

Acute Toxicity Study of Bioactive Galactomannans From Two Non-Traditional Leguminosae Sources

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Abstract

Galactomannans from *Caesalpinia pulcherrima* (GM-CP) and *Delonix regia* (GM-DR) are being pointed as potential therapeutic agents, but systematic evaluations on their acute toxicity are yet to be reported. In order to evaluate the occurrence of systemic toxicity, groups of three female rats received oral GM-CP or GM-DR (300 mg kg⁻¹), whereas the control group received vehicle. Since lethality was absent, other groups received doses of 2,000 mg kg⁻¹, which also did not cause lethality. Collection of organs and blood samples were done at day 14. Dermal toxicity of the galactomannans was also evaluated (2,000 mg kg⁻¹, n=3 per group), as well both mechanical hypernociception and inflammatory cell influx after administration of GM-CP or GM-DR via intra-articular route (200 µg, n = 5 per group). At the routes and doses employed, both galactomannans did not evoke physiological / behavioral changes or skin / joint inflammation. Since the LD50 was not inferior to 2,000 mg kg⁻¹, both galactomannans are in the class 5 of the Globally Harmonized System for Classification and Labelling of Chemicals.

Introduction

Galactomannans are water-soluble neutral polysaccharides found in the seed endosperm of numerous plants, particularly in the Leguminosae family. They are constituted by a (1→4) linked β-mannopyranosyl backbone partially substituted at O-6 with α-D-galactopyranosyl side groups (Meer et al. 1975; Srivastava and Kapoor 2005). Because of their high viscous water solutions, the galactomannans of *Ceratonia siliqua*, *Caesalpinia spinosa*, *Cyamopsis tetragonolobus* and *Trigonella foenum-graecum* are used as emulsion stabilizers in the food, cosmetic and pharmaceutical industries (Prajapati et al. 2013; Johnson et al. 2015). Despite of the galactomannans intake be conventionally assumed as safe, few systematic studies report the acute toxicology of such polysaccharides, especially those obtained from non-traditional sources (Pawar and Lalith 2015; Suryawanshi et al. 2015; Deshpande et al. 2016).

The Leguminosae species *Caesalpinia pulcherrima* (L.)Sw. and *Delonix regia* (Bojer ex Hook.)Raf. are native from Central America and Madagascar, respectively, but they are widely used as ornamental plants around the world (Singh and Kumar 2014; Santos et al. 2019). Their easily extractable galactomannans have been exploited as dietary fiber, fruit edible coating, preparation of mucoadhesive microspheres, drug delivery systems and injectable regenerative hydrogels (Cerqueira et al. 2009; Tamaki et al. 2010; Lima et al. 2010; Thombre and Gibe 2013; Buriti et al. 2014; Ogunjimi et al. 2017; Lima et al. 2019). Beneficial effects of these galactomannans were recently reported in rodent models of gastritis and osteoarthritis (Marques et al. 2019; Nascimento et al. 2021). Following international protocols preconized by Organization for Economic Co-operation and Development (OECD), this study evaluated the occurrence of systemic and local acute toxicity for these galactomannans in rats.

Methods

Seeds collection

Seeds of *Delonix regia* and *Caesalpinia pulcherrima* were collected in Limoeiro do Norte, Ceará, Brazil. The plants were identified at the Laboratory of Taxonomy of Angiospermae (Department of Biology/Federal University of Ceará) and the voucher specimens deposited at the Prisco Bezerra Herbarium / Federal University of Ceará (numbers 57951 and 57952, respectively). In accordance to 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 (SISGEN), code A54ED41.

Isolation and chemical characterization of the galactomannans

Galactomannans from *C. pulcherrima* (GM-CP) and *D. regia* (GM-DR) were isolated following the procedures respectively proposed by Marques et al. 2019 and Nascimento et al. 2021. Characterization were also done by such authors, and their results are summarized below. For each Leguminosae specie, 50 g of seeds were swelled under water vapour (1 atm, 30 min). Manually-removed endosperms were hydrated overnight at 4°C, and homogenized with 500 mL of distilled water. The first supernatant was collected after an overnight period of decantation. Remaining fragments were resubmitted to homogenization, and a second supernatant was collected. Both supernatants were mixed with two volumes of ethanol 96% (w/v), and the crude galactomannan was filtered under vacuum (glass filter no. 4), and dried at room temperature. Proteolysis was achieved by heating under reflux (85-90°C) of 1 g of crude sample in 200 ml of NaOH 5% (w/v) during 45 minutes under reflux. Alkali and low molar weight compounds were removed by dialysis against distilled water for 72 h, using a semipermeable cellulose membrane (cut off 10,000 g mol⁻¹). The dialysate was centrifuged for 15 minutes (1000 x *g*), and ethanol was added to the supernatant. The purified galactomannan was filtered under vacuum, and dried at room temperature until constant weight. Removal of proteins was confirmed in both samples through undetectable %N, using a 2400 Series II CHNS/O Elemental Analyzer. Thermogravimetric analysis revealed low levels of inorganic residues in the samples were found (0.24% for GM-CP and 0.56% for GM-DR). As expected for hygroscopic polysaccharides, moisture after desiccation was of 14.76% and 13.78%, respectively.

The purified samples displayed unimodal molar weight distribution in high-performance size-exclusion chromatography performed at room temperature (Shimadzu equipment, PolySep-GFC-P Phenomenex, 35 mm x 7.80 mm and PolySep-GFC-P linear Phenomenex, 300 mm x 7.80 mm columns, 0.1 mol L⁻¹ NaNO₃ as eluent and flow rate of 0.5 mL min⁻¹). Using standard neutral pullulan samples (ShodexDenko™, 10³ - 10⁶ g mol⁻¹), peak molar weights were in the magnitude order of 10⁶ for GM-CP and 10⁵ g mol⁻¹ for GM-DR. FT-IR spectroscopy in KBr pellets revealed typical polysaccharides signals; for GM-CP: 3000–3490 cm⁻¹ of O-H, 1016–1074 cm⁻¹ of C-O (alcohol), 1159 cm⁻¹ of C-O-C glycosidic linkage and 2923 cm⁻¹ of C-H; for GM-DR: 3403 cm⁻¹ of O-H, 2920 cm⁻¹ of C-H, 1158 cm⁻¹ of 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. As determined through hydrolysis with trifluoroacetic acid, derivatization with pyridine and N,O-bis (trimethylsilyl) trifluoroacetamide, analysis in Shimadzu GC2014 Chromatograph (30 m x 0.25 mm x 0.25 μm capillary column, flow of nitrogen as at 4 kgf cm⁻²), GM-CP was composed by

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 of 2.18:1 and 2.39:1, which were similar to those calculated by the intensity of signals at 4.73 ppm (H-1 of β -D-mannopyranose) and 5.02 ppm (H-1 of α -D-galactopyranose) in $^1\text{H-NMR}$ spectroscopy (Bruker DMX-500 spectrometer, D_2O as solvent).

Animals

Female Wistar rats (170–200 g) were obtained from the Federal University of Ceará, and maintained under padronized conditions (25 °C; cycle of 12 h light/12 h dark, and food and water *ad libitum*). The procedures were conducted according to the guidelines of the National Council for the Control of Animal Experimentation (CONCEA), under authorization by the ethics committee for animal research of the Federal University of Ceará (No. 52120510/2018, see supplementary material S1).

Evaluation of systemic toxicity

The acute systemic toxicity of the galactomannans was evaluated according to OECD Guideline No.423 (2002). Groups of three animals were subjected to 2-hour fasting, and received a single dose (300 mg kg^{-1} *per os*) of the galactomannans from *Caesalpinia pulcherrima* (GM-CP) or *Delonix regia* (GM-DR). Fasting was maintained until 2 hours after the administration. Since lethality was absent, other groups received doses of 2,000 mg kg^{-1} (p.o.) Control group received vehicle (10 mL kg^{-1} ; sterile saline).

Body weight measurement was recorded during a 14-day period, as well the occurrence of toxicity signals by the Malone's hippocratic screening (Malone and Robichaud 1962; Malone 1977). For evaluation of exploratory activity, animals were acclimated during 1 minute in a 30 x 30 x 15 cm acrylic box with transparent walls and black floor divided into 9 equal quadrants, and the exploratory activity measured by the number of quadrant crossings, self-cleaning (grooming) and rearing during 5 minutes (Archer 1973).

Animals were euthanized under anesthesia (xylazine 10 mg kg^{-1} + ketamine 100 mg kg^{-1} i.p.) for blood collection and removal of organs (kidneys, spleen, lungs, heart, liver and brain). Counting of blood figurative elements, as well cells volumes and hemoglobin content, were performed using an automated hematology analyzer (SDH-3, Labtest, Brazil). Labtest Diagnostics Kits were used to measure serum levels of urea, creatinine, total cholesterol, triglycerides, bilirubin, total proteins and albumin, and the activity of the enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphate. Organ samples were embedded in paraffin, sectioned at 4- μm -thick sections for hematoxylin & eosin staining, and examined under light microscopy by an experienced pathologist without knowledge of the treatments.

Evaluation of dermal toxicity

The dermal toxicity of the galactomannans was evaluated according to OECD Guideline No. 402 (2017) in groups of three animals receiving the highest doses of galactomannans (2,000 mg kg^{-1} *per os*).

Animals had their thoracic dorsal area shaved one day before the experiment. Antisepsis was performed with 2% (w/v) chlorhexidine, and GM-CP or GM-DR (2,000 mg/kg, 0.3 mL) was uniformly applied in an area of 3 cm². Control group received vehicle (saline). Inoculated skin was covered with a porous gauze dressing and non-irritating tape throughout 24-hour exposure period. Occurrence of skin reactions (hyperemia and edema) were monitored during 14 days, using the Draize score system (Draize et al. 1944).

Evaluation of intra-articular innocuity

Occurrence of intra-articular pro-inflammatory reactions was verified in the right tibiotarsal joints of rat groups receiving GM-CP or GM-DR (200 µg / 25 µL; n=5). Control group received vehicle (saline). For comparison, galactomannan not-submitted to alkaline hydrolysis step were also tested (protein contents of 0.89% and 1.00%, respectively).

After injection, animals were individually placed into Plexiglas boxes with malleable mesh net floors, and joint flexion was achieved by pressuring an algometer-coupled large probe (4.15 mm²) until the occurrence of paw withdrawal reflex. Reduced mechanical threshold is indicative of hypernociception (Bringel et al. 2020). The test was performed from hour 0 (immediately before the inoculation) to hour 6, in which animals were euthanized. Joint cavity was washed twice with 0.1 ml PBS containing 10 mmol L⁻¹ EDTA, and exudates were collected for total and differential cell counts using Neubauer chambers and stained smears, respectively.

Statistical analysis

Results were expressed as mean ± SEM. Statistical differences between parametric data were determined by one-way / two-way analysis of variance (ANOVA), followed by the Bonferroni's test. Non-parametric data were expressed as median (IQR) and analysed by the Kruskal-Wallis's test, followed by the Dunn's test. P <0.05 was considered significant. All data is available as supplementary material.

Results

Acute oral toxicity of the galactomannans

No deaths were observed after the oral administration of the galactomannans, which demonstrates an LD₅₀ greater than 2,000 mg kg⁻¹. Higher-doses of galactomannans had similar increase in body weight, as compared to the vehicle (GM-CP: + 1.0%; GM-DR: + 1.0%; control: + 2.75%, p>0.05) (Table 1).

Animals did not display alterations related to the central, somatosensory or autonomous nervous systems, as seen using the Malone's Hippocratic triage (Table 2). In all groups, it was observed normal respiration, vibrissae movement, lacrimation and salivation, but no signals of cyanosis, piloerection, analgesia / sedation, ataxia, prostration, catatonia, tail erection, stereotyped movements, tremors nor convulsion. Although exophthalmos seemed to be present at day 14 in the GM-DR group, and in various

milestones in the GM-CP group (60 min, day 7 and day 14), it was also found in the vehicle-treated after 60 min.

In all groups, the exploratory activity has decreased along the experiment (Fig. 1). The time of observation significantly influenced the data variability for crossings and rearings ($p=0.0241$ and 0.0354 , respectively), but not for groomings ($p=0.2274$). The treatments effect was not significant for such parameters ($p>0.05$).

No treatment-related gross pathological change was observed in the brain, liver, kidneys, heart, lungs and spleen (Table 3). Hystopathological alterations were also absent in such organs (Fig. 2), and no changes were detected on serum levels of the creatinine/urea and ALT/AST (Table 4). All groups displayed high blood glucose triglycerides levels, with significant hyperglycemia in the GM-DR group (+38.5% and +39.5% in comparison to control group, respectively). Other serum parameters remained unaltered, as well the hematological parameters (Table 5).

Dermal toxicity of the galactomannans

Topical application of *C. pulcherrima* or *D. regia* galactomannans did not evoke significant signals of skin irritation at $2,000 \text{ mg kg}^{-1}$ (Table 6). When measured 24 h or 7 days after induction, the Draize's scores values for hyperemia were slightly higher in the GM-CP and GM-DR groups, but they did not reach statistical significance, in comparison to the vehicle-treated group. On the other hand, no signals of edema were observed at any endpoint.

Intra-articular innocuity of the galactomannans

As compared to the control group (vehicle), the intra-articular administration of unpurified samples of GM-CP and GM-DR evoked both mechanical hypernociception and inflammatory cell influx (GM-CP: $18.6 \pm 3.0 \text{ g}$ and $11,060 \pm 3,835 \text{ cells mm}^{-3}$; GM-DR: $15.1 \pm 4.1 \text{ g}$ and $23,680 \pm 4,984 \text{ cells mm}^{-3}$; control: $47.6 \pm 6.1 \text{ g}$ and $151 \pm 44 \text{ cells mm}^{-3}$) ($p < 0.05$ vs. control) (Fig. 3). Neutrophils were the predominant in those inflammatory exsudates (>85%) (data not shown). On the other hand, administration of protein-free galactomannans was unable to cause adverse reactions (GM-CP: $38.0 \pm 4.6 \text{ g}$ and $41 \pm 18 \text{ cells mm}^{-3}$; GM-DR: $47.3 \pm 9.5 \text{ g}$ and $121 \pm 77 \text{ cells mm}^{-3}$, $p > 0.05$ vs. control).

Discussion

Safety of chemical compounds is an important issue for food or biomedical applications, and the conduction of acute toxicity studies has as the main objective the identification of side effects. In this study, bioactive and well-characterized galactomannans from *Caesalpinia pulcherrima* and *Delonix* had their acute toxicity systematically evaluated in rodents by the use of international preconized protocols, which comprises the administration of single initial doses of 300 mg kg^{-1} and a 14-day observation period.

As previously reported for others galactomannans, such as those of *Senna tora* and *Trigonella foenum-graecum* (Pawar and Lalith 2015; Deshpande et al. 2016), no lethality was observed among the animals treated with GM-CP or GM-DR, even at 2,000 mg kg⁻¹. Despite Suryawanshi et al. (2015) had already reported similar evidence for the *C. pulcherrima* galactomannan, such study did not investigate impairments on target organs or biochemical and haematological parameters, as we did (see below). Additionally, animals did not display alterations of spontaneous locomotory activity (Prut and Belzung 2003) or in the nervous systems. Although toxic reactions could alter the body weight (Teo et al. 2002), such parameter was not significantly different among the groups along the 14-day period.

Structural and weight changes in organs may reflect toxicity due to several factors, including changes in the enzyme activity, injuries and other physiological disorders, which are dependent on target organs, and make possible the knowledge of the general health status of the animals (Auletta 1995; Michael et al. 2007). No macroscopic lesions or weight organs alterations were induced by the galactomannans on vital organs. Nevertheless, functional events may arise prior to morphological damage, especially in organs such kidneys and liver (Ramaiah 2011; Alimba et al. 2012), but the serum levels of the creatinine/urea and ALT/AST were normal for the animals euthanized 14 days after the acute administration of galactomannans. Moreover, substances affecting bone marrow could inhibit certain steps in the production of hemoglobin, and reduce the ability of the blood to distribute oxygen throughout the body (Jain et al. 2009). Both galactomannans tested did not change the haematological parameters, so that erythrocytes were both normocytic and normochromic. However, animals in all groups displayed high blood glucose levels (>140 mg dL⁻¹), even in the control group. Such values of post-brandial capillary glycemia are not uncommon, especially when blood collection is taken after zylaxine/ketamine anesthesia (Saha et al. 2005; Wang et al. 2010), so that such hyperglycemia may not be due to the treatment with the galactomannan.

As evaluated using the Draize's scoring system, cutaneous administration of GM-CP or GM-DR did not cause hyperemia or edema at 2,000 mg kg⁻¹ and these findings agree with previous reports for innocuity of leaf polysaccharides of *D. regia* (Shewale et al. 2011; Wang et al. 2016). Regarding the intra-articular innocuity, the protein-free galactomannans did not evoke articular hypernociception or leukocyte influx. Similar to reported for the guar galactomannan (Castro et al. 2007), joint inflammatory responses were evoked solely by unpurified samples containing remaining proteins.

In conclusion, the lack of systemic and local reactions revealed that the seed galactomannan of *C. pulcherrima* and *D. regia* did not cause acute toxicity, and their LD50 values were superior to 2,000 mg kg⁻¹, being allocated in the class 5 of the Globally Harmonised System for Classification and Labelling of Chemicals (GHS) (UN, 2019). Further assessments involving different administration schemes, such as repetitive doses, and evaluations in humans are recommended for their safe consumption.

Declarations

Ethical approval This study was performed in accordance with the guidelines of the Brazilian Council of Control in Animal Experimentation (CONCEA). Approval was granted by the local ethics committee (No. 52120510/2018; October 22nd, 2018). Certificate is available as supplementary material.

Consent to participate Not applicable

Consent to publish Publication is consented by all authors.

Authors contribution RRC, EMCC and OVOM conceived and designed the research. FCJM, FGSN, DTTN and ICL conducted the experiments. PMGS and MRLM performed the histopathological analysis. AON and GFA performed the hematological and biochemical analyses. RRC and AMSA wrote the manuscript. All authors read and approved the manuscript and all data were generated in-house and that no paper mill was used.

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Competing interests The authors declare no conflict of interest.

Availability of data and material Availability is consented by all authors. All data is available as supplementary material.

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Tables

Table 1 Systemic administration GM-CP and GM-DR does not alter the body weight of female rats

Timepoint	Body weight (g)		
	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 = galactomannan from *C. pulcherrima* seeds (2,000 mg kg⁻¹ p.o.), GM-DR = galactomannan from *D. regia* seeds (2,000 mg kg⁻¹ p.o.). Mean ± SEM (n = 3 / group).

Table 2 Systemic administration of GM-CP and GM-DR does not alter the behavioral profile of female rats

Parameters	Control			GM-CP			GM-DR		
	60 min	day 7	day 14	60 min	day 7	day 14	60 min	day 7	day 14
Ataxia	-	-	-	-	-	-	-	-	-
Analgesia	-	-	-	-	-	-	-	-	-
Catatonica	-	-	-	-	-	-	-	-	-
Convulsions	-	-	-	-	-	-	-	-	-
Cyanosis	-	-	-	-	-	-	-	-	-
Diarrhoea	-	-	-	-	-	-	-	-	-
Diuresis	N	N	N	N	N	N	N	N	N
Escape reaction	-	-	-	-	-	-	-	-	-
Exophthalmus	P	-	-	P	P	P	-	-	P
Grooming	N	N	N	N	N	N	N	N	N
Lacrimation	-	-	-	-	-	-	-	-	-
Motor activity	N	N	N	N	N	N	N	N	N
Paw licking	N	N	N	N	N	N	N	N	N
Piloerection	-	-	-	-	-	-	-	-	-
Prostration	-	-	-	-	-	-	-	-	-
Respiration	N	N	N	N	N	N	N	N	N
Salivation	N	N	N	N	N	N	N	N	N
Sedation	-	-	-	-	-	-	-	-	-
Stereotyped movements	-	-	-	-	-	-	-	-	-
Tail erection	-	-	-	-	-	-	-	-	-
Tremors	-	-	-	-	-	-	-	-	-
Vibrissae movement	N	N	N	N	N	N	N	N	N

C.= control (vehicle) group, GM-CP = galactomannan from *C. pulcherrima* seeds (2,000 mg kg⁻¹ p.o.), GM-DR = galactomannan from *D. regia* seeds (2,000 mg kg⁻¹ p.o.). N= Normal, P = Present, - = Not found (n = 3 / group).

Table 3 Systemic administration of GM-CP and GM-DR does not alter the organs weight of female rats

Organ	Weight (g)		
	Control	GM-CP	GM-DR
Brain	1.84 ± 0.06	1.93 ± 0.03	1.86 ± 0.14
Heart	0.74 ± 0.06	0.69 ± 0.02	0.73 ± 0.02
Kidneys	1.43 ± 0.07	1.52 ± 0.07	1.37 ± 0.03
Liver	6.54 ± 0.19	6.28 ± 0.01	6.01 ± 0.38
Lungs	1.67 ± 0.18	1.51 ± 0.13	1.56 ± 0.11
Spleen	0.56 ± 0.06	0.50 ± 0.03	0.53 ± 0.04

GM-CP = galactomannan from *C. pulcherrima* seeds (2,000 mg kg⁻¹ p.o.), GM-DR = galactomannan from *D. regia* seeds (2,000 mg kg⁻¹ p.o.). Mean ± SEM (n = 3 / group).

Table 4 Systemic administration GM-CP and GM-DR does not alter serum markers of female rats

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

GM-CP = galactomannan from *C. pulcherrima* seeds (2,000 mg kg⁻¹ p.o.), GM-DR = galactomannan from *D. regia* seeds (2,000 mg kg⁻¹ p.o.). Mean ± SEM (n = 3 / group). *P<0.05 vs. Control (ANOVA, Bonferroni)

Table 5 Systemic administration of GM-CP and GM-DR does not alter the hematological parameters of female rats

Parameters	Unit	Control	GM-CP	GM-DR
<i>Red Blood Cells</i>	$\times 10^6 \mu\text{L}^{-1}$	8.41±0.14	8.24±0.10	8.10 ± 0.16
Hemoglobin	g dL^{-1}	14.0±0.83	13.8±0.1	13.8± 0.1
Hematocrit	%	49.71±0.39	48.38±0.40	45.3 ±1.78
MCV	fL	60±0	58±0	59±1
MCH	pg	16.6±0.3	16.8±0.3	17.1±0.3
MHCH	g dL^{-1}	28.0±0.4	28.5±0.2	30.6±1.4
RBC distribution	%	14.0±0.2	15.5±0.8	137±0.6
<i>Platelets</i>	$\times 10^3 \mu\text{L}^{-1}$	1784±86	1789 ± 22	1658±100
Platelet Mean Volume	fL	7.2±0.1	7.0 ± 0.1	7.2±0.1
Platelet distribution	%	36.1±0.5	36.0 ± 0.5	36.0±1.20
<i>Leukocytes (total count)</i>	$\times 10^3 \mu\text{L}^{-1}$	10.41±1.36	9.36±1.64	8.43 ± 0.30
Granulocytes	$\times 10^3 \mu\text{L}^{-1}$	2.01 ± 0.38	1.66 ± 0.51	0.80± 0.05
Lymphocytes	$\times 10^3 \mu\text{L}^{-1}$	7.74±1.05	6.98 ± 0.73	6.93±0.33
Monocytes, basophiles and eosinophiles	$10^3 \mu\text{L}^{-1}$	0.66 ± 0.05	0.63 ± 0.10	0.69± 0.08

GM-CP = galactomannan from *C. pulcherrima* seeds (2,000 mg kg⁻¹ p.o.), GM-DR = galactomannan from *D. regia* seeds (2,000 mg kg⁻¹ p.o.). MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; RBC = red blood cells distribution. Mean ± SEM (n = 3 / group).

Table 6 Topical administration of galactomannans of GM-CP and GM-DR does not evoke skin reactions in female Wistar rats

Reaction	Control	GM-CP	GM-DR
<i>Hyperemia</i>			
24 h	0 (0-2)	1 (1-2)	1 (0-1)
Day 7	0 (0-1)	1 (1-2)	1 (0-1)
Day 14	0 (0-1)	0 (0-1)	0(0-0)
<i>Edema</i>			
24h	0 (0-2)	0 (0-1)	0 (0-1)
Day 7	0(0-0)	1 (0-1)	0 (0-0)
Day 14	0(0-0)	0 (0-0)	0 (0-0)

GM-CP = galactomannan from *C. pulcherrima* seeds (2,000 mg kg⁻¹ p.o.), GM-DR = galactomannan from *D. regia* seeds (2,000 mg kg⁻¹ p.o.). Mean ± SEM (n = 3 / group).

Figures

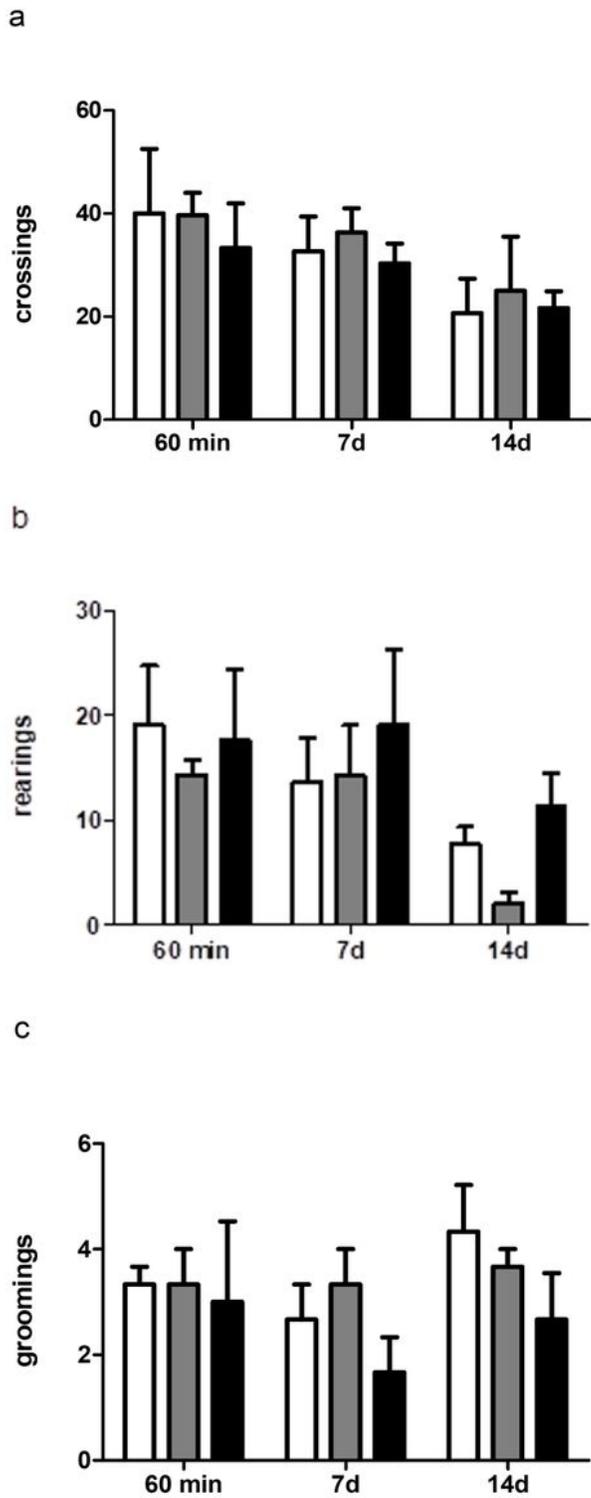


Figure 1

Systemic administration GM-DR or GM-DR does not alter the exploratory activity of female rats. Animals received vehicle (control, □) or galactomannans – (GM-CP, ■) / (GM-DR, ■) (2,000 mg kg⁻¹ p.o.). Mean ± SEM (n=3 / group) for number of crossings (a), rearings (b) and groomings (c). GM-CP = galactomannan of *C. pulcherrima* seeds; GM-DR = galactomannan of *D. regia* seeds

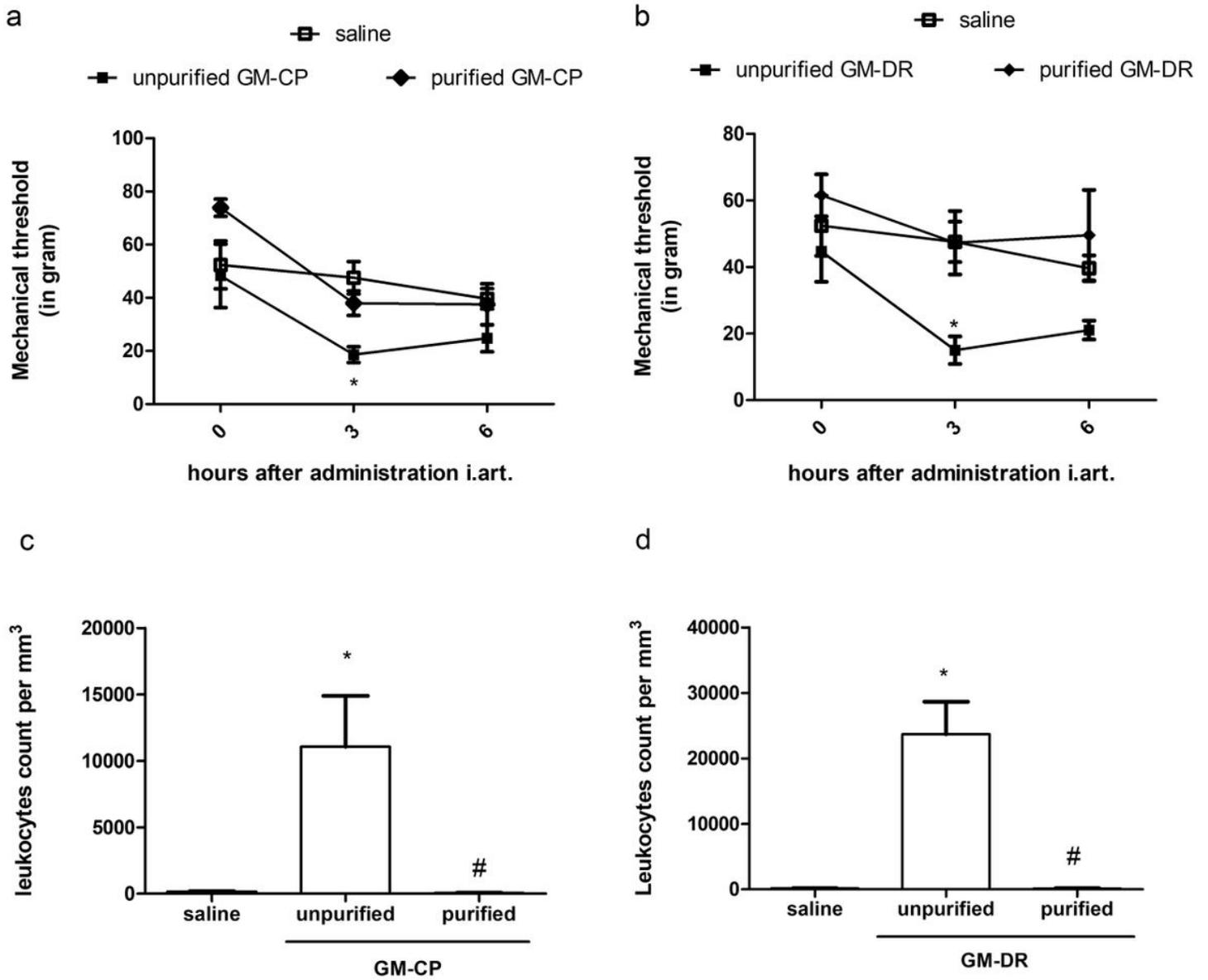


Figure 2

The purified galactomannans GM-DR or GM-DR do not evoke mechanical hypernociception (a, b) or leukocyte influx (c, d) in tibiotarsal joints of female rats. Mean \pm SEM (n = 5 /group). *p<0.05 vs. control (vehicle); #P<0.05 vs. unpurified galactomannan (ANOVA, Bonferroni). GM-CP = galactomannan of *C. pulcherrima* seeds; GM-DR = galactomannan of *D. regia* seeds

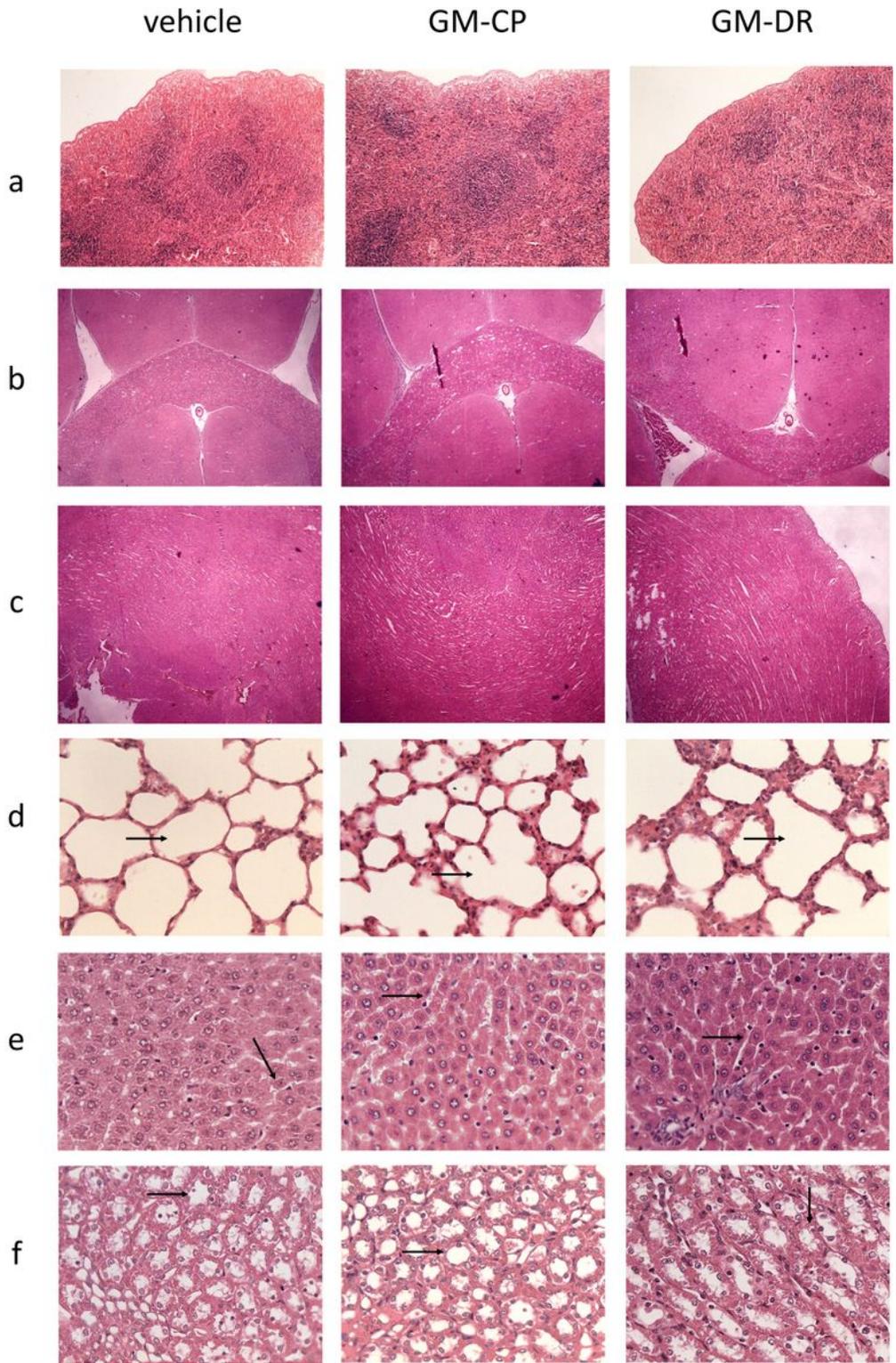


Figure 3

Photomicrographs of tissues from female rats treated with GM-DR or GM-DR. (a) Spleen with normal architecture (capsule, red and white pulps). (b) Brain, showing a normal section with the central channel surrounded by the substantia cinza. (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 = galactomannan of *C. pulcherrima* seeds (2,000 mg kg⁻¹ p.o.), GM-DR = galactomannan of *D. regia* seeds (2,000 mg kg⁻¹ p.o.)

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