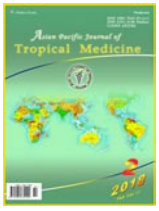

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Antidiabetic effects of galactomannans from *Adenanthera pavonina* L. in streptozotocin-induced diabetic mice

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ABSTRACT

Objective: To evaluate the antidiabetic effect of galactomannans extracted from *Adenanthera pavonina*'s L. seeds (GAP) in streptozotocin (STZ) induced diabetic mice. **Methods:** The preliminary galactomannan yield from *Adenanthera pavonina* L. plant and extraction products composition were evaluated. Various chemical characterization methods like thin layer chromatography, Fourier transform infrared spectroscopy, ^{1}H and ^{13}C nuclear magnetic resonance, and molecular weight by gel permeation chromatography have been employed to characterize the extracted galactomannan. The mice were divided in four groups: Normal control, diabetic control, GAP (1% and 2%) treated and standard drug treated groups. Diabetic mice received treatment daily for 30 d. Diabetes was induced by STZ at a single dose of 120 mg/kg. Body weight, water and food intake, fasting blood glucose, total cholesterol and triglycerides were measured. Histopathological analysis of pancreas and liver were performed to evaluate STZ-induced tissue injuries. **Results:** The isolated and extracted galactomannan from *Adenanthera pavonina* was confirmed by various chemical characterization methods. GAP exhibited a 1.46:1 mannose: galactose ratio, and high molar weight. Both GAP enriched food decreased glycaemia, total cholesterol and triacylglycerol. GAP didn't interfere on food intakes or body weight, although it increased water intake. Furthermore, the relative liver weight indicated toxic galactomannan effects on the histopathological changes of the pancreas in STZ induced diabetes. **Conclusions:** It is concluded that GAP is a natural product that contains potent galactomannan and is useful in preventing and treating diabetes.

1. Introduction

Diabetes mellitus (DM) is a chronic disease associated with metabolic dysfunction, generally followed by severe complications, which results in loss of life quality and premature mortality[1]. Hyperglycemia is the main DM symptom, and is responsible for tissue damage and vascular complications, which limits professional performance in diverse degrees and generats a social cost beyond the just of the outpatient. In 2015, according to the International Diabetes Federation, the diabetic population was 415 million people worldwide, with a prospect of increasing to 642 million by the year 2040.

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Therapeutic treatments include commercial insulin and oral hypoglycemic that help to control the patients glycaemia, however physical activity and healthy nourishment are important to their life quality. Among the nutrients highly relevant for the treatment of DM, dietary fibers, especially soluble fibers, stand out and represent an important part of a healthy diet because of their action in increasing peripheral insulin sensitivity and reducing blood lipid levels[2].

Galactomannans are soluble fibres, commonly found in the seeds of Fabaceae, although they may be obtained from other plants and fungi[3]. Their dietary consumption has hypoglycemic and hypocholesterolemic potential.

Adenanthera pavonina (*A. pavonina*) (Fabaceae family, subfamily Mimosoideae, known as dragon's eye) seeds are a promising source for galactomannans in Brazil, because, in addition to its richness in this polysaccharide[4], this plant is well adapted to Brazilian cultivation conditions[5]. Beacause of their chemical structures silmilar to galactomannans used in glycemic and metabolic DM control – guar gum and fenugreek[6], galactomannans from *A. pavonina* have potential to serve as a soluble fibre supplement for diabetic patients.

The objective of this study was to characterize and evaluate the effect of galactomannans extracted from *A. pavonina*'s L. seeds (GAP) on diabetic mice's glycaemia.

2. Materials and methods

2.1. Drugs and chemicals

Streptozotocin (STZ) was supplied by Sigma, Co (St Louis, MO, USA). Metformin hydrochloride was purchased from Medley Pharmaceutical, Co (Campinas, SP, Brazil). Total cholesterol (TC) and triacylglycerol (TAG) were determined by an enzymatic and colorimetric method using a commercial kit (Bioclin, Belo Horizonte, MG, Brazil). All other reagents of analytical grade were purchased locally.

2.2. Isolation and purification of galactomannans

A. pavonina seeds were collected in Fortaleza/Ceará/Brazil between September to October in 2014. The identification of the botanical species was performed by Herbarium Prisco Bezerra of the Federal University of Ceará, and deposited under number 56964. The isolation procedure of galactomannans from the seeds of *A. pavonina* was performed according to Vieira *et al*[7]. Initially, 150 g (seeds) were boiled in 500 mL distilled water for 2 h. After 12 h resting at 24 ℃, the endosperm was removed and lyophilized. For galactomannan's extraction, 2.5 g of the lyophilized endosperm were submitted to four consecutive extractions with water. At each extraction, 100 mL of distilled water at 80 ℃ for 4 h was used. After this procedure, the aqueous extract was vacuum-filtered with polyester net. The material retained in the net was submitted again to the extraction process repeatedly until the galactomannans were fully extracted. At the end of the aqueous extractions, the material obtained was filtered through a celite layer, frozen and lyophilized.

2.3. Moisture, ash, protein and carbohydrate content

Moisture and ash content were determined according to methods described by Instituto Adolfo Lutz (2008)[8]. Total carbohydrate content was quantified using the method reported by DuBois, Gilles, Hamilton, Rebers, & Smith (1956)[9] and protein content was determined according to Bradford (1976)[10].

2.4. Molar weight estimation through gel permeation chromatography

Gel permeation chromatography was performed in a SHIMADZU LC-10AD chromatograph with refraction index detector RID-10A at 40 ℃. A linear type ultrahydrogel 250 column (waters) with 7.8 mm × 300 mm was used, with a mobile phase of sodium nitrate solution (0.1 mol/L), and a flow rate 0.5 mL/min, a sample volume of 20 μL ran through the column. GAP sample was solubilized in 1.0 mL deionized water, resulting in a 1 mg/mL solution that was then filtered in a 0.45 μm Millipore® membrane. Pullulan (Shodex Denko®) samples of molecular weight (Mw) 5.90×10^3 g/mol, 4.73×10^4 g/mol , 1.12×10^5 g/mol, 4.04×10^5 g/mol and 7.88×10^5 g/ mol were used as molecular standard[11].

2.5. GAP monosaccharide content

GAP monosaccharide content was determined through its hydrolysis[12], followed by thin layer chromatography (TLC) analysis. In a silica gel 60 TLC chromatoplate, 4 μL of mannose and galactose standard solutions and hydrolyzed galactomannans at 10 mg/mL were applied. After elution with an acetic acid glacial, chloroform: Water as mobile phase (74:65:11 v/v), the plate was dried at 100 ℃ for 2 min and pulverized with orcinol-sulfuric acid spray reagent for detection.

2.6. Nuclear magnetic resonance (NMR) (^{1}H–NMR) and mannose: Galactose ratio determination

GAP sample (20 mg/mL) was dissolved in deuterium oxide (D_2O) and the solution was put in a 5 mm NMR tube at 85 ℃ under constant agitation. Proton resonance spectra were obtained in an Avance DRX-500 Bruker Spectrometers® operating in the hydrogen frequency, at 500 MHz. The spectra were registered at 85 ℃, and for its acquisition, 5 s relaxation periods and 30 pulse sequences were used[13].

2.7. Fourier transformation infrared absorption

The infrared spectra were obtained in a Perkin-Elmer 1320 Spectrometers®, with wavelengths of 400 cm^{-1} to 4 000 cm^{-1}. The GAP sample was analysed in KBr pellets[13].

2.8. Experimental animals

Fifty female Swiss mice (25 g – 30 g with ages ranging from 8 wk – 12 wk) were kept at ± 22 ℃, with 12/12 light/dark cycle[14]. All animals were kept in Polyacrylic cages and received water and food *ad libitum*. The experimental procedure performed on animals was in accordance with the policy of the Institutional Animal Ethical Committee of the State University of Ceará (UECE) (No. 0874146/2015.).

2.9. Diabetes induction

Diabetes was induced with an intraperitoneal injection of 120 mg/kg of STZ (Sigma®) in aqueous solution, at 24 mg/mL. After 7d, diabetes was confirmed by measuring the serum glucose level. Only mice whose post-induction glycaemia was above 200 mg/dL were considered diabetic[15].

2.10. Experimental design

Animals were distributed into five groups (n=10): NC group – normal control (healthy mice); DC Group – Diabetic control (diabetic mice); GAP 1% group – diabetic mice and treated with 1% GAP enriched food; GAP 2% group – diabetic mice and treated with 2% GAP enriched food; MET group – diabetic mice treated with metformin® (200 mg/kg).

2.11. Food preparation

The standard animal food used was from Presence Animal Nutrition®, purchased at a local market. Standard food presents 13% moisture, 23% total protein, 4% ethereal extract, 6% fibre and 10% mineral matter content. GAP 1% and GAP 2% were produced from crushed standard food with 1% (w/w) and 2% (w/w) GAP addition, respectively. After homogenization, food with GAP added was compressed into pellets and dried at 50 ℃ for 48 h.

2.12.Body weight water and food ingestion

The animals had their weight measured on the day before treatment, considered as day 0, 21,and 28 of treatment. Water and food ingestion were measured weekly during the 30 d of treatment.

2.13. Biochemical parameters analysis

Blood samples were collected on day 0, 21 and 30 of the treatment for evaluation of fasting glycaemia. TC and TAG were evaluated at the end of treatment. These biochemical parameters were analysed through enzymatic and colorimetric techniques using commercial Bioclin® specific kits.

2.14. Toxicity evaluation

Toxicity study was performed by relative liver weight (RLW) comparisons between groups and a long time and histological cuts from the animal's pancreas and liver. After being euthanised, the animal's organs were weighed, fixed with 10% formaldehyde for 24 h and treated with microscope slides preparation according to Michanaly[16].

2.15. Statistical analysis

Data were demonstrated as a mean ± standard deviation. Analysis of variance followed by a performance of Newman-Keuls test, as a P<0.05 being significant. GraphPad Prism® 6.0 software was used in the application of tests and graphic presentation.

3. Results

3.1. Isolation and characterization of galactomannan from the A. pavonina

The product of the extraction was a galactomannan free from insoluble fibres and with clear aspect in solution. A total of 11.00% yield of galactomannans was obtained in relation to seed total weight followed by endosperm/seed (13.83%) and GAP/endosperm (80.10%). The galactomannans were determined and the results revealed that the moisture percentage and ash content were 13.87% and 3.20%, respectively. For macronutrients, the values were 72.290% and 0.034% for carbohydrate and proteins respectively. High carbohydrate and low protein content confirmed the high purity of the extracted material. TLC showed that GAP was constituted of mannose and galactose unities. Gel permeation chromatography analysis presented a molar weight (Mw) for GAP of 1.45×10^7 g/mol. The ^{1}H NMR spectrum was 500 MHz, *A. pavonina* L. galactomannan and its anomeric region in the spectrum were shown in Figure 1. The resonances of the anomeric protons were well separated, and their identification was self-evident from the known monomeric compositions of the samples. Through ^{1}H NMR spectra interpretation, the M/G (D-mannose/D-galactose) ratio was calculated, resulting in a 1.46:1.00 value. By analyzing the ^{1}H NMR spectrum of galactomannan and expansion, it could be confirmed that the galacto pyranosyl obtained *A. pavonina* L. signals around were related to alpha-D-galacto pyranosyl: H-1, 5.02 ppm (G1); H-2, 3.80 ppm; H-3, 3.92 ppm; H-4, 4.00 ppm; H-5,3.89 ppm, and H-6, 3.76 ppm, and the beta -D-manopyranosyl, H-1, 4.74 ppm; H-2, 4.12 ppm; H-3, 3.79 ppm; H-4, 3.89 ppm; H-5,3.54 ppm (M5), and H-6, 3.90 ppm. The IR result showed that GAPs (Figure 2) presented relative absorption bands to hydroxyl groups (3 461 cm^{-1}), alkane groups (2 933 cm^{-1}), C-O and C-O-H groups (1 145 and 1 043 cm^{-1}) and D-galactopyranose unities (810 cm^{-1}).

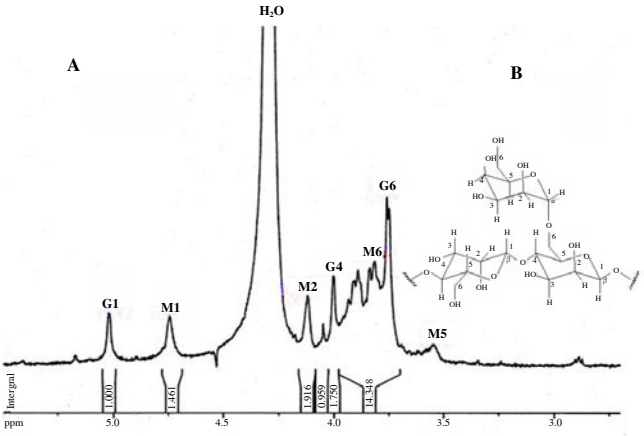

Figure 1. Spectra result.

A) ^{1}H NMR (500 MHz) spectra from a 20 mg/mL solution galactomannan and B) chemical structure of galactomannans obtained from *A. pavonina* L. respectively.

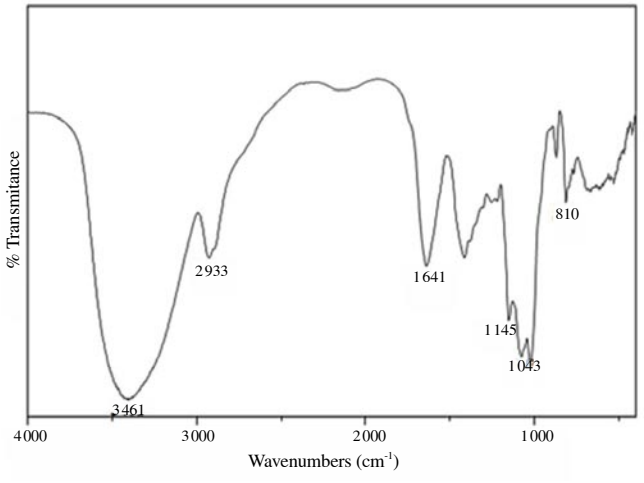

Figure 2. FT-IR spectra of galactomannan obtained from *A. pavonina* L. (cm^{-1} KBr).

3.2. Biochemical results

Glycaemia values (Table 1) showed that after 21 treatment days, both GAP 1% and GAP 2% exhibited significant glycaemia reduction in relation to diabetic control, which was kept until the end of the experiment, on the 30th day of treatment. GAP enriched food consumption during the 30 days resulted in lower TAG plasma levels (Table 2) in mice (P<0.001), although the only GAP 2% presented lower TC levels (Table 2) compared to diabetic control (P<0.05).

Table 1

Effect of *A. pavonina* galactomannan-enriched food on blood total cholesterol (mg/dL), triacylglycerols (mg/dL), water (mL/animal/day) and food ingestion (g/animal/day).

Groups	Total cholesterol	Triacylgliycerols	Water	Food
NC	144.00 ± 12.08	34.00 ± 9.22	5.00 ± 2.55	3.40 ± 0.29
DC	167.00 ± 9.88^{a}	73.00 ± 10.93^{a}	14.20 ± 2.45^{a}	5.80 ± 0.43^{a}
MET	136.00 ± 10.68^{b}	71.00 ± 7.44^{a}	12.30 ± 1.74^{a}	5.90 ± 0.68^{a}
GAP 1%	170.00 ± 10.75^{a}	30.00 ± 7.66^{b}	23.40 ± 2.56^{a,b}	6.10 ± 0.30^{a}
GAP 2%	152.00 ± 7.95^{b}	34.00 ± 8.15^{b}	21.20 ± 3.69^{a,b}	6.40 ± 0.15^{a}

Key: Value expressed as average ± standard deviation (n=10). a = significance of P<0.05 *vs.* NC; b = significance of P<0.05 *vs.* DC, One Way ANOVA, followed by *Newman Keuls* test.

Table 2

Effect of A. pavonina galactomannan-enriched food on fasting glycaemia of diabetic mice (mg/dL).

Groups	Day 0	Day 21	Day 30
CN	121.00 ± 8.83	110.00 ± 5.12	101.00 ± 10.59
CP	334.00 ± 17.10^{a}	441.00 ± 40.63^{a}	423.00 ± 31.55^{a}
MET	352.00 ± 13.96^{a}	397.00 ± 25.62^{a,b}	318.00 ± 25.30^{a,b}
GAP 1%	325.00 ± 17.16^{a}	372.00 ± 21.45^{a,b}	355.00 ± 30.03^{a,b}
GAP 2%	317.00 ± 16.24^{a}	388.00 ± 12.89^{a,b}	319.00 ± 40.57^{a,b}

Key: Value expressed as average ± standard deviation (n=10). a = significance of P<0.05 *vs.* NC; b = significance of P<0.05 *vs.* DC, One Way ANOVA, followed by *Newman Keuls* test.

3.3. Water and feed intake

Galactomannans food enrichment didn't change the animals feeding behavior (Table 1), yet both GAP 1% and GAP 2% group had higher water intake (23.40 ± 2.56) mL/animal/d and (21.20 ± 3.69) mL/animal/d, respectively, (Table 2) significantly superior (P<0.001; P<0.01, respectively) to DC group [(14.2 ± 2.45) mL/animal/d].

3.4. Body weight

Between diabetic groups, mice body weight (Table 3) wasn't significantly affected by GAP supplementation, however, it was seen that GAP 2% body weight was significantly similar to the NC group (P>0.05), revealing a tendency of these animals to recover the body lost due to the diabetic state. GAP 1% and GAP 2% groups presented a RLW (Table 4) higher than NC group, but similar to diabetic group (P>0.05) and inferior, to metformin, thus indicating hepatotoxicity lower than the standard drug to diabetes.

Table 3

Effect of *A. pavonina* galactomannan-enriched food on body weight (g).

Groups	Day 0	Day 21	Day 30
CN	30.40 ± 3.70	31.70 ± 4.05	31.00 ± 3.86
CP	27.30 ± 2.51[a]	27.70 ± 3.20[a]	27.10 ± 2.86[a]
MET	26.50 ± 1.69[a]	27.40 ± 2.38[a]	26.00 ± 2.82[a]
GAP 1%	28.10 ± 2.80	27.90 ± 3.40[a]	27.60 ± 3.24[a]
GAP 2%	28.40 ± 1.99	29.10 ± 2.46	28.70 ± 2.36

Key: Value expressed as average ± standard deviation (n=10). a = significance of P<0.05 *vs*. NC; b = significance of P<0.05 *vs*. DC, One Way ANOVA, followed by *Newman Keuls* test.

Table 4

Effect of *A. pavonina* galactomannan-enriched food on liver weight.

Groups	Absolute liver weight (g)	Relative liver weight (%)
CN	1.32 ± 0.25	4.33 ± 0.28
CP	1.63 ± 0.25	5.72 ± 0.44[a]
MET	1.66 ± 0.21	7.41 ± 0.48[a,b]
GAP 1%	1.76 ± 0.25	5.84 ± 0.31[a]
GAP 2%	1.69 ± 0.33	5.17 ± 0.38[a,b]

Key: Value expressed as average ± standard deviation (n=10). a = significance of P<0.05 *vs*. NC; b = significance of P<0.05 *vs*. DC, One Way ANOVA, followed by *Newman Keuls* test.

3.5. Histopathological results

The pancreatic tissue showed no architectural alteration in the NC animals. In the DC group, there was only a slight decrease in the size of the islets with cell degeneration. In the MET group, there was a drastic reduction in the number and size of islets with evident cellular degeneration. In the GAP-1 group, discrete cell degeneration was visualised in all animals, however, only 2 animals showed an apparent decrease in the number of pancreatic islets. In the GAP-2 group, there was discrete degeneration in all animals, however, 4 animals showed an apparent decrease in the number of islets (Figure 3). Regarding the hepatic tissue, there were no architectural changes in the animals of the NC group, however, some animals in the group exhibited hydropic degeneration that varied from mild to moderate. There were also discrete inflammatory foci consisting of lymphocytes and plasma cells (Figure 4).

In 13 animals from this experiment, 2 animals in the DC group, 3 in the MET group, all from the GAP-1 group and 3 in the GAP-2 group, there was neoplasia that reproduced malignant hepatocytes of severe anisocytosis, with a massive cytoplasm and nucleus with irregular contours, grossly distributed chromatin with evident nucleoli (irregular and sometimes macronuclei). There were also binucleation, the presence of atypical mitoses and multiple foci of apoptosis. Bile duct hyperplasia was also seen in 1 of GAP-1 and 1 of GAP-2 animals. The remaining liver tissue was thirsty for hydropic degeneration (Figure 4).

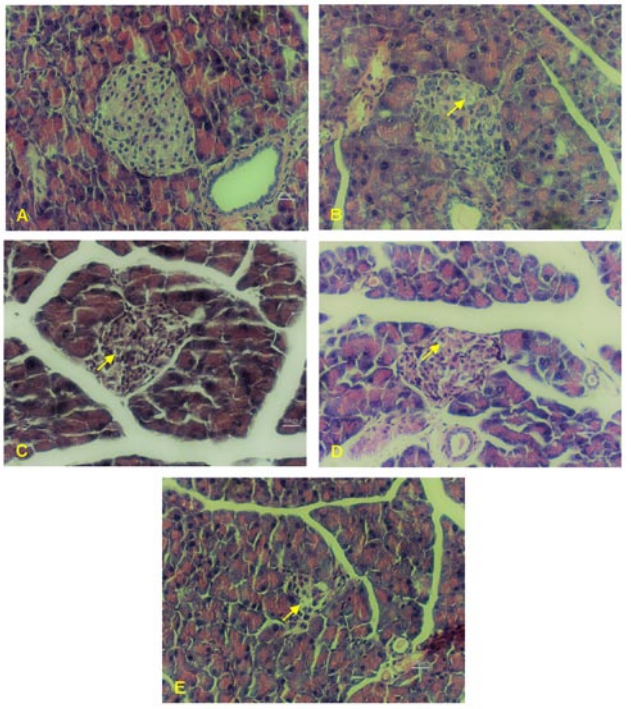

Figure 3. Pancreas photomicrograph of diabetic animals treated with galactomannan enriched food.

(A): Normal control; (B): Diabetic control; (C): GAP 1%; (D): GAP 2%; (E): MET-Cell degeneration areas. HE, 200×.

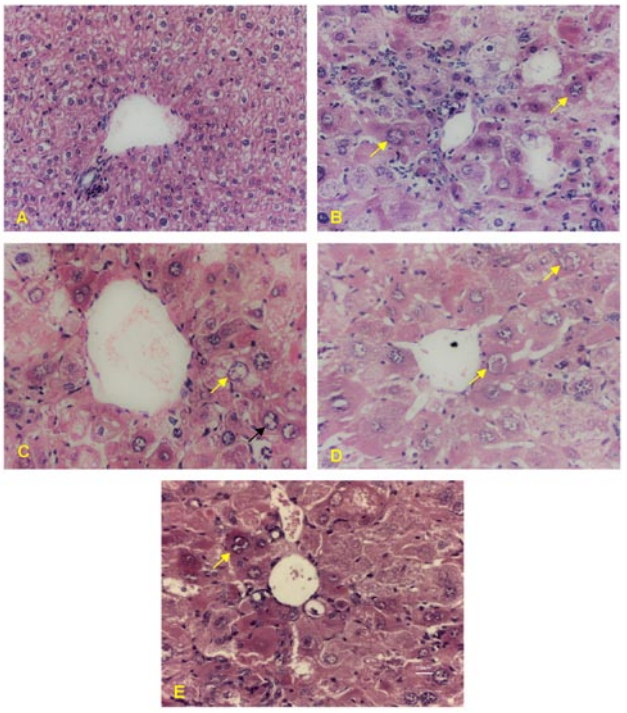

Figure 4. Liver photomicrograph of diabetic animals treated with galactomannan enriched food.

(A): Normal control; (B): Diabetic control; (C): GAP 1%; (D): GAP 2%; (E): MET-Cell degeneration areas. HE, 200× .

4. Discussion

DM is chronic, and becomes the most common disease, especially in developing nations. The disease is featured for the severity of its complications, and has been considered as a public health issue regarding populace development and maturing, more prominent urbanization, the expanding pervasiveness of obesity and sedentarism, and the expanded survival rate from individuals with DM[17]. Traditional plant remedies have been used for centuries in the treatment of diabetes[18], but only a few of these plants have been scientifically evaluated by researchers[19-21]. Therefore, we have investigated the effect of galactomannans from *A. pavonina* L. on STZ-induced diabetic mice.

In the present study, isolated endosperm of *A. pavonina* L. seeds contains water-soluble galactomannan. The product extracted from *A. pavonina* L. seed comprises a galactomannan free of insoluble fibers in solution, and with clear appearance. The yield was 11.00% galactomannan based on the total seed weight. This value is different from the yield reported in the literature for *A. pavonina* - 17.1%[4]. The differences in yield may be related to factors such as the source species of galactomannan, the seed source or maturation stage of the endosperm[13].

TLC shows that chemical structure of GAP is compatible with galactomannan, which has a main chain composed of mannose with ramifications formed by one unity of galactose[22]. The higher molar weight presented by GAP implies a higher interaction between polysaccharide chains, which results in increased molecular aggregation, and a decrease on intrinsic galactomannans viscosity. However GAP presents a Mw similar to guar gum – 1.3×10^7 g/mol[23], to carob gum – 1.2×10^6 g/mol and to fenugreek galactomannans – 1.4×10^6 g/mol[24]. The M/G ratio of GAP is similar to the ones found for galactomannans with known hypoglycemic activity, like fenugreek galactomannans[25], which has a 1:1 M/G ratio[26,27], and guar gum[26] whose M/G ratio is 1.43:1 or 1.2:1[28]. Lateral galactose groups interfere in galactomannan's water solubility, as they make polysaccharide crystallisation and interchain interactions harder, to ease its water solubilization. The detection of absorption bands, 810 cm^{-1}, in the GAPs Fourier transformation infrared absorption suggest the presence of D-galactopyranose unities[29]. And a study with *A. pavonina* (L.) films detected two absorption bands, 812 cm^{-1} and 871 cm^{-1}, suggesting the presence of α-D-galactopyranose units and β-linked D-mannopyranose units, respectively. Glycaemia values show that both GAP 1% and GAP 2% exhibit significant glycaemia reduction in relation to diabetic control. Studies imply that concentration and viscosity contribute directly to hypoglycemic activity because it slows digestive enzymatic activity and glucose absorption in the gut[25,30]. Galactomannans viscosity is influenced by its M/G ratio. The higher the galactose content in the molecule, the easier the water access to the molecule. Galactomannan prevents interactions with other polysaccharides and their crystallisation[3]. The lower TAG plasma levels and lower TC levels may be a reflex of animals glycaemia diminution, due to a lower intestinal glucose absorption tax. Once minor blood glucose reduces insulin excretion and consequently makes hepatic TAG and cholesterol production lower. Other studies indicate that the decrease of digestive lipases activity and high 3-hydroxy-3-methylglutaryl-CoA reductase activity, hepatic enzyme are responsible for bile acid production and neutral steroids[31], this explains the hypocholesterolemic action of galactomannans.

The weight of the organs provides information for preliminary evaluations of toxicological tests. It may indicate lesions to the systems, as we can observe, for example, elevation of liver weight due to hepatocellular hypertrophy[32]. The results of RLW indicated GAP hepatotoxicity was lower than the standard drug of diabetes, and these results were confirmed by the histological analysis of hepatic and pancreatic tissue. These changes may be justified by administration of STZ, a drug with diabetogenic properties because of its pancreatic β-cell selectivity. However, STZ induces sub-lethal DNA alkylation in important organs such as the liver and can promote carcinogenesis by inducing sub-lethal DNA alkylation[33]. In conclusion, our results in this study suggest that galactomannans extracted from *A. pavonina* L. seeds have antidiabetic activity, by regulating glycaemia and diabetic mice lipedema, not interfering with the animals food behaviour and body weight, beyond that no toxicity signals are observed. This work evidences that *A. pavonina* L. galactomannans have potential as a therapeutic alternative for the control and treatment of DM. For being soluble fibres, the antidiabetic effect of galactomannans may be related to intestinal glucose and lipid absorption inhibition, and is potentially involved in the reabsorption inhibition of biliary salts, but further studies are necessary to perform this analysis.

Conflict of interest statement

The authors declared that there is no conflict of interest.

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