NEW BIOMEDICAL TECHNOLOGIES

Effects of Pectin from *Amaranthus cruentus* on Isolated Rat Heart

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Experiments with isolated rat heart showed that pectin isolated from amaranth causes coronary spasm without changing contractile tone.

Key Words: *pectin; isolated heart; pressure; coronary blood vessels*

Pectin is the major component of the heteropolysaccharides pectin substances. The main polymeric chain of pectins consists of the residues of digalacturonic acid or its methyl esters linked by the α(1→4) glycoside bond. Pectin substances are found in citrus plants, beetroot, apples, and some other plants and used as setting agents, emulsifiers, and stabilizers in food and pharmacological industries. Pectins facilitate excretion of heavy metals from human and animal organism, normalize metabolic processes, and are used for prevention and therapy of gout and chronic colitis. They inhibit tumorigenesis in rats [5,11], normalize blood cholesterol levels [7-9], and improve the function of digestive [7,12] and cardiovascular [10] systems. However, it was reported that pectin substances produce some undesirable effects, such as retardation [11] and bronchial asthma [12].

In the present study we examined the effects of pectin on isolated perfused heart. Pectin was isolated from the stem and leaves of *Amaranthus cruentus*. The content of pectins in this plant is 10% of dry weight, which is much higher than in traditional raw materials for pectin isolation.

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MATERIALS AND METHODS

Pectin substances were isolated by the conventional method [5]: grinding of the raw material→acid hydrolysis→filtration→ethanol precipitation→drying.

The size of the raw material particles varied from 0.1 to 0.8 mm. Oxalic acid (0.25% aqueous solution) was used as a hydrolyzing agent. The hydromodule was equal to 1:10-15. Hydrolysis was carried out for 3 h at 45-50°C and 760 mm Hg. Hydrolyzate was filtered, and polysaccharides were concentrated by filtration in an AUF-01 apparatus (membrane filters with a filtration border of 50 kD). Pectin substances were precipitated from the residue with ethanol (1:2). The precipitate was separated and lyophilized.

Molecular mass of the precipitate determined by viscosimetry varied from 20 to 25 kD. Thin-layer and high efficiency liquid chromatography showed that the pectin substance consisted of galacturonic acid (67%), rhamnose (4.1%), arabinose (6.6%), glucose (8.3%), galactose (7.7%), fructose (4.1%), and xylose (2.1%). The degree of esterification determined by titration [6] and infrared spectroscopy (Fig. 1, *b*) was 65-70%. Static capacity by calcium chloride estimated by the method [1] was 0.08 mol/kg.

The effects of pectin on the coronary flow rate (CFR) and maximum pressure in the left ventricle

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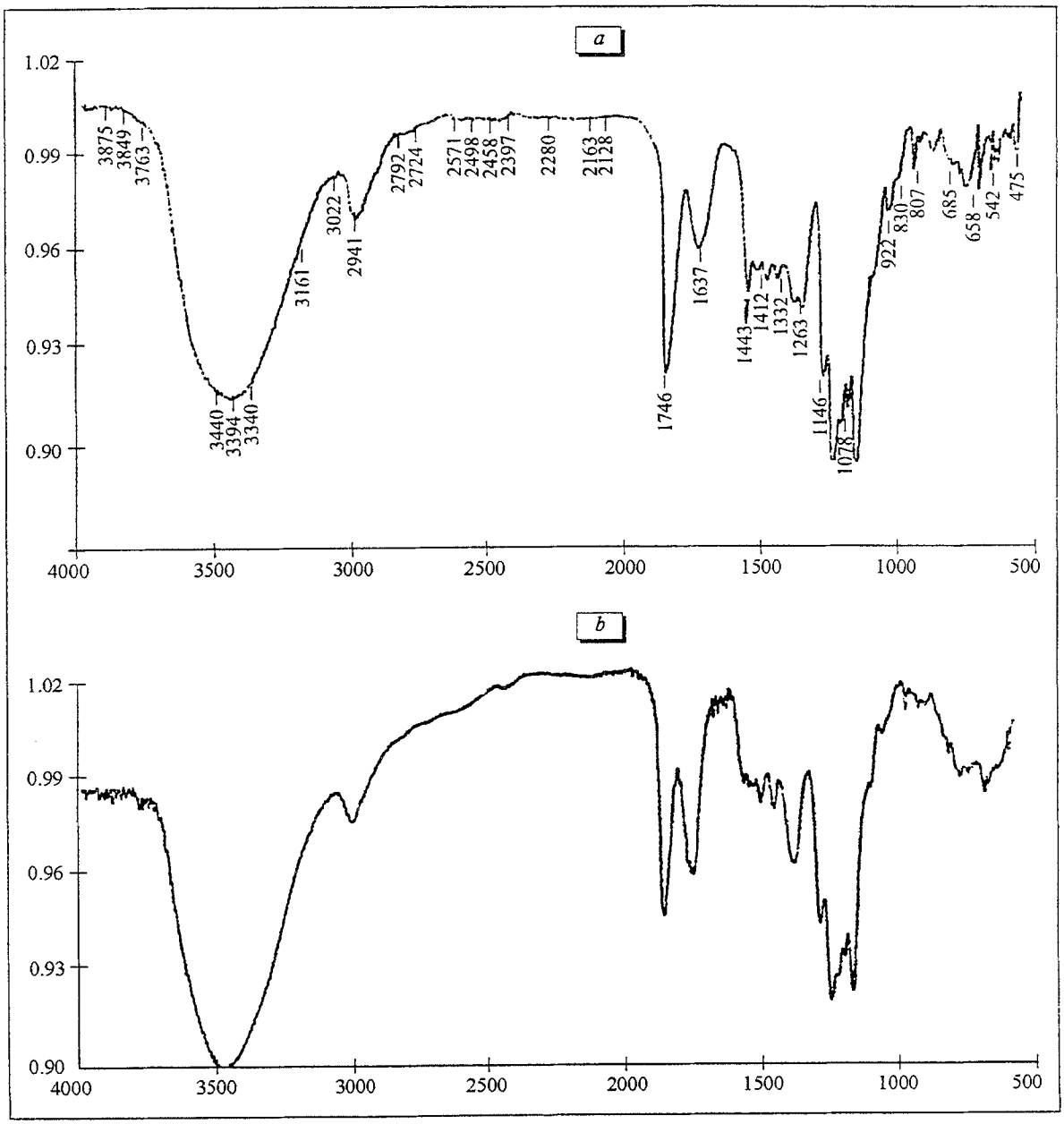

Fig. 1. Infrared spectrum of commercial pectin preparations (*a*) and pectin isolated from *Amaranthus cruentus* (*b*). Pectin was preformed with KBr. Abscissa: wavelength, cm^{-1}; ordinate: light absorbance, optical density units.

(P_{max}) were studied on isolated rat hearts perfused by the method of Langendorff with Fallen's modifications. The heart with an essentially long segment of ascending aorta was incised under Nembutal (0.04 g/kg) anesthesia and placed in cold (4°C) physiological saline. Perfusion with Krebs—Henseleit buffer (pH 7.4) was started immediately via a cannula inserted in the aorta. It was carried out in the noncirculation regime at 37.5°C and constant pressure (65 mm Hg). The perfusate was bubbled with an O_2(96%)/CO_2(4%) mixture. Glucose (11 mM) was used as a substrate [10].

The coronary flow rate was assessed by the perfusate outflow and expressed in arbitrary units. The maximum pressure in the left ventricle was measured by the method [2] with the use of a latex balloon (500 μl) inserted into the left ventricle via the left atrium auricle and the atrioventricular opening and connected via the standard system to a P37B pressure sensor of an SP-1-400 blood pressure monitor (Statham).

Five series of experiments were performed; two male Wistar rats (body weight 230-280 g) were used in control and experimental groups in each series. In each experiment, the hearts were perfused with Krebs—Henseleit buffer for 25-30 min (an initial restoring perfusion). Control hearts were perfused with the same buffer for 95 min. Experimental hearts were

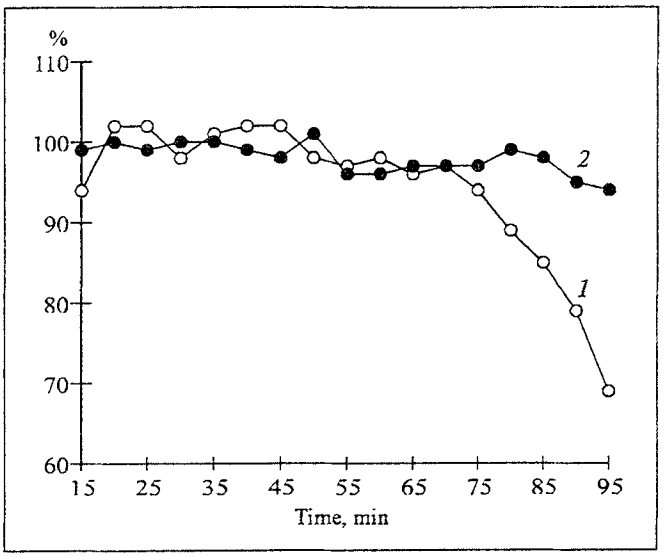

Fig. 2. Effect of pectin on the maximum pressure in the left ventricle. Here and in Fig. 3: *1*) control; *2*) experiment.

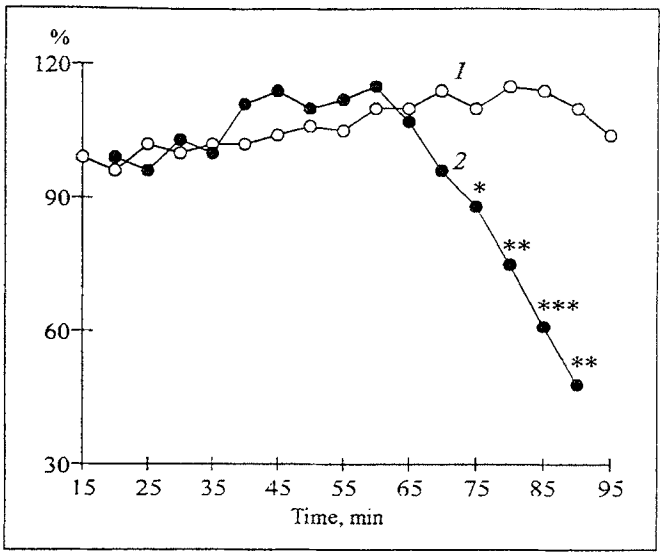

Fig. 3. Effect of pectin on the coronary flow rate. *$p<0.05$, **$p<0.01$, ***$p<0.001$ compared with the control.

perfused for 60 min with the buffer containing 0.004 g/liter pectin, on the 65th min of perfusion pectin concentration was increased to 0.04 g/liter. In preliminary experiments, pectin produced no effect on CFR at concentrations of 0.001 and 0.01 g/liter.

RESULTS

In the control hearts, CFR remained virtually unchanged throughout the observation period, while P_{max} gradually decreased (Figs. 2 and 3). There was no statistically significant difference between these parameters in hearts perfused for 60 min with a buffer containing 0.004 g/liter pectin and in control hearts, while an increase in the pectin concentration to 0.04 g/liter led to a pronounced decrease in CFR ($p<0.05$). The maximum pressure in the left ventricle was constant during the entire period of heart perfusion. It should be mentioned that in previous studies pectin isolated from amaranth caused a simultaneous decrease in CFR and P_{max}. However, in the present study P_{max} remained unchanged irrespective of the drop in CFR.

From this observation it can be suggested that at 0.004 g/liter pectin does not affect the function of isolated rat heart, while at 0.04 g/liter it exhibits a vasoconstrictor activity toward coronary blood vessels without changing myocardial contractile tone.

Thus, the pectin substance isolated from *Amaranthus cruentus* produces a positive inotropic effect on the myocardium. Although pectins reduce calcium concentration by binding calcium ions to free carboxyl groups of galacturonic acid residues, which at least theoretically reduces P_{max} due to esterification of most carboxyl groups, in the present study we did not observe any reduction in P_{max}.

Further investigations are required to elucidate the mechanism of pectin effects on isolated rat heart, central and peripheral circulation, and on hepatic, digestive, and excretion functions.

REFERENCES

1. A. A. Arasimovich, S. V. Baltaga, and N. P. Ponamareva, in: *Methods of Analysis of Pectin Substances, Hemicellulose, and Pectolytic Enzymes in Fruits and Vegetables* [in Russian], Kishinev (1970), p. 84.
2. N. P. Sheluknina, P. Sh. Abaeva, and G. B. Aimukhamedova, in: *Pectin and Parameters of Its Preparation* [in Russian], Frunze (1987), p. 108.
3. T. Bamba, W. Chun, and S. Hosoda, *J. Nutr. Sci. Vitaminol. (Tokyo)*, **38**, No. 2, 197-201 (1992).
4. J. Doherty and A. A. Jackson, *Acta Paediatr.*, **81**, No. 8, 514-517 (1992).
5. W. E. Hardman and I. L. Cameron, *Carcinogenesis*, **16**, No. 6, 1425-1431 (1995).
6. A. Kraut, Z. K. Peng, A. B. Becker, *et al.*, *Chest*, **102**, No. 5, 1605-1607 (1992).
7. J. W. Lampe, J. L. Slavin, K. S. Baglien, *et al.*, *Am. J. Clin. Nutr.*, **53**, No. 5, 1235-1241 (1991).
8. G. H. McIntosh, J. Whyte, R. McArthur, *et al.*, *Ibid.*, pp. 1205-1209.
9. N. E. Miller, O. H. Thelle, and D. S. Forde, *Lancet*, No. 1, 965-968 (1977).
10. J. R. Neely and M. J. Rovetto, *Methods Physiol.*, **39**, 43-60 (1975).
11. K. J. Pienta, H. Naik, A. Akhtar, *et al.*, *J. Natl. Cancer Inst.*, **87**, No. 5, 348-353 (1995).
12. J. A. Roth, W. L. Frankel, W. Zhang, *et al.*, *J. Surg. Res.*, **58**, No. 2, 240-246 (1995).
13. L. F. Tinker, B. O. Schneeman, P. A. Davis, *et al.*, *Am. J. Clin. Nutr.*, **53**, No. 5, 1259-1265 (1991).
14. H. Trowell, *Ibid.*, **25**, 926-932 (1972).