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Gastroprotective Effect of a Pectin from *AMARANTHUS CRUENTUS* L

Key words: *pectin of Amaranthus cruentus L., gastropathy, indomethacin, meloxicam*

Annotation: *This article present an analysis of data protective effect of amaranth pectin from Amaranthus cruentus L. (fam. Amaranthaceae) in case of indomethacin and meloxicam nonsteroidal anti-inflammatory drugs (NSAID)-induced stomach lesions in male Wistar rats. Oral administration of amaranth pectin (100 mg/kg) suppress formation of the gastric mucosal lesions. The findings confirmed that amaranth pectin enhanced resistance of the stomach tissue to NSAIDs and had gastroprotective effect in minimizing the number and sizes of destructive regions, reducing ATP and glycogen deficiency, decreasing excess lactate, and normalizing energy balance in the gastric tissue. Based upon its antiulcer effect, amaranth pectin may be recommended for preventing and treating stomach diseases, combined with the basic therapy.*

Over the past twenty years, there has been greatly increasing research interest focused by pharmacologists, practicing physicians and pharmacists on pectic polysaccharides (pectins), a group of biologically active natural substances commonly found in higher plants (3). At present, pectins are generally acknowledged and included in the FAO/WHO Codex Alimentarius.

Pectins are known to exhibit antimicrobial, antitumor, antibacterial, anti-inflammatory and hypoglycemic activities, showing positive effects as immunostimulants, prebiotics, good heavy metal and radionuclide chelators (4, 14, 17, 18). Pectins are widely used to produce complex medicinal products intended for the targeted drug delivery owing to digestion process simulation (11). Pectins have been shown to be effective in treating gastropathy caused by emotional stress, xenobiotic contamination, various bacterial infections or side effects of drugs (5, 15, 16, 19).

The research is aimed at investigating the effect of amaranth pectin in non-steroidal anti-inflammatory drug-induced gastropathy (NSAID-induced gastropathy) and its influence on energy metabolism in the gastric tissue.

Materials and methods

This experiment was carried out on male Wistar rats (laboratory animal farm “Stolbovaya”, RAMS, Russia), weighing 180-200 g. The animals were kept under standard vivarium conditions, with free access to feeding stuffs (OOO “Laboratorkorm”, Russia) and tap water. Each experimental group contained 8 animals. NSAID-induced gastropathy was induced by intragastric administration of indomethacin (40 mg/kg, Balkanpharma, Bulgaria) or meloxicam (30 mg/kg, Boehringer Ingelheim, Germany) in the form of water suspension.

The rats were starved but given water for twenty four hours before NSAID exposure. During starvation, the rats were kept in metabolic cages provided with a wide wire –mesh floor to avoid coprophagy or eating cuttings.

The monosaccharide composition of amaranth (*Amaranthus cruentus* L.) pectin contained 60% galacturonic acid, having molecular weights of 25 kD with 65% degree of esterification. Pectin was administered intragastrically, as a single dose, on an empty stomach, as a 2% aqueous pectin solution (100 mg/kg) during 6 days, and one hour before NSAID exposure – on the seventh day. Omeprazole (30 mg/kg, OAO “Sintez”, Russia) was used as reference drug according to the dosage scheme. The control group of animals received an equivolume of normal saline instead of pectin, omeprazole and NSAID.

The rats were kept and euthanized as recommended by the Directive 2010/63/EU of the European Parliament and of the Council of 22 september 2010 on the protection of animals used for scientific purposes. The animals were decapitated under ether anaesthesia upon the expiry of 5 hours after intragastric administration of indomethacin or meloxicam. Each stomach was opened along the greater curvature to determine a number of animals with gastric mucosa lesions, degree of ulceration (number of destructive changes per one animal) and Pauls index as an integral indicator of the amount of destruction defined using the formula: $PI = \text{mean degree of ulceration} \times \text{per cent of the animals ulcerated} / 100$ (13). The total ulcerated area (punctate hemorrhages, erosions, and strip-like lesions on the stomach lining) was determined in millimeters. The gastroprotective activity was defined as the ratio of PI of the NSAID group divided to PI of the NSAID and pectin group. The stomach tissue intended for biochemical tests was kept in liquid nitrogen. The contents of integral indicators of tissue supply of adenosine triphosphate (ATP), glycogen and lactate were determined by commonly used in experimental pharmacology biochemical methods in the gastric tissue with the most damaged regions of stomach lining.

The statistical data were processed using “Statistica, v.6.0” software. The statistical significance of differences between the control and experimental groups was estimated using Student's *t*-test. The data were represented as mean \pm standard error of mean.

Results

The maximum stomach lesions are known to exhibit 5 hours after administration of indomethacin. The stomach lining was characterized by the presence of hyperemic areas and multiple punctate and linear surface hemorrhagic erosive damages mostly observed in the antrum. PI was high (19.6 units) that confirmed considerable structural changes on the stomach lining. There were also metabolic disorders in the gastric tissue, such as deficiency of ATP and glycogen production and excess lactate. In the indomethacin group, the levels of ATP and glycogen were 49% and 42% lower than those of the control group, respectively, the quantity of lactate exceeded by 25% of the control group value, thus confirming the shift to anaerobic metabolic pathway in the stomach lining (Table 1). The tissue energy failure is known to precede the structural damages.

Using amaranth pectin, the number of structural damages and their length on the stomach lining of rats were 64% and 42% less than in the indomethacin group. PI was 2.1 times less than in rats exposed to ulcerogenic action of indomethacin. The gastroprotective action of pectin accounted for 2.2 units (the drug is assumed effective, if the index exceeds 2 units). Using pectin, the energy deficiency of the stomach tissue has appeared to be less visible: the contents of ATP and glycogen only differed from the control indices by 28% and

17%, compared to the indomethacin group indices of 49% and 42%, respectively. At the same time, pectin prevented acidosis in the gastric tissue: the lactate level approached standard value. The lactate level found in untreated rats was higher by 25% than that of the control group (Table 1).

The paper does not review therapeutic effects of omeprazole (reference drug) though it is true that its gastroprotective effect and PI exceed 4 times those of amaranth pectin.

Table 1
Protective effect of amaranth pectin against indomethacin-induced gastric lesions

Indicators	Animal groups			
	Control	Indomethacin	Indomethacin + pectin	Indomethacin + omeprazole
Number of animals with gastric mucosal lesions, %		100	100	75
Degree of ulceration, unit		19.6±1.79	9.1±1.10*	3.0±1.05**
Total length of stomach lesions, mm		34.6±2.71	20.1±1.95*	4.0±1.19**
Pauls index, unit		19.6	9.1	2.2
Gastroprotective activity, unit			2.2	8.9
ATP, μmol/g	2.38±0.11	1.22±0.09*	1.71±0.10**	1.97±0.12**
Glycogen, μmol/g	20.0±1.54	11.6±1.13*	16.7±0.76**	19.2±1.26**
Lactate, μmol/g	1.07±0.07	1.30±0.03*	1.06±0.06**	1.01±0.04**

*- Significant differences from control; **- significant differences from indomethacin; ATP – adenosine triphosphate.

Meloxicam (movalis) is well-known selective NSAID that causes minimum of digestive tract complications, as contrasted with nonselective NSAID indomethacin. This experiment confirms that, when using meloxicam, the intensity of the lesions in the stomach lining appears to be weaker than that of the indomethacin group: its PI is considerably lower – 2.8 times. The gastric lesions were found to be punctuate hemorrhages, PI constituted 7.0 units. Meloxicam caused noticeable metabolic changes of the stomach tissue. The contents of ATP, glycogen and lactate differed from the intact control by 26%, 24% and 22%, respectively (Table 2).

Pectin increased resistance of the mucosal lining to this NSAID. The number of alterations and their length were 62% and 58% lower than those of the meloxicam group. PI differed between treated and untreated groups by 2.8 times, in favor of pectin. When using pectin, energy reserve in the gastric tissue remained at higher level. The contents of ATP and glycogen differed from the control group by 8% and 11% (26% and 24%, respectively, in the meloxicam group).

Administration of omeprazole was capable of keeping the stomach lining undamaged because the stomach tissue was, to a lesser extent, involved in pathological processes. The degree of ulceration was 4 times less than that of the meloxicam group. At the same time, omeprazole appeared to be more efficient than amaranth pectin in preventing destructive lesions and inhibiting regenerative processes in the stomach lining caused by meloxicam. Its gastroprotective activity was found to be 2 times higher than that of pectin. In fact,

omeprazole and amaranth pectin were almost similarly capable of stabilizing energy supply of the stomach lining.

Table 2

Effects of amaranth pectin against meloxicam-induced gastropathy in rats

Indicators	Animal groups			
	control	meloxicam	meloxicam + pectin	meloxicam + omeprazole
Number of animals with gastric mucosal lesions, %		100	75	62.5
Degree of ulceration, unit		7.0±0.84	3.4±1.14*	1.7±0.56**
Length of stomach lesions, mm		10.1±1.14	4.2±1.03	1.9±0.58**
Pauls index, unit		7.0	2.5	1.1
Gastroprotective activity, unit			2.8	6.4
ATP, µmol/g	1.95±0.07	1.45±0.06*	1.79±0.05**	1.84±0.05**
Glycogen, µmol/g	20.4±1.24	15.6±0.90*	18.3±0.61**	19.2±0.59**
Lactate, µmol/g	1.06±0.15	1.28±0.12*	1.02±0.13**	0.99±0.12**

*- Significant differences from control; ** - significant differences from meloxicam; ATP – adenosine triphosphate.

Discussion

Indomethacin and meloxicam-induced adverse effects on the stomach tissue have been shown to cause multiple glandular defects, such as hyperemia, multiple punctuate or strip-like hemorrhagic mucosal erosions. At the same time, as reported, the selective NSAID meloxicam appears to be less aggressive than nonselective NSAID indomethacin. Prolonged administration of nonselective NSAID is known to cause the risk of gastropathy to a greater extent, as compared with the selective NSAID. In this paper, no consideration is given to NSAID pharmacology, mechanism and side effects because all these aspects are well-known (12).

The administration of amaranth pectin did not allow to completely prevent NSAID-induced destructive lesions and trophic disorders of stomach but reduced the risk for pronounced pathology: extent of gastric lesions was reliably lower in animals, which had been administered the drug, than in the indomethacin and meloxicam groups. The gastroprotective action of pectin was higher against the less aggressive meloxicam than indomethacin but in both simulation groups it was considerably inferior to that of omeprazole (official anti-ulcer drug). At the same time, administration of pectin and omeprazole allowed to reduce energy substrate deficiency in the gastric tissue, as compared with groups of rats, which had been administered both NSAIDs without correction.

There is almost no information about pectin-induced effects on energy metabolism in the gastric tissue in animals and humans that ensures its adequate functioning. Although, as reported, there is an energy stabilizing action of pectic polysaccharides, such as lemnan, a pectin from *Lemna minor* L. (6), and zosterin from the eelgrass *Zostera marina* L. (7) under immobilization stress conditions with 2,4-D herbicide intoxication. The capability of pectins to maintain optimum level of energy support of metabolic processes in gastric tissue is indirectly confirmed by the, *Cola cordifolia* (Cav.) R. Br. (1), *Angelica sinensis* (Oliv.) Diels (2), *Ribes nigrum* L. (9), *Acmella oleracea* (L.) R. K. Jansen (10), *Decalepis hamiltonii* Wight & Arn. (15) and many others. In case of gastric lesions (immobilization, NSAID, *Helicobacter pylori*, ethanol, acetic acid), pectins stimulate gastric mucin production and

synthesis of prostaglandin E₂, inhibit free radical lipid peroxidation, reduce inflammatory markers galectin-3 and pro-inflammatory cytokine TNF- α levels. A number of clinical studies are indicative of the efficiency of pectins in case of erosive and ulcerative digestive tract lesions (1, 5, 8). Analyzing data about gastroprotective effect of pectins allows to confirm its non-specificity and multicomponent mechanism.

Conclusion

The research findings confirm that amaranth pectin reduces the risk for adverse NSAID-induced effects on animal stomach, considerably decreasing destructive erosive defects and metabolic lesions on its wall. In case of NSAID-induced ulceration, one of the gastroprotective mechanisms of pectin is found to be its energy stabilizing effect. This allows recommending amaranth pectin as anti-ulcer drug combined with NSAID to achieve preventive and therapeutic goals during the background treatment.

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