Anti-inflammatory and anti-necrotic effects of lectins from *Canavalia ensiformis* and *Canavalia brasiliensis* in experimental acute pancreatitis

Samara Rodrigues Bonfim Damasceno Oliveira¹ · Álvaro Xavier Franco¹ · Marielle Pires Quaresma¹ · Cecília Mendes Morais de Carvalho¹ · Fabrícia da Cunha Jácome Marques¹ · Patrícia da Silva Pantoja¹ · Vanessa Azevedo Mendonça¹ · Vinicius José da Silva Osterne² · Jorge Luis Almeida Correia² · Ana Maria Sampaio Assreuy³ · Marcellus Henrique Loiola Pontes de Souza¹ · Kyria Santiago do Nascimento² · Benildo Sousa Cavada² · David Neil Criddle⁴ · Pedro Marcos Gomes Soares¹

Received: 9 November 2021 / Revised: 14 January 2022 / Accepted: 9 February 2022 / Published online: 3 March 2022

© The Author(s), under exclusive licence to Springer Science+Business Media, LLC, part of Springer Nature 2022

**Abstract**

Lectins isolated from *Canavalia ensiformis* (ConA) and *Canavalia brasiliensis* (ConBr) are promising molecules to prevent cell death. Acute pancreatitis, characterized by acinar cell necrosis and inflammation, presents significant morbidity and mortality. This study has investigated the effects of ConA and ConBr in experimental acute pancreatitis and pancreatic acinar cell death induced by bile acid. Pancreatitis was induced by retrograde pancreatic ductal injection of 3% sodium taurocholate (Na-TC) in male Swiss mice. ConA or ConBr (0.1, 1 or 10 mg/kg) were intravenously applied to mice 1 h and 12 h after induction. After 24 h, the severity of pancreatitis was evaluated by serum amylase and lipase, histopathological changes and myeloperoxidase assay. Pancreatic acinar cells were incubated with ConA (200 µg/ml) or ConBr (200 µg/ml) and taurolithocholic acid 3-sulfate (TLCS; 500 µM). Necrosis and changes in mitochondrial membrane potential (ΔѰm) were detected by fluorescence confocal microscopy. Treatment (post-insult) with ConA and ConBr decreased pancreatic damage caused by retrograde injection of Na-TC in mice, reducing pancreatic neutrophil infiltration, edema and necrosis. In addition, ConA and ConBr decreased pancreatic acinar cell necrosis and depolarization of ΔѰm caused by TLCS. The inhibition of necrosis was prevented by the lectin domain blockade. In conclusion, ConA and ConBr markedly inhibited *in vitro* and *in vivo* damage, effects partly dependent on the interaction with mannose residues on acinar cells. These data support the potential application of these proteins for treatment of acute pancreatitis.

**Keywords** Lectins · Anti-inflammatory · Anti-necrotic · Acute Pancreatitis

**Introduction**

Acute pancreatitis is a potentially severe inflammatory disease of the exocrine pancreas presenting significant morbidity and mortality. The most common cause is cholelithiasis, accounting for 35%-60% of cases [1]. Acute biliary pancreatitis is characterized by elevated pancreatic amylase and lipase, tissue necrosis and severe pancreatic inflammation, which may lead to a systemic inflammatory response syndrome, multiple organ dysfunction and death [2].

According to the revised 2012 Atlanta Classification of Acute Pancreatitis, the presence of necrosis and the number of organs affected by the subsequent inflammatory response determines the disease severity (mild, moderate, severe) and dictates the short-term and long-term management of the patients [3]. The severity of experimental pancreatitis is correlated with the extent of cell necrosis and inversely with apoptosis [4–6].

In isolated pancreatic acinar cells, bile acids such as taurolithocholic acid 3-sulfate (TLC-S), cause necrosis due to a sustained increase of cytosolic calcium ([Ca2+]C) via activation...
of the Gpbar1 G-protein receptor [7] Ca2+ release from intracellular stores and inhibition of the sarco-endoplasmic reticulum Ca2+–adenosine triphosphatase (SERCA) pump [8] leading to premature intracellular enzyme activation, cell vacuolization, mitochondrial depolarization and decrease in ATP production [9–11]. However, currently there is no specific therapy to treat the disease.

Leguminous lectins of the Diocleinae subtribe, a class of carbohydrate-binding proteins, have received increasing attention because of their various biological properties [12]. ConA and ConBr, isolated from the seeds of Canavalia ensiformis and Canavalia brasiliensis, possess binding specificity to residues of glucose/mannose and share 90% structural similarity, and are considered promising molecules since they possess a variety of biological functions, including neuroplasticity [13–17], modulation of cell death, and antiproliferative effects on tumor cells [18, 19]. They promote apoptosis in human leukemic cells lines, in a mitochondrial-dependent manner, while preserving healthy cells [20]. In addition, these lectins exert immunomodulatory and antinoceptive properties in experimental models in vivo [21–24]. However, the potential of these lectins to prevent cell necrosis and inflammation in acute pancreatitis has not been fully investigated. This study aimed to evaluate the effects of ConA and ConBr in the mice experimental acute biliary pancreatitis induced by tauroliothocholic acid 3-sulphate (TLC-S) and assess the involvement of the lectin-carbohydrate interaction.

Materials and methods

Chemicals

Collagenase was purchased from Worthington Biochemical Corporation (Lakewood, NJ, USA); TMRM and Hoechst 33,342 from Molecular Probes (Eugene, Oregon, USA); TLC-S (tauroliothocholic acid 3-sulphate), Na-TC (sodium taurocholate), PI (propidium iodide) and α-methylmannoside from Sigma (St. Louis, MO).

Lectin purification and dissolution

The lectins were purified from seeds of the leguminous plants Canavalia ensiformis (L.) DC. (Jack bean) (ConA) and Canavalia brasiliensis MART (ConBr) by affinity chromatography [25, 26]) both possessing binding-specificity to D-glucose and D-mannose. After purification, the lectins were dialyzed in distilled water, lyophilized and dissolved in 0.9% NaCl (sterile saline) immediately prior to use.

Animals

Male Swiss mice (20–25 g), provided by the Animal Housing Facility of the Department of Physiology and Pharmacology of the Medical School of the Federal University of Ceará, were kept in ventilated racks maintained at 23 ± 2 °C, with a 12 h light/dark cycle and free access to a balanced diet (PURINA®) and purified water. All procedures were in accordance with the guidelines of the National Council for the Control of Animal Experimentation (CONCEA) and approved by the Animal Use Ethics Committee (CEUA) of the Federal University of Ceará (protocol n° 99/2013).

Experimental acute pancreatitis

Acute pancreatitis was induced by retrograde pancreatic ductal injection of 3% Na-TC (5 μL/min for 10 min via infusion pump, survival rate was 80% on average) in adult mice (20–25 g). The control groups received retrograde pancreatic ductal infusion of saline (Sal Group) alone or the surgical procedure without infusion, denominated Sham group [27]. ConA or ConBr (0.1, 1 or 10 mg/kg i.v.) were applied as treatment to mice 1 h and 12 h after pancreatitis induction. After 24 h, the animals were sacrificed, and the blood was collected for estimation of pancreatic enzymes amylase and lipase and the pancreas removed for myeloperoxidase assay (MPO) and histopathological analysis.

Histopathological evaluation

The pancreas was fixed in 10% formalin, embedded in paraffin by standard methods, cut into sections of 5 μm through a microtome, and subsequently stained with hematoxylin–eosin (HE). The morphological alterations characteristic of acute pancreatitis were evaluated as previously [28]: edema, inflammatory infiltration and necrosis. The degree of edema was determined using a scale of 0 to 3 (0 = absent, 1 = interlobular edema, 2 = interlobular edema and moderate intralobular edema, and 3 = interlobular edema and severe intralobular edema). The presence of inflammatory infiltrate was also analyzed, using a grid of 0 to 3 (0 = absent 1 = scarce perivascular infiltration, 2 = moderate perivascular infiltration and scarce diffuse infiltration, and 3 = abundant diffuse infiltration). Parenchymal necrosis was analyzed by assigning scores from 0 to 3 (0 = absent, 1 = less than 15% of pancreatic cells involved, 2 = 15 to 35% of pancreatic cells involved, and 3 = more than 35% of cells involved). Evaluation was performed on 10 random fields (×400) by 1 blinded investigator, calculating the means ± SEM (≥ 8 mice/group).
Myeloperoxidase activity

Myeloperoxidase (MPO) activity was determined as described [29]. Pancreatic tissue was homogenized, resuspended in 100 mM phosphate buffer (pH 6.0) containing 0.5% hexadecyltrimethyl ammonium bromide and centrifuged for 20 min at 16,000 g. MPO activity was measured in supernatants (3,3,5,5-tetramethylbenzidine substrate with 1% H2O2). Absorbance was measured at 450 nm and MPO calculated as the difference between absorbance at 0 and 2 min.

Amylase measurement

Serum amylase was determined by a colorimetric assay (Labtest®, Brazil) according to the manufacturer’s instructions. Blood samples were taken and centrifuged at 3500 rpm for 10 min as previously [30]. For the amylase assay, 500 µl of the substrate was added to the samples (10 µl). They were incubated in a water bath at 37 °C for 2 min, followed by addition of 500 µl of the color reagent and 4 ml of distilled water. After mixing and waiting 5 min, absorbance was determined at 660 nm.

Lipase measurement

Serum lipase was determined by colorimetric assays (Bioclin®, Brazil), according to the manufacturer’s instructions. For this, 1 ml of the reagent 1 (Buffer—Tris (Hydroxymethyl) Aminemethane), 50 µl of reagent 2 (Enzymatic Inhibitor – PMSF/ Phenylmethylsulfonyl fluoride) and 100 µl of reagent 3 (Color Reagent – DTNB/ (5,5-dithiobis-(2-nitrobenzoic acid) were added to 50 µl of the sample. Samples were placed in 37 °C water bath for 2 min with 100 µl of reagent 4 (2,3-Dimercapto-1-propanol tributyrate), homogenized and incubated at 37 °C for 30 min. Two milliliters of Reagent 5 (Acetone) were added, and samples homogenized and allowed to stand for 3 min at room temperature. The material was centrifuged at 3500 rpm for 5 min and the absorbance of the supernatants determined at 410 nm [30].

Cell preparation and solutions

Acinar cells were isolated from the excised pancreas of male CD1 mice (8–12 weeks old) with purified CLSPA collagenase (Worthington Biochemical Corp.®) as previously described [5]. The experiments were performed at room temperature (23–25 °C) and the cells were used within 4 h after isolation. The extracellular solution contained (in mM): 140 NaCl; 4.7 KCl; 1.13 MgCl2; 1 CaCl2; 10 D-glucose; and 10 HEPES (4- (2-hydroxyethyl) -1-piperazinoethanesulfonic acid), adjusted to pH 7.35 ± 0.1 [31].

Roles of ConA and ConBr on cellular necrosis and mitochondrial membrane potential (ΔΨm): involvement of lectin domain

Cells were incubated for 1 h with ConA (200 µg/ml) or ConBr (200 µg/ml) and stimulated for 30 min with TLCS (500 µM). Necrotic cell death was detected by confocal microscopy (FluoViewTM 1000 – Olympus) using propidium iodide (PI; 1 µM: excitation 488 nm, emission 630–693 nm), cell membrane impermeable nucleic acid marker. In separate experiments changes in mitochondrial membrane potential (ΔΨm) were performed using the fluorescent probe tetramethylrhodamine methyl ester (TMRM 100 nM—excitation/emission: 543/550–650, 15 min incubation), the accumulation of which in mitochondria is driven by the highly negative inner mitochondrial membrane potential. Total cell number was detected using nuclear Hoechst 33,342 (50 µg/mL: excitation 364 nm, emission 405–450 nm) [10]. An average of 100 cells was analyzed from each test group. The percentage (%) of cells exhibiting mitochondrial depolarization, detected as a relative decrease in TMRM fluorescence, was calculated from total number of cells labeled with Hoechst 33,342 and TMRM.. Cell counts were performed in triplicate within 15 high-power fields. The involvement of the lectin domain on cellular necrosis was assessed by the prior incubation of ConA and ConBr (37 °C; 1 h) with 0.2 M of their binding sugar α -methyl-D-mannoside (α-MM).

Statistical analysis

The results were expressed as mean ± SEM (Standard Error of the Mean). Statistical analysis between groups was performed using Analysis of Variance ANOVA, followed by Bonferroni multiple comparisons test. The differences were considered statistically significant when P < 0.05. GraphPad Prism® Software version 5.0 was used.

Results

Effects of ConA and ConBr on histopathological changes of experimental acute pancreatitis

In vivo, lectins given after induction of pancreatitis protected the animals against acute pancreatitis caused by Na-TC. Thus ConA (10 mg/kg) and ConBr (10 mg/kg) protected the pancreatic tissue against histopathological alterations, comprising increased neutrophilic infiltration – (Fig. 1a), oedema (Fig. 1b) and tissue necrosis (Fig. 1c) caused by Na-TC. ConA and ConBr reduced the total histological changes induced by Na-TC by 43.6% and 71.8%, respectively, compared to controls (Fig. 1d). Photomicrographs show the histopathological changes caused by Na-TC (Fig. 1f) (black arrow: neutrophilic...
ConA and ConBr protect against histopathological damage caused by Na-TC in experimental acute pancreatitis in mice. Histopathological analysis of the aspects of inflammation (a), Oedema (b), Necrosis (c) and Total scores (d). Data were expressed as mean ± standard error of the mean (SEM) of an experimental “n” of at least 8 animals. * p<0.05 vs. Sal group; # p<0.05 vs. Na-TC group. One-way ANOVA followed by Bonferroni post-test. Photomicrographs of the histopathological analysis (increase of 400 X) are shown in E: representing the Saline group; F: representing the Na-TC group; G: representing the ConA + Na-TC group and H: representing the ConBr + Na-TC group. Black arrow: indicates presence of neutrophil infiltrate. Red arrow: indicates edema.

Role of ConA and ConBr on MPO activity in the Na-TC-induced experimental acute pancreatitis

Having observed the protective effects of lectins at a dose of 10 mg/kg on histopathological damage, a range of concentrations (0.1; 1 and 10 mg/kg) were analyzed on pancreatic myeloperoxidase changes. Both lectins decreased the sodium taurocholate Na-TC-induced elevation of myeloperoxidase in a dose-dependent manner; the results of this analysis confirmed that the 10 mg/kg dose of ConA and ConBr showed higher efficacy with both molecules decreasing the MPO levels by 94.4% and 98.1%, respectively, compared to Na-TC group, justifying its choice for subsequent analysis (Fig. 2a, b).
Effects of ConA and ConBr on amylase and lipase assay in the Na-TC-induced experimental acute pancreatitis

Na-TC administration increased amylase and lipase by 81.3% and 73.0%, respectively, compared to controls. ConA and ConBr treatment was protective, decreasing the elevated amylase by 42.0% and 31.4%, respectively. Similarly, the Na-TC-induced rise of lipase was reduced by 55.0% and 63.0% by ConA and ConBr treatment, respectively (Fig. 3a, b).

ConA and ConBr decrease necrosis and depolarization of mitochondrial membrane potential (Δѱm) induced by TLCS in pancreatic acinar cells

Pancreatic acinar cells that were treated with lectins prior to administration of TLCS showed a percentage of necrotic cells similar to controls. Application of TLCS increased acinar cell necrosis by 66.9% compared to control. Incubation with ConA and ConBr decreased this rise by 45.0% and 44.9%, respectively (Fig. 4a, b). In separate experiments, the intense mitochondrial depolarization of acinar cells induced by TLCS was reduced by 62.1% and 56.5% by prior incubation with ConA and ConBr, respectively (Fig. 5a, b).

Protective effects of ConA and ConBr on TLCS-induced necrosis is dependent on interaction with mannone residues

The anti-necrotic effects of the lectins were abolished by the association of both molecules with their specific sugar ligand α-methyl mannoside (α-MM). No statistical difference between lectin and TLCS groups was observed. In addition, application of α-MM per se did not induce acinar cell injury (Fig. 6).

Discussion

This study has shown for the first time that treatment with ConA and ConBr lectins is protective against the detrimental inflammatory, biochemical and histopathological changes that occur during biliary acute pancreatitis. It is likely that a significant component of this beneficial activity resides in local actions of these biological molecules, since both protected pancreatic acinar cells against mitochondrial dysfunction and necrosis caused by TLCS. The protective actions were dependent, at least in part, on specific interactions with the lectin domain.

Acute pancreatitis is a necro-inflammatory disorder the main causal factor of which is the presence of gallstones within the distal common bile duct, allowing consequent reflux of bile into the pancreatic duct [32, 33] accounting for 30–60% of cases [34]. Retrograde administration of Na-TC into the pancreas of mice is a reliable, established experimental model of biliary acute pancreatitis, characterized by pancreatic inflammation with defined histopathological, inflammatory and biochemical changes [27]. Toxic precipitating agents such as bile acids cause acinar cell lesions; after intense stimulation with Na-TC the pancreatic tissue becomes swollen, with tissue necrosis and prominent leukocyte infiltration apparent [11]. In the present study, ConA and ConBr protected against histopathological damage of the pancreas caused by Na-TC, with significantly reduced edema, necrosis and neutrophil infiltration. In accord, both lectins decreased MPO levels elevated in biliary AP, consistent with protection against neutrophil infiltration that causes tissue damage and aggravates the inflammatory lesions [35]. A marked increase in serum pancreatic lipase and amylase levels at 24 h, characteristic of acinar cell damage during acute pancreatitis [36–38] was also reduced by treatment with ConA and ConBr.

Antinociceptive [22, 24]; and anti-inflammatory [23, 39]; properties of Dioeleinae lectins, have previously been reported in animal experimental models. However, to-date...
there has been no study showing anti-inflammatory activities of ConA and ConBr. Our current results demonstrate novel protective actions of ConA and ConBr to combat inflammation in experimental acute pancreatitis. Since the lectins were applied after acute pancreatitis had been instigated, rather than as pretreatment, new possibilities for therapeutic application are suggested; an important finding since there is currently no specific treatment for this debilitating and sometimes fatal disease. Systemically administered lectins bind to pancreatic acinar cells and modulate their metabolism. However, it cannot be excluded that the protection may also involve other properties of the lectin proteins and indirect effects mediated through lectin-driven changes to the immune response [40].

Pancreatic acinar cell necrosis is considered the initiating event of cell damage fundamental for the onset of an inflammatory cascade in acute pancreatitis [41–44]. Bile acids cause local cell injury by the induction of sustained increases in cytosolic [Ca$^{2+}$] that reduce mitochondrial membrane potential and deplete cellular ATP, leading to acinar cell necrosis [5–10]. Mitochondrial dysfunction, involving formation of the permeability transition pore, is a core feature of acute pancreatitis. [11] In isolated acinar cells ConA and ConBr protected against mitochondrial dysfunction, partially inhibiting mitochondrial depolarization induced by TLCS. These data are supported by studies showing the involvement of these lectins with mitochondrial function in other cell types e.g. after associating with the mannose moiety residing on a cell membrane glycoprotein in a liver tumor cell line, ConA was internalized to mitochondria via clathrin-mediated endocytosis and promoted autophagic cell death [45]. In addition, ConA and ConBr promoted partial $\Delta \psi_m$ loss that triggered apoptosis in leukemic cells lines [20]. Consistent with an inhibition of $\Delta \psi_m$ depolarization induced by TLC-S, ConA and ConBr significantly prevented TLC-S-induced necrosis. Previously ConBr was found to protect against hippocampal cell death in mice induced by quinolinic acid, an action that involved inhibition of necrosis [46].

![Image](image_url)

**Fig. 4** ConA and ConBr protect against pancreatic acinar cells necrosis induced by TLCS. The image panel in a illustrates in the first column: representative pancreatic acinar cells in transmitted light; second column: nuclei of total cells labeled with Hoechst 33,342 (blue staining); third column: necrotic cells, labeled nuclei with PI (red staining) and fourth column: overlapping images (merge), facilitating visualization and quantification of cell death. Images obtained with FluoViewTM 1000 confocal microscope—Olympus, 400X magnification. (b) Necrosis percentage (ConA and ConBr+TLCS). Mean ± SEM of 15 fields in triplicate. Necrosis (%) was given by the ratio of cells stained with Hoechst 33,342 and PI. *p<0.05 vs. control (C); #p<0.05 vs. TLCS. One-way ANOVA and Bonferroni test.
The surface of the pancreatic acinar cell contains several sugar residues including N-acetyl-glucosamine, galactose and possibly also N-acetyl-neuraminic acid in the apical region, and fucose, galactose, mannose and glucose residues in the basolateral region [47, 48]. Molecules such as ConA and ConBr possess binding specificity to glucose/mannose residues, suggesting binding to sugars present on the surface of the acinar cell as a possible mechanism of action of lectins. The non-catalytic domain or carbohydrate recognition domain (CRD) of the lectins, also called the lectin domain, is the site at which lectins bind specifically and reversibly to carbohydrates and other substances that contain sugar moieties. Many of the biological activities of lectins in general are related to an interaction with sugars present on the cell surface [49]. Proof of participation of the lectin domain in processes of cell recognition and interaction has been shown by inhibition of the lectin effect by binding to its specific sugar/glycoconjugate [22, 23, 24, 38]. ConA and ConBr are glucose/mannose ligands lectins which have

Fig. 5 ConA and ConBr reduce the depolarization of mitochondrial membrane potential of acinar cells induced by TLCS. The image panel illustrates in the first column: representative pancreatic acinar cells in transmitted light; second column: nuclei of total cells labeled with Hoechst 33,342 (blue staining); third column: mitochondria labeled with TMRM (red staining); fourth column: overlapping images (merge), facilitating visualization and quantification of cells. Images obtained with FluoViewTM 1000 confocal microscope—Olympus, 400X magnification. (b) Percentage (%) of mitochondrial depolarization (ConA and ConBr+TLCS). Mean±SEM of 15 fields in triplicate. The percentage (%) of mitochondrial depolarization is given by the ratio of cells labeled with Hoechst 33,342 and TMRM, where a decrease in relative fluorescence of TMRM represents mitochondrial depolarization. *p<0.05 vs. control (C); #p<0.05 vs. TLCS. One-way ANOVA and Bonferroni test

Fig. 6 Antinecrotic effect of ConA and ConBr is lectin domain dependent. Data are expressed as mean±standard error of the mean (SEM) of the count of 15 image fields obtained under a FluoViewTM 1000—Olympus confocal microscope, repeated in triplicate. The percentage of necrosis is given by the ratio of cells labeled with Hoechst 33,342 and propidium iodide (PI). *p<0.05 vs control group (C); #p<0.05 vs TLCS group; #p<0.05 vs ConA and ConBr groups. One-way ANOVA followed by Bonferroni posttest
had biological activities previously attributed to their lectin domain [23, 50, 51]. In this study, protective effects against TLC-S-induced cell death were abolished after blocking the ConA and ConBr lectin domain, indicating that their actions are, at least partly, due to a direct interaction of the lectin domain with pancreatic acinar cells. Dicoleinae lectins exhibit high degree of homology in their primary structures and share many biochemical and structural features, such as evolutionarily conserved regions that characterize this group [52]. Differences in their biological activity may reflect not only small changes in the amino acid sequence of the CRD, but also different conformations of the site itself and adjacent loops [52, 53]. The comparative study of lectins from the same subfamily is useful to broaden knowledge about biological structure–activity relationships of these proteins. The lectins evaluated in this study exhibit 99% structural similarity with only two amino acid residues different: glycine (Gly) 58 and Gly 70 in ConBr are replaced by aspartate (Asp) and alanine (Ala) in ConA, respectively, with none of these residues close to the carbohydrate binding site. Both are tetramers at neutral pH or above 5.5, and dimers below 5.5 [54, 55]. ConA and ConBr exhibited a very similar pattern of results in all assays, in agreement with prior analyses with ConA previously reported despite the high structural similarity of these lectins [12, 55–57]. However, in the present study, ConA and ConBr exhibited a very similar pattern of results in all assays, in agreement with prior analyses with ConA and ConBr in leukemia cell lines [20].

In summary, our findings demonstrate for the first time that ConA and ConBr, applied after the initiation of biliary acute pancreatitis, are protective against inflammation and tissue damage. These actions are partly mediated via interaction of the lectin domain with sugar residues present on the acinar cells, leading to protection against mitochondrial dysfunction and acinar cell necrosis. Further studies are warranted to better understand the underlying mechanisms of action of these anti-inflammatory molecules and assess their potential for therapeutic development.

Acknowledgements This research was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico (FUNCAP).

Author contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Samara Rodrigues Bonfim Damasceno Oliveira; Marielle Pires Quaresma; Patrícia da Silva Pantoja; Vinicius José da Silva Osterne; Jorge Luís Almeida Correia. The first draft of the manuscript was written by Samara Rodrigues Bonfim Damasceno Oliveira; Pedro Marcos Gomes Soares and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding This research was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), under Grant Numbers PNE-0112–00,038.01.00/16, SPU 4,509,534/2016 and 428,713/2018–1.

Data availability The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

Compliance with ethical standards

Ethics approval All procedures were approved by the Animal Use Ethics Committee (CEUA) of the Federal University of Ceará (protocol no 99/2013).

Consent to participate All authors declare participation in the study.

Consent for publication All authors declare consent for publication of study.

Conflict of interests The authors have no conflicts of interest to declare that are relevant to the content of this article.

References

1. Dedemagi, G., Nikolopoulos, M., Kalaitzopoulos, I., Sgourakis, G.: Management of patients after recovering from acute severe biliary pancreatitis. World J. Gastroenterol. 22, 7708–7717 (2016)
2. Huber, H., Algül, H.: Treatment of acute necrotizing pancreatitis. Internist. (Berl) 60, 226–234 (2019)
3. Banks, P.A., Bollet, T.L., Dervenis, C., Gooszen, H.G., Johnson, C.D., Sarr, M.G., Tsitotos, G.G., Vege, S.S.: Classification of acute pancreatitis–2012: revision of the Atlanta classification and definitions by international consensus. Gut 62, 102–111 (2013)
4. Saluja, A., Hofbauer, B., Yamaguchi, Y., Yamanaka, K., Steer, M.: Induction of apoptosis reduces the severity of caerulein-induced pancreatitis in mice. Biochem. Biophys. Res. Commun. 220, 875–878 (1996)
5. Booth, D.M., Murphy, J.A., Mukherjee, R., Awais, M., Neoptolemos, J.P., Gerasimenko, O.V., Tepikin, A.V., Petersen, O.H., Sutton, R., Criddle, D.N.: Reactive oxygen species induced by bile acid induce apoptosis and protect against necrosis in pancreatic acinar cells. Gastroenterology 140, 2116–2125 (2011)
6. Meyrignac, O., Lagier, S., Bourret, B., Mokrane, F.Z., Buscail, L., Rousseau, H., Otel, P.: Acute pancreatitis: extrapancreatic necrosis volume as early predictor of severity. Radiology. 276, 119–128 (2015)
7. Perides, G., Laukkarien, J.M., Vassileva, G., Steer, M.L.: Biliary acute pancreatitis in mice is mediated by the G-protein-coupled cell surface bile acid receptor Gpbar1. Gastroenterology. 138, 715–725 (2010)
8. Gerasimenko, J.V., Flowerdew, S.E., Voronina, S.G., Sukhomlin, T.K., Tepikin, A.V., Petersen, O.H., Gerasimenko, O.V.: Bile acids induce Ca (2+) release from both the endoplasmic reticulum and acidic intracellular calcium stores through activation of inositol trisphosphate receptors and ryanodine receptors. J. Biol. Chem. 281, 40154–40163 (2006)
9. Voronina, S.G., Gryshchenko, O.V., Gerasimenko, O.V., Green, A.K., Petersen, O.H., Tepikin, A.V.: Bile Acids Induce a Cationic Current. Depolarizing Pancreatic Acinar Cells and Increasing the Intracellular Na+ Concentration. J. Biol. Chem. 280, 1764–1770 (2005)
10. Booth, D.M., Mukherjee, R., Sutton, R., Cridde, D.N.: Calcium and reactive oxygen species in acute pancreatitis: friend or foe? Antioxid. Redox Signal. 15, 2683–2698 (2011)
11. Mukherjee, R., Mareninova, O.A., Odinokova, I.V., Huang, W., Murphy, J., Chovan, M., Javed, M.A., Wen, L., Booth, D.M., Cane, M.C., Awaiz, M., Gaviíllet, B., Pruss, R.M., Schaller, S. Molkentin, J.D., Tepikin, A.V., Petersen, O.H., Pandol, S.J., Gukovsky, I., Cridde, D.N., Gukovskaya, A., Sutton, R.: Mechanism of mitochondrial permeability transition pore induction and damage in the pancreas: inhibition prevents acute pancreatitis by protecting production of ATP. Gut 65, 1333–1346 (2016)
12. Cavada, B.S., Barbosa, T., Arruda, S., Grangeiro, T.B., Barral-Netto, M.: Revisiting proteins: do minor changes in lectin structure matter in biological activity? Lessons from and potential biotechnological uses of the Diocleinae subtribe lectins. Curr. Protein. Pept. Sci. 2, 123–135 (2001)
13. Cavada, B.S., Osterne, V.J.S., Lossio, C.F., Pinto-Junior, V.R., Oliveira, M.V., Silva, M.T.L., Leal, R.B., Nascimento. K.S.: One century of ConA and 40 years of ConBr research: A structural review. Int. J. Biol. Macromol. 1, 901–911 (2019)
14. Lin, S.S., Levitan, I.B.: Concanavalin A: a tool to investigate neuronal plasticity. Trends Neurosci. 14, 273–277 (1991)
15. Scherer, W.J., Udin, S.B.: Concanavalin A reduces habituation in the tectum of the frog. Brain Res. 667, 209–215 (1994)
16. Everts, J., Petroski, R., Kielzelskien, P., Teichberg, V.L., Heinemann, S.F., Hollmann, M.: Lectin-induced inhibition of desensitization of the kainate receptor GluR6 depends on the activation state and can be mediated by a single native or ectopic N-linked carbohydrate side chain. J. Neurosci. 19, 916–927 (1999)
17. Jacques, A.V., Rieger, D.K., Maestri, M., Lopes, M.W., Peres, T.V., Gonçalves, F.M., Pedro, D.Z., Tasca, C.I., López, M.G., Egea, J., Nascimento, K.S., Cavada, B.S., Leal, R.B.: Lectin from Canavalia brasiliensis (ConBr) protects hippocampal slices against glutamate neurotoxicity in a manner dependent of PI3K/Akt pathway. Neurochem. Int. 62, 836–842 (2013)
18. Liu, B., Li, C.Y. Bian, H.J., Min, M.W., Chen, L.F., Bao. J.K.: Antiproliferative activity and apoptosis-inducing mechanism of Concanavalin A on human melanoma A375 cells. Arch. Biochem. Biophys. 457, 1–6 (2009)
19. Silva, F.O., Santos, P.D., Figueirôa, E.O., Do Melo, C.M., De A., L.N.J.K., Arruda, F.V., Cajazeiras, J.B., Do Nascimento, K.S., Teixeira, E.H., Cavada, B.S., Porto, A.L., Pereira, V.R.: Antiproliferative effect of Canavalia brasiliensis lectin on B16F10 cells. Res. Vet. Sci. 96, 276–282 (2014)
20. Faheina-Martins, G.V., Da Silveira, A.L., Cavalcanti, B.C., Ramos, M.V., Moraes, M.O., Pessoa, C., Aratú, D.M.A.: Antiproliferative effects of lectins from Canavalia ensiformis and Canavalia brasiliensis in human leukemia cell lines. Toxicol. In Vitro. 26, 1161–1169 (2012)
21. Silva, A.F., Matos, M.P., Ralph, M.T., Silva, D.L., De Alencar, N.M., Ramos, M.V., Lima-Filho, J.V.: Comparison of immunomodulatory properties of mannose-binding lectins from Canavalia brasiliensis and Cratyx argentea in a mice model of Salmonella infection. Int. Immunopharmacol. 31, 233–238 (2016)
22. Pinto, N.V., Cavada, B.S., Brito, L.F., Pereira, R.I., Da Silva, M.T., Castro, R.R., Freitas Pires, A., Assreuy, A.M.: Effects of Canavalia lectins on acute inflammation in sensitized and non-sensitized rats. Inflammation 36, 713–722 (2013)
23. Pinto, N.V., Santos, C.F., Cavada, B.S., Do Nascimento, K.S., Pereira, J.F.N., Pires, A.D.F., Assreuy A.M.: Homologous Canavalia lectins elicit different patterns of antiinflammatory responses. Nat. Prod. Commun. 11, 1621–1614 (2013b)
24. Pires, A.F., Assreuy, A.M., Lopes, É.A., Celedônio, N.R., Soares, C.E., Rodrigues, N.V., Sousa, P.L., Benevides, R.G., Nagano, C.S., Cavada, B.S., Leal-Cardoso, J.H., Coelho-De-Souza, A.N., Santos, C.F.: Opioid-like antiinflammatory effects of oral administration of a lectin purified from the seeds of Canavalia brasiliensis. Fundam. Clin. Pharmacol. 27, 201–209 (2013)
25. Carlini, C.R., Guimarães, J.A.: Isolation and characterization of a toxic protein from Canavalia ensiformis (jack bean) seeds, distinct from concanavalin A. Toxicon 19, 667–675 (1981)
26. Moreira, R.A., Cavada, B.S.: Lectin from Canavalia brasiliensis Mart. Isolation, characterization and behavior during germination. Biologia Plantarum. 26, 113–120 (1984)
27. Laukkanen, J.M., Van Acker, G.J., Weiss, E.R., Steer, M.L., Perides, G.A.: Mouse model of acute biliary pancreatitis induced by retrograde pancreatic duct infusion of Na-taurocholate. Gut 56, 1590–1598 (2007)
28. Ouyang, Y., Wen, L., Armstrong, J.A., Chovan, M., Latawiec, D., Cai, W., Awaiz, M., Mukherjee, R., Huang, W., Gough, P.J., Bertin, J., Tepikin, A.V., Sutton, R., Cridde, D.N.: Protective Effects of Necrostatin-1 in Acute Pancreatitis: Partial Involvement of Receptor Interacting Protein Kinase 1. Cells 10(5), 1035 (2021). https://doi.org/10.3390/cells10051035
29. Dawra, R., Ku, Y.S., Sharif, R.: An improved method for extracting myeloperoxidase and determining its activity in the pancreas and lungs during pancreatitis. Pancreas 37, 62–68 (2008)
30. da Silva-Leite K.E.S., Giroão D.K.F.B., de Freitas Pires A., Assreuy A.M.S., de Moraes P.A.F., Cunha A.P., Ricardo N.M.P.S., Cridde D.N., de Souza M.H.L.P., Pereira M.G., Soares P.M.G.: Xenima americana heteropolysaccharides ameliorate inflammation and visceral hypernociception in murine caerulein-induced acute pancreatitis: Involvement of CB2 receptors. Biomed. Pharmacother. 106, 1317–1324 (2018).
31. Huang, W., Booth, D.M., Cane, M.C., Chovan, M., Javed, M.A., Elliott, V.L., Armstrong, J.A., Dingsdale, H., Cash, N., Li, Y., Greenhalw, F., Mukherjee, R., Kaphalia, B.S., Jaffar, M., Petersen, O.H., Tepikin, A.V., Sutton, R., Cridde, D.N.: Fatty acid ethyl ester synthase inhibition ameliorates ethanol-induced Ca2+-dependent mitochondrial dysfunction and acute pancreatitis. Gut 63, 1313–1324 (2014)
32. Opie, E.L., Meakins, J.C.: Data concerning the etiology and pathology of hemorrhagic necrosis of the pancreas (acute hemorrhagic pancreatitis). J. Exp. Med. 11, 561–578 (1909)
33. Shah, A.P., Mourad, M.M., Bramhall, S.R.: Acute pancreatitis: current perspectives on diagnosis and management. J. Inflamm. Res. 11, 77–85 (2018)
34. Leppäniemi, A., Tolonen, M., Tarasconi, A., Segovia-Lohse, H., Gamberini, E., Kirkpatrick, A.W., Ball, C.G., Parry, N., Martelli, S., Wolbrick, D.H., Van Goor, G., Baiocchi, L., Ansalon, W., Biffi, F., Coccolini, S., Di Saverio, Y., Kluger, E., Moore, F. Catena.: WSES guidelines for the management of severe acute pancreatitis. World. J. Emerg. Surg. 14, 2–7 (2019)
35. Aratani, Y.: Myeloperoxidase: Its role for host defense, inflammation, and neutrophil function. Arch. Biochem. Biophys. 640, 47–52 (2018)
36. Lippi, G., Valentino, M., Cervellini, G.: Laboratory diagnosis of acute pancreatitis: in search of the holy grail. Crit. Rev. Clin. Lab. Sci. 49, 18–31 (2012)
37. Kompaniens, G., Hahn, A., Komolafe, O., Pereira, S.P., Davidson, B.R., Gurusamy K.S.: Serum amylose and lipase and urinary trypsinogen and amylose for diagnosis of acute pancreatitis. Cochrane Database Syst. Rev. 4, CD012010 (2017)
38. Huang, W., Cane, M.C., Mukherjee, R., Szatmary, P., Zhang, X., Elliott, V., Ouyang, Y., Chovan, M., Latawiec, D., Wen, L., Booth, D.M., Haynes, A.C., Petersen, O.H., Tepikin, A.V., Cridde, D.N., Sutton, R.: Caffeine protects against experimental acute pancreatitis
by inhibition of inositol 1,4,5-trisphosphate receptor-mediated Ca2+ release. Gut. 66, 301–313 (2017)
39. Assreuy, A.M., Shibuya, M.D., Martins, G.J., De Souza, M.L., Cavada, B.S., Moreira, R.A., Oliveira, J.T., Ribeiro, R.A., Flores, C.A.: Anti-inflammatory effect of glucose-mannose binding lectins isolated from Brazilian beans. Mediators Inflamm. 6, 201–210 (1997)
40. Silva, A.F., Matos, M.P., Ralph, M.T., Silva, D.L., de Alencar, N.M., Ramos, M.V., Lima-Filho, J.V.: Comparison of immunomodulatory properties of mannose-binding lectins from Canavalia brasiliensis and Cratylia argentea in a mice model of Salmonella infection. Int Immunopharmacol. 31, 233–238 (2016)
41. Criddle, D.N., Ratary, M.G.T., Neoptolemos, J.P., Tepikin, A.V., Petersen, O.H., Sutton, R.: Ethanol toxicity in pancreatic acinar cells: Mediation by nonoxidative fatty acid metabolites. PNAS 10, 10738–10743 (2004)
42. Criddle, D.N., Gerasimenko, J.V., Baumgartner, H.K., Jaffar, M., Voronina, S., Sutton, R., Petersen, O.H., Gerasimenko, O.V.: Calcium signalling and pancreatic cell death: apoptosis or necrosis? Cell Death Differ. 14, 1285–1294 (2007)
43. Mareninova, O.A., Sung, K.F., Hong, P., Lugea, A., Pandol, S.J., Gukovsky, I., Gukovskaya, A.S.: Cell death in pancreatitis: caspases protect from necrotizing pancreatitis. J. Biol. Chem. 281, 3370–3381 (2006)
44. Szatmary, P., Liu, T., Abrams, S.T., Voronina, S., Wen, L., Chvanov, M., Huang, W., Wang, G., Criddle, D.N., Tepikin, A.V., Toh, C.H., Sutton, R.: Systemic histone release disrupts plasmalemma and contributes to necrosis in acute pancreatitis. Pancreatology 17, 884–892 (2017)
45. Lei, H.Y., Chang, C.: Lectin of Concanavalin A as an anti-hepatoma therapeutic agent. J. Biomed. Sci. 16, 10 (2009)
46. Russi, M.A., Vandresen-Filho, S., Rieger, D.K., Costa, A.P., Lopes, M.W., Cunha, R.M., Teixeira, E.H., Nascimento, K.S., Cavada, B.S., Tasca, C.I., Leal, R.B.: ConBr, a lectin from Canavalia brasiliensis seeds, protects against quinolinic acid-induced seizures in mice. Neurochem. Res. 37, 288–297 (2012)
47. Jonas, L., Ostwald, C., Griethe, W., Letko, G.: Light and electron microscopic studies of the lectin binding on the glycoalix of rat pancreatic cells. I. Normal tissue and isolated cells. Acta histochem. 91, 213–214 (1991)
48. Jonas, L., Putzke, H.P.: Light and electron microscopic studies of lectin binding to the glycoalix of rat pancreatic cells. II. Light microscopic changes after induction of an olive-oil pancreatitis. Acta histochem. 93, 388–396 (1992)
49. Mishra, A., Behura, A., Mawatwal, S., Kumar, A., Naik, L., Mohanty, S.S., Manna, D., Dokania, P., Mishra, A., Patra, S.K., Dhim, R.: Structure-function and application of plant lectins in disease biology and immunity. Food Chem Toxicol. 134: 110827 (2019)
50. Assreuy, A.M., Fontenele, S.R., Pires, A.D.F., Fernandes, D.C., Rodrigues, N.V., Bezerra, E.H., Moura, T.R., Do Nascimento, K.S., Cavada B.S.: Vasodilator effects of Diocleinae lectins from the Canavalia genus. Naunyn-Schmied Arch. Pharmacol. 380, 509–521 (2009)
51. Burgess, A., Monorn, J.P., De Saint-Basile, G., Callebaut, I.: A concanavalin A-like lectin domain in the CHS1/LYST protein, shared by members of the BEACH family. Bioinformatics 25, 1219–1222 (2009)
52. Delatorre, P., Rocha, B.A.M., Gadelha, C.A.A., Santi-Gadelha, T., Cajaizeiras, J.B., Souza, E.P., Nascimento, K.S.: Crystal structure of a lectin from Canavalia maritima (ConM) in complex with trehalose and maltose reveals relevant mutation in ConA-like lectins. J. Struct. Biol. 154, 280–286 (2006)
53. Delatorre, P., Rocha, B.A.M., Souza, E.P., Oliveira, T.M., Bezerra, G.A., Moreno Freitas F.B.B.T.: Structure of a lectin from Canavalia gladiata seeds: new structural insights for old molecules. BMC Struct. Biol. 7, 52–60 (2007)
54. Agrawal, B.B.L., Goldstein, L.J.: Specific binding of concanavalin A to cross linked dextran gels. Biochem. J. 96, 23–25 (1965)
55. Andrade, J.L., Arruda, S., Barbosa, T., Palm, L., Ramos, M.V., Cavada, B.S.: Lecitin-induced nitric oxide production. Cell Immunol. 194, 98–102 (2009)
56. Barauna, A.C., Kaster, M.P., Heckert, B.T., Nascimento, K.S., Rossi, F.M., Teixeira, E.H., Cavada, B.S., Rodrigues, A.L.S., Leal, R.B.: Antidepressant-like effect of lectin from Canavalia brasiliensis (ConBr) administered centrally in mice. Pharmacol Biochem Behav 85, 160–169 (2006)
57. Barbosa, T., Arruda, S., Cavada, B.S., Grangeiro, T.B., Freitas, L.A.R., Barral Netto, M.: In vivo lymphocyte activation and apoptosis by lectins of the Diocleinae subtribe. Mem. Inst. Oswaldo Cruz 96, 673–678 (2001)

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.