Chemistry of Natural Compounds, Vol. 41, No. 1, 2005

GALACTOMANNAN FROM *Gleditsia macracantha* SEEDS AND ITS BIOLOGICAL ACTIVITY

R. K. Rakhmanberdyeva, D. A. Rakhimov, A. A. Vakhabov, Z. A. Khushbaktova, and V. N. Syrov

UDC 547.917

Galactomannan, a nontoxic compound that exhibits in experiments with intact rats a slight hypocholesterinemic and hypoglycemic activity, was isolated from the coating of Gleditsia macracantha *seeds. It prevents significantly the development of experimental hyperglycemia and diabetes when administered simultaneously with alloxan.*

Key words: *Gleditsia macracantha*, galactomannan, hypoglycemic activity.

Eight species and varieties of *Gleditsia* have been introduced to the Rusanov Botanical Garden of the Academy of Sciences of the Republic of Uzbekistan. Seeds of *Gleditsia* have a high content of galactomannan (GM) water-soluble polysaccharides [1].

Water-soluble polysaccharide isolated from whole *Gleditsia macracantha* Desf. seeds (large-thorned locust) contains 1.97% nitrogen, which indicates the presence of proteinaceous substances. About 25% of the whole seeds consists of the germinating part, which is rich in proteinaceous substances (48% of the dry mass). A large fraction (40%) of these are reserve proteins such as globulins; the remainder, functional proteins (various enzymes, inhibitors, etc.). It was found that 3% are inhibitors of proteolytic enzymes such as trypsin and chymotrypsin, which are high-molecular-weight inhibitors.

Therefore, we used samples of GM isolated from seed coating, which does not contain protein, for further chemical and biological investigations.

The isolation of GM, as described before [1, 2], includes triple extraction of the raw material with water and precipitation by alcohol in a 1:2 ratio. The GM yield was 21.7% of the air-dried mass of raw material.

GM is a friable, white, amorphous, and odorless powder, mp 275-280°C (dec.), insoluble in alcohol and acetone, and soluble in water. It forms viscous solutions $\eta_{char} = 7.5$ (*c* 0.5%, H_2O) with specific rotation $[\alpha]_D^{22}$ +14.0° (*c* 0.1%, H_2O). The monosaccharide content of GM was determined by total acid hydrolysis. Mannose and galactose were identified in a 5:1 ratio in the hydrolysate by paper chromatography (PC) and GC.

The polysaccharide structure consists of long linear chains of D-mannose with a side chain of D-galactose [2].

Investigations of the biological activity of GM isolated from *G. macracantha* found that this polysaccharide is nontoxic. Single administration s.c. and i.p. to rats at doses up to 500 mg/kg and orally up to 1000 mg/kg did not cause behavorial changes or death (observation for 14 d). Multiple administration of GM at a dose of 50 mg/kg (for 3 wk) also did not produce any noticeable negative behavorial effects in the animals. In addition, it was found during the biochemical tests that GM exhibits in experiments with intact animals a slight hypocholesterinemic (12-14%, $P > 0.05$) and hypoglycemic (15-17%,, $P < 0.05$) action. Considering literature data on the ability of compounds of this class to elicit a more distinct hypoglycemic effect under pathological disruption of carbohydrate exchange [3], we studied the corresponding action of GM from *G. macracantha* seeds on the course of alloxan hyperglycemia and alloxan diabetes. The results in general confirmed this hypothesis. Thus, control rats remaining alive 3 d after alloxan administration can be divided into three groups according to the extent of developed hyperglycemia and the subsequent course of diabetes. Table 1 shows that mild diabetes developed in 10 rats. The blood glucose content in these did not exceed 200 mg% during the experiments.

S. Yu. Yunusov Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax (99871) 120 64 75. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 10-11, January-February, 2005. Original article submitted September 13, 2004.

0009-3130/05/4101-0011

TABLE 1. Effect of Galactomannan from *Gleditsia macracantha* Seeds on Glucose Content in Blood Serum of Rats with Alloxan Hyperglycemia and Alloxan Diabetes (M ± m)

Experimental conditions	Number of deceased animals	Developed forms of diabetes	Glucose content in blood serum (mg, %) after days				
			Initial	3	7	14	21
Control, alloxan	18 (36%)	Mild (10 rats)	95.4±7.2	166±10.2*	170±9.8*	178±20.2*	180±18.6*
		Moderate (13 rats)	93.2±6.4	248±19.4*	252±16.6*	280±28.4*	276±30.4*
		Severe (9 rats)	96.4±4.8	368±28.4*	370±32.4*	360±36.4*	354±46.8*
Exptl. (alloxan +	10 (20%)	Not developed (6 rats)	98.2±8.4	126±10.2	130±12.8	118±7.6	100±7.4
galactomannan from		Mild (22 rats)	96.2±6.4	136±12.4*	140±9.8*,**	132±8.8*,**	106±8.2**
Gleditsia macracantha		Moderate (7 rats)	94.2±7.8	216±18.6*	210±10.4*,**	140±12.4*,**	116±6.4**
seeds)		Severe (5 rats)	98.2±5.2	320±30.2*	276±14.6*,**	260±24.2*,**	158±13.6*,**

*Reliable relative to initial values, **relative to the corresponding control ($P < 0.05$). Number of animals in experiment - 50.

The glucose level in 13 specimens varied in the range 200-300 mg%. These animals belong to the group with moderate diabetes. A severe form of alloxan diabetes was observed with a blood glucose content elevated above 300 mg%. As a rule, hyperglycemia that developed after alloxan administration was stable and persisted through the experiment.

The group of experimental rats that received GM and alloxan exhibited, first of all, a much lower death rate and a distinctly lower sugar level in the blood of those that remained alive. Six rats exhibited brief hyperglycemia although diabetes did not develop. The blood glucose level rose to the level of mild diabetes in 22 rats. However, it was 18.1, 17.7, and 25.8% lower on the 3rd, 7th, and 14th day than in the control. It did not significantly differ from the initial level on the 21st day (the blood sugar content was 41.1% lower than the corresponding control in this period). Moderate diabetes developed in 7 rats. However, continued administration of GM to these animals also helped to reduce the blood glucose level. This was especially evident on the 14th and 21st day of observation (the hypoglycemic effect was 50.0 and 42.0%, respectively, of the control). Severe diabetes developed in only 5 rats of the experimental group. In this instance, although administration of GM prevented an increase in the blood sugar level, the value was nevertheless higher than the initial one by 60.8% at the end of observation (Table 1). A comparison of the blood sugar concentration in control and experimental rats with severe diabetes showed that it was 13.1 ($P < 0.5$), 25.4 ($P < 0.05$), 27.8 ($P < 0.05$), and 55.4% ($P < 0.002$) lower in animals receiving GM after 3, 7, 14, and 21 d, respectively.

Thus, GM from *G. macracantha* seeds is nontoxic, has a significant effect on the overall behavior of animals (for different administration pathways), and possesses a slight hypocholesterinemic and hypoglycemic effect in experiments on intact animals. It has a rather marked hypoglycemic action on animals with alloxan hyperglycemia and diabetes and also in general alleviates the toxic action of alloxan on an organism.

EXPERIMENTAL

PC was carried out on Filtrak FN-12,15 paper using *n*-butanol:pyridine:water (6:4:3) with anilinium acid phthalate developer.

GC was performed on a Chrom-5 instrument with a flame-ionization detector, a stainless-steel column (200 × 0.3 cm) packed with 5% Silicone XE-60 on Chromaton NAW-0.200-0.250 mesh, 210°C, N_2 carrier gas at 60 mL/min, and samples as aldonitrile acetates [4].

Viscosity of GM was measured in an Ostwald viscometer with a capillary 0.75 mm in diameter at 22°C.

Specific rotation of GM was determined on a Zeiss polarimeter in tubes 1 dm in length of 10 mL volume and 0.5 dm in length of 1 mL volume at 20-23°C.

Isolation of GM. Seed coating of *G. macracantha* was isolated from the germinating part (350.0 g), ground and passed through a sieve with 2-mm openings, and extracted three times with water at room temperature in the ratios 1:20, 1:15, and 1:10 for 24 h. The extract was separated from pulp by filtration through fine paper on a Buchner vacuum funnel. The total volume

of aqueous extract was 10 L. GM was isolated from the thick, mucilaginous, and transparent extract by precipitation with alcohol in a 1:2 ratio. The solid was separated, washed with alcohol of increasing strength (80-90°), and dried in air. Yield of GM, 71 g.

Monosaccharide Composition of GM. GM (0.05 g) was hydrolyzed with H_2SO_4 (2 N) for 8 h at 100°C. The hydrolysate was neutralized with $BaCO_3$, deionized with cation exchanger KU-2 (H^+), evaporated, and studied by PC and GC.

The overall toxicity of GM was studied in experiments on white rats of both sexes and 20-23 g mass. Hypocholesterinemic and hypoglycemic activity of the studied polysaccharide was studied using male rats of mass 160-180 g. Cholesterol and glucose in blood serum were determined as before [5, 6]. Alloxan was administered once s.c. at 150 mg/kg as a freshly prepared solution (5%); GM, orally at 50 mg/kg once with alloxan and then daily until the completion of the experiment. Blood samples were taken from the end of the tail for determination of the blood glucose level during the development of the pathological disease. Results were treated statistically as before [7].

REFERENCES

1. M. R. Mirzaeva, R. K. Rakhmanberdyeva, E. L. Kristallovich, D. A. Rakhimov, and N. I. Shtonda, *Khim. Prir. Soedin.*, 727 (1998).
2. M. R. Mirzaeva, R. K. Rakhmanberdyeva, and D. A. Rakhimov, *Khim. Prir. Soedin.*, 573 (1999).
3. T. Kiho, S. Itahashi, and M. Sakushima, *Biol. Pharm. Bull.*, **20**, No. 2, 118 (1997).
4. D. G. Lance and J. K. N. Jones, *Can. J. Chem.*, **45**, 17, 1995 (1967).
5. L. L. Abell, B. B. Levy, B. B. Brodie, and F. E. Kendall, *J. Biol. Chem.*, **195**, 357 (1952).
6. A. V. Karakashov and E. P. Vichev, *Micromethods in the Clinical Laboratory* [Russian translation], Meditsina i Fizkul′tura, Sofia (1968).
7. M. L. Belen′kii, *Elements of Quantitative Evaluation of the Pharmacological Effect*, Medgiz, Leningrad (1963), p. 125.