive neurons to innocuous mechanical stimuli [33–34]. It has been demonstrated that the intraplantar administration of carrageenan in rats induce hypernociception mediated initially by TNF- α [35] and in our study, the hypernociception induced by intraplantar administration of TNF- α was inhibited AAL, suggesting a direct involvement of this pathway in the sensitization of nociceptors. It is well established that inflammatory hypernociception, such as that

induced by PGE₂ in EP receptors, depends on signalling pathways, requiring activation of PKA and PKC in neurons, in presence of cAMP [32, 36–38]. In the present study, AAL prevented hypernociception induced by the cAMP analog db-cAMP that elicits hypernociception via PGE₂ pathway [32] and also directly by PGE₂, suggesting that the lectin presents antinociceptive property via direct action on PGE₂ receptor or by intracellular mechanisms involving the pathway PGE₂/PKA/PKC.

PKA and PKC mediate hypernociception following activation of EP receptors (4 and 1) in peripheral nociceptors [32, 34]. Studies suggest that PGE₂ involves sensitization of the potential transient receptor vanilloid-1 (TRPV1) channels by PKA and PKC activation [34]. The sensitization of TRPV1 via PGE₂/EP1/PKC and PGE₂/EP4/PKA cascades is considered to be involved in inflammatory processes [39]. In rats, the local injection of capsaicin into the plantar hind paw produces thermal and mechanical hypernociception at the site of injection [40]. The capsaicin model is well understood in the literature due to the known molecular events (activation of TRPV1 receptors, cation entry, C-fibers depolarization) [41]. Thus, it is possible to be speculated that the TRPV1 channel is the target of the lectin antinociceptive effect since it inhibited nociception induced by PGE₂ and capsaicin.

Our data support the idea that AAL may inhibit several stages of the prostanoids formation, either directly on the enzyme cyclooxygenase or direct on PGE₂ receptors. This result allows us to speculate that AAL may be inhibiting other prostanoids that participate in acute inflammation such as PGD₂ and PGI₂. It is also possible that the lectin is modulating the expression of inflammatory pathways as COX and TNF-α via the nuclear factor kappa B (NF-κB). Other Dalbergieae lectin also show modulator effect on the gene expression of inflammatory pathways such as *Lonchocarpus araripensis* [42].

Plant lectins are also capable to interact with Toll-like receptors, responsible for activating the NF-κB pathway [43], which regulates the expression of several cytokines, such as TNF-α, IL-1, IL-6, IL-8, IL-12 and chemokines. These data allows us to open perspectives for even more to be discovered about the interactions of lectins with cellular structures involved in inflammation. It has been observed for lectins isolated from the Dalbergieae tribe (mannoside affinity) the ability to bind glycan structures involved in cell-cell recognition process by molecular docking [44–46]. Studies with a lectin-like domain of TNF-α have shown improve in lung function in transplanted rats, including the of reduction in reactive oxygen species [47]. Therefore, the presence of this domain in TNF-α opens the possibility that AAL can occupy the carbohydrate site preventing the binding of TNF-α by competitive mechanisms.

In conclusion, *Andira anthelmia* lectin demonstrates anti-inflammatory effect, via lectin domain, with the participation of TNF-α and PGE₂/cAMP/TRVP1 pathway.

Declarations

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Conflict of interest The authors declare no conflicts of interest.

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Figures

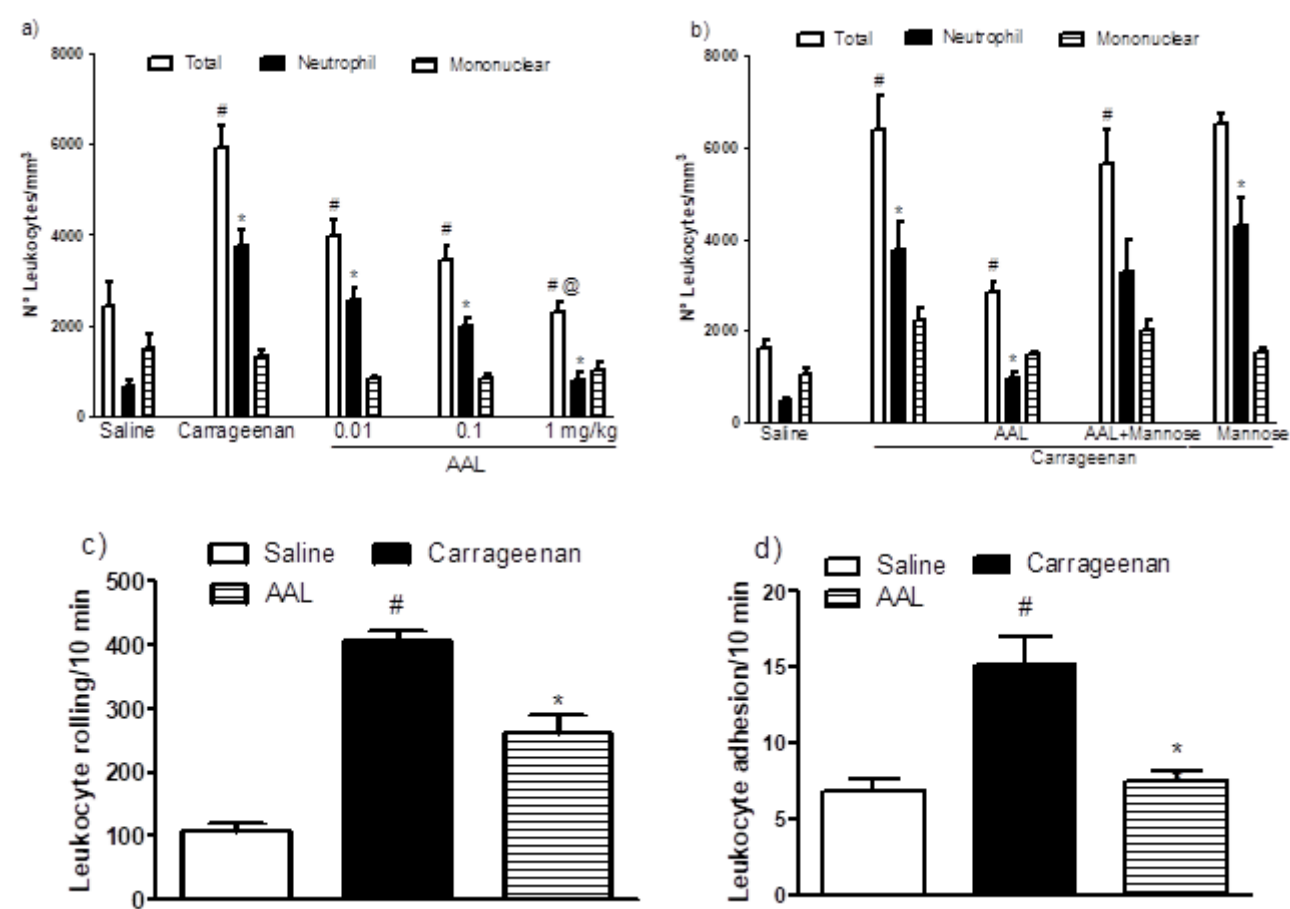


Figure 1

AAL reduces leukocyte-migration induced by carrageenan. (A) Animals received AAL (0.01-1 mg/kg; i.v.), (B) mannose (0.1 M; i.v.) or AAL (1 mg/kg i.v.) associated to mannose 30 min before peritonitis induction by carrageenan (500 µg/cavity). Control animals received 0.9% saline (0.1 ml/100 g; s.c.). Total and differential cell counts of peritoneal fluid was performed 4 h after peritonitis induction. (C) Rolled and (D)

adhered leukocytes were evaluated by intravital microscopy for 10 min. Mean $\pm$ S.E.M. (n = 6). ANOVA and Bonferroni tests. #p<0.05 vs. saline, *p<0.05 vs. carrageenan, @p<0.05 vs. AAL 0.01

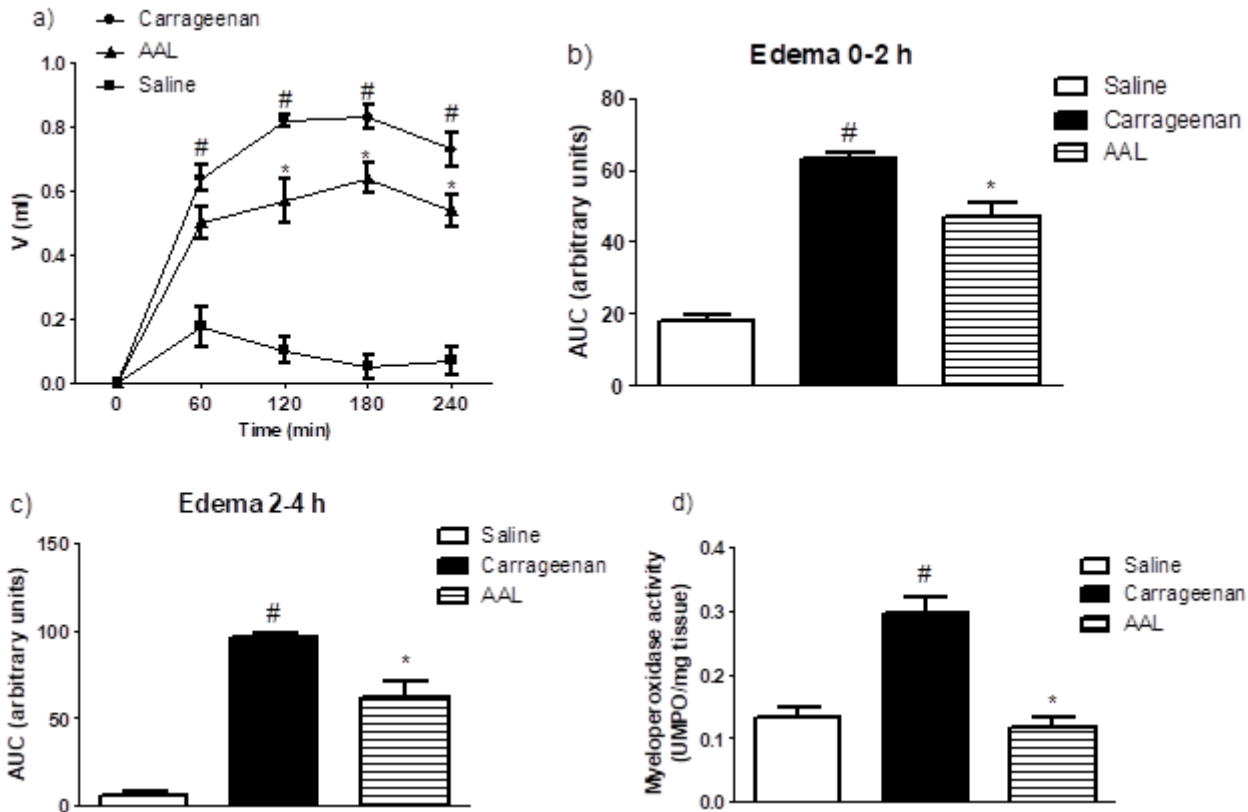


Figure 2

AAL inhibits paw edema and the increased myeloperoxidase activity induced by carrageenan. Animals received AAL (1 mg/kg; i.v.) 30 min before injection of carrageenan (300 μ g/paw) or 0.9% saline (100 μ l/paw) (A) Paw edema was measured by hydroplethysmometry at 0-240 min after carrageenan and expressed as the variation in paw volume displacement (ml) or area under curve (B, C). After 4 h, samples of subcutaneous plantar tissue were collected for evaluation of (D) myeloperoxidase activity (A405nm). ANOVA and Bonferroni tests. #p<0.05 vs. saline, *p<0.05 vs. carrageenan

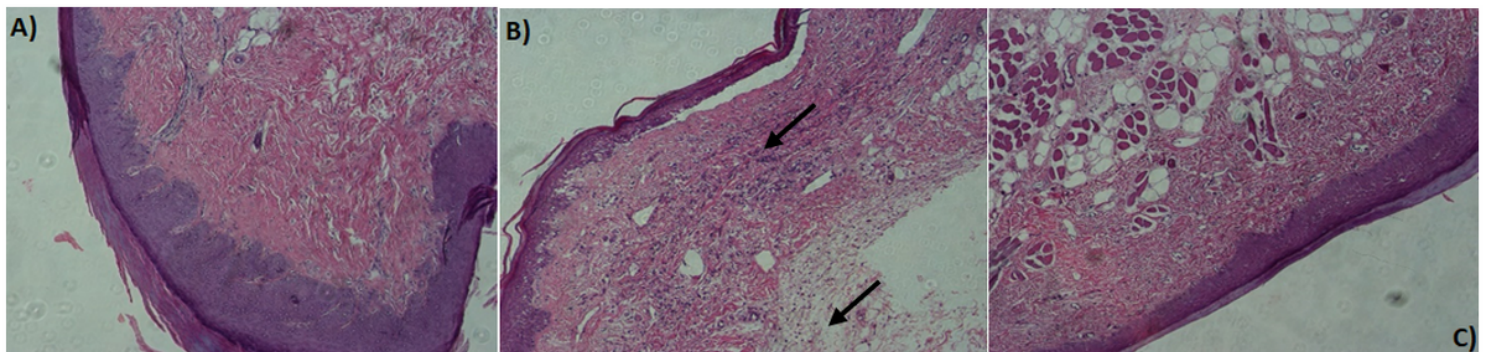


Figure 3

AAL attenuates the histological profile of carrageenan-induced paw edema. Cross section; HE (20X). (A) Saline: absence of inflammation (Median score: 0); (B) Carrageenan: severe cellular infiltrate (Median score: 3); (C) AAL: reduction of inflammatory parameters (Median score: 0). Arrows: cellular infiltrate or edema

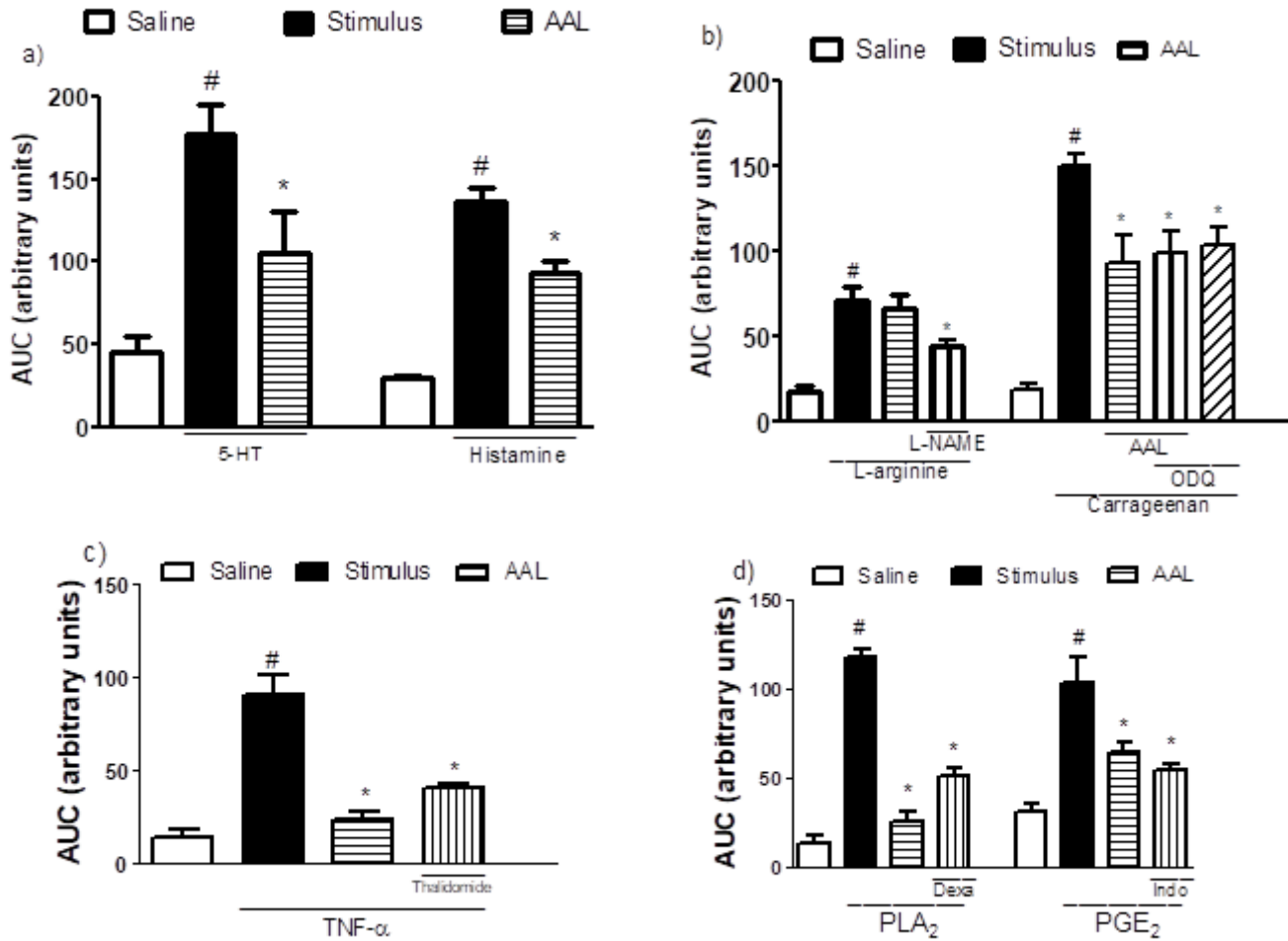


Figure 4

AAL inhibits paw edema induced by serotonin, histamine, TNF- α , phospholipase A2 (PLA₂) and PGE₂. Animals received AAL (1 mg/kg i.v.) 30 min before stimuli injection, and paw edema measured by hydroplethysmometry and expressed as area under curve. (A) Serotonin (5-HT: 100 μ g/paw), histamine (1 μ g/paw), (B) L-arginine (15 nmol/paw), carrageenan (300 μ g/paw), (C) TNF- α (5 ng/paw), (D) PLA₂ (30 μ g/paw) and PGE₂ (30 μ g/paw). L-NAME (30 mg/kg; s.c.), ODQ (8 μ g/kg; s.c.), thalidomide (45 mg/kg; s.c.), dexamethasone (0.5 mg/kg; s.c.) and indomethacin (5 mg/kg; s.c.) were used as anti-inflammatory control. ANOVA and Bonferroni tests. #p<0.05 vs. saline, *p<0.05 vs. stimuli

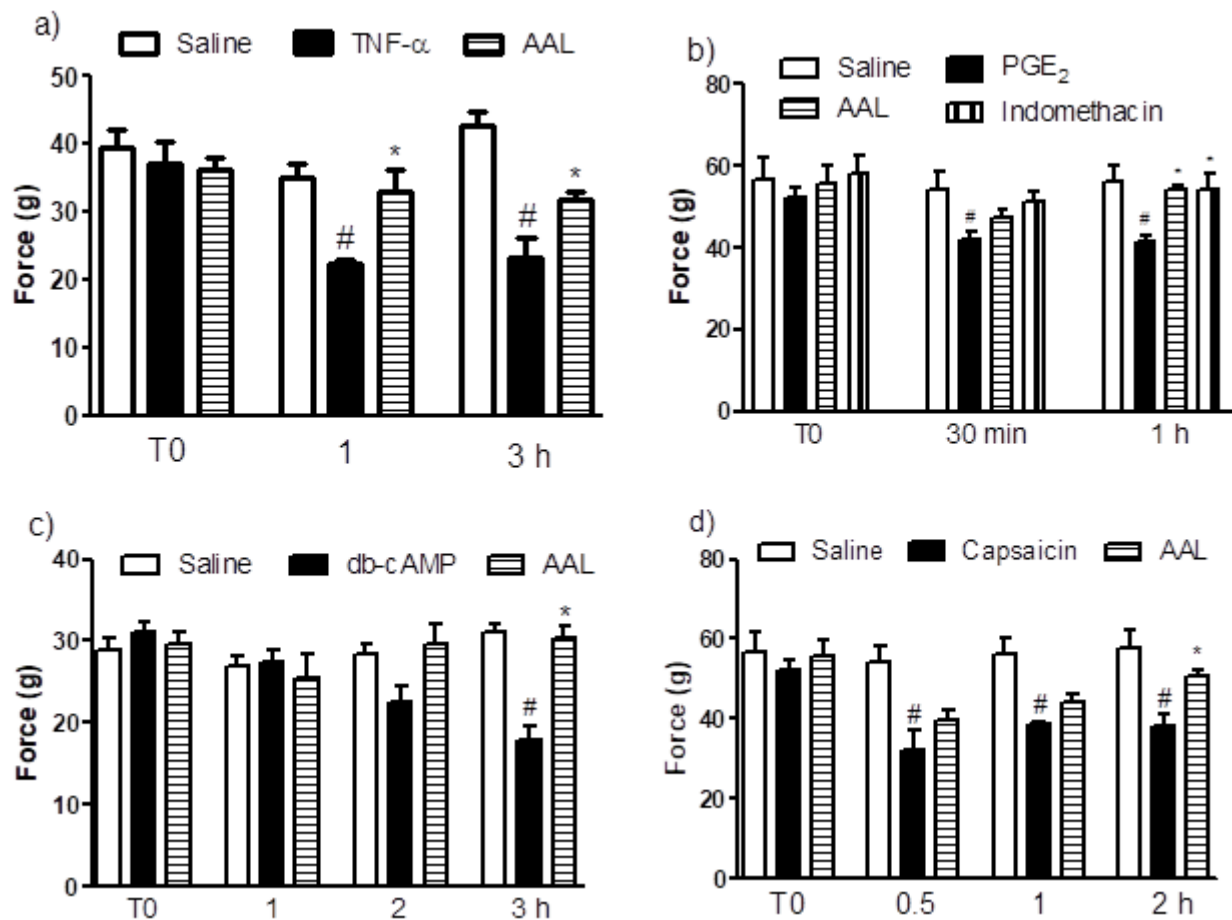


Figure 5

AAL inhibits mechanical hypernociception induced by TNF- α , PGE₂, db-cAMP and capsaicin. Animals received AAL (1 mg/kg; i.v.) 30 min before injection of the stimuli and evaluated for paw withdrawal response (g) analgesimetry. (A) TNF- α (5 ng/paw), (B) PGE₂ (30 μ g/paw), (C) db-cAMP (100 μ g/paw) and (D) Capsaicin (1 μ g/paw). Indomethacin (5 mg/kg; s.c.) was used as antihypernociceptive control. ANOVA and Bonferroni tests. # p <0.05 vs. saline, * p <0.05 vs. stimuli

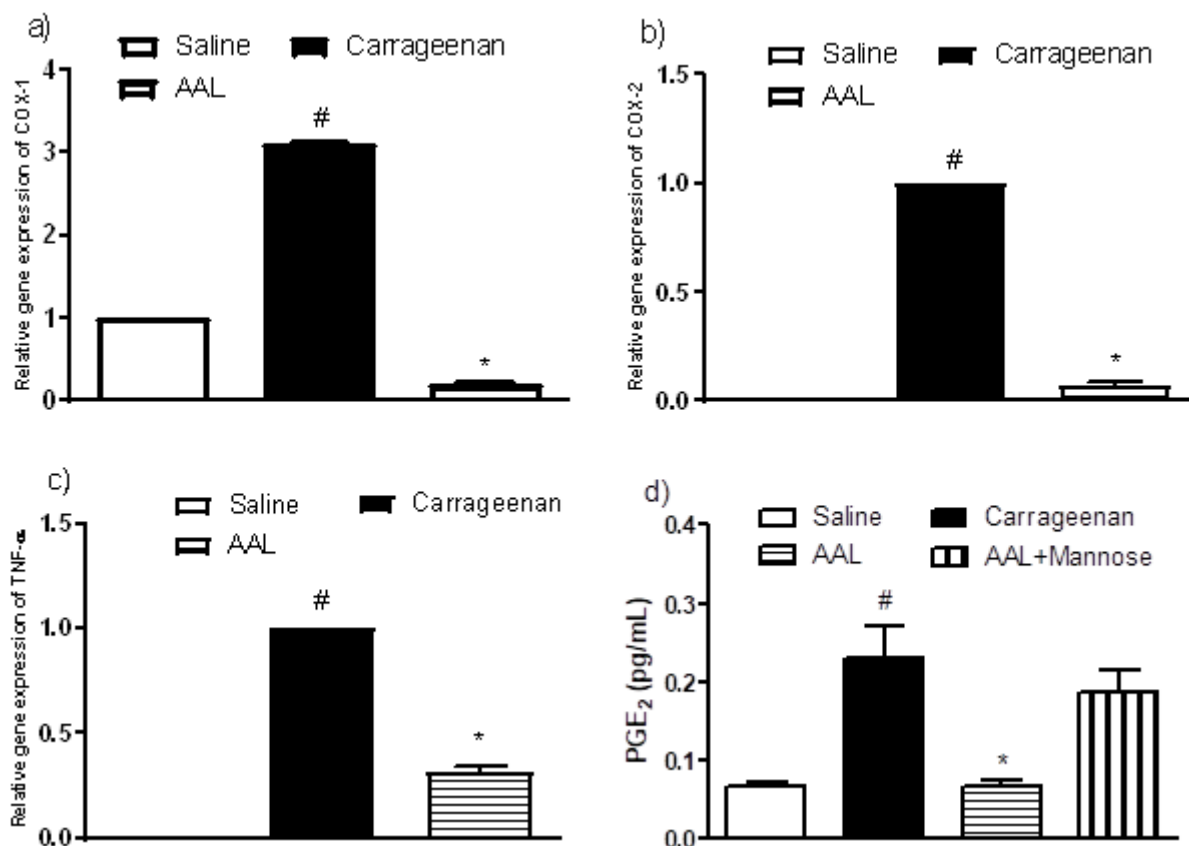


Figure 6

AAL inhibits mechanical hypernociception induced by TNF- α , PGE₂, db-cAMP and capsaicin. Animals received AAL (1 mg/kg; i.v.) 30 min before injection of the stimuli and evaluated for paw withdrawal response (g) analgesimetry. (A) TNF- α (5 ng/paw), (B) PGE₂ (30 μ g/paw), (C) db-cAMP (100 μ g/paw) and (D) Capsaicin (1 μ g/paw). Indomethacin (5 mg/kg; s.c.) was used as antihypernociceptive control. ANOVA and Bonferroni tests. #p<0.05 vs. saline, *p<0.05 vs. stimuli