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Scientific Article

Acute toxicity study of bioactive galactomannans from seeds of two non-traditional leguminosae

Estudo de toxicidade aguda de galactomannas bioativas de sementes de duas leguminosas não tradicionais

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ABSTRACT - Non-traditional galactomannans sources are widely cultivated in the Brazilian Northeast Region, such as the species *Caesalpinia pulcherrima* (peacock flower) and *Delonix regia* (flamboyant). The galactomannans GM-CP and GM-DR were extracted from the respective seeds and are being studied as potential therapeutic agents, but systematic evaluations on their acute toxicity are yet to be reported. Groups of three female rats received oral GM-CP or GM-DR (300 mg kg^{-1}), whereas a control group received vehicle (saline). Since there was no lethality, other groups received doses of 2000 mg kg^{-1}, which also did not cause lethality. Organs and blood samples were collected on day 14. Mechanical hypernociception and inflammatory cell influx were measured in other groups receiving intra-articular doses of GM-CP or GM-DR (200 µg, n = 5 per group). Neither galactomannan evoked physiological / behavioral changes or joint inflammation. Since the LD50 was less than 2000 mg kg^{-1}, such polysaccharides may be allocated in the class 5 of the Globally Harmonized System for Classification and Labelling of Chemicals.

Keywords: Polysaccharide. Galactomannan. Safety. Peacock flower. Flamboyant.

RESUMO - Fontes não tradicionais de galactomananas são amplamente cultivadas na Região Nordeste do Brasil, como as sementes das espécies ornamentais *Caesalpinia pulcherrima* (flor-de-pavão) e *Delonix regia* (flamboiã). As galactomananas GM-CP e GM -DR, extraídas respectivamente dessas sementes, estão sendo estudadas como potenciais agentes terapêuticos, mas avaliações sistemáticas sobre a toxicidade aguda das mesmas ainda não foram relatadas. Grupos de três ratas receberam GM-CP ou GM-DR oral (300 mg kg^{-1}), enquanto um grupo controle recebeu veículo (solução salina). Como não houve letalidade, outros grupos receberam doses de 2000 mg kg^{-1}, o que também não causou letalidade. A coleta de órgãos e amostras de sangue foi feita no dia 14. A hipernocicepção mecânica e o influxo de células inflamatórias foram medidos em outros grupos que receberam doses intra-articulares de GM-CP ou GM-DR (200 µg, n = 5 por grupo). Ambas as galactomananas não evocaram alterações fisiológicas/comportamentais ou inflamação articular. Como a DL50 não foi inferior a 2000 mg kg^{-1}, tais polissacarídeos podem ser alocados na classe 5 do Sistema Globalmente Harmonizado para Classificação e Rotulagem de Produtos Químicos.

Palavras-chave: Polissacarídeo. Galactomanana. Segurança. Flor-de-pavão. Flamboiã.

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INTRODUCTION

Galactomannans are neutral polysaccharides commonly used in the food, cosmetic and pharmaceutical industries due to viscoelastic properties in aqueous dispersions. They are constituted by a (1→4) linked β-mannopyranosyl backbone partially substituted at O-6 with α-D-galactopyranosyl side groups (SHARMA et al., 2022) and are obtained from seed endosperm of Leguminosae species, especially *Ceratonia siliqua*, *Caesalpinia spinosa*, *Cyamopsis tetragonolobus* and *Trigonella foenum-graecum* (JOHNSON et al., 2015; WANG et al., 2023) (Figure 1). However, non-traditional sources of galactomannans are widely cultivated in the Brazilian Northeast Region, such as the ornamental species *Caesalpinia pulcherrima* (L.)Sw. and *Delonix regia* (Bojer ex Hook.)Raf. (CERQUEIRA et al., 2009; MODI et al., 2016).

Biomedical applications for *C. pulcherrima* and *D. regia* galactomannans have been reported, such as dietary fiber, preparation of mucoadhesive microspheres, drug delivery systems, injectable regenerative hydrogels and edible films and coatings (BURITI et al., 2014; OGUNJIMI et al., 2017; LIMA et al., 2019, JOSEPH et al., 2020; CHEL-GUERRERO et al., 2024). Moreover, oral administration of *C. pulcherrima* galactomannan protects mice against indomethacin-induced gastric lesions, and the local administration of *D. regia* galactomannan inhibits cartilage damage in rats subjected to monoiodoacetate-

induced osteoarthritis in rat tibiotarsal joints, besides promoting healing of excisional cutaneous wounds in mice (MARQUES et al.,2019; NASCIMENTO et al., 2021; LIMA et al., 2022).

Although the galactomannan intake is conventionally assumed as safe, there are few toxicological studies focusing on such polysaccharides (PAWAR; LALITHA, 2015; SURYAWANSHI et al., 2015; DESHPANDE; MOHAN; THAKURDESAI, 2016). In order to ensure the safety of chemicals, international protocols have been preconized by the Organization for Economic Co-operation and Development (OECD). Using the Guideline No. 423, this study aimed to investigate the occurrence of harmful events elicited by *C. pulcherrima* and *D. regia* galactomannans after administration via single oral route. Additionally, an approach for intra-articular reactions was also performed.

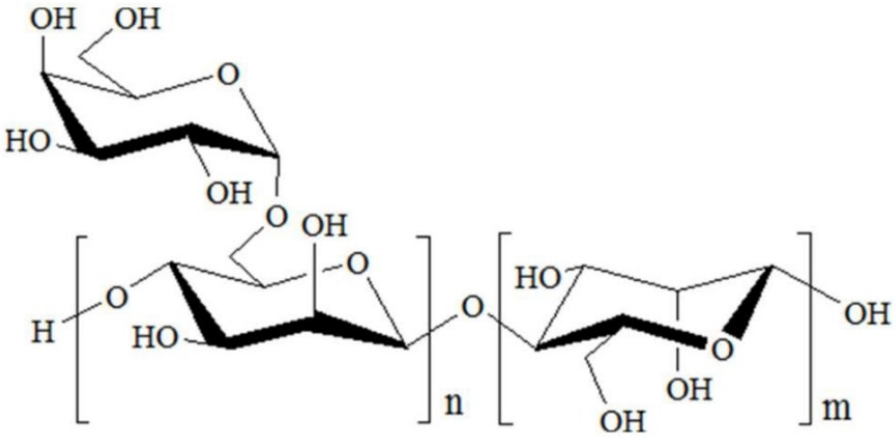

Figure 1. Structural representation of a typical galactomannan segment. m = non-substituted mannose residues in the backbone; n = partially substituted at O-6 with α-D-galactopyranosyl side groups (WANG et al., 2023).

MATERIAL AND METHODS

Seed collection

Seeds of *Delonix regia* and *Caesalpinia pulcherrima* were collected in Limoeiro do Norte, Ceará, Brazil (5.1467° S, 38.0965° W). Voucher specimens were deposited at the Prisco Bezerra Herbarium / Federal University of Ceará (numbers 57951 and 57952, respectively). In accordance with the Brazilian Federal Law No. 13123/2015, the assessment activity was registered at the National System for the Management of the Genetic Heritage and the Associated Traditional Knowledge (Code A54ED41).

Galactomannan isolation

The galactomannans of *C. pulcherrima* (GM-CP) and *D. regia* (GM-DR) were obtained by following procedures previously described (MARQUES et al., 2019; NASCIMENTO et al., 2021). Briefly, seeds (50 g) were swelled under water vapor (1 atm, 30 min) in order to allow manual removal of endosperm, which was homogenized with water (500 mL). Supernatant was collected after an overnight period of decantation, and the remaining fragments were resubjected to homogenization. Both supernatants were mixed with two volumes of ethanol in order to precipitate the crude galactomannans, which were filtered under vacuum, washed with ethanol and acetone, and dried until reaching constant weight. Proteins were removed through alkaline hydrolysis under reflux (1 g of sample in 200 mL of 5% (w/v) NaOH, 85 -90 °C, 45 min), followed by dialysis against water for 72 h (cut off 10000 g mol^{-1}) and centrifugation (1000 x *g*, 15 min). The purified galactomannan was recovered by addition of ethanol and filtration under vacuum.

Animals and protocols

Female Wistar rats (0.170 - 0.200 kg) were maintained under standardized conditions (25 °C; cycle of 12 h light/12 h dark, and food and water *ad libitum*). A total of 36 animals were used. The procedures were conducted according to the guidelines of the National Council for the Control of Animal Experimentation (CONCEA), authorized by the Ethics Committee for Animal Research / Federal University of Ceará (CEUA/UFC, No. 52120510/2018).

Following the guideline No. 423 (OECD, 2002), groups of 3 animals were subjected to 2-hour fasting, and received a single dose of GM-CP or GM-DR (300 mg kg^{-1} *per os*). Control group received vehicle (10 mL kg^{-1}; sterile saline). Fasting was maintained until 2 hours after administration. Since lethality was absent, other groups received higher doses of galactomannans (2000 mg kg^{-1} *per os*).

Body weight, toxicity signals and spontaneous exploratory activity were monitored for 14 days (MALONE, 1977). Animals were anesthetized with xylazine / ketamine (10 / 100 mg kg^{-1} i.p.) for blood collection and euthanasia with removal of organs (kidneys, spleen, lungs, heart, liver and brain). Blood cells count and hemoglobin content were determined by automated hematology analyzer (SDH-3, Labtest, Brazil). Labtest Diagnostics Kits were used for measurement of serum urea, creatinine, total cholesterol, triglycerides, bilirubin, total proteins and albumin, as well as enzyme activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase. Organs were embedded in paraffin for hematoxylin & eosin staining (4-µm-thick), and examined under light microscopy by an observer without knowledge of the treatments.

Administration of GM-CP or GM-DR (200 µg / 25 µL

saline; n=5) was done in the right tibiotarsal joints of rats (n=5 per group). Control group received vehicle. Animals were individually placed into Plexiglas® boxes with malleable mesh net floors, and joint flexion was achieved by pressing an algesimeter-coupled large probe (4.15 mm^2) until the occurrence of paw withdrawal reflex. Reduced mechanical threshold is indicative of hypernociception (BRINGEL et al., 2020). Animals were euthanized at hour 6, and joint exudates were obtained by washing with 2 x 0.1 mL sodium EDTA (10 mmol L^{-1}), dissolved in saline-phosphate buffer. Total and differential cell counts were done using Neubauer chamber and stained smears, respectively.

Statistical analysis

Parametric data were expressed as mean ± standard error of mean. Statistical differences between parametric data were determined by one-way / two-way analysis of variance, followed by the Bonferroni's test. P <0.05 was considered significant. GraphPad Prism v. 5.00 was used for data analysis and graph creation.

RESULTS AND DISCUSSION

Safety of chemicals is an important issue for biomedical applications. In this study, the bioactive galactomannans of *Caesalpinia pulcherrima* (GM-CP) and *Delonix regia* (GM-DR) had their acute toxicity systematically evaluated in rodents after administration through oral or intra-articular routes.

Chemical characterization of GM-CP and GM-DR were published elsewhere (MARQUES et al., 2019; NASCIMENTO et al., 2021). Briefly, the peak molar weights were estimated by high-performance size-exclusion chromatography, and displayed magnitude orders of 10^6 and 10^5 g mol^{-1}, respectively. Fourier Transform Infrared spectroscopy in KBr pellets revealed typical polysaccharides signals; for GM-CP: 3000-3490 cm^{-1} (O-H), 1016-1074 cm^{-1} (C-O) (alcohol), 1159 cm^{-1} (C-O-C) glycosidic linkage and 2923 cm^{-1} (C-H); for GM-DR: 3403 cm^{-1} (O-H), 2920 cm^{-1} (C-H), 1158 cm^{-1} (C-O) flexion in the pyranose ring, and the 1160-950 cm^{-1} region, which includes signals for vibration of glycosidic C-O and C-O-C and primary alcohol C-O-H bonds. Thermogravimetric analysis revealed low levels of inorganic residues in the samples (0.24% for GM-CP and 0.56% for GM -DR). As expected for hygroscopic polysaccharides, moisture after desiccation was 14.76% and 13.78%, respectively. Gas chromatography revealed that GM-CP was composed of mannose (64.5%), galactose (29.1%) and glucose (7.4%), whereas GM-DR contained mannose (64.5%), galactose (27.0%) and glucose (8.4%); respective mannose:galactose ratios were 2.18:1 and 2.39:1, which were similar to signal intensities at 4.73 ppm (H-1 of β-D-mannopyranose) and 5.02 ppm (H-1 of α-D-galactopyranose) measured through ^{1}H-Nuclear Magnetic Resonance spectroscopy. Protein content was below the detection limit (<0.40%), as determined by elemental nitrogen analysis.

No lethality was observed after high oral doses of GM-CP or GM-DR, so that their median lethal doses (LD50) are > 2000 mg kg^{-1}. Although LD50 has been previously reported for other seed galactomannans, including those from the species *Senna tora*, *Trigonella foenum-graecum*, *Caesalpinia ferrea*, and even *C. pulcherrima* (LIJU et al., 2015; PAWAR; LALITHA, 2015; SURYAWANSHI et al., 2015; DESHPANDE; MOHAN; THAKURDESAI, 2016), this study also focused on effects over target organs or biochemical / hematological parameters. Nevertheless, studies regarding GM-DR toxicology are yet to be performed.

The animals treated with higher doses of galactomannans had body weight increase similar to those in the control group (GM-CP: + 1.0%; GM-DR: + 1.0%; control: + 2.75%, p>0.05) (Table 1). Spontaneous exploratory activity significantly decreased along the experiment, but it was not related to the treatments (Figure 2). Exophthalmos was observed in the control group 1h after vehicle administration, as well as in the GM-CP at some timepoints (60 min, day 7 and day 14) and in the GM-DR group at day 14. However, all animals displayed normal respiration rate, vibrissae movement, lacrimation and salivation, and absence of cyanosis, piloerection, analgesia / sedation, ataxia, prostration, catatonia, tail erection, stereotyped movements, tremors or convulsion was observed.

Table 1. Systemic administration of *Caesalpinia pulcherrima* and *Delonix regia* galactomannans does not alter the body weight of female rats.

Timepoint	Body weight (x 10^{-3} kg)		
	Control	GM-CP	GM-DR
60 min	182± 4	182± 3	184± 7
Day 7	183± 3	182± 4	186± 7
Day 14	187± 7	185± 5	186± 7

GM-CP = *Caesalpinia pulcherrima* galactomannan (2000 mg kg^{-1} *per os*), GM-DR = *Delonix regia* galactomannan (2000 mg kg^{-1} *per os)*. Mean ± standard error of mean (n = 3 / group). Sources of variations (interaction P=0.0968, timepoint P=0.6109, treatment P=0.2810 and matching P<0.0001) (Two-way ANOVA, Bonferroni).

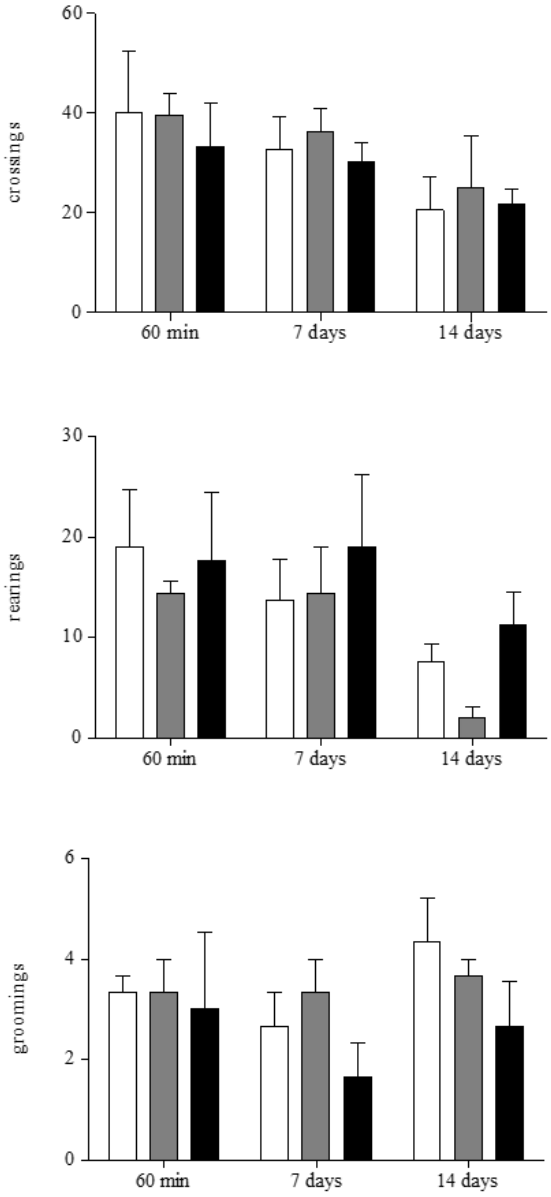

Figure 2. Systemic administration of *Caesalpinia pulcherrima* and *Delonix regia* galactomannans does not alter spontaneous exploratory activity of female rats. Animals received orally vehicle (control, □), *Caesalpinia pulcherrima* (2000 mg kg^{-1} *per os,* ■) or *Delonix regia* galactomannnan (2000 mg kg^{-1} *per os,* ■). Mean ± standard error of mean (n = 3 / group. Sources of variation for crossings (interaction P=0.9759, timepoint P=0.0291, treatment P=0.8130 and matching P=0.0682), rearings (interaction P=0.8836, timepoint P=0.0354, treatment P=0.3747 and matching P=0.4190), and groomings (interaction P=0.7501, timepoint P=0.2274, treatment P=0.4334 and matching P=0.1105) (Two-ANOVA, Bonferroni).

The weights of organs were not altered by the treatments (Table 2). Although no histopathological lesions were reported (Figure 3), some functional events could arise prior to morphological damage, but seric levels of creatinine/urea and ALT/AST or creatinine/urea remained normal (Table 3). It is known that fenugreek seeds galactomannan intake did not alter cytokines (TNF-alpha and IL-6), reactive oxygen/nitrogen species, superoxide dismutase or malondialdehyde, in either liver or kidney tissues (ALSULIAM et al., 2022). Absence of inflammatory reactions may be inferred to *C. pulcherrima* and *D. regia* galactomannans, so that renal and liver parameters were preserved. All animals displayed blood glucose levels >140 mg dL^{-1} (control: 143±4 mg dL^{-1}; GM-CP: 182±4 mg dL^{-1}; GM-DR: 198±8 mg dL^{-1}, $p<0.05$ *vs* control), but post-prandial capillary hyperglycemia is not uncommon, especially under xylazine/ketamine anesthesia (WANG et al., 2010). Since antidiabetic activity by *Caesalpinia ferrea* galactomannan was demonstrated in rats subjected to streptozotocin (CUNHA et al., 2017), hyperglycemia could be due to experimental procedures, rather than the galactomannans intake. Moreover, substances affecting bone marrow could inhibit certain steps in the production of hemoglobin, reducing the ability of the blood to distribute oxygen throughout the body (JAIN et al., 2009), but both galactomannans did not change the hematological parameters, so that erythrocytes were both normocytic and normochromic (Table 4).

Table 2. Systemic administration of *Caesalpinia pulcherrima* and *Delonix regia* galactomannans does not alter the weight of organs of female rats.

Organ	Weight (x 10^{-3} kg)			
	Control	GM-CP	GM-DR	P
Brain	1.84 ± 0.06	1.93 ± 0.03	1.86 ± 0.14	0.7712
Heart	0.74 ± 0.06	0.69 ± 0.02	0.73 ± 0.02	0.3948
Kidneys	1.43 ± 0.07	1.52 ± 0.07	1.37 ± 0.03	0.7310
Liver	6.54 ± 0.19	6.28 ± 0.01	6.01 ± 0.38	0.7658
Lungs	1.67 ± 0.18	1.51 ± 0.13	1.56 ± 0.11	0.7401
Spleen	0.56 ± 0.06	0.50 ± 0.03	0.53 ± 0.04	0.3009

GM-CP = *Caesalpinia pulcherrima* galactomannan (2000 mg kg^{-1} *per os*), GM-DR = *Delonix regia* galactomannan (2000 mg kg^{-1} *per os*). Mean ± standard error of mean (n = 3 / group, one-way ANOVA, Bonferroni).

Table 3. Systemic administration of *Caesalpinia pulcherrima* and *Delonix regia* galactomannans does not alter serum markers of female rats.

Parameter	unit	Control	GM-CP	GM-DR	P
Alkaline phosphatase	U L^{-1}	70 ± 34	136 ± 53	70 ± 12	0.5769
ALT	U L^{-1}	95 ± 4	74 ± 2	74 ± 13	0.1833
AST	U L^{-1}	28 ± 3	35 ± 1	33 ± 2	0.1365
Albumin	mg dL^{-1}	4.53 ± 0.34	5.14 ± 0.29	5.41 ± 0.19	0.0783
Bilirubin	mg dL^{-1}	0.07 ± 0.03	0.04 ± 0.03	0.20 ± 0.10	0.4702
Creatinine	mg dL^{-1}	0.66 ± 0.02	0.67 ± 0.10	0.57 ± 0.03	0.1790
Glucose	mg dL^{-1}	143 ± 14	182 ± 4	198 ± 8*	0.0285
Protein	mg dL^{-1}	10.83 ± 0.00	11.93 ± 0.43	11.50 ± 0.34	0.1570
Total cholesterol	mg dL^{-1}	62 ± 4	68 ± 7	68 ± 9	0.7676
Triglycerides	mg dL^{-1}	128 ± 1	119 ± 14	137 ± 2.	0.3515
Urea	mg dL^{-1}	75 ± 2	78 ± 3	69 ± 3	0.1610

GM-CP = *Caesalpinia pulcherrima* galactomannan (2000 mg kg^{-1} *per os*), GM-DR = *Delonix regia* galactomannan (2000 mg kg^{-1} *per os*). Mean ± standard error of mean (n = 3 / group). *P<0.05 *vs.* control (One-way ANOVA, Bonferroni).

Table 4. Systemic administration of *Caesalpinia pulcherrima* and *Delonix regia* galactomannans does not alter the hematological parameters of female rats.

Parameters	Unit	Control	GM-CP	GM-DR	P
Red Blood Cells	x 10^{6} μL^{-1}	8.41 ± 0.14	8.24 ± 0.10	8.10 ± 0.16	0.3422
Hemoglobin	g dL^{-1}	14.0 ± 0.83	13.8±0.1	13.8 ± 0.1	0.8543
Hematocrit	%	49.71 ± 0.39	48.38 ± 0.40	45.3 ±1.78	0.0648
MCV	fL	60 ± 0	58 ± 0	59 ± 1	0.3499
MCH	pg	16.6 ± 0.3	16.8 ± 0.3	17.1 ± 0.3	0.4858
MHCH	g dL^{-1}	28.0 ± 0.4	28.5 ± 0.2	30.6 ± 1.4	0.1553
RBC distribution	%	14.0 ± 0.2	15.5 ± 0.8	13.7 ± 0.6	0.7623
Platelets	x 10^{3} μL^{-1}	1784 ± 86	1789 ± 22	1658 ± 100	0.2844
Platelet Mean Volume	fL	7.2 ± 0.1	7.0 ± 0.1	7.2 ± 0.1	0.2783
Platelet distribution	%	36.1 ± 0.5	36.0 ± 0.5	36.0 ± 1.20	0.9183
Leukocytes (total count)	x 10^{3} μL^{-1}	10.41 ± 1.36	9.36 ± 1.64	8.43 ± 0.30	0.504
Granulocytes	x 10^{3} μL^{-1}	2.01 ± 0.38	1.66 ± 0.51	0.80 ± 0.05	0.1352
Lymphocytes	x10^{3} μL^{-1}	7.74 ± 1.05	6.98 ± 0.73	6.93 ± 0.33	0.7240
Monocytes, basophiles and eosinophiles	10^{3} μL^{-1}	0.66 ± 0.05	0.63 ± 0.10	0.69 ± 0.08	0.8509

GM-CP = *Caesalpinia pulcherrima* galactomannan (2000 mg kg^{-1} p.o.), GM-DR = *Delonix regia* galactomannan (2000 mg kg^{-1} p.o.). MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; RBC = red blood cells distribution. Mean ± standard error of mean (n = 3 / group, one-way ANOVA, Bonferroni).

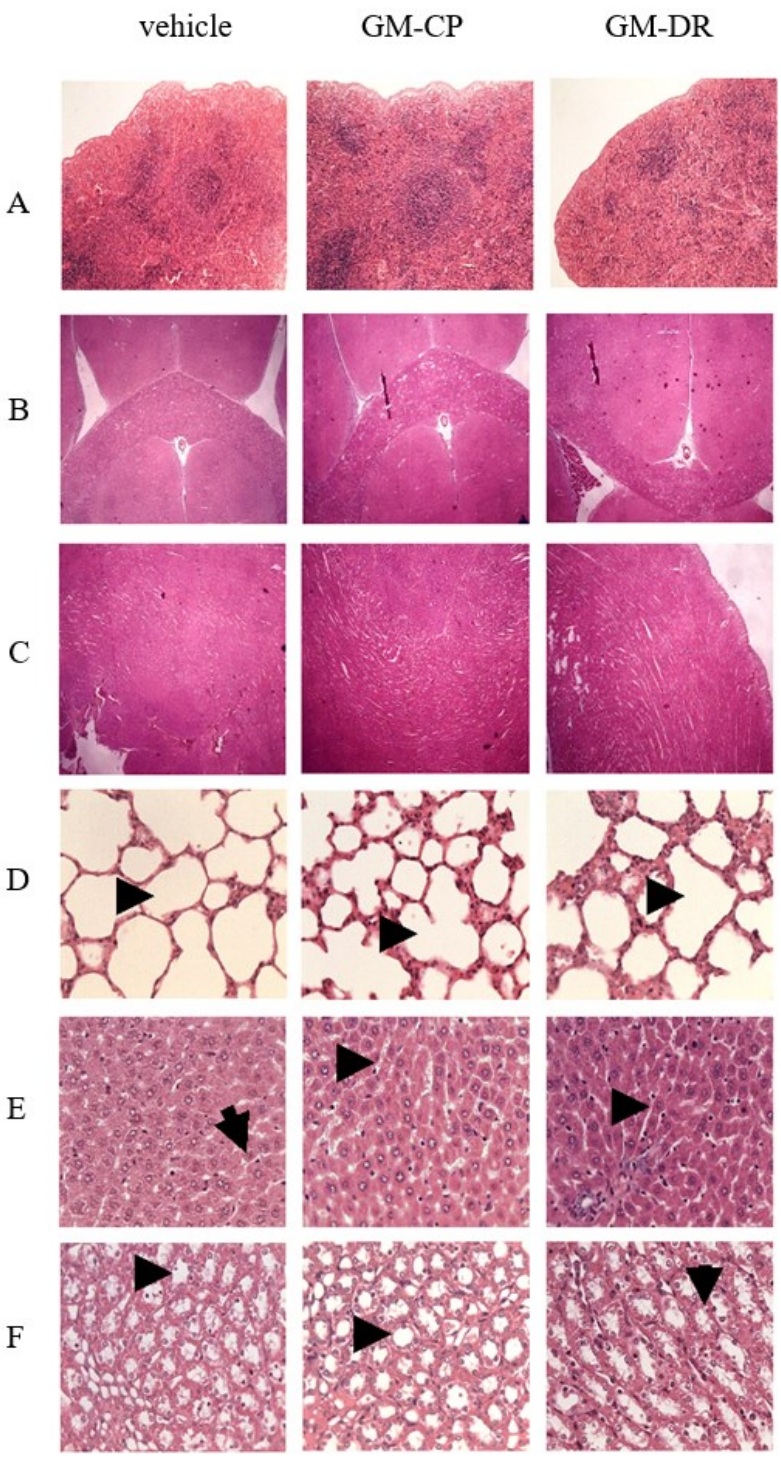

Figure 3. Photomicrographs of organs from female rats treated with Systemic administration of *Caesalpinia pulcherrima* and *Delonix regia* galactomannans. (A) Spleen with normal architecture (capsule, red and white pulps). (B) Brain, showing a normal section with the central channel surrounded by the grey matter. (C) Heart. (D) Lung with normal alveolar sacs (arrow). (E) Liver with hepatocytes arranged in thin plates and sinusoids (arrow). (F) Kidney with normal architecture of proximal tubules (arrow) and absence of inflammation or necrosis. H&E staining. Magnification of 100 x (A, B, C) or 400 x (D, E, F). vehicle= control group, GM-CP = *Caesalpinia pulcherrima* galactomannan (2000 mg kg^{-1} *per os*.), GM-DR = *Delonix regia* galactomannan (2000 mg kg^{-1} *per os*).

In comparison with vehicle-treated animals, the intra-articular administration of galactomannans did not alter the mechanical threshold for the paw withdrawal reflex (Figure 4). Besides absence of hypernociception, no inflammatory cell influx was detected (vehicle: 111 ± 43 cells / mm^3; GM-CP: 62 ± 45 cells / mm^3; GM-DR: 61 ± 44 cells / mm^3).

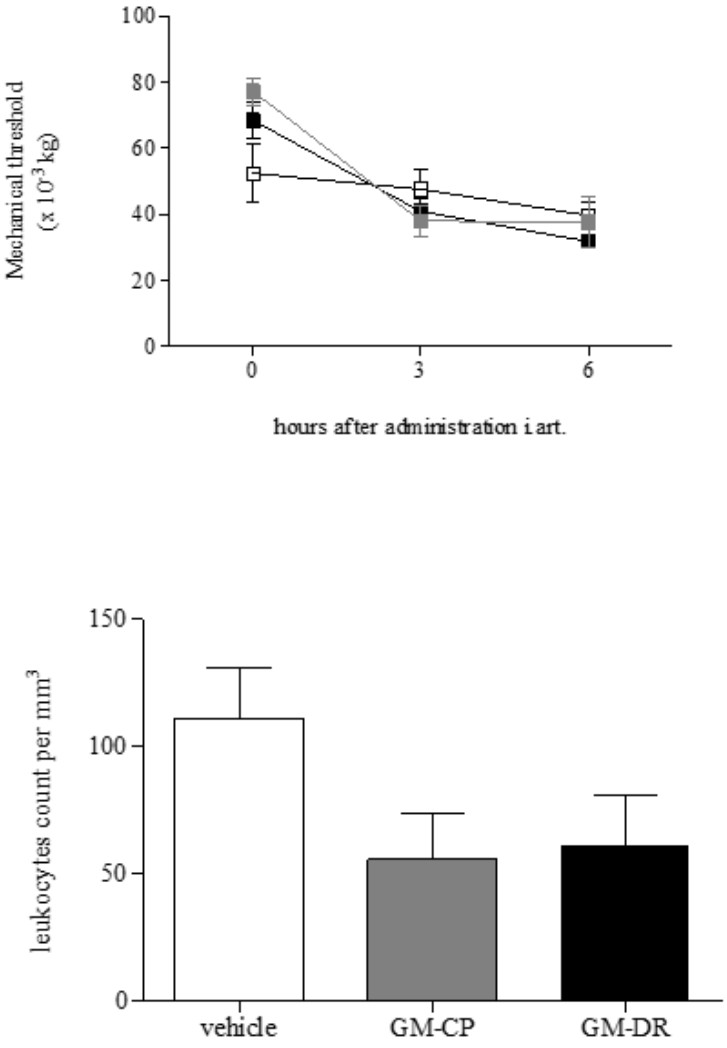

Figure 4. Galactomannans of *Caesalpinia pulcherrima* or *Delonix regia* do not evoke inflammatory reactions in joints of female rats. Animals received intra-articular injection of vehicle (25 µL, □), *Caesalpinia pulcherrima* (200 µg / 25 µL, ■) or *Delonix regia* galactomannnan (200 µg / 25 µL, ■). Mean ± standard error of mean (n = 5 /group). Sources of variation for hypernociception (interaction P=0.0222, timepoint P<0.0001, treatment P=0.6625 and matching P=01656) (Two-ANOVA, Bonferroni). P=0.1257 for cell influx (One-way ANOVA, Bonferroni).

CONCLUSION

The lack of systemic and local reactions revealed that the *Caesalpinia pulcherrima* and *Delonix regia* galactomannans are innocuous, and may be allocated in the class 5 of the Globally Harmonized System for Classification and Labelling of Chemicals (LD50 > 2000 mg kg^{-1}) (UN, 2019), so that our findings are useful in order to promote exploitation of species well adapted to the Caatinga biome and consequent regional development.

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