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Healing and preventive effects of low-esterified pectin on liver injury induced by carbon tetrachloride in rats

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SUMMARY

The purpose of this study was to investigate the pharmacological effects of low-esterified pectin on carbon tetrachloride (CCL_4)-induced hepatotoxicity in rats. The study included two experiments. In the first experiment the animals were given daily CCL_4 through gavage for 7 days and then 10, 50, or 250 mg/kg b.w. of pectin for 21 days. At the end of experiment rats were killed within 24 hours. The increased bilirubin level, enhanced alanine aminotransferase and aspartate aminotransferase activity in plasma induced by CCL_4 were partly normalized by pectin administration in a dose-dependent manner. The pectin treatment also resulted in significant recovery of CCl_4-induced decrease of the liver glycogen content. In addition, pectin significantly improved CCL_4-induced alterations of pro-oxidant and antioxidant biochemical parameters in liver and plasma compared to those of rats administered CCL_4. In the second experiment the animals were given daily 10, 50 or 250 mg/kg b.w. of pectin for 21 days before a 7-day administration of CCL_4. Rats were killed 24 hours after the end of experiment. Pretreatment with pectin before CCL_4 administration resulted in significantly inhibited increase of the blood enzymatic activities of alanine and aspartate aminotransferases and bilirubin level in a dose-dependent manner. Also, preliminary administration of pectin prevented elevation of malondialdehyde and conjugated diene levels in liver and plasma as well as a reduction of glutathione content in liver of rats given CCL_4. These results suggest that low-esterified pectin exert healing and preventive effects on CCL_4-induced hepatotoxicity in rats.

Key words: Pectin; Liver injury; Carbon tetrachloride; Hepatoprotective activity

INTRODUCTION

Pectins are the ionic plant polysaccharides functioning as hydrating agents and cementing substances for the cellulose network (Thakur et al, 1997). They are widely used in food industry because of their gelling and thickening properties (May, 1990). The main structural features of pectin are the linear chains containing more than 100 of (1→4)-linked α-D-galacturonic acid units (Thibault *et al.*, 1993) representing non-branched blocks of molecule. These "smooth" homogalacturonic regions are interrupted with "hairy" rhamnogalacturonic parts in which galacturonic acids are interspersed with (1→2)-linked α-L-rhamnopyranosyl residues carrying neutral side sugar chains (Schols and Voragen, 1996). The long linear chains of homogalacturonan are partially esterified with methanol. For the most part natural pectins are highly esterified while pectins with lower degree of esterification can be prepared (Ridley *et al.*, 2001).

A number of physiological as well as pharmacological effects of pectin and pectin-containing products have been described in studies with laboratory animals. These effects include reduction of serum cholesterol levels (Gonzalez *et al*, 1998; Vergara-Jimenez *et al*, 1998), interaction with metal ions (Dongowski *et al*, 1997), lowering of sphingomielin concentration in very low density lipoproteins (Bladergroen *et al*, 1999), enhanced fecal bile acid excretion, increased hepatic

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synthesis of bile acids, and depletion of cholesterol in liver (Garcia-Diez *et al.* 1996), suppression of colon carcinogenesis (Heitman *et al.*, 1992) as well as exhibition *in vitro* antimutagenic activity against nitroaromatic mutagens (Hensel and Meier, 1999) and inhibition of cancer cell proliferation (Hsieh and Wu, 1995).

Clinical studies have showed the beneficial effects of pectin on human health. Dietary supplementation of pectin decreased blood cholesterol level in subjects with mild and moderate hypercholesterolemia (Groudeva *et al.*, 1996; Knopp *et al.*, 1999), and reduced serum glucose concentration in patients with diabetes mellitus (Levitt *et al.*, 1980). It exerted therapeutic effects in children with persistent diarrhea (Rabbani *et al.*, 2001), and had a significantly slowed down the rate of catch-up body weight gain. Also, pectin was shown to lower the urea production in children recovering from severe cachexia (Doherty and Jackson, 1992).

Analysis of the complete spectrum of pharmacological effects exerted by pectin and elaboration of drugs based on pectins are an important problem of pharmacy (Khotimchenko *et al.*, 2001). Some recent studies suggest that physiological effects of pectins are closely related to their structural characteristics and physical and chemical properties. In particular, pectins with greater methoxyl content and higher molecular weight or higher viscosity are considered as better cholesterol-lowering agents (Yamaguchi *et al.*, 1995; Tepstra *et al.*, 1998). Interaction of pectins with bile acids is diminished as degree of esterification decreases (Dongowski *et al.*, 1995). And finally, activity of pectin inhibiting the attachment of fibroblast growth factor to its receptor was shown to correlate significantly with sugar content, methoxyl content, and size of pectin (Lin *et al.*, 2001).

In the present study we investigated the effects of low-esterified pectin with the degree of esterifica-tion less than 1% on rats with carbon tetrachloride-induced liver injury. Before experiments the main physical and chemical properties such as molecular weight, methyl ester group content, galacturonan content, and intrinsic viscosity of the pectin fraction were characterized. During this study two experiments were carried out. The purpose of the first experiment was to estimate healing effects of low-esterified pectin. In the second experiment preventive influence of low-esterified pectin was studied.

MATERIALS AND METHODS

Pectin preparation

High-esterified citrus pectin without additives was obtained from Copenhagen Pectin A/S, Lille Skensved, Denmark. The degree of esterification of this preparation was 66%. The pectin preparation contained no acetyl or amide groups. For the preparation of low-esterified pectin, 100 g of high-esterified citrus pectin was de-esterified in 1600 ml 50° EtOH containing 20 g NaOH (30 min at 20°C). After acidification pectin was isolated from EtOH.

Pectin analysis

The galacturonan content of the pectin preparation was determined colorimetrically by the *m*-hydroxydiphenyl method (Blumenkrantz and Asboe-Haunsen, 1973). The degree of esterification was characterized using titrimetric analysis (Afanasyev *et al.*, 1984). Intrinsic viscosity of pectin was determined in 0.05 M NaCl/0.005 M Na-oxalate at 25.0°C and pH 6.0 using an Ubbelohde viscosimeter. The intrinsic viscosity is related empirically to the molecular weight by the Mark-Howink relation (Kravtchenko, Pilnik, 1990).

Animals and diet

Male Wistar rats were obtained from the Pacific Institute of Bioorganic Chemistry (Vladivostok, Russia). The rats weighing 130-160 g were housed in stainless steel, wired cages (in groups of three-four per cage) and kept in an isolated room at a controlled temperature (20-22°C) and ambient humidity (60-65%). Lights were maintained on a 12-h light-dark cycle. Animals were first adapted to the facility for one week and provided with water and standard feed *ad libitum*. The composition of the standard diet was as follows (g/100 g): casein, 21.0; cellulose, 5.3; sunflower oil, 7.0; cholesterol, 1.0; sucrose, 15.0; starch, 45.9; methionine, 0.3; minerals, 3.5; vitamin mixture 1.0. The rats were cared for according to the guide for the care and use of laboratory animals of Vladivostok State

Medical University.

Experimental design

In experiment 1 50 rats were randomized into five groups. Animals of group 1 (control 1 –CCl_4) were daily fed the standard diet and one hour before feeding (8:00) administered orally 1 ml of olive oil through gastric gavage for seven days. During this period of time animals of group 2 (control 1 +CCl_4), group 3 (pectin 10), group 4 (pectin 50), and group 5 (pectin 250) were daily fed the standard diet and one hour before feeding (8:00) administered orally 300 mg/kg of body weight of carbon tetrachloride in 1 ml of the olive oil solution through gastric gavage. Then half of rats of group 1 (control 1 –CCL_4) and group 2 (control 1 +CCl_4) were killed by decapitation, blood samples were collected and liver tissue was obtained. Following four days all animals were fed the standard diet only. Then animals of group 1 (control 2 –CCL_4) were fed the standard diet for 21 days. During this period of time animals of group 2 (control 2 +CCl_4) were fed the standard diet and one hour before feeding they were additionally given 1 ml of distilled water through gastric tube. Animals of group 3 in addition to standard diet were given 10 mg/kg b.w. of pectin in 1 ml of water solution through oral gavage. Animals of group 4 were administered 50 mg/kg b.w. of pectin in a form of 1 ml of water solution whereas animals of group 5 were given through gavage 1 ml of pectin solution containing 250 mg/kg b.w. of dry pectin. At the end of experiment 1 animals of all groups were given light ether anesthesia and collection of aortic blood was performed. The rats were killed by decapitation, and liver was immediately removed. Sodium citrate was added to blood samples to prevent coagulation, afterwards blood and liver were stored at -30°C until analysis.

In experiment 2 62 rats were divided into five groups and fed the standard diet for three weeks. One hour before feeding animals of group 1 (control 1 –CCl_4) and group 2 (control 1 +CCl_4) were daily administered orally 1 ml of distilled water through a gastric tube. During the same period of time animals of group 3 (pectin 10), group 4 (pectin 50), and group 5 (pectin 250) were given orally through gavage 1 ml of pectin solution containing 10, 50 and 250 mg/kg b.w. of dry pectin, respectively. On the 22nd day of experiment half of rats of all groups were killed by decapitation, and blood and liver samples were collected. Then animals of group 1 (control 2 –CCL_4) were fed standard diet and one hour before feeding were given 1 ml of olive oil for seven days. Animals of group 2 (control 2 +CCl_4), groups 3, 4, and 5 were administered through a gastric tube 1 ml of the olive oil solution containing 300 mg/kg b.w. of carbon tetrachloride for seven days. At the end of experiment all rats were killed by decapitation under light anesthesia, aortic blood was collected and liver was removed and stored until analysis.

Biochemical analysis

The activities of plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) as well as total and conjugated bilirubin were measured spectrophotometrically using commercially available kits (Lachema, a.s., Czech Republic) by the method of Reitman and Frankel (1957). The enzyme activity was expressed as μkat/ min · L plasma. Reduced glutathione was measured according to mode described by Anderson (Anderson, 1985). Total protein concentration was calculated with bromophenol blue (Greenberg and Gaddock, 1982). Total thiols were measured spectrophotometrically after treatment with 5,59-dithiobis(2-nitrobenzoic acid) (Jocelyn, 1989), which generates a yellow chromophore (λ_{max} = 412 nm). Antioxidant blood activity (AOA) was assayed using lipoprotein suspension of chicken eggs. 1 ml of plasma was mixed with 1 ml of lipoprotein suspension in a test-tube, and then 7 ml of phosphate buffer were added. Lipid peroxidation was initiated by addition of 1 ml of 25 mM $FeSO_4 \cdot 7H_2O$. Test-tubes were incubated for 15 minutes at 37°C. Lipid peroxidation was estimated by the appearance of thiobarbituric acid reactive substances spectrophotometrically quantified at 535 nm formed according to (Rohn *et al.*,1993) with some modifications. Before measurement all samples were mixed up with 0.1 ml of 10^{-2} solute of ethanol, centrifuged at 900 g, and then mixed with 1.8 ml of TBARS reagent (9% thiobarbituric acid, 0.6 N HCl, 0.0056% butylated hydroxytoluene). After that 2 ml of chloroform were added into the test-tube and optical density was measured in a water phase of

each sample. Total blood antioxidant activity was calculated using the following equation: AOA= $\frac{\Delta C_c - \Delta D_{dens}}{\Delta D_c} \cdot 100\%$, where $\Delta D_c = D^t_c - D^0_c$, $\Delta D_{debs} = D^t_{dens} - D^0_{dens}$, D^0_c, D^0_{dens} - optical density of free lipoprotein suspension and lipoprotein suspension with plasma, respectively, estimated before incubation; D^t_c, D^t_{dens} - optical density measured in the same samples at the time t (after 15 minutes of incubation). Blood conjugated diene content was measured by the second-derivative UV spectroscopy method (Corongiu and Banni, 1994). Plasma and liver malondialdehyde contents were assayed by thiobarbituric acid reaction (Yagy, 1984).

Statistical analysis

Values of biochemical parameters are given in the text as mean ± SEM (Standard Error of Mean). Statistical evaluation was performed using the two-tailed Student's-*t* test; a value of $P < 0.05$ was accepted as statistically significant.

RESULTS

Healing effects of low esterified pectin on toxic liver injury in rats (Experiment 1)

As it was expected 7-days oral administration of carbon tetrachloride resulted in significant alterations of biochemical markers indicating damage of liver cells. Activities of blood ALT and AST were enhanced and the level of total and reduced bilirubin was increased. The glycogen content in liver was low. Simultaneously the level of malondialdehyde and conjugated diene in blood became higher, whereas reduced glutathione and thiol group contents were lowered. There was found a more than two-fold reduction of blood antioxidant activity (Table 1, 2; control 1+CCl_4 group). Within 21 days after the end of carbon tetrachloride administration all registered parameters in liver and blood did not change (control 2 +CCl_4 group), suggesting no self-healing influences in animals during the experiment.

Administration of pectin influenced and altered all registered parameters of liver damage depending however on a dose of polysaccharide given. 10 mg/kg of pectin significantly increased the glycogen amount by 33.4% in comparison with positive control 2 group. The other values did not markedly change. Administration of 50 mg/kg of pectin resulted in significantly increased glycogen level (46.4%) and reduced activities of ALT (21.8%) and AST (24.4%). Oral administration of 250 mg/kg of pectin significantly changed all registered parameters of liver damage. There was a 71.5% increase of glycogen content, a 51.7% and 68.7% reduction of activities of ALT and AST, respectively, as well as decrease in levels of total and reduced bilirubin by 64.2% and 63.7%, respectively (Table 1).

Pectin administration in rats with liver injury made an influence upon parameters of prooxidant and antioxidant systems. 10 mg/kg of pectin slightly but significantly caused an increase of blood antioxidant activity and a 33.5% reduction of the blood malondialdehyde level in comparison

Table 1. Healing effects of low-esterified pectin on markers of liver tissue damage in CCl_4-induced liver injury (experiment 1)

Group	ALT (μkat/ min·L plasma)	AST (μkat/ min·L plasma)	Total bilirubin (μmol/L plasma)	Conjugated bilirubin (μmol/L plasma)	Glycogen (μmol/g liver tissue)
Control 1 (– CCl_4) n=5	0.62±0.05	0.28±0.03	9.44±0.48	4.73±0.29	254.6±14.3
Control 1 (+CCl_4) n=6	4.12±0.28	2.88±0.14	42.75±3.82	18.2±0.91	84.0±6.7
Control 2 (– CCl_4) n=5	0.52±0.04	0.34±0.03	10.71±1.23	5.08±0.36	232.9±18.1
Control 2 (+CCl_4) n=8	3.62±0.24	2.17±0.18	48.31±4.29	22.9±1.94	108.6±9.4
Pectin 10 n=8	4.08±0.39	1.84±0.15	40.51±3.25	19.3±2.02	144.9±12.0[a]
Pectin 50 n=8	2.83±0.26[a]	1.64±0.15[a]	39.26±3.49	17.6±2.08	159.0±13.7[b]
Pectin 250 n=8	1.74±0.22[c]	0.68±0.63[a]	17.28±1.89[c]	8.31±0.96[c]	186.3±19.2[c]

The data show M±SEM, [a]$P<0.05$, [b]$P<0.01$, [c]$P<0.001$, significantly different from the control 2 (+CCl_4) value. n=number of rats.

with the control 2 +CCL_4 group. Pectin in a dose of 50 mg/kg significantly altered all values registered excluding the level of thiol groups. The liver malondialdehyde content was reduced by 30.0%, the blood malondialdehyde and conjugated diene levels were decreased by 46.5% and 38.1%, respectively whereas glutathione content and total blood antioxidant activity were increased by 116.1% and 38.0%. 250 mg/kg of pectin resulted in more pronounced alterations of all parameters compared to those of the control 2 +CCl_4 group, although some values were not similar to those of the control 2 –CCl_4 group (Table 2).

Preventive effects of low esterified pectin on toxic liver injury in rats (Experiment 2)

A 21-day administration of 10 and 50 mg/kg of pectin to healthy rats did not change activities of ALT and AST, the level of total and reduced bilirubin as well as parameters indicating activity of prooxidant and antioxidant systems. Only a dose of 250 mg/kg of pectin significantly reduced the level of malondialdehyde in blood and liver. However, advance administration of pectin substantially influenced the pathologic alterations in liver induced by carbon tetrachloride. In the group of rats that were fed 250 mg/kg of pectin for 21 days before administration of carbon tetrachloride all markers of liver damage differed significantly from those of the control 2 +CCl_4 group. ALT and AST activities were 68.8% and 60.6% lower, respectively. Total and reduced bilirubin levels were decreased by 50.6% and 40.7%, respectively. Values of activity of prooxidant and antioxidant systems were altered as follows: liver malondialdehyde level was 40.1% lower, blood malondialdehyde level was 57.7% lower, conjugated diene was 39.2% lower, reduced glutathione was 75.9% higher, thiol groups was –65.6% higher, and total blood antioxidant activity was 83.25 higher. In rats that were preliminary fed 50 mg/kg of pectin only 4 parameters out of 10 differed significantly, in particular, ALT activity was 22.5% lower, AST activity –27.1% lower, glutathione –34.6% higher, and total blood antioxidant activity was increased by 37.7%. Advance administration of 10 mg/kg of pectin did not influence the subsequent course of liver injury induced by carbon tetrachloride (Tables 3 and 4).

DISCUSSION

Various agents such as viruses, chemicals, alcohol as well as autoimmune diseases have been found

Table 2. Healing effects of low-esterified pectin on the prooxidant and antioxidant systems parameters in rats with CCL_4-induced liver injury (experiment 1)

Group	MDA nmol/mg protein	MDA (nmol/ml plasma)	Conjugated dienes (nmol/ml plasma)	Glutathione-GSH (μg/mg protein)	Thiol groups (μg/mg protein)	AOA (%)
Control 1 (– CCl_4) n=5	1.86±0.24	3.45±0.31	3.36±0.29	15.12±1.17	39.07±3.53	62.51±4.72
Control 1 (+CCl_4) n=6	6.39±0.64	12.08±0.89	8.84±0.73	4.64±0.35	19.44±2.02	28.22±1.67
Control 2 (– CCl_4) n=5	1.17±0.11	3.04±0.21	2.45±0.16	17.34±1.68	44.13±4.84	64.78±4.93
Control 2 (+CCl_4) n=8	4.87±0.42	9.57±1.07	6.59±0.52	5.22±0.48	27.13±2.84	32.08±2.81
Pectin 10 n=8	5.07±0.47	6.36±0.60[a]	5.33±0.48	6.43±0.71	30.05±3.42	42.11±3.60[a]
Pectin 50 n=8	3.46±0.41[a]	5.12±0.55[b]	4.08±0.61[b]	11.28±1.34[b]	33.52±3.68	44.26±4.57[a]
Pectin 250 n=8	2.63±0.24[c]	4.23±0.36[c]	3.60±0.44[c]	14.34±1.52[c]	38.43±4.12[a]	53.78±5.93[b]

The data show M±SEM, [a]$P<0.05$, [b]$P<0.01$, [c]$P<0.001$, significantly different from the control 2 (+CCl_4) value.
n = number of rats.

Table 3. Preventive effects of low-esterified pectin on markers of liver tissue damage in CCl_4-induced liver injury (experiment 2)

Group	ALT (μkat/ min·L plasma)	AST (μkat/ min·L plasma)	Total bilirubin (μmol/L plasma)	Conjugated bilirubin (μmol/L plasma)
Control 1 (– CCl_4) n=6	0.72±0.05	0.29±0.03	13.24±1.44	5.86±0.41
Pectin 10 n=6	0.59±0.07	0.32±0.04	12.91±0.95	6.02±0.48
Pectin 50 n=6	0.60±0.06	0.38±0.04	12.18±0.97	6.39±0.49
Pectin 250 n=6	0.71±0.07	0.38±0.05	14.02±1.34	7.12±0.61
Control 2 (– CCl_4) n=6	0.79±0.06	0.36±0.04	11.32±1.16	6.58±0.63
Control 2 (+CCl_4) n=8	4.00±0.32	2.36±0.22	37.19±3.26	17.20±1.49
Pectin 10 n=8	3.71±0.28	2.11±0.20	36.43±3.85	17.14±1.86
Pectin 50 n=8	3.10±0.25[a]	1.72±0.18[a]	31.67±3.01	14.38±1.92
Pectin 250 n=8	1.25±0.09[b]	0.93±0.10[b]	18.38±1.84[c]	10.20±0.81[b]

The data show M±SEM, [a]$P<0.05$, [b]$P<0.01$, [c]$P<0.001$, significantly different from the control 2 (+CCl_4) value.
n=number of rats.

Table 4. Preventive effects of low-esterified pectin on the prooxidant and antioxidant systems parameters in rats with CCL_4-induced liver injury (experiment 2)

Group	MDA nmol/mg protein	MDA (nmol/ml plasma)	Conjugated dienes (nmol/ml plasma)	Glutathione-GSH (μg/mg protein)	Thiol groups (μg/mg protein)	AOA (%)
Control 1 (– CCl_4) n=6	2.43±0.21	3.49±0.22	3.66±0.32	12.11±0.84	45.31±3.26	60.37±4.17
Pectin 10 n=6	2.75±0.22	3.80±0.21	4.04±0.37	11.54±0.89	48.02±3.66	58.29±3.96
Pectin 50 n=6	2.23±0.19	3.51±0.43	5.13±0.42	15.29±1.25	50.13±4.97	55.14±4.79
Pectin 250 n=6	1.79±0.16[d]	2.48±0.27[d]	4.17±0.48	14.30±1.52	46.41±3.79	64.11±6.01
Control 2 (– CCl_4) n=6	2.98±0.25	3.96±0.19	4.10±0.39	11.70±0.69	42.63±3.13	55.73±3.65
Control 2 (+CCl_4) n=8	7.20±0.83	10.97±1.15	8.16±0.87	5.11±0.46	22.72±2.44	23.17±1.84
Pectin 10 n=8	7.06±0.48	10.55±0.58	7.92±0.68	5.25±0.21	23.69±2.68	24.88±2.13
Pectin 50 n=8	6.49±0.54	9.58±0.47	7.32±0.77	6.88±0.52[a]	26.90±2.35	31.90±3.32[a]
Pectin 250 n=8	4.31±0.41[b]	4.64±0.23[c]	4.96±0.43[b]	8.99±0.78[b]	37.63±3.88[b]	42.44±4.71[b]

The data show M±SEM, [a]$P<0.05$, [b]$P<0.01$, [c]$P<0.001$, significantly different from the control 2 (+CCl_4) value; [d]$P<0.05$, significantly different from the control 1 (– CCl_4) group.
n=number of rats.

to cause a liver injury. Also it was pointed out that liver injury induced by carbon tetrachloride in rats and mice is similar to that induced by hepatotoxic agents in man regarding its morphological and biochemical parameters. Therefore, carbon tetrachloride induced liver damage is widely used in experimental studies as a convenient model for the evaluation of hepatoprotective activity of various substances including drugs, food additives and dietary supplements. This model allows one to make an analysis of cellular and molecular links of pathogenesis of liver failure and portal-systemic encephalopathy and to ascertain the mechanism of healing and preventive effects exerted by hepatoprotectors. Carbon tetrachloride is thought to cause hepatotoxicity, which finally leads to the necrosis of liver cells. (CCL_4 converted into free radicals and electrophilic intermediates during reaction of

homolitic disintegration proceeding with participation of cytochrome P-450 causes severe liver injury.) (centrilobular hepatic necrosis) (necrosis, inflammation, and fibrosis). (Kim *et al.*, 1996). Covalent binding of reactive carbon tetrachloride metabolites with liver cell components initiates an inhibition of lipoprotein resulting in steatosis and provokes lipid peroxidation. These two processes are not dependent on each other. Lipoprotein secretion may result in adduct formation and, finally, cancer initiation, whereas the latter results in the loss of calcium homeostasis and leads to apoptosis and death of a cell (Boll *et al.*, 2001; Hemmings *et al*, 2002). It was also shown also that carbon tetrachloride administration causes an increase of the hepatic tumor necrosis factor-alpha mRNA expression and serum tumor necrosis factor-alpha levels as well as inducible nitric oxide synthase protein expression in liver (Morio *et al.*, 2001).

In the present study the effects of pectin on the course and prevention of toxic liver injury induced by oral administration of carbon tetrachloride were examined in rats. It was found that at the end of experiments the tested sample of pectin exerts dose-dependent both healing and preventive effects in rats. In the first experiment animals were given repeated oral doses of carbon tetrachloride for 7 days followed by repeated oral doses of pectin for 21 days and then were killed in 24 hours. Healing influence of pectin was manifested as a tendency towards normalization of such parameters of liver damage as activities of ALT and AST, levels of total and reduced bilirubin as well as improvement of the glycogen content in liver. At the same time, pectin contributed to reduction of malondialdehyde and conjugated diene levels and a rise of glutathione GSH and thiol group content in liver simultaniously increasing total blood antioxidant activity. These results suggest that low-esterified pectin protects liver cells from destructive influence of carbon tetrachloride inhibiting lipid peroxidation and stimulating antioxidant activity. Conceivably, the more mechanisms are involved in the aforementioned processes but this yet to be studied.

In the second experiment rats were administered by oral gavage repeated doses of pectin for 21 days and then repeated oral doses of carbon tetrachloride for 7 days and were killed in 24 hours after the end of experiment. Advance administration of pectin influenced the subsequent development of hepatitis induced by the administration of CCL_4. These findings show that low-esterified pectin under in vivo conditions possesses a hepatoprotective potential which could be attributed to conservation of the integrity of biological membranes because it was associated with a decrease of lipid peroxidation intensity increasing ultimately the resistance of liver cells to destructive influence of toxicants. At least partly this effect may be explained by the capacity of pectin to reduce the lipid peroxidation level in healthy rats as it was shown in the present work. Furthermore, a number of studies have shown that in colon pectin is fermented more or less completely by the microflora to short-chain fatty acids, such as acetate, propionate and butyrate (Titgemeyer *et al.*, 1991; Dongowski *et al.*, 2000). That is why of great interest are the experiments in which animals are fed the diet supplemented with pectin, or sodium acetate, sodium propionate, or sodium butyrate for 14 days and then are injected intraperitoneally with D-galactosamine. Pectin and acetate suppressed the D-galactosamine induced enhancement of blood ALT and AST activities (Sugiyama *et al.*, 1999). Regarding this ability of intestinal microflora, it is interesting that acetate added to diet was found to exert preventive effects against toxic liver injury. These findings should be taken into consideration stating that the pectin fibers may exert their preventive effects at least partly by means of specific short-chain fatty acids e.g. acetate that is one of the products of pectin fermentation by intestinal bacterial flora. However, this assumption should be further examined. Nevertheless, regardless of the exact mechanisms providing beneficial effects of pectin, it is evident that pectin may be useful in treatment or prevention of liver failures especially those manifesting with increased liver and serum transaminase activities and elevated plasma bilirubin level.

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