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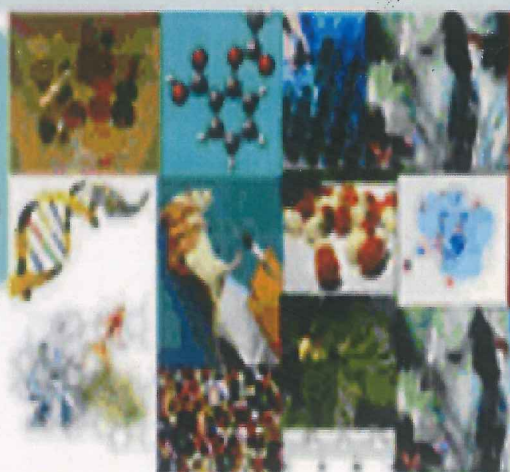
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Extraction of pectin from peels of (*Citrus sinensis*) to study its hypolipidemic effects

Tayseer Ali Talab

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Abstract: Pectin, which is a family of complex polysaccharides that contains 1, 4-linked α and β galactosyluronic acid residues, was extracted using alcohol precipitation method from peels of *Citrus sinensis*. The result showed that the color of the pectin is, soluble in hot and cold alkali and water. The results showed that the pectin of *Citrus sinensis* induced hypolipidemic effect; it significantly decreased total cholesterol, triglycerides, LDL and VLDL serum levels and significantly increased serum HDL level in hyperlipidemic mice. Pectin could be inhibited lipid parameters by many mechanisms. Accordingly, because of the safety of pectin and its significant hypolipidemic effects, it might be considered as therapeutic alternative in hyperlipidemia.

Keywords: pectin, pharmacology, hyperlipidemia.

Introduction:

Pectin is a purified carbohydrate product obtained from the inner portion of the rind/peels of citrus fruits. Pectin extracted from various fruits can be different in molecular structure (i.e., molecular weight degree of esterification, acetyl content) and therefore possesses different functional properties. Typically, a whole mature fruit contains 3-7% pectin substances on a dry weight basis and 0.1-1.1% on a fresh weight basis. The relatively high pectin and low caloric content of citrus fruits make them a good source of soluble dietary fiber ⁽¹⁾.

The chemistry and gel-forming characteristics of pectin have enabled this naturally occurring biopolymer to be used in pharmaceutical industry, health promotion and treatment. It was used in the treatment of disorders related to overeating. Pectin reduces rate of digestion by immobilizing food components in the intestine. This results in less absorption of food. The thickness of the pectin layer influences the absorption by prohibiting contact between the intestinal enzyme and the food, thus reducing the latter's availability ⁽²⁻⁴⁾.

Due to its large water binding capacity, pectin gives a feeling of satiety, thus reducing food consumption. Experiments showed a prolongation of the gastric emptying half-time from 23 to 50 minutes of a meal fortified with pectin ⁽⁵⁾.

Pectin was used as carrier material in colon-specific drug delivery systems (for systemic action or a topical treatment of as ulcerative colitis, Crohn's disease and colon carcinomas ⁽⁶⁻¹⁰⁾.

Pectin favorably influences cholesterol levels in blood. It has been reported to help reduce blood cholesterol in a wide variety of subjects and experimental conditions ⁽¹¹⁾.

Consumption of at least 6 g/day of pectin is necessary to have a significant effect in cholesterol reduction. Amounts less than 6 g/day of pectin are not effective ⁽¹²⁾.

A 13% reduction in serum cholesterol was recorded within 2 weeks of treatment ⁽¹³⁾.

This study was designed for isolation of pectin from and studying its hypolipidemic effects in experimentally induced hyperlipidemia in mice.

Materials and Methods:

Sample preparation:

Mature *Citrus sinensis* fruits were purchased from the local Market. Each of the fruits was cut into four parts and the peel removed (a soft white substance inside the skin of citrus fruits), then the peels were further cut into smaller pieces for easy drying and washed with large quantity of water to remove the Glycosides the bitter taste of the peels and then weighed with a digital weighing balance and air dried.

Pectin extraction:

The dried peels were separately transferred into a beaker containing 500 ml of water, 2.5 ml hydrochloric acid was added to give a pH of 2, then boiled for 45 min. Thereafter, the peels were removed from the extracts by filtering through a what man No. 1 filter study. The cake was washed with 250 ml boiled water and the combined filter allowed to cool to 25°C to minimize heat degradation of the pectin. The extracted pectin was precipitated by adding 200 ml absolute ethanol to 100 ml of the extracted pectin with thorough stirring, left for 30 min to allow the pectin float on the surface. The gelatinous pectin flocculants was then skimmed off. The extracted pectin was purified by washing in 200 ml acetone and then pressed on a nylon cloth to remove the residual HCl and universal salt. The dried pectin was powdered using a pestle and mortar and weighed using a digital weighing balance ⁽¹⁴⁾.

Induction of hyperlipidemia:

The experiment was conducted on 40 male mice weighing approximately 25g. The animals were divided into two groups and the first group was fed hyper-cholesterolemic diet (standard diet 97% , 2% cholesterol and 1% pectin) , while the second group was fed hypercholesterolemic diet (standard diet 98 % and 2% cholesterol) without pectin to serve as control. The treatment continued for 8 weeks. After the treatment period, blood samples were collected via cardiac puncture under light anesthesia ⁽¹⁵⁾.

Determination of lipid profile:

Lipids parameters, total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL) and very low- density lipoprotein cholesterol (VLDL) were determined according to methods enzymatic and mathematic methods described previously ⁽¹⁶⁻¹⁹⁾.

Statistical analysis:

Student -t- test was used to determine the significancy between groups ⁽²⁰⁾.

The results:

Citrus sinensis peels contained 12.4% pectin. It was white in color, odorless, viscous and mucilaginous, soluble in pure water, partially soluble in cold water. It is insoluble in alcohol and organic solvents. Dry powdered pectin, when added to water, has a tendency to hydrate, very rapidly, forming gels.

Pectin 1% induced significant ($p < 0.001$) decline in the level of total cholesterol (164.9 ± 43.7 vs 224.9 ± 34.5 in control group), triglycerides (100.4 ± 38.8 vs 225.9 ± 30.4 in control group), LDL (85.2 ± 39.0 vs 152.7 ± 22.3 in control group) and VLDL (10.3 ± 3.9 vs 20.1 ± 4.2 in control group), while it significantly ($p < 0.01$) increased HDL (68.8 ± 13.3 vs 51.6 ± 12.3 in control group)

Table 1: Effect of pectin isolated from *Citrus sinensis* peels on lipid profile in hyperlipidemia induced in mice.

Groups	Total Cholesterol mg/dl	Triglycerides mg/dl	LDL mg/dl	VLDL mg/dl	HDL mg/dl
Pectin treated group	164.9 ± 43.7^b	100.4 ± 38.8^b	85.2 ± 39.0^b	10.3 ± 3.9^b	68.8 ± 13.3^a
Control group	224.9 ± 34.5	225.9 ± 30.4	152.7 ± 22.3	20.1 ± 4.2	51.6 ± 12.3

a ($p < 0.01$), b ($p < 0.001$) in comparison with control

Discussion:

As recorded in this study, the percent of pectin extracted from *Citrus sinensis* peels, and its general characteristics were also recorded previously. However the characteristics of *Citrus sinensis* peels pectin were differ than pectin isolated from other sources⁽²¹⁻²⁴⁾.

Coronary heart disease (CHD) remains the main leading cause of death all over the world. The most important clinical risk indicators for CHD are increased serum total cholesterol, low-density lipoprotein cholesterol and decreased high-density lipoprotein cholesterol. Dietary modification therefore is the first line of preventive strategy against development of CHD. The association between hyperlipidemia and increased risk of heart diseases has made the scientific community aware of dietary sources that might effectively reduce plasma lipids level⁽²⁵⁻²⁶⁾.

The hypolipidemic effects of pectin were also previously recorded in different animal models⁽²⁷⁻²⁹⁾.

The mechanisms by which dietary pectin lowers plasma and liver cholesterol levels in cholesterol-fed rats were previously studied. Pectin feeding increased fecal bile acid excretion in cholesterol-fed rats. In vitro studies with inverted intestinal sacs demonstrated that pectin decreased taurocholic acid transport by approximately 50%. Rats responded to dietary pectin and cholestyramine, a known inhibitor of bile acid absorption, similarly. Cholesterol-14-⁽¹⁴⁾C absorption was somewhat depressed by dietary pectin as evidenced by fecal radioactive cholesterol excretion and deposition of cholesterol-14 in liver. The effect of pectin on plasma and liver cholesterol was not altered by dietary sucinyl sulfathiazole. Accordingly, these results indicate that pectin lowers plasma and liver cholesterol levels in cholesterol-fed rats primarily by inhibiting bile acid absorption and also by reducing cholesterol absorption⁽³⁰⁾. However, Garcia-Diez et al., found that addition of pectin to the diet of rats (7 g/100 g diet for 4 wk) resulted in lower serum and liver cholesterol concentrations (-27 and -17%, respectively). Fecal bile acid excretion (+168%) and the hepatic activity of cholesterol 7 α -hydroxylase (+70%) were significantly higher in pectin-fed animals. HMG-CoA reductase activity was also significantly greater (+11%) in the presence of dietary pectin. These results indicated that pectin, by enhancing fecal bile acid excretion, may cause increased hepatic synthesis of bile acids and liver depletion of cholesterol in rats, which results in a higher rate of cholesterol synthesis and reduced serum cholesterol concentrations⁽³¹⁾.

On the other hand, the oral administration of pectin to rats reduced and delayed the peak plasma triacylglycerol concentration. Pectin inhibited the hydrolysis of trioleoylglycerol emulsified with soybean phosphatidylcholine by pancreatic, carboxylester, and lingual lipases in a concentration-dependent manner. However, the effective concentration of pectin for lingual lipase was 100 times lower than that for pancreatic lipase. Pectin did not inhibit the tributyrin- and p-nitrophenylbutyrate-hydrolyzing activities by pancreatic and carboxylester lipase. When low molecular weight pectin was assayed, pectin at a molecular weight of 90,000 (MW 90) most strongly inhibited three lipase activities. When the effect of pH on pectin inhibition was analyzed using pancreatic lipase, strong inhibition was observed at an acidic pH (below pH 7.0). Pectin reduced the amount of pancreatic lipase protein in the fat layer in a concentration-dependent manner and concomitantly increased that in the supernatant. These results suggest that pectin may interact with emulsified substrates and inhibit the adsorption of lipase to the surface of substrate emulsion⁽³²⁾.

Therefore, pectin could be inhibited lipid parameters by many mechanisms. Accordingly, because of the safety of pectin and its significant hypolipidemic effects, it might be considered as therapeutic alternative in hyperlipidemia.

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