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Experimental Evaluation of the Anti-Inflammatory and Anti-Ulcer Effects Pectin of *Scutellaria Comosa* and Galactomannan of *Crotalaria Alata*

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Abstract Pectin substances Scutellaria comosa - PS and galactomannan Crotalaria alata - GM, in experiments on rats (males, 180-200 g), when administered orally at a dose of 250 mg/kg have a pronounced antiinflammatory effect (inhibit the development of edema of the extremities when injected under the aponeurosis of the foot of one of the them 0.1 ml of 25% formalin solution) and also has an anti-ulcer effect in the modeling of "acetylsalicylic", "ethanol" gastric ulcers, as well as ulcers caused by strong stress and butadion.

Keywords Scutellaria comosa, Crotalaria alata, Pectin substances, Galactomannan, Anti-inflammatory and anti-ulcer actions

1. Introduction

Polysaccharides - pectin substances and galactomannans are fairly widespread plant metabolites. Pectins have recently attracted the growing interest of researchers in various fields of medicine in the treatment of diseases associated with irritation of the mucous membranes, especially in gastroenterological practice. They can be used as a physical barrier to protect the epithelium from opportunistic microbial invasion and other irritants in the gastrointestinal tract [1-3].

Galactomannan (GM) - polyfunctional phytopolysaccharides due to their unique physicochemical properties of hydrocolloids and lack of toxicity are also widely used in various fields of medicine as food additives, stabilizers, thickeners and gelling agents. They have hypocholesterolemic, hypoglycemic activity [4,5]. On the basis of galactomannan at the Institute of Chemistry of Plant Substances of the Academy of Sciences of the Republic of Uzbekistan, the gledol medium was created for the precipitation of peripheral blood lymphocytes [6].

The aim of this work was to study the pectin of *Scutellaria comosa* (PS) and galactomannan *Crotalaria alata* (GM) for the presence of antiinflammatory and antiulcer activity. This is due to the high frequency of the corresponding pathological conditions and the frequent development of side effects, including those from the gastrointestinal tract with the enteral use of traditional drugs [7].

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2. Materials and Methods

2.1. The Chemical Part of the Work

Pectin (PS) was isolated from the aerial part of *Scutellaria comosa*, galactomannan (GM) from the seeds of *Crotalaria alata* [8–10].

PS and GM are amorphous powders that dissolve in water to form viscous solutions.

The viscosity of PS and GM solutions was measured on an Ostwald viscometer with a capillary diameter of 0.73 mm at a temperature of 20-22℃.

The content of galacturonic acid in PS was determined by the carbazole method [11]. The molecular weights of PS and GM were determined by high performance size exclusion chromatography (Table 1).

The monosaccharide compositions of PS and GM were studied by a complete acid hydrolysis. PS was hydrolyzed with 2N H_2SO_4 for 24 hours, GM with 1N H_2SO_4 for 8 hours at 100℃. The hydrolysates were neutralized with $BaCO_3$, deionized with KY-2(H+) cation exchanger, and evaporated. The indication of PS and GM monosaccharides was carried out in the system n-butanol-pyridine-water (6:4:3), developer acid aniline phthalate. GC analysis of the samples was carried out on a Shimadzu GC-2010 chromatograph (Japan) with a flame ionization detector, a Shimadzu

Rxi-624Sil MS quartz capillary column (30m × 0.25mm × 1.40 μm), a mobile phase rate (N2) of 1.5 ml/min, an injector temperature 260℃, detector temperature 280℃ and column temperature 230℃. Samples were taken as aldonitrile acetates [12] (Table 1).

Table 1. Physicochemical characteristics of PS and GM

Type of polysaccharides	PS	GM
Мм, kDa	288	540
Relative solution viscosity	5.25 (c1%; H_2O)	49.86 (c 75; H_2O)
DE,%	44,4	-
Monosaccharide composition, %:		
Gal	23.5	17.5
Glu	4.2	1.5
Man	-	76.3
Ara	49.1	2.5
Rha	23.5	-
Gal A, %	85.0	-

Note: PS- pectin from Scutellaria comosa, GM – galactomannan from Crotalaria alata, DE-degree of esterification, *Gal*-galactose, *Glu*-glucose, *Man*-mannose, *Ara*-arabinose, *Rha*-rhamnose, *Gal A*-galacturonic acid

As can be seen from Table 1, according to the monosaccharide composition, PS is rhamnogalacturonan with a degree of esterification of 44.4%, that is, the studied PS is a low-esterified pectin. GM has a higher molecular weight and a relative viscosity. The main monosaccharide in GM is mannose (76.3%) and galactose (17.5%).

2.2. Pharmacological Part of the Work

The experiments were carried out on male rats weighing 180-200 g. The maintenance and manipulations performed with animals corresponded to generally accepted world standards with laboratory animals (Directive 2010/63/EU of the European Parliament, September 22, 2010). Edema caused by the injection of 0.1 ml of a 2.5% formalin solution under the aponeurosis of the foot of the right hind limb with an oncometric evaluation of the results served as a model of inflammation [13]. The antiinflammatory activity of the studied polysaccharides in this case was studied in comparison with butadione, one of the effective representatives of non-steroidal anti-inflammatory drugs [7].

Polysaccharides were administered at a dose of 250 mg/kg (in preliminary experiments, these doses are established as the most effective), butadione - 25 mg/kg orally throughout the entire observation period (7 days), starting 3 days before formalin administration.

The antiulcer activity of polysaccharides was studied on models of "acetylsalicylic" ulcer of the gastric mucosa, reproduced by double intragastric administration of aspirin at a dose of 150 mg/kg with an interval of 4 hours, "ethanol" gastric ulcer caused by the administration of absolute ethanol to animals in a volume of 1 ml per 200 g body weight; stress ulcers caused by immobilization of animals in the supine position for 16 hours, as well as gastric ulcers caused by butadion (injected intramuscularly at 0.2 ml per 100 g of a 15% butadione solution in chilled acetone) [14]. When playing "acetylsalicylic" and "ethanol" gastric ulcers, rats were deprived of food and water 24 hours before exposure to ulcerogens. When playing a "butadione" ulcer, rats were deprived of food two days before exposure to the ulcerogen, leaving free access to water. Destructive lesions of the stomach were counted one day after the onset of ulcerogenic exposure (animals were slaughtered by instantaneous decapitation under light ether anesthesia). In the case of the development of a "butadion" ulcer, in addition to calculating the total number of destructions of the gastric mucosa, the Pauls index (PI) was calculated using the formula:

$$PI = A \times B/100,$$

where A is the average number of ulcers per animal,

B-number of animals with ulcers in the group (%).

The severity of antiulcer activity was judged by the ratio of PI in the control and experimental groups.

The studied polysaccharides in this case were administered orally during the previous 7 days, the last time 3 hours before slaughter, using a gastric tube, also at the rate of 250 mg/kg. Control animals received an adequate amount of distilled water.

The results obtained were processed by the method of variation statistics using Student's t-test.

3. Results and Discussion

The experiments performed showed that the administration of a phlogogenic agent to rats promotes the development of hyperemia and gradual swelling of the limb with a maximum development 3 hours after the administration of formalin, when the volume of the limbs of rats was increased by an average of 59.2% compared to the initial one. In subsequent periods of observation, the edema gradually decreased and the initial volume of the limbs was restored by the 7th day of the experiment (Fig. 1).

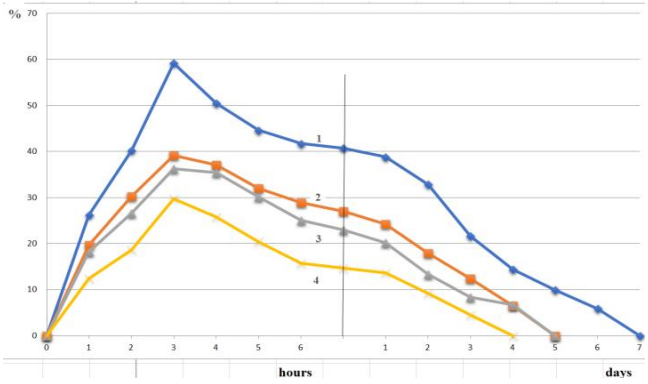

Figure 1. Influence of pectin substances from Scutellaria comosa (4), galactomannan Crotalaria alata (3) and butadiene (2) on the dynamics of inflammatory edema caused by formalin, compared with control (1). The abscissa axis (X) - time (hour, days), the ordinate axis (Y) - the development of edema (in% of the initial volume)

Table 2. Influence pectin of Scutellaria comosa, galactomannan of Crotalaria alata and butadione on the inflammatory process in rats at the time of maximum development of edema (after 3 hours), as well as a day and 3 days after formalin administration (M±m, n=6)

The nature of the experiment	Increase in the volume of the limb,% of the original	The difference in increase in volume compared to				Increase in the volume of the limb,% of the original	The difference in increase in volume compared to				Increase in the volume of the limb,% of the original	The difference in increase in volume compared to			
		Control	P	Butadion	P		Control	P	Butadion	P		Control	P	Butadion	P
	After 3 hours					In a day					After 3 days				
Control	59,2 ± 4,6	-	-	-	-	38,8 ± 2,5	-	-	-	-	21,6 ± 2,2	-	-	-	-
PS	29,7 ± 4,2	29,5	<0,001	9,5	<0,25	13,7 ± 1,9	25,1	<0,001	10,5	<0,25	4,5 ± 2,0	17,1	<0,001	7,9	<0,05
GM	36,3 ± 2,7	22,9	<0,002	3,4	>0,5	20,2 ± 4,9	18,6	<0,01	4,0	>0,5	8,4 ± 3,1	13,2	<0,01	4,0	<0,5
Butadion	39,2 ± 5,4	20,9	<0,02	-	-	24,2 ± 5,7	14,6	<0,05	-	-	12,4 ± 2,2	9,2	<0,02	-	-

Table 3. Influence of pectin Scutellaria comosa and galactomannan of Crotalaria alata on the healing process of experimental gastric ulcers in rats, * (M±m, n=6)

Experiment conditions	The number of destructions of the gastric mucosa caused by		
	Acetylsalicylic acid	ethanol	Stress impact
Control	17,8 ± 0,87	11,5 ± 0,56	4,7 ± 0,67
PS	2,7 ± 0,42[1,2]	3,0 ± 0,36 [1,2]	1,8 ± 0,48[1]
GM	4,7 ± 0,49[1]	5,0 ± 0,36[1]	2,5 ± 0,43[1]

Note. [1-]Significant in relation to the corresponding control,
[2-]significant between the groups receiving the studied pectin *Scutellaria comosa* and galactomannan *Crotalaria alata* (p□0.05) *caused by acetylsalicylic acid, ethanol and stress

Table 4. Influence of pectin substances of *Scutellaria comosa* and galactomannan of *Crotalaria alata* on the healing process of experimental gastric ulcers in rats caused by butadione (M±m, n=6)

Experiment conditions	Number of animals in the group	Number of animals (%) with ulcers	Destruction of the stomach					Pauls index	Antiulcer activity compared to control
			Small (d<1,5 mm)	Medium (d<1,5-2,5mm)	Large (d<2,5-5,0mm)	Striped	Total		
Control (butadione)	10	90	20,0±2,38	7,7±0,96	6,5±0,87	5,5±0,67	39,7±4,71	35,7	-
Butadione+PS S. *comosa*	10	70	12,2±2,67[1]	3,6±0,89	3,5±0,85[1]	2,9±0,72[1]	22,2±4,96[1]	15,5	2,30
Butadione + GM *C. alata*	10	70	13,9±3,1	5,1±1,13	4,2±0,93	4,0±0,89	27,2±5,96	19,0	1,88

The prophylactic-therapeutic administration of the studied PS and GM polysaccharides did not change the dynamics of edema development, but contributed to curbing the process of exudate accumulation after formalin administration. After 3 hours, the limb volume of animals treated with PS was only 29.7% and those receiving GM - 36.3% of the original. The similar effect of butadione in this period was 39.7%. A day later, the increase in the volume of the limb of rats in the control was 38.8% and in those who received PS, GM and butadion - 13.7; 20.2 and 24.2%, after 3 days - in the control 21.6, under the influence of drugs 4.5; 8.4 and 12.4%, respectively (Fig. 1, Table 2).

The initial volume of the limb in the control animals was restored after 7 days, and in those receiving PS, GM and butadione at an earlier time (on days 4 and 5, respectively) (Fig. 1).

The obtained data show that the studied pectin substances and galactomannan have a significant anti-inflammatory effect. PS shows a slightly more pronounced effect than butadione, GM has a similar anti-inflammatory effect.

An important point in the experiments was the establishment of the fact that PS and GM, having an anti-inflammatory effect are similar to butadione, in contrast to it, they have a clear antiulcer effect when various ulcerogens (including butadione itself) are administered to animals. The data obtained in this regard are shown in table 3.

As can be seen from the presented results of the study, PS, GM in the conditions of the development of "acetylsalicylic" ulcers showed a significant gastroprotective effect, reducing the number of destructive formations by 84.8 and 72.7%. In the group of animals that were injected with ethanol, control rats showed a large number of point and strip-like destructions, as well as small hemorrhagic erosions. In rats, which were previously injected with the studied PS, GM the introduction of ethanol was accompanied only by the development of erosive destructions, and their total number was significantly less than in the control ones.

The gastroprotective effect of PS and GM in this case was 73.9 and 56.5%. A clear gastroprotective effect was also revealed in the development of a "stress ulcer" (the effect was 61.7 and 46.8%). Extremely favorable results were obtained with the introduction of PS and GM under conditions where the occurrence of ulcers was initiated by the introduction of butadione. A decrease in the number of all types of resulting destructions was revealed. As a consequence, their total number in the experimental groups was significantly less than the control values. As a result, calculated on the basis of the Pauls index, the antiulcer activity of the studied polysaccharides was quite pronounced. The anti-ulcerogenic effect of PS in almost all cases was higher than that of GM (Table 3.4). As for the mechanism of the revealed effects of PS and GM, in experiments with the introduction of acetylsalicylic acid as an ulcerogen, their antiulcer effect can be explained by a local enveloping effect, since acetylsalicylic acid, breaking through the natural protective barrier of gastromucopolysaccharides, damages the cells of the mucous membrane. The enveloping action of PS and GM may also underlie their antiulcerogenic effect upon oral administration of ethanol. At the same time, the results of experiments on models of a “stress” gastric ulcer and an ulcer caused by butadion do not allow us to speak of a purely local enveloping effect of the studied polysaccharides, since in order to prevent an ulcerogenic effect in these cases, it is not enough to protect the mucous membrane with enveloping agents (starch, in these experiments was inefficient). The most probable is the increase under the influence of PS and GM of nonspecific resistance of the gastroduodenal zone due to the activation of some protective reactions of the body, as well as a certain influence aimed at enhancing reparative processes in tissues [15-16]. Such a conclusion can be quite justified, given that many plant extracts and individual substances isolated from them, which increase the general nonspecific resistance of the body, also have a noticeable antiulcer effect [17-20]. The presence of anti-inflammatory activity in them can be largely associated with an increase in the general nonspecific resistance of the organism.

4. Conclusions

Pectic substances (PS) isolated from the aerial part of *Scutellaria comosa* and galactomannan (GM) from the seeds of *Crotalaria alata* have a significant anti-inflammatory effect, superior to that of butadione, affecting mainly the exudative component of the inflammatory process.

In experiments on rats, it was also found that the studied polysaccharides, due to the local enveloping effect on the gastric mucosa and the increase in nonspecific resistance of the gastroduodenal zone as a whole, have a noticeable positive effect on a variety of ulcerogenic factors. The anti-inflammatory and antiulcer activity of the studied pectin substances (PS) and galactomannan (GM) can also be associated with their physicochemical parameters, i.e., mainly with gel-forming properties. The anti-inflammatory and anti-ulcer activity of the studied pectin substances (PS) and galactomannan (GM) is probably related to their physico-chemical parameters, i.e., mainly to gel-forming properties. PS, which are characterized by the predominant presence of free hydroxyl groups and having a low degree of esterification, promote penetration into the area of the wall of the gastrointestinal tract. It is likely that galactomannans, due to their high molecular weight (540 kDa) and viscosity (49.86 mg/dL), similar to high esterified pectins, can interact with mucosal gastromucopolysaccharides, forming gel-like tangles that help reduce penetration into the intestinal wall.

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