Pectin

Pectin is a naturally occurring complex substance (known as a heteropolysaccharide) found in plants. Compared to cellulose and hemicellulose fibers which do not dissolve in water, pectin is water soluble and is often used as a gelling agent in foods like jams and jellies.

Pectin was discovered and isolated by French chemist Henri Braconnot in 1825.

Its physiological functions are to provide mechanical strength and plasticity to plant bodies to form a barrier against the external environment, and to control the movement of water and fluids through the rapidly growing plant parts[51, 90].

From a chemical standpoint, pectin is a complex three-dimensional structure of multiple saccharide units (polysaccharide) found at the highest concentration in a plant's middle lamella, i.e., an intercellular layer which cements the primary cell walls of adjoining cells. It is also found within the primary cell wall gradually decreasing in concentration towards the plasma membrane[90].

The exact chemical composition and structure of pectin is still under debate due to the high complexity of this molecule. Pectin's molecular backbone is mostly composed of D-galacturonic acid units (at least 65%) joined in chain by α (1-4) glycosidic linkage[86]. D-galacturonic acid is an oxidized form of the sugar D-galactose. Other components of pectin include neutral sugars like rhamnose that introduces a kink into the straight galacturonan (linear chain of α(1-4)-linked D-galacturonic acids) chains and arabinose, galactose or xylose which construct the side chains[8, 86]. Based on structural similarities, several regions or structural elements of pectin have now been identified, distinguished and named (Figure 1)[97].

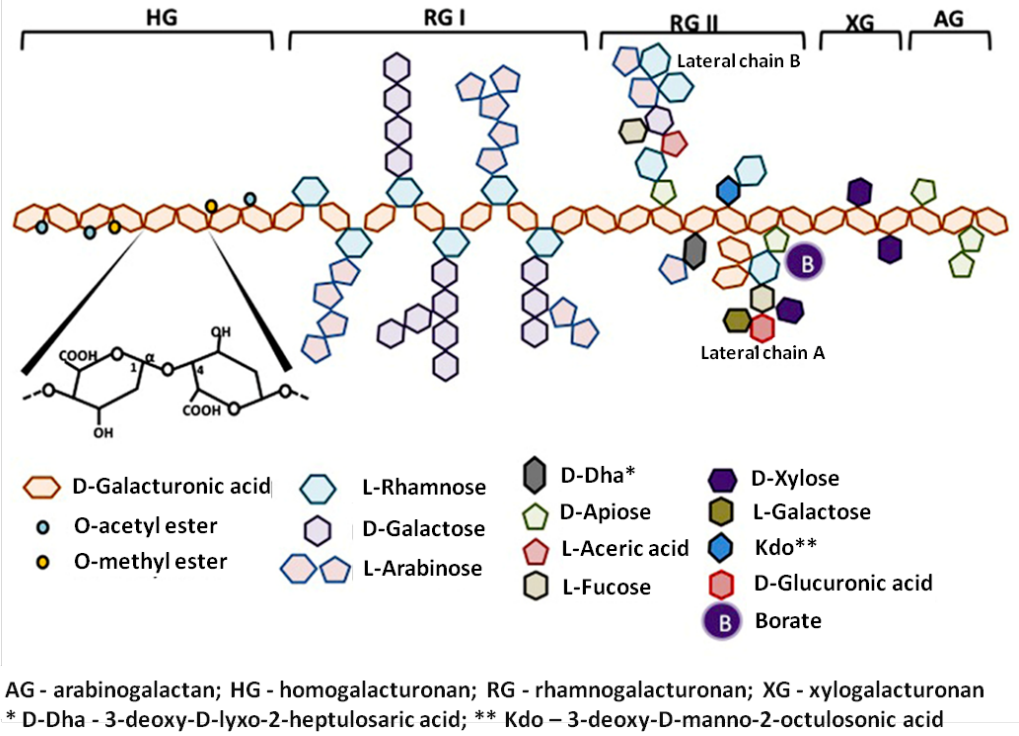

Fig.1. Schematic representation of pectin structure [51, modified]

An important feature that determines both the gelling capacity and the therapeutic properties of pectin is the degree of methoxylation (DM)[64, 76, 84, 86]. DM defines a percentage of methyl groups (-CH3) to the carboxyl groups (-COOH) in the polygalacturonic acid chain which classifies pectin into low-methoxyl (LM, up to 50%) or high-methoxyl (HM, above/over 50%)[8, 90].

The structural, quantitative and qualitative variations among different pectins are related to the source of the pectin, i.e., its origin in a particular plant (e.g., apple, carrot, peach, etc.), and to the physicochemical (e.g., temperature, pH) and biological (e.g., enzyme activity) conditions created either in nature or in the laboratory[51, 86, 90, 97]. Pectin is found across the plant kingdom, however, among edible plants the citrus fruits and especially their peels seem to be the richest sources of this polysaccharide followed by pears and apples (Figure 2).

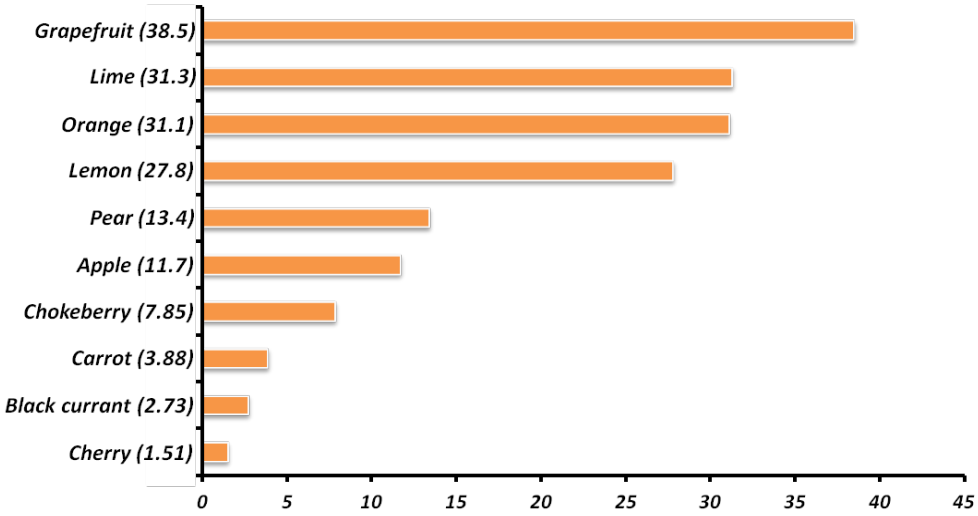

Fig.2. Pectin content in pulp of selected fruits and vegetables [g/100g; Dry Mass] [50,64]

According to animal and human studies, the majority of orally administrated pectin passes through the stomach and small intestine to be degraded or metabolized in the large intestine[40]. Because pectin is not depolymerized (digested) by intestinal enzymes in animals and humans, a partial degradation that occurs in the stomach and small intestine may be due to the physicochemical conditions within these organs[2, 40]. For instance, intestinal microorganisms have enzymes (e.g..pectate lyase, polygalacturonase, pectinesterase) that cut the long chain of pectin resulting in shorter, more absorbable oligomers. However, it is unclear whether those oligosaccharides are stable metabolites and can be absorbed, or if they are further degraded yielding a variety of short chain fatty acids (SCFA) such as acetate, butyrate, propionate, and valerate as well as other end products and gases. An animal study has shown that no or very little galacturonan was found in the contents of colon and feces as a result of more or less intense enzymatic activity of intestinal microorganisms[22].

From the therapeutic standpoint, both long (not absorbable) and short (absorbable) chains (fragments) of pectin are important and have been used in different therapies. For instance, the functions of long polymers of pectin are limited mostly to the gastrointestinal region, however specific depolymerization conducted under laboratory conditions allows the smaller fragments to enter the bloodstream. These smaller pectin units are being utilized in a broader spectrum of clinical conditions including protection against heavy metal toxicity or even cancer[51, 86].

In addition, pectin has been found to be effective against obesity, metabolic syndrome and diabetes[57, 80]. It can ameliorate inflammatory disorders, reduce cardiovascular risk factors, and even protect the body against poisoning with radioactive elements like Cesium 137[5, 16, 39, 65, 80]. Pectin also promotes the growth of normal intestinal microflora while inhibiting the activity of pathogens[22, 32, 76].

Health benefits

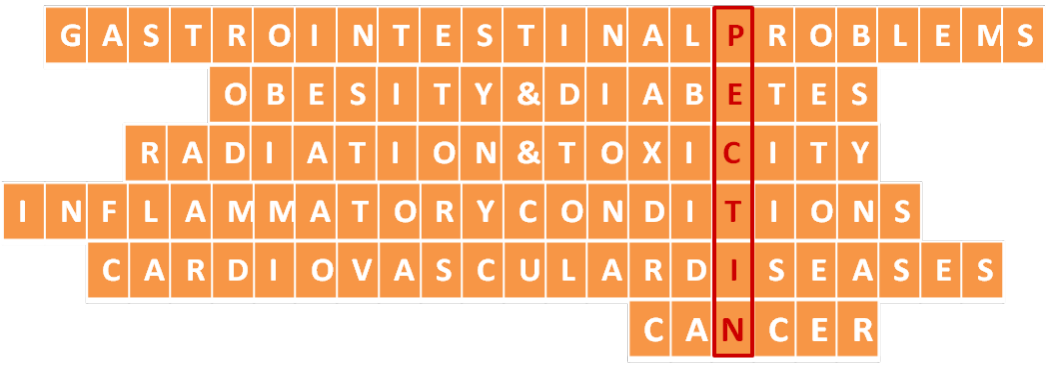

Fig.3. Selected health issues targeted by pectin

Years of research on pectin have been confirming its various health-promoting properties. As mentioned earlier, its therapeutic applications are mostly related to the source and form of pectin, i.e., if it is modified and, if so, in what way (temperature, pH, enzymes)[51].

Natural pectin found in fresh fruits and vegetables cannot be digested or absorbed due to its high molecular weight which limits its "work place" to the gastrointestinal tract. Obviously, since the body is a system of connected vessels, pectin in the digestive system may still positively affect distant organs such as the heart or brain and support many other aspects of the body's physiology. In contrast, properly modified pectin that is composed of short and non-branched carbohydrate chains can be absorbed into the bloodstream and may directly interact with its targets virtually all over the body[51, 102].

Therefore, the choice of pectin is determined by the body's physiological needs.

Gastrointestinal effects: Apple pectin has been demonstrated to be an effective anti-diarrhea agent[7, 21, 99].

READ MORE This is because as a water-soluble fiber, pectin has a gel-forming effect when mixed with water, while as a prebiotic it stimulates proliferation of beneficial intestinal microorganisms that add bulk to the stool. Additionally, pectin counteracts bacteria that often cause diarrhea in the first place such as *Salmonella*, *Shigella*, *Klebsiella*, *Enterobacter*, *Proteus* and *Citobacter*[76]. Not surprisingly, pectin is also effective in combating the opposite condition - constipation[98] - which is achieved by the very same mechanism, i.e., water absorption and stimulation of intestinal bacteria proliferation. As a consequence, the volume and viscosity of stool increases and bowel regularity is improved.

Cholesterol lowering effect: Another important feature of pectin action in the gastrointestinal tract is its ability to trap different molecules within its matrix and carry them through the colon for excretion. One such molecule is cholesterol.

READ MORE Apple pectin binds with and removes cholesterol before it is absorbed in the body[30]. The results from *in vitro* studies show that pectins are able to adsorb over 90% of the total cholesterol with higher affinity than cholestyramine (a synthetic drug) which was effective only in adsorption of cholate (salt of cholic acid which is a primary bile acid)[19, 85]. This is in agreement with animal and human studies that consistently confirm the cholesterol lowering effect of apple pectin[12, 31, 33, 49, 71].

It is worth noting that pectin not only prevents the absorption of digested cholesterol but also the reabsorption of cholesterol that is released into the intestine with bile as one of its components. In one study involving adjustment of the diet of the participants, decrease of both total cholesterol and LDL cholesterol levels was observed in the ranges between 11 to 77 mg/dL and 7 to 67 mg/dL, respectively in a 3-week period[12]. Moreover, the authors reported a significant reduction in body weight ranging from 5.2 to 19.9 pounds with an average of 11.7 pounds. Such improvements in lipid profile and weight loss may also lessen the risk of arteriosclerosis, heart ailments, stroke and diabetes.

Glucose metabolism: Another benefit of pectin intake is related to its slower absorption of dietary sugars.

READ MORE It was found that animals fed highly methoxylated apple pectin experienced a decrease in body weight as well as plasma glucose and insulin levels[80]. It is believed that the gel-forming properties of pectin are responsible for the delay in gastric emptying and also for forming a thick layer

that hinders intestinal glucose absorption[80]. It is thought that lower insulin levels are a consequence of the reduction in the rate of glucose absorption. Similar results were observed in patients with type 2 diabetes whose diet was enriched with apple pectin (20 g throughout the day)[82]. Healthy volunteers also experienced anti-hyperglycemic effects after consuming dried and powdered apple pomace[57]. This preparation, rich in pectin, improved glucose metabolism by reducing the postprandial glucose response by approximately two timesand by increasing urinary glucose excretion by five times.

Intestinal health: Pectin greatly affects gastrointestinal tissue. It is well known that dietary pectin can stimulate proliferation of intestinal cells and activity of the brush border membrane (intestinal microvilli) enzymes[17, 28].

READ MORE In animals, pectin supplementation resulted in significant increases in length, weight, and number of cells in both the small and large intestines[28]. It is believed that this effect is induced by increased concentration of glucagon-like peptide-2 (GLP-2) in plasma which was observed after pectin supplementation. GLP-2 is a 33 amino acid long peptide secreted from gut endocrine cells that inter alia regulates gastric motility, gastric acid secretion, and intestinal hexose transport and increases intestinal surface area via stimulation of cell proliferation[24]. In animals it is shown to have reparative and cytoprotective effects, thereby suggesting it might be useful in treating human disorders characterized by injury and/or dysfunction of the intestinal mucosal epithelium (the innermost absorptive and secretory layer of cells in the gastrointestinal tract), e.g., during chemotherapy[46, 89].

Colitis: One of diseases of the digestive tract successfully ameliorated by pectin is colitis. Colitis refers to an inflammation of the colon that can develop into ulcerative colitis and even colon cancer[55].

READ MORE Animal experiments demonstrated the protective effect of pectin, especially pectin derived from apples, but also from cranberries or shrubs like *Rauvolfia verticillata* and *Comarum palustre*[52, 55, 61, 74, 75, 79]. Application of these pectins was found to decrease free radical damage and inflammation, and enhance the amount of mucus in the colon of animals with colitis. Greater insight into the studies uncovered multifactorial activities of pectin metabolites that activated and/or inhibited many processes in colon tissue and in colon microbiota[6, 55, 70, 91].

Inflammation: One of the major metabolites of pectin is butyrate. Butyrate is the main energy source for the colonocytes (colon cells) that also triggers differentiation and apoptosis of colon cells[91]. It also

exerts potent anti-inflammatory effects by inhibiting the activation of a pro-inflammatory cell-signaling component called nuclear factor kappa B (NFkB)[3, 83].

READ MORE Apart from the direct healing properties of pectin in ulcerative colitis, researchers realized that due to its indigestible character pectin can be used to create a protective coating for other medicines such as anti-inflammatory mesalazine or probiotics that need to be delivered to the colon[77, 96]. Furthermore, the protective properties of pectin against colitis also extend to colon cancer[6, 51, 55, 70, 91].

Colon cancer: Multiple research data show that animals treated with a chemical cancer inducer developed fewer colon tumors if their diet was enriched in pectin[51]. At the same time pectin acts on the colonocytes inducing their apoptosis (cell death) and on colonic microorganisms decreasing activity of bacterial enzyme β-glucuronidase which is considered to be a prime factor in the etiology of colon cancer[44, 51].

Protection against radiation: An important discovery was made with Chernobyl victims, which showed that intake of apple pectin greatly reduced the radiation load in children from Ukraine and Belarus afflicted by the Chernobyl disaster[5, 39, 65]. In one study the average reduction of cesium 137 levels in children receiving oral pectin powder was 62.6% after a month-long trial[65].

Removal of Toxic Metals: It has been shown that reduced molecular mass pectin that passes into the bloodstream such as modified citrus pectin MCP (average molar mass 15400, consisted mostly of linear homogalacturonan with a 3.8% degree of esterification and approximately 10% rhamnogalacturonan II) can increase urinary excretion of toxic metals[26, 27, 101].

READ MORE In a 6-day pilot study healthy subjects with a "normal" body load of metals ingested 15 g of MCP for 5 days, and 20 g on day 6. Their average increase in urinary excretion of arsenic, cadmium, and lead on day 6 was 130%, 150%, and 560%, respectively[27]. The ability of MCP to chelate heavy metals has been reproduced in other trials involving hospitalized patients due to lead poisoning[26, 101]. In contrast to other chelators, MCP is claimed to act gently and safely selectively binding heavy metals without removing essential minerals like magnesium, calcium or zinc[102]. Interestingly, an earlier study investigating apple pectin effects on some electrolyte levels (sodium, potassium, chloride, calcium and magnesium) and trace elements (iron and copper) in patients with hyperlipoproteinemia did not

find any adverse effects and changes in serum levels of measured electrolytes and trace elements during the 3-month period of observation[34].

Counteracting lectin effects: Another beneficial effect of modified pectin is its capability to bind and block galectin-3 in the body[47]. Galectin-3 is a carbohydrate-binding protein (lectin). Because of its broad biological functions (e.g., cell adhesion, cell activation and chemo-attraction, cell growth, differentiation, and apoptosis) its over expression has been associated with inflammation, fibrosis (formation of excess fibrous connective tissue in an organ or tissue in a reparative or reactive process), heart disease, stroke, cancer and other pathologies[4, 13, 14, 47, 51, 58, 59].

Inflammation: Recent research demonstrated that galectin-3 plays a role in aggravating joint inflammation and destruction in experimental arthritis[4]. In one study scientists treated human synovial fibroblasts obtained from rheumatoid arthritis and osteoarthritis patients with MCP and found that it could inhibit pro-inflammatory cytokine secretion (IL-6).

READ MORE Another *in vitro* study demonstrated that pectin and modified pectin, by mechanisms independent of galectin-3 inhibition, suppress the pro-inflammatory responses of activated macrophages (immune cells)[16]. They significantly inhibited the expression of two key enzymes engaged in inflammatory processesy: inducible nitric oxide synthase (iNOS), which yields excess nitric oxide (NO) exacerbating symptoms and pathology, and cyclooxygenase-2 (COX-2) which is involved in the synthesis of inflammatory mediators such as prostaglandins and which, for that reason, is the main target for non-steroidal anti-inflammatory drugs (NSAIDs). Pectin may therefore play an important role in helping reduce inflammation.

Fibrosis and aldosterone: As indicated earlier, galectin-3 is upregulated in fibrosis including vascular, cardiac, renal, liver, and lung fibrosis[13, 14, 56, 58, 59] leading to serious complications in the structure and function of these organs. For cardiovascular and renal fibrosis, it is known that increased galectin-3 synthesis can be related to higher plasma aldosterone[58]. Aldosterone is a hormone produced by the adrenal glands and helps in controlling the amount of fluid/electrolytes in the body. It stimulates the kidneys to retain more sodium and water while excreting potassium. Thus, Aldosterone secretion is induced by low blood pressure, reduced blood flow to the kidneys, increased blood acidity and high serum potassium concentration. In contrast, increased blood

volume and blood flow to the kidneys, falling potassium levels, and rising sodium levels serve to decrease aldosterone secretion.

READ MORE However, this feedback mechanism fails in conditions like hyperaldosteronism where the adrenal glands produce too much aldosterone, or in the case of so-called "aldosterone breakthrough" in which plasma aldosterone remains elevated. Aldosterone levels also increase in patients taking certain medications (e.g., angiotensin-converting enzyme inhibitors and/or angiotensin receptor blockers) leading to cardiovascular and renal fibrosis[10, 14]. It has been demonstrated that animals treated with aldosterone and salt had an increased level of galectin-3 which resulted specifically in enhanced collagen type I synthesis[13]. In addition, they developed inflammation, fibrosis, and vascular, cardiac, and renal hypertrophy (an increase in the volume of tissue or organ caused by enlargement of cells in contrast to hyperplasia in which the cell size is not changed but the number of cells increases)[13, 14, 58]. As expected, administration of MCP inhibited galectin-3 and prevented aldosterone induced pathological changes indicating that MCP may be a natural and effective option in hyperaldosteronism therapy.

Obesity: Over expression of galectin-3 is also promoted by obesity which further adds to the inflammation and fibrosis in the cardiovascular system[59]. In morbidly obese patients galectin-3 levels have been associated with diastolic dysfunction. Diastolic dysfunction refers to abnormal stiffening and hypertrophy of the left ventricular wall which impairs adequate filling of the ventricles with oxygenated blood coming from the lungs via the pulmonary vein and left atrium. This causes the blood to be drawn back into the left atrium, the pulmonary vein, and eventually into the lungs raising the pressure in the pulmonary vessels and leaking fluid into the lung alveoli causing pulmonary edema. In a normotensive animal model of diet-induced obesity, MCP treatment resulted in lowered cardiovascular levels of galectin-3, total collagen, and collagen type I as well as other factors associated with inflammation and fibrosis in the heart and aorta in these obese animals[59].

Kidney oxidative stress injury: MCP has also been shown to be useful in ameliorating renal injury induced by oxidative stress[47]. In an animal study with experimental acute kidney injury, MCP lessened the unfavorable changes during the initial injury phase. At the recovery phase, MCP treated mice had reduced galectin-3 in association with decreased renal fibrosis, macrophages,

pro-inflammatory cytokine expression and apoptosis. These data point to the protective role of MCP against nephropathy and indicate a possible novel strategy for reducing kidney injury.

Cancer: Anticancer activities of pectin have been known for several decades and they are still being explored because of the highly complex structure of pectin[51]. Different types of pectin display different cancer fighting mechanisms. MCP, which is the most often studied pectin, has demonstrated significant inhibition of the growth of breast cancer, multiple myeloma (cancer that forms in antibody producing cells called plasma cells) and colon and prostate cancer cells[51]. In animal studies, MCP presented strong anti-metastatic properties by decreasing the metastasis of highly metastatic mouse melanoma to the lungs by more 90%[73]. Also, feeding MCP to rats injected with prostate cancer cells and to mice with implanted breast or colon tumors resulted in significantly fewer metastatic colonies in the lungs[63, 68].

READ MORE A more detailed study revealed that MCP blocked the pleiotropic activity of galectin-3 over expressed in cancer cells by reducing metastasis, angiogenesis (development of new blood vessels) and even tumor growth since galectin-3 is involved in all these processes[63]. Scientists identified the rhamnogalacturonan-I (RG-I) region as one responsible for binding to and inhibiting galectin-3[37, 51]. Not surprisingly okra pectin mainly composed of RG-I was able to inhibit proliferation and induce apoptosis of melanoma (skin cancer) cells by interaction with galectin-3[95]. In a phase II human clinical trial in prostate cancer patients who were treated with MCP, the researchers noted a marked increase in so-called prostate specific antigen doubling time (PSADT)[35]. PSA is an enzyme produced by the prostate gland which has been used as prostate tumor marker, while PSADT is the time it takes for PSA value to double. Therefore, PSADT reflects the speed at which cancer is growing, and lengthening of PSADT represents a suppression in cancer growth rate.

Regarding colon cancer, a number of animal studies showed that apple pectin is effective in reducing both tumor numbers and activity of β-glucuronidase (an enzyme produced by fecal bacteria whose activity is linked to colon cancer development)[70]. Other studies demonstrated the activation of pro-apoptotic and inhibition of anti-apoptotic genes by pectin[51]. Also butyrate, a product of pectin fermentation, can induce the colonocytes apoptosis[51]. These are just a few of the many examples of indirect anti-tumor activities of pectin.

There is a growing field of evidence that some polysaccharides in pectins isolated from different plants can act as immunopotentiating agents. For instance, polysaccharides named angelans isolated from *Angelica gigas* (a Chinese medicinal plant) can increase immune functions of dendritic cells (antigen-presenting cells), macrophages, natural killer cells, lymphocytes B and T, potentiating the immune activity against tumor cells[38, 45]. In a mouse model of colitis-associated colon cancer, apple oligogalactan (AOG, composed of five galacturonic acids) was found to be highly effective against carcinogenesis[55]. AOG decreased inflammation, which is an important effect since evidence strongly supports a link between inflammation and cancer. The authors of this work proposed AOG as useful for clinical treatment of colitis as well as prevention of carcinogenesis.

Human studies

Table 1 summarizes human studies involving pectin supplementation.

Abbreviations used in Table 1: DE - degree of esterification; HDL-C - high density lipoprotein cholesterol; LDL-C - low density lipoprotein cholesterol; MCP - modified citrus pectin; MW - molecular weight; PSA - prostate specific antigen; PSADT - prostate specific antigen doubling time; TGs - triglycerides; VLDL-C - very low density lipoprotein cholesterol.

Table 1.

OBJECIVE	SUBJECTS	FORM & DOSE OF PECTIN	PRIMARY RESULTS
[21] To assess the efficacy of the apple pectin-chamomile extract (Diarrhoesan) on children with acute non-complicated diarrhea.	79 children (6 months to 5.5 years of age) with acute non-complicated diarrhea	39 children received Diarrhoesan, and 40 the placebo in addition to the usual rehydration and realimentation diet for three days.	Diarrhea had ended significantly more frequently (33/39) in Diarrhoesan group than in placebo (23/40). Diarrhoesan reduced the duration of diarrhea by at least 5.2 hours.
[98] To explore the effect of pectin on colonic transit time, clinical symptoms and gut microbiota in adults with slow-transit constipation.	80 patients with slow-transit constipation	Patients received 24 g/day of pectin or placebo (maltodextrin) for 4 weeks.	After treatment in pectin group, colonic transit time decreased from 80.3 hours to 60.2 hours. Constipation score was also decreased. Content of *Bifidobacterium sp.* and *Lactobacillus sp.* (healthy microbiota) increased whereas *Clostridium sp.* significantly decreased.
[15] To evaluate the effect of grapefruit pectin on plasma cholesterol, TGs, VLDL-C, LDL-C, HDL-C and LDL-C:HDL-C	27 healthy volunteers (9 males, 18 females, mean age 59.1 years) screened to be at medium to high risk of	Subjects were instructed to take 27 capsules (15 g) per day (3 x 9 with meals) of grapefruit pectin (high	Grapefruit pectin supplementation decreased plasma cholesterol 7.6%, LDL-C 10.8%, and LDL-C:HDL-

ratio without diet or lifestyle changes.	coronary heart disease due to hypercholesterolemia with mean plasma cholesterol 275 mg/dL (range 208-420 mg/dL).	metoxyl - 70%) or placebo (flour) for 4 weeks .	C ratio 9.8%.
[11] To evaluate different sources and types of well-characterized pectin on LDL-C and inflammatory markers.	Mildly hyper-cholesterolemic persons	Subjects received 15 g/day pectin or cellulose with food for 4 weeks.	Relative LDL-C lowering was as follows: citrus pectin DE-70 = apple pectin DE-70 (7-10% reduction of LDL-C) > citrus pectin DE-35 = apple pectin DE-35 > orange pulp fiber DE-70 and low-MW pectin DE-70 > citrus DE-0. Conclusion: high DE and high MW are important for cholesterol lowering. Pectin did not affect inflammatory markers.
[9] To evaluate lipid-lowering effect of mixture of fiber consisting of guar and apple pectin in combination with apple pomaces in type II diabetic patients.	15 female type II diabetics (age mean 62 years) with hypercholesterolemia (total cholesterol > 240 mg/dL and LDL-C > 130 mg/dL).	Patient received the fiber mixture (1 package of 17 g with about 5.9 g water-soluble fiber) dissolved in 250 ml water for the next 9 weeks: during the first 3 weeks 2 portions per day, the next 3 weeks twice 1/2 portion and the last 3 weeks one 1/2 portion daily. The fiber mixture had to be consumed 30 minutes before a main meal.	During the first 3 weeks total cholesterol level decreased by 11.3%, during the next 3 weeks by 12.6%, and by 9.6% during the last 3 weeks (under reduced fiber intake). Plasma TGs during these three 3-week periods decreased by 15.5%, 19.2% and 12.3%. HDL-C levels remained unchanged.
[65] To compare the efficacy of a dry and milled apple-extract containing 15-16% pectin with a similar placebo-powder in 64 "Chernobyl" children.	64 children from radiologically contaminated areas with the average 137 cesium load of about 30 Bq/kg body weight	Children received 5g of apple pectin (n=32) or placebo (n=32) powder diluted in water twice a day, at meals, for 3 weeks. All children stayed in sanatorium in clean radiological environment and received radiologically clean food.	The average reduction of the 137 cesium levels in apple pectin group was 62.6%; the reduction with "clean" food and placebo only was 13.9%.
[27] To evaluate the effect of MCP on the urinary excretion of toxic elements in healthy individuals.	Healthy volunteers	Subjects ingested 15 g of MCP (PectaSol) each day for 5 days and 20 g on day 6.	MCP administration significantly increased urinary excretion of arsenic (130% on day 1), cadmium (150% on day 6), and lead (560% on day 6).
[35] To investigate the tolerability and effect of MCP (PectaSol) in 13 men with prostate cancer and biochemical PSA failure after localized treatment, that is prostatectomy, radiation, or cryosurgery.	A total of 13 men with prostate cancer were evaluated for tolerability, and 10 for efficacy.	Patients ingested 18 capsules (14.4 g) of MCP (PectaSol) daily divided in 3 doses for the 12 months.	PSADT increased in 70% of patients after 12 months of taking MCP. In one case PSADT increased up to 867.7%.

Synergy

Biochemical reactions in the body are orderly and complex. Final biological effects are usually achieved through participation of many compounds (e.g., substrates, cofactors) and affected by various factors (e.g., temperature, pH). Often the same result may be accomplished through alternative biochemical routes and pathways. For healthy metabolism, both optimal physiological conditions and the presence of all required compounds (to avoid missing links) are necessary. Substances that work in synergy may be directly involved in the same pathway or act indirectly on alternative pathways leading to the same physiochemical response. Often they have a supporting function in the process, e.g., by increasing absorption or bioavailability of molecules involved and helping them to get to the reaction site at the optimum amount.

Thus by applying the principles of synergy in nutritional supplementation, the targeted biological effect can be obtained with lower, non-toxic doses of the participating compounds and result in better efficacy than when using a high dose of one of the compounds in a particular pathway.

Pectin has been shown to potentiate the efficacy of various therapeutic approaches via synergistic or additive interactions with natural compounds (Table 2) and drugs (Table 3).

Abbreviations used in Table 2 and Table 3: AP - apple pectin; IC50 - half maximal inhibitory concentration; LMP - low-methoxylated pectin; MCP - modified citrus pectin.

Table 2. Synergistic or additive effects of pectin and some natural compounds

Natural compound(s) + pectin	Effects
[43] BreastDefend + MCP	↓ adhesion and migration of breast cancer cells
[43] ProstaCaid + MCP	↓ adhesion and migration of prostate cancer cells
[67,68] quercetin + AP	↑ absorption of quercetin
[71] pea protein + AP	↓ plasma cholesterol concentration

Table 3. Synergistic or additive effects of pectin with some drugs

Drug + pectin	Effects
[92] cisplatin + MCP	↑ cisplatin-induced apoptosis of prostate cancer cells
[41] paclitaxel + MCP	↑ apoptosis of ovarian cancer cells
[42] doxorubicin + MCP	↑ cytotoxicity of doxorubicin (allowed to reduce the in vitro IC50 of doxorubicin by 10.7-fold)
[88] adriamycin + LMP	↓ tumor growth

Safety

Pectin is abundant in many fruits and vegetables and is a natural component of the human diet. Based on typical consumption of fruits and vegetables, the average daily intake of pectin has been estimated to be around 5 g[40]. The US Food and Drug Administration classified pectin as food and Generally Recognized as Safe (GRAS)[103]. Various decades-long clinical applications of pectin show that it is well tolerated and safe. Scientific data from different studies indicate pectin use in dosages ranging from 6 to 36 grams per day taken for several weeks to several months[9, 11, 15, 20, 27, 98]. The typical therapeutic dose is 5 g taken three times daily. This amount of MCP was ingested by prostate cancer patients for 12 months without any serious side effects observed[35]. Comparative assessment of serum levels of the electrolytes such as sodium, potassium, chloride, calcium and magnesium as well as trace elements like iron and copper, showed no statistically significant differences during the three-month administration of apple pectin (15 g/day)[20, 34]. In conclusion, the authors stated that daily intake of 15 g of high-esterified apple pectin for 3 months has no adverse effects. However, the absorption and therapeutic efficacy of vitamin E, β-carotene (pro-vitamin A), and epigallocatechin gallate (EGCG, extracted from green tea) have been found to be hindered by ingestion of pectin[78, 81, 87]. This should be taken into account in determining the cause of deficiency or poor therapeutic response to the aforementioned compounds or supplements.

READ MORE Generally, pectin is a soluble fiber and it is unlikely to cause any serious adverse reactions. An exception to this could be allergies, which some people may have to citrus fruits or any other ingredient contained in or used as a pectin source. Mild side effects may include gas, abdominal pain, loose stools, and bloating especially with the first high dose pectin supplementation which can be resolved by gradual introduction of pectin into the diet[60, 62]. It is important to indicate that excessive consumption of regular pectin may increase the methanol levels in the blood, which may contribute to the development of nonalcoholic liver cirrhosis[53]. However, this effect was related mostly to a higher degree of esterification (DE) found in regular pectin (60-90%, esterified with methyl alcohol) in contrast to low DE found in modified citrus pectin (less than 10%)[86, 102]. Evaluation of the occupational exposure to pectin has indicated that some workers could develop asthma after prolonged inhalation of pectin dust[18, 48, 93]. Hence, according to the authors, pectin dust should be added to the list of the substances known to induce occupational asthma.

Finally, due to its adsorptive character pectin was found to interfere with absorption of some pharmaceutical drugs. As such, concomitant pectin administration decreased the absorption of digoxin (cardiac glycoside used for heart failure and/or irregular heartbeat - to avoid the interaction take pectin 2 hours after digoxin)[2], tetracycline antibiotics (to avoid the interaction take pectin 2 hours before or 4 hours after tetracycline antibiotics)[1] and Lovastatin (Mevacor; used for lowering cholesterol - to avoid the interaction take pectin 1 hour after Lovastatin)[94]. For this reason, it is recommended that patients on medications seek professional advice from qualified health care providers before introducing any additional treatment.

Mechanism of action in more detail

Various *in vitro* and *in vivo* studies indicate anti-inflammatory properties of pectin[16, 55]. These include decreased expression of protein and mRNA of inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX-2) in lipopolysaccharide (LPS)-activated macrophages[16]. LPSs (aka endotoxins) are found in the outer membrane of gram-negative bacteria and can trigger strong immune responses in animals after binding to Toll-like receptor 4 (TLR4) present on many cell types, especially immune cells (monocytes, macrophages, dendritic cells and B cells). Further investigation of the anti-inflammatory mechanism of pectin revealed that it can inhibit phosphorylation of mitogen-activated protein kinases (MAPKs) and I kappa B kinase (IKK) activity as well as activate a downstream nuclear factor kappa B (NFkB, transcription factor) and activator protein-1 (AP-1, transcription factor) (Figure 4). In addition it has been found that pectin could bind with LPS which may result in a decreased binding of LPS to TLR4. A study used apple oligogalactan (AOG, composed of five galacturonic acids) and demonstrated strong inhibition of all biomarkers induced by LPS when AOG was combined with LPS[55]. Subsequent *in vitro* studies showed that concomitant administration of LPS and AOG resulted in an increase of TLR4 level in cytoplasm and its significant decrease, even elimination, from the cell membrane. According to the authors, this indicates that AOG and LPS bind to different sites of TLR4 and that the presence of AOG promotes distribution of TLR4 from cell membrane into the cytoplasm causing hyporesponsiveness to LPS and attenuates inflammation.

Since inflammation is a key factor in cancer development and facilitates growth, invasion and metastasis of tumor cells, a decrease in inflammatory response has subsequent anti-cancer effects[84]. Interestingly, pectin's metabolite butyrate, which is a short chain fatty acid, was found to inhibit not only NFkB but also histone-deacytelase (HDAC) enzyme activity[91, 100]. It is important to stress that gene expression requires controlled coiling and uncoiling of DNA around histones. This process is regulated through acetylation (uncoiling) and deacetylation (coiling) of the lysine residues in core histones by histone acetyl transferases (HAT) and histone deacetylases (HDAC), respectively. In addition, HDAC deacetylate a number of non-histone proteins including transcription factors, hormones, and receptors. Therefore, HDAC inhibitors have wide-ranging clinical usage from psychiatry and neurology to inflammatory diseases and cancer. As such, the formation of butyrate in the colon due to bacterial fermentation of dietary fiber/pectin is vital for colonocytes as it is both their primary energy source as well as anti-inflammatory and anti-cancer agent[23, 100].

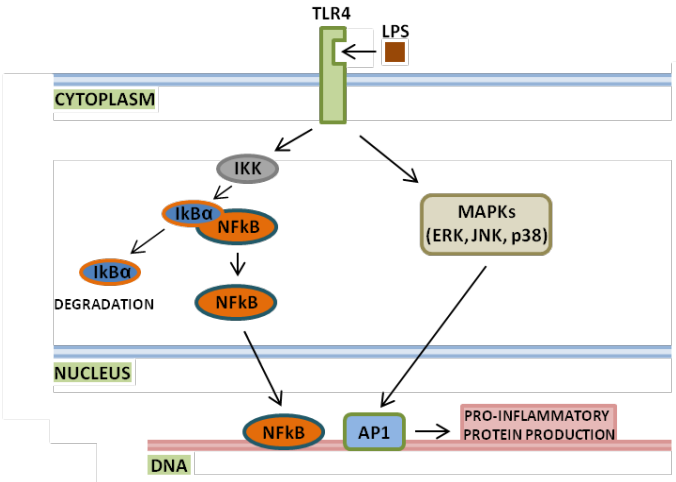

Fig.4. Overview of TLR4-triggered MAPK/NFkB signaling pathways after LPS stimulation

Another anti-cancer (also anti-inflammatory) mechanism of pectin, especially characteristic for modified citrus pectin (MCP), is blocking of the galectin-3 activity[51]. Galectin-3 is over expressed in most types of cancer cells and plays a role in cell-cell and cell-extracellular matrix adhesion through binding to glycoconjugates. Recent studies suggest that the functional motif that binds to galectin-3 resides in the neutral sugar side chains (rich in galactose and arabinose) rather than in the internal region of the chain[29, 36]. Moreover, force spectroscopy showed the specific interaction between disaccharide β-galactobiose (two molecules of galactose linked by β-1,4 or β-1,3 glycosidic bond) and galectin-3, again confirming the role of pectin-derived galactan side chains in blocking galectin-3[37]. Interestingly, these "active fragments" can be obtained from potato pectin after controlled enzymatic hydrolysis of potato β-galactan[36, 104].

This mechanism of blocking galectin-3 has been associated with many other health benefits of MCP. This is due to divergent effects of galectin-3 on different cells and cell activities that derive from its

inter- and sub-cellular interactions with a range of diverse binding partners, although its normal plasma concentration is very low (less than 17.8 ng/ml)[25, 66, 69]. In addition to cancer, overexpression of galectin-3 is a critical aspect in vascular, cardiac and renal fibrosis as well as inflammatory arthritis and colitis[4, 13, 14, 47, 54, 58, 59]. Consequently, as research shows, inhibition of galectin-3 by MCP improves the symptoms and prevents the pathological changes in affected tissues and organs.

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