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Purification and Biological Activities of *Abelmoschus esculentus* Seed Lectin

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Abstract The *Abelmoschus esculentus* (Malvaceae) plant originated in Africa and has spread across a number of tropic countries, including northeastern Brazil. The plant has been used to treat various disorders, such as cancer, microbial infections, hypoglycemia, constipation, urine retention and inflammation. The lectin of *A. esculentus* (AEL) was isolated by precipitation with ammonium sulfate at a saturation level of 30/60 and purified by ion exchange chromatography (Sephacel-DEAE). The electrophoresis (SDS-PAGE) profile of the AEL showed two protein bands of apparent molecular mass of approximately 15.0 and 21.0 kDa. The homogenity of the protein was confirmed by electrospray mass spectrometry (ESI-MS), which revealed the presence of a 10.29-kDa monomer and a 20.58-kDa dimer. The AEL exhibits agglutinating activity against rabbit (74.41 UH/mP) and human type ABO erythrocytes (21.00 UH/mP). This activity does not require the presence of divalent cations and is specifically inhibited by lactose, fructose and mannose. The intravenous treatment with 0.01, 0.1 and 1 mg/kg of AEL inhibited the paw edema elicited by carrageenan by approximately 15, 22 and 44 %, respectively, but not that induced by dextran. In addition, treatment with 0.1, 1 and 10 mg/kg of AEL also inhibited the abdominal writhing induced by acetic acid by approximately 52, 57 and 69 %, respectively. In conclusion, AEL is a new lectin with a molecular mass of 20.0 kDa, which is -composed of a 10.291-Da monomer and a 20.582-kDa dimer, that exhibits anti-inflammatory, antinociceptive and hemagglutinating activities. In addition, the lectin hemagglutinating property is both metallo-independent and associated with the lectin domain.

Keywords Plant lectin · *Abelmoschus esculentus* · Seeds · Purification · Anti-inflammatory · Antinociceptive

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1 Introduction

Historically, the use of plants for medicinal purposes is universal [28]. Even today, many communities utilize plants as the main, and sometimes the only, alternative for ailment relief [5]. The *Abelmoschus esculentus* (Malvaceae) plant originated in Africa and has spread across the tropic countries, including northeast Brazil, where it is now a common component of the native diet [36]. In addition, this plant has been used to treat a variety of disorders, such as cancer, microbial infection, hypoglycemia, constipation, urine retention and inflammation [16, 19, 25, 34].

Lectins are proteins that are capable of specifically and reversibly binding to carbohydrates in their mono- or oligosaccharide forms, thereby agglutinating cells and precipitating the oligosaccharides and glycoproteins [26].

These proteins participate in cellular communication, development, host defense, inflammation, tumor metastasis, agglutination of erythrocytes and lymphocytes, and the inhibition of bacteria and fungi [6]. Studies of the interactions in the lectin-oligosaccharide complex provide a molecular basis for the understanding of their biological effects [30, 32].

Plant lectins possess at least one carbohydrate-binding site and a conserved metal binding site for divalent cations, such as calcium and manganese. Some of these lectins also exhibit the ability of binding to specific hydrophobic compounds, which could be related to the transport of secondary metabolites [12]. This interaction between plant lectins and plant secondary metabolites occurs through the conserved binding site for α-aminobutyric acid found in the lectin of *Canavalia gladiata* seeds. The co-purification of Abu with *C. gladiata* lectin was demonstrated by crystal structure and mass spectrometry [10]. Plant lectins have been used to modulate a number of biological processes, such as the receptor binding of insulin and insulin-like growth factor 1 [23], nitric oxide synthesis [15] and the inhibition of the central nervous system involving the GABAergic mechanism [35]. They have also been extensively used as tools for the study of inflammatory [1, 2, 27, 33], anti-inflammatory [3, 4, 24, 29] and antinociceptive [9, 14, 17] processes. Most of these biological effects can be inhibited by the association of the lectins with their specific ligand sugars.

Although some lectins have being well characterized, the structural and biological properties of many plant lectins remain unknown. Thus, this study describes the isolation, chemical characterization and evaluation of the agglutinating, antinociceptive and anti-inflammatory activities of the *A. esculentus* seed lectin.

2 Materials and Methods

2.1 Animals

Male and female Wistar rats (*Rattus norvegicus*, 150–250 g), male Swiss mice (*Mus musculus*, 25–35 g) and adult white rabbits (New Zealand) were maintained under proper conditions (circadian cycle, 20 ± 3 °C) and provided with water and food ad libitum. The animals were brought to the laboratory for an adjustment period of at least 1 h before the experiments, which were conducted between 7 a.m. and 4 p.m. All protocols were approved by the Ethics Committee of the State University of Ceara (No. 10130208-8/40).

2.2 Drugs and Reagents

Acrylamide, *N′N′*-methylenebisacrylamide, bovine serum albumin, coomassie brilliant blue (G-250 and R-250), molecular markers, carrageenan, dextran, acetic acid, indomethacin, D-galactose, D-lactose, D-glucose, D-mannose and *N*-acetyl-D-glucosamine were obtained from Sigma Chemical Co., St. Louis, USA. Sodium dodecyl sulfate and 2-mercaptoetanol from Merck, Darmstadt, Germany and sephacel-DEAE was obtained from Pharmacia Fine Chemicals, Uppsala, Sweden. The lectin and drugs were solubilized in sterile 0.15 M NaCl (saline).

2.3 Plant Collection and Lectin Extraction

Abelmoschus esculentus was collected in the state of Paraiba, Brazil. Its mature seeds were grounded into a fine powder using an electric mill (Wiley) coupled with a 60-mesh sieve. The flour was defatted with n-hexane and air-dried at room temperature. The resultant powder was extracted at room temperature by continuous stirring for 3 h with a solution of 1:10 (w/v) 0.1 M Tris–HCl, pH 7.4, and 0.15 M NaCl. After centrifugation at 5,000*g* for 20 min, the insoluble fraction was discharged, and the supernatant was used to measure the hemagglutinating activity. The amount of soluble protein present in the extracts or fractions was analyzed by spectrophotometry at 280 nm.

The lectin was extracted using the seed meal (80 g) treatment with a solution of 0.1 M Tris–HCl buffer, pH 7.4, and 0.15 M NaCl for 3 h at 18 °C. The material was centrifuged at 5,000*g* for 20 min at 4 °C. The supernatant was precipitated with ammonium sulfate at a saturation level of 30/60 for 4 h at 18 °C prior to centrifugation at 5,000*g* for 20 min; the pellet then was collected at 4 °C. The fractions were exhaustively dialyzed against H_2O before being freeze-dried [31].

2.4 Ion Exchange Chromatography

The 30/60 fraction, which contains the hemagglutinating activity, was applied to an ion-exchange chromatography column (DEAE-Sephacel, 2.0 × 20 cm) and equilibrated with 0.02 M phosphate buffer, pH 7.5. The adsorbed proteins were eluted using a linear gradient of 0–1.0 M NaCl in PBS buffer. The elution was monitored at 280 nm, and fractions of 1.5 mL were tested for hemagglutinating activity on rabbit and human erythrocytes.

2.5 Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Electrophoresis was performed on 2-mm vertical gel slabs (one 12 % polyacrylamide separation gel with 3.0 M Tris–HCl, pH 8.8, with 1 % SDS buffer and one 3.5 % stacking gel with 0.5 M Tris–HCl, pH 6.8, with 1 % SDS buffer) at 20 mA for 4 h [20]. The samples were dissolved in a solution of 0.0625 M Tris–HCl, pH 8.3, with 1 % SDS

buffer 5 % 2-mercaptoetanol and 0.02 % 0.01 M bromophenol blue and incubated for 10 min at 100 °C. The protein bands were stained with Coomassie Brilliant Blue R-250, and their sizes (in kDA) were compared with the following molecular markers: beta-galactosidase 116, phosphorylase B 97.4, bovine serum albumin 66.2, ovalbumin 45, carbonic anidrase 31, trypsin inhibitor 21.4 and lysozyme 14.4.

2.6 Mass Spectrometry

The molecular mass was determined by electrospray ionization using a hybrid quadrupole/ion mobility separator/orthogonal acceleration time of flight instrument (Synapt HDMS system-Waters Corp., Milford, USA). The capillary and cone voltages were set to 3 kV and 40 V, respectively. The protein sample at 10 pmol was infused into the system using a flow rate of 10 µL/min. The source temperature was maintained at 100 °C, and nitrogen was used as the drying gas at a flow rate of approximately 150 l/h. The data were acquired by the Mass Lynx 4.0 software, and the multiple charged spectra were deconvoluted using maximum entropy techniques [13].

2.7 Biological Activities

The hemagglutinating activity was determined using 2 % rabbit and human erythrocytes; human cells were obtained from healthy blood donors at the Hematology Center in Paraiba, Brazil. A volume of 100 µL of a solution of 0.1 M Tris–HCl buffer, pH 7.4, and 0.15 M NaCl was added to a series of 10 tubes. In addition, 100 µL of lectin was added to the first tube. The second tube received 100 µL from the mixture of the first tube, and so on until the tenth tube, which accumulated a total volume of 200 µL and was eliminated from the assay. A volume of 100 µL of 2 % rabbit erythrocyte suspension (native or previously trypsinized or papainized) was then added to each tube; the tubes were then incubated for 30 min at 37 °C and for 30 min at room temperature. The hemagglutinating activity was expressed as the hemagglutinating unit (HU)/mL, which is defined as the reciprocal of the highest dilution that shows positive results [30]. In the carbohydrate inhibition test, the lectin was previously incubated with the sugar of interest for 30 min at 37 °C for 30 min.

The anti-inflammatory activity was evaluated using the paw edema model. This model involves the intravenous (i.v.) injection of 0.01, 0.1 or 1 mg/kg of lectin or saline into the rats; after 30 min, edema was induced with subcutaneous (s.c.) intraplantar administration of carrageenan (2 mg/paw) or dextran (300 µg/paw). The paw volumes were measured by plethysmometry. The differences between the volumes (mL) of the pre- and post-injected paws (1/2, 1–5 h) were then calculated, and the area under the time-course curve was determined [21].

The antinociceptive activity was evaluated using the writhing test. The mice received an injection of lectin (0.1, 1 or 10 mg/kg; i.v.), saline (i.v.) or indomethacin (10 mg/kg; i.p.) 30 min before receiving an i.p. injection of 0.6 % (v/v) acetic acid (0.1 mL/10 g body weight). The number of abdominal writhes was recorded starting 10 min after the administration of the acetic acid and for the next 20 min [18].

2.8 Statistical Analysis

The data are presented as the Mean ± SEM of the n animals per group and analyzed by ANOVA and a subsequent Bonferroni test. $P < 0.05$ was defined as significant.

3 Results

3.1 Lectin Isolation and Purification

The ion-exchange chromatography of the aqueous extract of *A. esculentus*, resulted in two peaks. Peak I (not retained on the column) was eluted with a 0.02 M phosphate buffer at pH 7. 5, whereas Peak II (lectin of *A. esculentus*) was obtained by a linear gradient of NaCl (0–1 M) in PBS buffer, which was monitored at 280 nm. The AEL electrophoresis profile showed two protein bands with apparent molecular masses of 15.0 and 21.0 kDa (Fig. 1). The mass spectrometry analysis demonstrated that the lectin can be found in two oligomerization forms: a monomer with 10.29 kDa and a dimer with 20.58 kDa (Fig. 2).

3.2 Biological Assays

3.2.1 Hemagglutinating Activity

The aqueous extracts (native and proteolytic enzyme-treated) from *A. esculentus* seeds exhibited hemagglutinating activity on rabbit erythrocytes (74.41 UH/mP), human type O red blood cells (21.00 UH/mP) and human type B native erythrocytes (24.00 UH/mP) (Table 1). In addition, the *A. esculentus* lectin (AEL) showed no metallo-dependence, and its hemagglutinating activity was specifically inhibited by lactose, fructose and mannose (6.25 mM) (Table 2).

3.2.2 Anti-inflammatory Activity

The injection of carrageenan (2 mg/paw; s.c.) in rats elicited intense paw edema (5.90 ± 0.31 AUC) for a short duration compared with the saline injection (0.37 ± 0.08

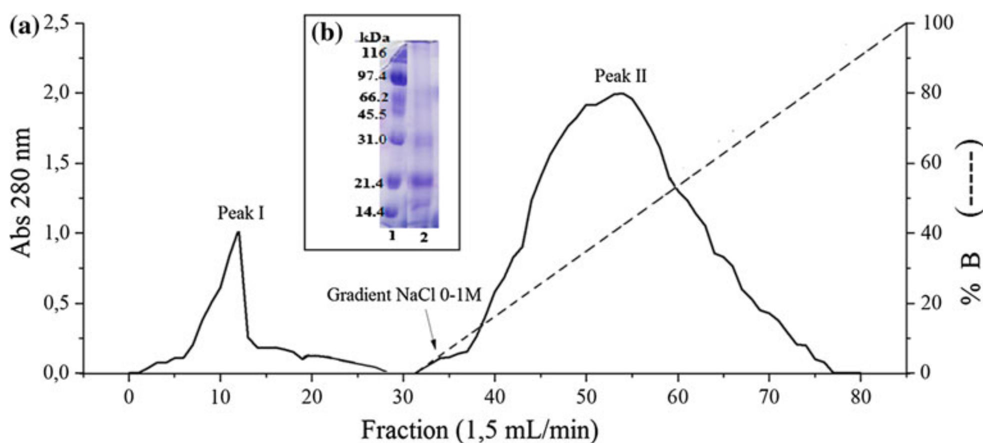

Fig. 1 Isolation of the lectin from *A. esculentus* seeds. (*a*) Ion-exchange chromatography. The lyophilized fractions (30/60) were applied to DEAE-Sephacel (2.0 × 20 cm), equilibrated with 0.02 M phosphate buffer, pH 7.5. Proteins were eluted (Peak II) with a linear gradient of 0–1 M NaCl in PBS buffer and monitored at 280 nm. Fractions of 1.5 mL were collected for hemagglutinating assay. (*b*) SDS–polyacrylamide gel electrophoresis of AEL in presence of 2-mercaptoethanol. *Lane 1*: molecular mass markers (kDa): beta-galactosidase 116, phosphorylase B 97.4, bovine serum albumin 66.2, ovalbumin 45, carbonic anidrase 31, trypsin inhibitor 21.4, Lysozyme 14.4. *Lane 2*: Peak II of ion exchange chromatography

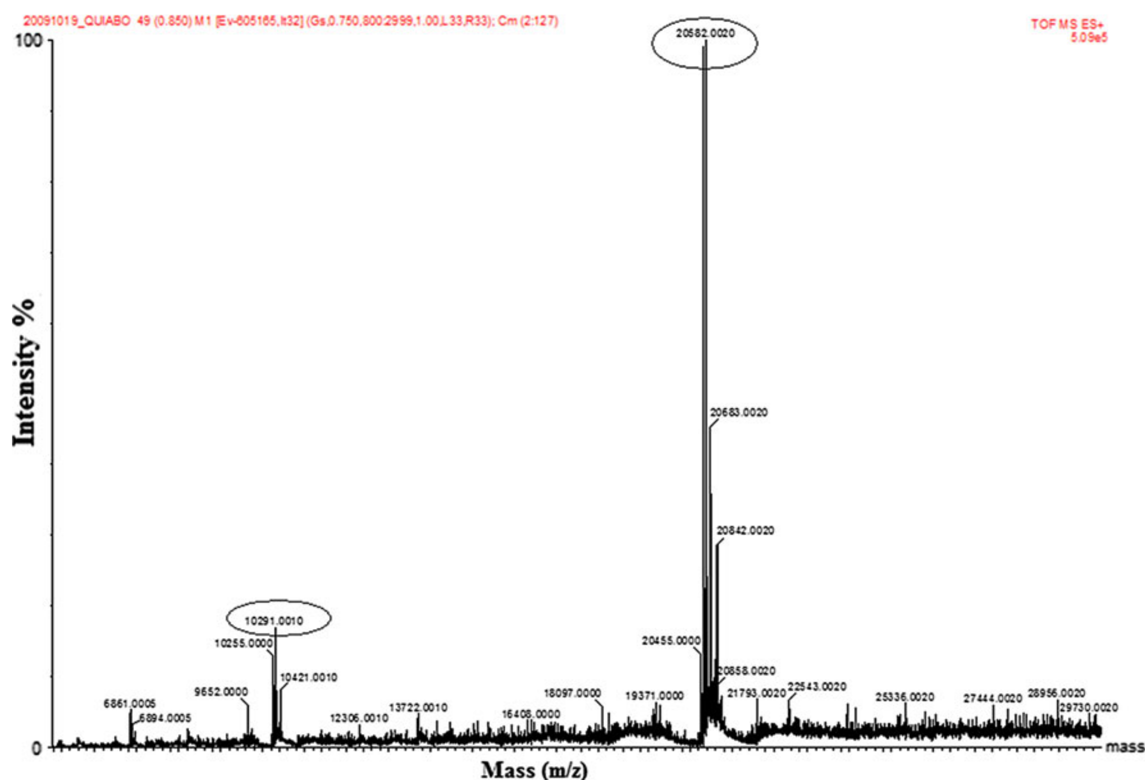

Fig. 2 Hybrid quadrupole/ion mobility separator/orthogonal acceleration mass spectra. Lectin from Peak II (AEL) of ion exchange chromatography shows apparent molecular mass of 10.29 (monomer) and 20.58 kDa (dimer)

AUC). The i.v. treatment with 0.01, 0.1 and 1 mg/kg of AEL 30 min before the administration of carrageenan significantly reduced this edematogenic effect by approximately 15 % (5.01 ± 0.31 AUC), 22 % (4.62 ± 0.28 AUC) and 44 % (3.31 ± 0.28 AUC), respectively (Fig. 3a, b). However, the i.v. treatment of animals with 1 mg/kg of AEL 30 min before a 300-µg/paw dextran injection did not alter the edematogenic effect (dextran: 148.52 ± 7.06 vs.

Table 1 Hemagglutinating activity of AEL against rabbit and human erythrocytes

Minimum hemagglutinating concentration (UH mg^{-1})			
Erythrocytes (2 %)	Native	Trypsin	Papain
Human			
A	ND	ND	ND
B	24.00	ND	ND
O	21.00	21.00	21.00
Rabbit	74.41	74.41	74.41

AEL with 0.025 M Tris buffer, pH 7.4. was incubated for 30 min at 37 °C and 30 min at room temperature with a 2 % suspension of trypsinized, papainized or native erythrocytes

ND not determined

Table 2 Influence of carbohydrate on the hemagglutinating activity of AEL

Sugar	Minimum inhibitory concentration (mM)
D-Galactose	NI
D-Frutose	62.5
D-Lactose	62.5
D-Maltose	NI
D-Trealose	NI
D-Mannose	62.5

AEL was incubated for 30 min at 37 °C with carbohydrates (0.5 M–62.5 mM) before incubation in the same conditions with a 2 % suspension of native rabbit erythrocytes

NI no inhibition at a concentration of 0.5 M

AEL + dextran: 143.19 ± 8.59 vs. saline: 32.27 ± 6.75 AUC) (Fig. 3c).

3.2.3 Antinociceptive Activity

The i.p. injection of 0.6 % acetic acid into mice induced abdominal writing (64.62 ± 3.09 writhes). The negative controls were injected with indomethacin and exhibited a significantly reduced number of writhes (2.12 ± 0.58). The administration of 0.1, 1 and 10 mg/kg of AEL 30 min before the acetic acid injection also inhibited the number of writhes by 52 % (31.25 ± 3.94), 57 % (27.5 ± 3.82) and 69 % (20.12 ± 3.55), respectively (Fig. 4).

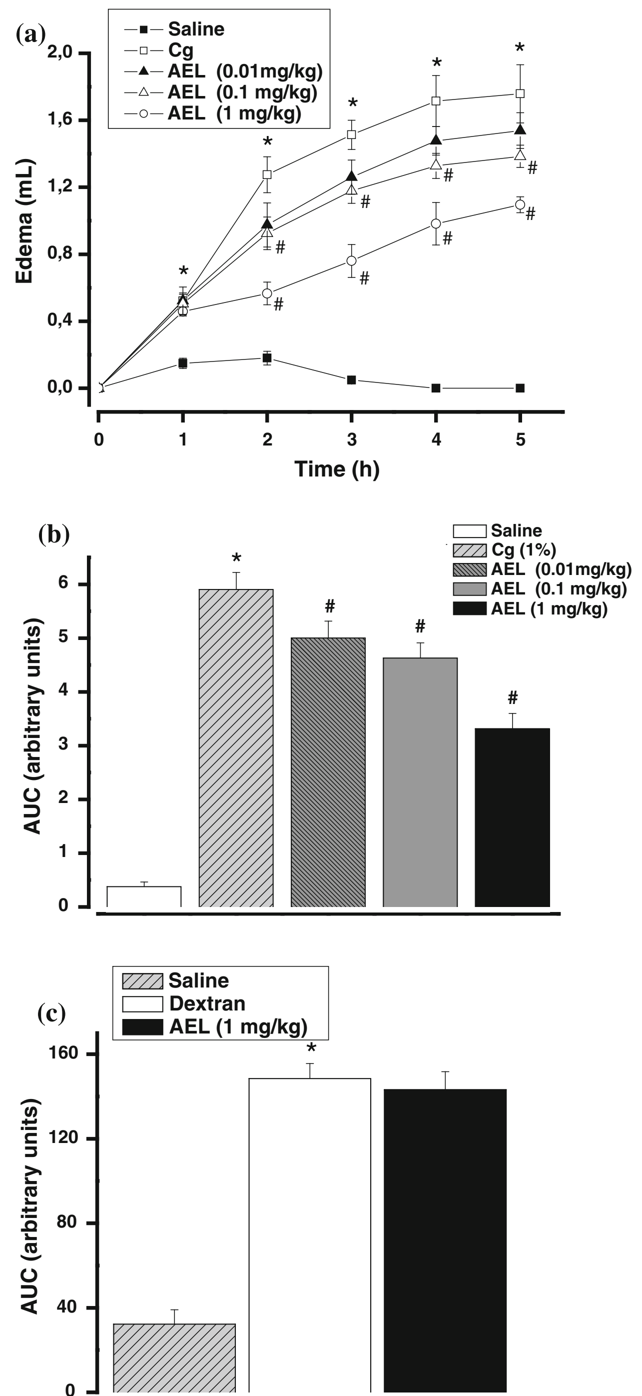

Fig. 3 Anti-edematogenic activities of AEL. AEL (0.01; 0.1; 1 mg/kg) or saline was injected i.v in rats 30 min before s.c. intraplantar injection of carrageenan (2 mg/paw) (**a**, **b**) or dextran (300 μg/paw) (**c**). Paw volumes were measured by plethysmometry (1/2, 1–5 h) and expressed in mL or AUC. Mean ± S.E.M. (n = 6–10). *$P < 0.05$ compared to Saline; # $P < 0.05$ compared to carrageenan

4 Discussion

The extracts from *A. esculentus* were able to agglutinate human and rabbit erythrocytes, which is a physico-chemical property of many lectins [11]. This observed agglutination did not require the presence of divalent cations, which is in contrast with the seed lectins of *Bauhinia pentandra* and *B. purpurea*, and was inhibited by galactose and galactose-

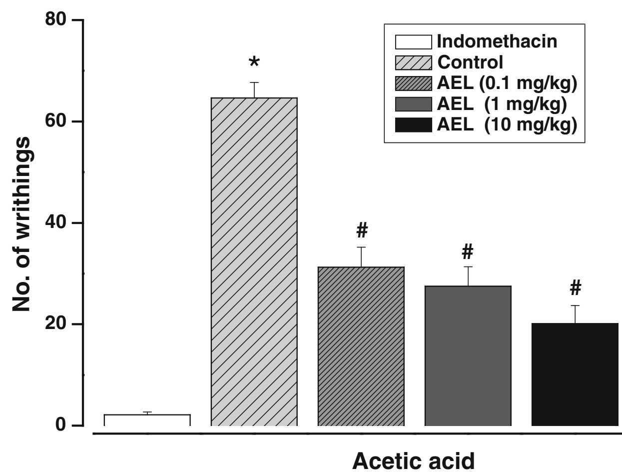

Fig. 4 Antinociceptive activities of AEL. AEL (0.1, 1, 10 mg/kg; i.v.) or indomethacin (10 mg/kg; i.p.) was injected in mice 30 min before i.p. injection of acetic acid 0.6 % (v/v) (0.1 mL/10 g body weight). Abdominal writhes were counted 10 min after stimuli during 20 min. Mean ± S.E.M. (n = 6–10). *$P < 0.05$ compared to indomethacin; # $P < 0.05$ compared to acetic acid

containing sugars and derivatives, such as galactosamine, α-D-melibiose, lactose and raffinose [8, 32]. Similarly, the lectin of *Araucaria angustifolia* exhibits hemagglutinating activity in either its native form or treated [29]. The AEL, which was analyzed in an experimental model of acute inflammation, showed inhibitory effects against the rat paw edema elicited by carrageenan but not in that induced by dextran. Carrageenan is a substance that elicits edema that is characterized by intense neutrophil infiltration and is associated with the release of inflammatory mediators, such as prostaglandins and nitric oxide [11]. However, dextran elicits osmotic edema involving histamine, serotonin and bradykinin [22]. These data suggest that the AEL anti-inflammatory effect occurs only in edema that involves cell infiltrates. Similar results have been found for other leguminous lectins [4]. All doses of AEL tested also reduced the number of writhes induced by acetic acid in mice. Similarly, the *Canavalia boliviana* lectin (administered intravenously) [14] and other Diocleinae lectins (administered orally) [17] have been found to inhibit the writhes induced by acetic acid in mice. This model involves the generation of pain by the release of bradykinin, serotonin and capsaicin, which in turn stimulate the peripheral nociceptive neurons that are sensitive to non-steroidal anti-inflammatory drugs [7] (Fig. 4).

5 Conclusion

The *A. esculentus* lectin is a 20.0 kDa protein that can be found as a 10.291-kDa monomer or a 20.582-kDa dimer. This protein exhibits anti-inflammatory, antinociceptive and hemagglutinating activities. The lectin hemagglutinating property is both metallo-independent and associated with the lectin domain.

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