

Healing and Preventive Effects of Calcium Alginate on Carbon Tetrachloride Induced Liver Injury in Rats

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Abstract: The purpose of this study was to investigate the pharmacological effects of calcium alginate on carbon tetrachloride (CCL₄)-induced hepatotoxicity in rats. The study included two experiments. In the first experiment the animals were given daily CCL₄ through gavage for 7 days and then 10, 50, or 250 mg/kg b.w. of calcium alginate for 21 days. The increased bilirubin level, enhanced alanine and aspartate aminotransferase activity in plasma and reduced liver glycogen content induced by CCL₄ were partly normalized by alginate administration in a dose-dependent manner. In addition, alginate significantly improved CCL₄-induced alterations of pro-oxidant and antioxidant biochemical parameters in liver and plasma compared to those of rats administered CCL₄. In the second experiment the animals were given daily 10, 50 or 250 mg/kg b.w. of calcium alginate for 21 days before 7-day administration of CCL₄. Pretreatment with alginate before CCL₄ 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 alginate prevented elevation of lipid peroxidation products and reduction of liver glutathione content in rats given CCL₄. These results suggest that calcium alginate exerts healing and preventive effects on CCL₄-induced hepatotoxicity in rats.

Key words: calcium alginate; liver injury; carbon tetrachloride; hepatoprotective activity; rats.

Introduction

Alginic acid and its salts (alginates) occur mainly in marine brown seaweeds making main part of their polysaccharides and comprising up to 40% of the dry matter [1]. Red seaweeds belonging to the *Corallinacea* family also contain these substances [2], and bacteria of the genera *Pseudomonas* and *Acetobacter* are known to contain acetylated alginates [3]. By their chemical nature, alginates consist of a linear chain of (1–4)-linked residues of β -D-mannuronic acid and α -L-guluronic acid in different proportions and sequential arrangements. The most common arrangement is that of a block copolymer, in which long homopolymeric sequences of guluronic acid residues (G–G blocks) and similar sequences of mannuronic acid residues (M–M blocks) are intercalated between sequences of mixed composition (M–G blocks) [4].

In seaweeds polysaccharides are the primary components of both the cell walls and the intercellular matrix. The biological functions of alginates in the plants include prevention of desiccation, maintaining of integrity of cells, and providing of mechanical strength and flexibility of the algal tissue. Ion-exchange functions are also important [5]. Alginates, extracted from brown seaweed with an acid and an alkali, are used in a wide range of applications, particularly in the food, industrial, and pharmaceutical fields because of their water holding and gel forming capacities and ability to form and stabilize emulsions [6]. In biotechnology alginate gel beads are used as a matrix to entrap molecules of biological significance [7], such as enzymes [8, 9] or whole microbial [10], plant [11] or animal cells [12, 13, 14]. In experimental pharmacy alginate microparticles are developed as sustained delivery carriers for drugs [15, 16, 17], antigens [18] and recombinant gene products [19]. Cell-interactive alginate hydrogel is an agent for bone tissue engineering [20, 21] and a promising material for use as an implant for peripheral nerve regeneration [22].

A number of pharmacological effects of alginates and alginate-containing products have been described in studies with laboratory animals and under *in vitro* conditions. These effects include reduction of serum cholesterol level [23], interaction with metal ions, and suppression of intestinal absorption of heavy metals and radioactive strontium, barium, and radium [24]. They also include prevention of growth of murine tumor cells [25], stimulation of production of nitric oxide, hydrogen peroxide, and tumor necrosis factor by macrophages in mice [26], and inhibition of activity against tobacco mosaic virus infection [27]. Alginates containing zinc ions were shown to possess potentiating effects on prothrombotic coagulation and platelet activation [28]. Alginate sulfate prepared from sodium alginate was found to have a high anticoagulant activity [29].

In clinical trials alginate had been shown to exert beneficial effects on human health. Sodium alginate helped in lowering significantly postprandial rises of blood glucose, insulin and C-peptide in humans with noninsulin-dependent diabetes [30]. Sodium alginate exerted hemostatic effect on

utero-cervical hemorrhage [31], and calcium alginate swabs decreased per-operative blood loss and post-operative suction drainage loss [32]. In pharmaceutical field alginates have been used to treat wounds and gastric ulcers for many years [33].

In this study we investigated the effects of calcium alginate in rats with carbon tetrachloride-induced liver injury. The purpose of the first experiment was to estimate healing effects of calcium alginate. In the second experiment preventive influence of calcium alginate was studied.

Results and Discussion

Calcium alginate substrate

The uronic acid concentration of the calcium alginate preparation used in experiment was 77.3%. The calcium content was 72.5%. This indicates 82.5% of carboxyl groups in preparation are present in a form of calcium salt. The intrinsic viscosity of original sodium alginate used for preparation of calcium alginate was 1270 ml/g. Calculated molecular weight of the original sodium alginate and calcium alginate used in experiments was $403 \cdot 10^3$ Da.

Healing effects of calcium alginate on toxic liver injury in rats (Experiment 1)

As expected 7-days oral administration of carbon tetrachloride resulted in statistically significant alterations of biochemical markers of damage to hepatocytes. Activities of blood ALT and AST were elevated and the serum concentrations of total and reduced bilirubin were increased. The glycogen content in liver was low (Table 1, positive control 1 group). Simultaneously the level of malondialdehyde and conjugated dienes in blood became higher whereas reduced glutathione and thiol group contents were lowered. There was more than a two-fold reduction of blood antioxidant activity (Table 2, positive control 1 group). For 21 days after the end of carbon tetrachloride administration all registered parameters in liver and blood did not change (positive control 2 group), suggesting continued damage and lack of self-healing.

Administration of calcium alginate influenced and altered all registered parameters of liver damage in a dose-dependent manner. The dose of 10 mg/kg was not effective. Oral administration of 50 mg/kg of calcium alginate statistically significantly changed all parameters of liver damage measured. Observations included increased of glycogen content by 57.8%, reduced activities of ALT and AST by 40.3% and 51.3%, respectively, as well as decreased concentrations of total and reduced bilirubin by 33.5% and 29.4%, respectively. Administration of 250 mg/kg of calcium alginate resulted in increased liver glycogen content by 71.6%, reduced activities of ALT and AST by 62.9% and 62.6%, respectively, and decreased blood total bilirubin concentration by 64.3% and blood conjugated bilirubin concentration by 46.0% (Table 1).

Administration of calcium alginate to rats with liver injury influenced parameters of pro-oxidant and antioxidant systems. The dose of 10 mg/kg calcium alginate caused slight but statistically significant reduction of blood malondialdehyde concentrations (27.7%) compared with positive control 2. Calcium alginate in a dose of 50 mg/kg statistically significantly altered all values

recorded except for thiol group concentrations. The liver malondialdehyde content was reduced by 45.2%, the blood malondialdehyde and conjugated diene levels were decreased by 38.5% and 32.0%, respectively, whereas reduced glutathione content and total blood antioxidant activity were increased by 66.7% and 55.4%, respectively. Treatment with 250 mg/kg of calcium alginate resulted in more pronounced alterations of all parameters compared to those of positive control 2 although some values were not similar to those of negative control 2 group (Table 2).

Table 1. Healing effects of calcium alginate on markers of liver tissue damage in CCl₄-induced liver injury (experiment 1).

Group	ALT (μ kat/L plasma)	AST (μ cat/L plasma)	Total bilirubin (μ mol/L plasma)	Conjugated bilirubin (μ mol/L plasma)	Glycogen (μ mol/g liver tissue)
Control 1 (– CCl ₄) n=6	0.69±0.06	0.38±0.03	11.82±0.96	5.21±0.43	237.0±24.6
Control 1 (+CCl ₄) n=5	4.86±0.36	3.06±0.24	39.21±4.18	24.32±2.04	93.1±8.3
Control 2 (– CCl ₄) n=5	0.61±0.05	0.35±0.03	12.67±1.48	4.34±0.38	250.2±25.7
Control 2 (+CCl ₄) n=8	4.39±0.31	2.94±0.22	45.65±4.73	20.82±1.71	112.3±12.8
Ca alginate 10 n=8	4.42±0.34	2.86±0.19	38.63±3.50	23.26±2.27	117.3±14.4
Ca alginate 50 n=8	2.62±0.25 ^c	1.42±0.13 ^c	30.35±3.58 ^a	14.70±1.79 ^a	177.2±16.5 ^b
Ca alginate 250 n=8	1.63±0.19 ^c	1.13±0.11 ^c	16.30±1.57 ^c	11.25±1.37 ^b	192.7±20.1 ^b

The data show Mean±SEM, ^a $P<0.05$, ^b $P<0.01$, ^c $P<0.001$, when compared with control 2 (+CCl₄) by one-way ANOVA and following Tukey's test, n = number of rats.

Table 2. Healing effects of calcium alginate on the pro-oxidant and antioxidant system parameters in rats with CCL₄-induced liver injury (experiment 1).

Group	MDA nmol/mg protein	MDA (nmol/ml plasma)	Conjugated dienes (nmol/ml plasma)	GSH (µg/mg protein)	Thiol groups (µg/mg protein)	AOA (%)
Control 1 (– CCl ₄) n=6	2.14±0.20	3.95±0.37	4.17±0.35	13.26±1.12	46.22±4.85	60.62±5.83
Control 1 (+CCl ₄) n=5	6.84±0.69	11.49±1.10	9.20±0.86	5.72±0.48	21.35±1.87	21.46±2.04
Control 2 (– CCl ₄) n=5	1.68±0.17	4.14±0.37	3.54±0.32	15.19±1.50	44.85±4.29	56.30±4.98
Control 2 (+CCl ₄) n=8	5.38±0.45	11.26±1.16	7.76±0.68	5.68±0.55	25.28±2.36	29.35±3.34
Ca alginate 10 n=8	5.18±0.49	8.14±0.81 ^a	7.82±0.81	5.24±0.51	24.59±2.72	36.29±3.28
Ca alginate 50 n=8	2.95±0.38 ^b	6.92±0.68 ^b	5.28±0.50 ^a	9.47±1.03 ^b	28.22±2.71	45.61±4.70 ^a
Ca alginate 250 n=8	2.48±0.31 ^c	5.63±0.58 ^c	3.88±0.44 ^c	10.63±1.19 ^b	36.8±3.88 ^b	50.82±5.78 ^b

The data show Mean±SEM, ^a $P < 0.05$, ^b $P < 0.01$, ^c $P < 0.001$, when compared with control 2 (+CCl₄) by one-way ANOVA and following Tukey's test, n = number of rats.

Preventive effects of calcium alginate on toxic liver injury in rats (Experiment 2)

A 21-days administration of 10 or 50 mg/kg of calcium alginate to healthy rats did not change the blood activities of ALT and AST, the blood concentrations of total and reduced bilirubin or parameters indicating activity of pro-oxidant and antioxidant systems. Only a dose of 250 mg/kg slightly although statistically significantly reduced the level of malondialdehyde in blood and liver. However preliminary administration of calcium alginate influenced the pathologic alterations in liver induced by carbon tetrachloride. Advance administration of 10 mg/kg of calcium alginate did not influence the subsequent course of liver injury induced by carbon tetrachloride. In rats that were preliminary given 50 mg/kg of calcium alginate only 4 parameters out of 10 differed significantly in comparison with positive control 2 (+CCl₄) group, in particular, the blood ALT activity was 24.0% lower, the blood AST activity was 30.7% lower, liver glutathione concentration was 59.6% higher, and total blood antioxidant activity was 39.2% higher. In the group of rats given 250 mg/kg of calcium alginate for 21 days before administration of carbon tetrachloride all markers of liver

damage differed statistically significantly from those of the positive control 2 (+CCl₄) group. ALT and AST activities were 62.1% and 59.2% lower, respectively. Total and reduced bilirubin concentrations were decreased by 30.1% and 32.2%, respectively. Parameters indicating activity of pro-oxidant and antioxidant systems differed as follows: liver malondialdehyde concentration was 42.5% lower, blood malondialdehyde concentration was 52.9% lower, plasma conjugated diene concentration was 41.4% lower, reduced glutathione was 76.6% higher, thiol groups was 57.2% higher, and total blood antioxidant activity was 71.1% higher (tables 3 and 4).

The data obtained through this study demonstrate that calcium alginate has beneficial effects on CCl₄-induced hepatotoxicity in a dose-dependent manner. Liver injury induced by carbon tetrachloride administration in rats and mice is similar to that caused by some more common hepatodestructive agents such as viruses, chemicals, alcohol or autoimmune diseases in humans based on morphological and biochemical markers of liver disease [34]. Therefore a tetrachloride induced liver damage is widely used in experimental studies as a convenient model for evaluation of hepatoprotective activity of various substances including drugs 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 mechanisms of healing and preventive effects exerted by hepatoprotectors [35]. Carbon tetrachloride is thought to induce hepatotoxicity finally leading to necrosis of liver cells. CCl₄ converted into free radicals and electrophilic intermediates during reaction of homolytic disintegration proceeding with participation of cytochrome P-450 causes severe liver injury resulting in centrilobular hepatic necrosis, inflammation, and fibrosis [36]. Covalent binding of reactive carbon tetrachloride metabolites with liver cell components initiates inhibition of lipoprotein secretion resulting in steatosis while reaction with oxygen provokes lipid peroxidation leading to apoptosis and death of cells [37, 38]. The results obtained in our study show that calcium alginate has two effects on CCl₄-induced liver damage in rats. Firstly, it exerts a healing effect manifesting as a tendency towards faster normalization of such parameters of liver damage as activities of ALT and AST, blood concentrations of total and reduced bilirubin as well as improvement of the liver glycogen content after carbon tetrachloride administration. At the same time alginate contributes to reduction of blood malondialdehyde and conjugated diene concentrations and a rise of reduced glutathione and thiol group contents in liver simultaneously increasing the total blood antioxidant activity. These results suggest that calcium alginate protects liver cells from the destructive influence of carbon tetrachloride by inhibiting lipid peroxidation and stimulating antioxidant activity. Conceivably additional mechanisms are involved in the aforementioned processes but they are not studied yet. Secondly calcium alginate possesses a preventive effect. So preliminary intake of alginate influences subsequent development of liver injury induced by the administration of CCl₄. These findings show that calcium alginate under in vivo conditions possesses a hepatoprotective potential activity, which could be attributed to conservation of integrity of biological membranes because it was associated with a decrease of lipid peroxidation intensity enhancing ultimately the resistance of liver cells to destructive influence of toxicants. At least partly this effect may be explained by the capacity of calcium alginate to reduce

the lipid peroxidation level in healthy rats as was shown in the present work. Furthermore a number of studies have shown that alginate is fermented more or less completely by the microflora to short-chain fatty acids such as acetate, propionate and butyrate in the colon [39]. Acetate suppressed the D-galactosamine induced enhancement of blood ALT and AST activities in animals fed a diet supplemented with sodium acetate for 14 days [40]. These findings should be taken into consideration stating that the alginate fibers may exert their preventive effects at least partly by means of specific short-chain fatty acids. This hypothesis should be further examined.

Table 3. Preventive effects of calcium alginate on markers of liver tissue damage in CCl₄-induced liver injury (experiment 2).

Group	ALT (μ kat/L plasma)	AST (μ kat/L plasma)	Total bilirubin (μ mol/L plasma)	Conjugated bilirubin (μ mol/L plasma)
Control 1 (- CCl ₄) n=6	0.81 \pm 0.07	0.32 \pm 0.03	12.24 \pm 1.44	6.13 \pm 0.59
Ca alginate 10 n=5	0.79 \pm 0.08	0.34 \pm 0.03	13.52 \pm 1.08	7.74 \pm 0.62
Ca alginate 50 n=5	0.73 \pm 0.07	0.36 \pm 0.04	12.64 \pm 0.98	7.51 \pm 0.60
Ca alginate 250 n=6	0.77 \pm 0.08	0.37 \pm 0.05	13.17 \pm 1.36	6.39 \pm 0.62
Control 2 (- CCl ₄) n=6	0.83 \pm 0.08	0.37 \pm 0.04	14.22 \pm 1.38	6.58 \pm 0.65
Control 2 (+CCl ₄) n=8	4.12 \pm 0.31	2.38 \pm 0.20	35.65 \pm 3.28	18.36 \pm 1.66
Ca alginate 10 n=8	4.03 \pm 0.34	1.96 \pm 0.19	36.37 \pm 3.61	16.22 \pm 1.73
Ca alginate 50 n=8	3.13 \pm 0.28 ^a	1.65 \pm 0.22 ^a	30.46 \pm 2.94	14.28 \pm 1.57
Ca alginate 250 n=8	1.56 \pm 0.17 ^c	0.97 \pm 0.10 ^b	24.91 \pm 2.74 ^a	12.44 \pm 1.36 ^a

The data show Mean \pm SEM, ^a P <0.05, ^b P <0.01, ^c P <0.001, when compared with control 2 (+CCl₄) by one-way ANOVA and following Tukey's test, n = number of rats.

Table 4. Preventive effects of calcium alginate on the pro-oxidant and antioxidant system parameters in rats with CCL₄-induced liver injury (experiment 2).

Group	MDA nmol/mg protein	MDA (nmol/ml plasma)	Conjugated dienes (nmol/ml plasma)	GSH (µg/mg protein)	Thiol groups (µg/mg protein)	AOA (%)
Control 1 (– CCL ₄) n=6	2.64±0.23	4.55±0.49	4.55±0.39	12.64±0.93	48.76±3.63	56.36±5.29
Ca alginate 10 n=5	2.55±0.24	5.11±0.53	5.11±0.48	12.28±0.98	44.32±3.89	54.39±5.11
Ca alginate 50 n=5	2.71±0.26	4.56±0.42	5.73±0.56	13.25±1.22	49.27±4.85	59.26±5.81
Ca alginate 250 n=6	1.96±0.18 ^d	3.04±0.31 ^d	4.42±0.45	11.79±0.92	44.38±4.74	53.36±5.03
Control 2 (– CCL ₄) n=6	2.79±0.27	4.22±0.40	4.84±0.52	12.31±1.03	47.26±4.82	59.21±6.02
Control 2 (+CCL ₄) n=8	7.97±0.74	12.37±1.23	8.65±0.77	5.13±0.49	21.73±2.36	27.10±2.75
Ca alginate 10 n=8	7.13±0.68	10.34±1.28	8.15±0.73	5.48±0.31	20.46±1.98	27.64±2.29
Ca alginate 50 n=8	6.44±0.57	9.94±0.78	7.28±0.67	8.19±0.81 ^b	28.29±2.44	37.72±3.34 ^a
Ca alginate 250 n=8	4.58±0.42 ^b	5.83±0.59 ^c	5.07±0.49 ^b	9.06±0.87 ^b	34.17±3.67 ^a	46.38±4.85 ^b

The data show Mean±SEM, ^a $P < 0.05$, ^b $P < 0.01$, ^c $P < 0.001$, when compared with control 2 (+CCL₄); ^d $P < 0.05$, when compared with control 1 (– CCL₄) by one-way ANOVA and following Tukey's test, n = number of rats.

Conclusions

In this paper beneficial effects of polysaccharide from brown seaweeds calcium alginate were examined using a CCL₄-induced liver injury model in rats. The results indicate that calcium alginate has both healing and preventive effects on some of the parameters indicating hepatotoxic damages in CCL₄-exposed rats. Both phenomena were accompanied by a normalization of biochemical

parameters of oxidative processes in the blood and liver suggesting that ameliorative effects of calcium alginate may be related to its antioxidant properties. Regardless of the exact mechanisms providing beneficial affects of alginates it is evident that calcium alginate may be useful in treatment or prevention of liver failures especially those manifesting with increased liver and serum transaminase activities and elevated blood bilirubin concentrations.

Experimental

Calcium alginate preparation

Sodium alginate (type HV) without additives was obtained from Kelco (California, USA). Calcium alginate was prepared as follows. 200 g of sodium alginate was suspended in 1000 ml of 70% ethyl alcohol. Shaking intensively 8 g of $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$ diluted preliminary in 100 ml of 70% ethyl alcohol were added. Calcium alginate obtained was separated with a porous glass filter with a mesh 40 μm , rinsed with 500 ml of 70% ethyl alcohol, and dried at 60°C.

Calcium and sodium alginate analysis.

The uronic acid content of original sodium alginate and prepared calcium alginate was determined colorimetrically by the *m*-hydroxydiphenyl method and expressed in percent [41]. The calcium content in the preparation studied was assayed by atomic-absorption and expressed in mg/g and mg-equivalents/g of sample [42]. The ratio of carboxyl groups forming a calcium salt was calculated as a calcium : uronic acid content ratio expressed in mg-equivalents/g. Intrinsic viscosity of original sodium alginate 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 [43].

Animals and diet

Male Wistar rats were obtained from 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. All animal experiments were conducted in accordance with the guide for the care and use of laboratory animals of Vladivostok State Medical University.

Experimental design

In the first experiment 48 rats were randomized into five groups. Animals of group 1 (control 1 – CCl₄) were fed the standard diet daily and one hour before feeding (8:00 AM) given 1 ml of olive oil orally through gastric gavage for seven days. During this time animals of group 2 (control 1 +CCl₄), group 3 (alginate 10), group 4 (alginate 50), and group 5 (alginate 250) were daily fed the standard diet and one hour before feeding (8:00 AM) administered 300 mg/kg of body weight (b. w.) of carbon tetrachloride diluted in 1 ml of the olive oil solution orally through gastric gavage. Then half of rats of group 1 (control 1 –CCl₄) and group 2 (control 1 +CCl₄) were killed by decapitation under light ether anesthesia, blood samples were collected and liver tissue was obtained. For the next four days all animals were fed the standard diet only. Then animals of group 1 (control 2 –CCl₄) and group 2 (control 2 +CCl₄) were fed the standard diet and one hour before feeding they were additionally given 1 ml of distilled water through gastric tube for 21 days. Animals of group 3 in addition to standard diet were given 1 ml of water suspension containing 10 mg/kg b.w. of calcium alginate through oral gavage. Animals of group 4 were administered 1 ml of water suspension containing 50 mg/kg b.w. of calcium alginate whereas animals of group 5 were given 1 ml of calcium alginate suspension containing 250 mg/kg b.w. of dry alginate by gavage. At the end of the first experiment animals of all groups were given light ether anesthesia and aortic blood was collected. 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 for 12-72 hours until analysis.

In the second experiment 60 rats were divided into five groups and fed the standard diet for three weeks. One hour before feeding animals of group 1 (control 1 –CCl₄) and group 2 (control 1 +CCl₄) were administered 1 ml of distilled water through gastric tube daily. During the same period of time animals of group 3 (alginate 10), group 4 (alginate 50), and group 5 (alginate 250) were given 1 ml of calcium alginate suspension containing 10, 50 and 250 mg/kg b.w. of dry alginate, respectively by gavage. On 22nd day of the 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 –CCl₄) 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 +CCl₄), groups 3, 4, and 5 were administered 1 ml of the olive oil solution containing 300 mg/kg b.w. of carbon tetrachloride through a gastric tube 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 for 12-72 hours.

Biochemical analysis

The activities of plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) as well as total and conjugated bilirubin levels in blood were measured spectrophotometrically using commercially available kits (Lachema, a.s., Czech Republic). The enzyme activity was expressed as $\mu\text{kat/L}$ plasma. A liver reduced glutathione content was measured according to

methods described by Anderson [44]. Liver glycogen content was assessed using method described by Van Handel [45]. Total protein concentrations in livers were calculated using a bromophenol blue rapid assay [46]. Total thiols in liver were measured spectrophotometrically after treatment with 5, 5-dithio-bis(2-nitrobenzoic acid) [47], which generates a yellow chromophore ($\lambda_{\max} = 412$ nm). Blood antioxidant 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 $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$. Test-tubes were incubated for 15 minutes at 37°C. Lipid peroxidation was estimated spectrophotometrically at 535 nm by the appearance of the thiobarbituric acid reactive substances formed according to method described by Rohn [48] with follows modifications. Before measurement all samples were mixed with 0.1 ml of 0.1 M ethanol solute, 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 mixed by volume). 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 follows equation: $\text{AOA} = \frac{\Delta D_c - \Delta D_{dens}}{\Delta D_c} \cdot 100\%$, where $\Delta D_c = D_c^t - D_c^0$, $\Delta D_{dens} = D_{dens}^t - D_{dens}^0$, D_c^0, D_{dens}^0 - optical density of free lipoprotein suspension and lipoprotein suspension with plasma, respectively, estimated before incubation; D_c^t, D_{dens}^t - 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 [49]. Plasma and liver malondialdehyde contents were assayed using thiobarbituric acid reactions [50].

Statistical analysis

Values of biochemical parameters are given as mean \pm SEM (Standard Error of Mean). All values given in the work were normally distributed. Statistical evaluation was performed using one-way ANOVA test followed by Tukey's post hoc test. Values were accepted as statistically significant at $P < 0.05$ levels.

References and Notes

1. Rossel, K.G.; Srivastava, L.M. Seasonal variation in the chemical constituents of the brown algae *Marocystis integrifolia* and *Noveocystis luetkeana*. *Can. J. Bot.* **1984**, *62*, 2229-2236.
2. Okazaki, M.; Furuya, K.; Tsukayama, K.; Nisizawa, K. Isolation and identification of alginic acid from calcareous red alga *Serraticardia maxima*. *Bot. Mar.* **1982**, *25*, 123-131.
3. Sabra, W.; Zeng, A.P.; Deckwer, D. Bacterial alginate: physiology, product quality and process aspects. *Appl. Microbiol. Biotechnol.* **2001**, *56*, 315-325.
4. Sidsrod, O.; Draget, K.I. Chemistry and physical properties of alginates. *Carbohydr. Eur.* **1996**, *14*, 6-13.
5. Skjak-Brak, G. Alginate: biosynthesis and structure-function relationship relevant to biomedical and biotechnological applications. *Biochem. Plant Polysacch.* **1992**, *20*, 27-33.
6. Pilnic, W.; Rombouts, F. Polysaccharides and food processing. *Carbohydr. Res.* **1985**, *142*, 93-105.
7. Chan, L.W.; Lee, H.Y.; Heng, P.W.S. Production of alginate microspheres by internal gelation using an emulsification method. *Int. J. Pharm.* **2002**, *242*, 259-262.
8. Coppi, G.; Iannuccelli, V.; Bernabei, M.T.; Cameroni, R. Alginate microparticles for enzyme peroral administration. *Int. J. Pharm.* **2002**, *242*, 263-266.
9. Blandino, A.; Macias, A.; Cantero, D. Calcium alginate gel as encapsulation matrix for coimmobilized enzyme systems. *Appl. Biochem. Biotechnol.* **2003**, *110*, 53-60.
10. Norton, S.; Watson, K.; Damore, T. Ethanol tolerance of immobilized brewers' yeast cells. *Appl. Microbiol. Biotechnol.* **1995**, *43*, 18-24.
11. Declerck, S.; Strullu, D.; Plenchette, C.; Guillemette, T. Entrapment of in vitro produced spores of *Glomus versiforme* in alginate beads: *in vitro* and *in vivo* inoculum potentials. *J. Biotechnol.* **1996**, *48*, 51-57.
12. Torre, M.L.; Maggi, L.; Vigo, D.; Galli, A.; Bornaghi, V.; Maffeo, G.; Conte, U. Controlled release of swine semen encapsulated in calcium alginate beads. *Biomaterials* **2000**, *21*, 1493-1498.
13. Tatarkiewicz, K.; Garcia, M.; Lopez-Avalos, M.; BonnerWeir, S.; Weir, G.C. Porcine neonatal pancreatic cell clusters in tissue culture: Benefits of serum and immobilization in alginate hydrogel. *Transplantation* **2001**, *71*, 1518-1526.
14. Simpson, N.E.; Grant, S.C.; Blackband, S.J.; Constantinidis, I. NMR properties of alginate microbeads. *Biomaterials* **2003**, *24*, 4941-4948.
15. Tonnesen, H.H.; Karlsen, J. Alginate in drug delivery systems. *Drug Develop. Indust. Pharmacy* **2002**, *28*, 621-630.
16. Ain, Q.; Sharma, S.; Khuller, G.K.; Garg, S.K. Alginate-based oral drug delivery system for tuberculosis: pharmacokinetics and therapeutic effects. *J. Antimicrob. Chemother* **2003**, *51*, 931-938.

17. Murata, Y.; Kofuji, K.; Kawashima, S. Preparation of floating alginate gel beads for drug delivery to the gastric mucosa. *J. Biomater. Sci. Polymer Edition* **2003**, *14*, 581-588.
18. Kim, B.; Bowersock, T.; Griebel, P.; Kidane, A.; Babiuk, L.A.; Sanchez, M.; Attah-Poku, S.; Kaushik, R.S.; Mutwiri, G.K. Mucosal immune responses following oral immunization with rotavirus antigens encapsulated in alginate microspheres. *J. Control. Release* **2002**, *85*, 191-202.
19. Ross, C.J.D.; Chang P.L. Development of small alginate microcapsules for recombinant gene product delivery to the rodent brain. *J. Biomater. Sci. - Polymer Edition* **2002**, *13*, 953-962.
20. Alsberg, E.; Anderson, K.W.; Albeiruti, A.; Franceschi, R.T.; Mooney, D.J. Cell-interactive alginate hydrogels for bone tissue engineering. *J. Dental Res.* **2001**, *80*, 2025-2029.
21. Ueyama, Y.; Ishikawa, K.; Mano, T.; Koyama, T.; Nagatsuka, H.; Suzuki, K.; Ryoike, K. Usefulness as guided bone regeneration membrane of the alginate membrane. *Biomaterials* **2002**, *23*, 2027-2033.
22. Hashimoto, T.; Suzuki, Y.; Kitada, M.; Kataoka, K.; Wu, S.; Suzuki, K.; Endo, K.; Nishimura, Y.; Ide, C. Peripheral nerve regeneration through alginate gel: analysis of early outgrowth and late increase in diameter of regenerating axons. *Exp. Brain Res.* **2002**, *146*, 356-368.
23. Seal, C.J.; Mathers, J.C. Comparative gastrointestinal and plasma cholesterol responses of rats fed on cholesterol-free diets supplemented with guar gum and sodium alginate. *Br. J. Nutr.* **2001**, *85*, 317-324.
24. Khotimchenko, Yu.S.; Kovalev, V.V.; Savchenko, O.V.; Ziganshina, O.A. Physical-chemical properties, physiological activity, and usage of alginates, the polysaccharides of brown algae. *Rus. J. Mar. Biol.* **2001**, *27*, S53-S64.
25. Fujihara, M.; Nagumo, T. An influence of the structure of alginate on the chemotactic activity of macrophages and the antitumor activity. *Carbohydr. Res.* **1993**, *243*, 211-216.
26. Son, E.H.; Moon, E.Y.; Rhee, D.K.; Pyo, S. Stimulation of various functions in murine peritoneal macrophages by high mannuronic acid-containing alginate (HMA) exposure in vivo. *Int. Immunopharmacol.* **2001**, *1*, 147-154.
27. Sano, Y. Antiviral activity of alginate against infection by tobacco mosaic virus. *Carbohydr. Polym.* **1999**, *38*, 183-186.
28. Segal, H.C.; Hunt, B.J.; Gilding, K. The effect of alginate and non-alginate wound dressings on blood coagulation and platelet activation. *J. Biomater. Appl.* **1998**, *12*, 249-257.
29. Huang, R.H.; Du, Y.M.; Yang, J.H. Preparation and in vitro anticoagulant activities of alginate sulfate and its quaterized derivatives. *Carbohydr. Polym.* **2003**, *52*, 19-24.
30. Torsdottir, I.; Alpsten, M.; Holm, G.; Sandberg, A. S.; Tolli, J. A small dose of soluble alginate-fiber affects postprandial glycemia and gastric emptying in humans with diabetes. *J. Nutr.* **1991**, *121*, 795-799.

31. Nishiya, M.; Hareyama, H.; Makinoda, S.; Fujimoto, S. A study on the hemostatic effect of sodium alginate on uterocervical hemorrhage. *Asia Oceania J. Obstet. Gynecol.* **1994**, *20*, 203-208.
32. Davis, M.S.; Flannery, M.C.; McCollum, C.N. Calcium alginate as haemostatic swabs in hip fracture surgery. *J. R. Coll. Surg. Edinb.* **1997**, *42*, 31-32.
33. Nagaoka, M.; Shibata, H.; Kimura-Takagi, I.; Hashimoto, S.; Aiyama, R.; Ueyama, S.; Yokokura, T. Anti-ulcer effects and biological activities of polysaccharides from marine algae. *BioFactors* **2000**, *12*, 267-274.
34. Rechnagel, R.O.; Glende, E.A. Jr. Carbon tetrachloride hepatotoxicity: an example of lethal cleavage. *CRC Crit Rev Toxicol* **1973**, *2*, 263-297.
35. Zimmerman, H. J. *Hepatotoxicity*, Appleton-Century-Crofts, N.Y. **1978**
36. Kim, S.; Chung, H.; Cho, J. Molecular mechanism for alkyl sulfide-modulated carbon tetrachloride-induced hepatotoxicity: the role of cytochrome P450E1, P450B and glutathione S-transferase expression. *J. Pharmacol. Exp. Ther.* **1996**, *277*, 1058-1066.
37. Boll, M.; Weber, L.W.D.; Becker, E.; Stampfl, A. Mechanism of carbon tetrachloride-induced hepatotoxicity. Hepatocellular damage by reactive carbon tetrachloride metabolites. *J. Biosci.* **2001**, *56*, 649-659.
38. Hemmings, S.J.; Pulga, V.B.; Tran, S.T.; Uwiera, R.R.E. Differential inhibitory effects of carbon tetrachloride on the hepatic plasma membrane, mitochondrial and endoplasmic reticular calcium transport systems: implications to hepatotoxicity. *Cell Bioch. Funct.* **2002**, *20*, 47-59.
39. Michel, C.; Lahaye, M.; Bonnet, C.; Mabeau, S.; Barry, J.L. *In vitro* fermentation by human faecal bacteria of total and purified dietary fibres from brown seaweeds. *Br. J. Nutr.* **1996**, *75*, 263-280.
40. Sugiyama, K.; Puming, H.; Shingo, W.; Shigeru, S. Teas and other beverages suppress D-galactosamine-induced liver injury in rats. *J. Nutr.* **1999**, *129*, 1361-1367.
41. Blumenkrantz, S.; Asboe-Haunsen, G. New method for quantitative determination of uronic acids. *Anal. Biochem.* **1973**, *54*, 484-489.
42. KostECKA, K. S. Atomic Absorption Spectroscopy of Calcium in Foodstuffs in Non-Science-Major Courses *J. Chem. Educ.* **2000**, *77*, 1321.
43. Kravtchenko, T. P.; Pilnik, A. A simplified method for the determination of the intrinsic viscosity of pectin solutions by classical viscosimetry. In: *Gums and Stabilizers in the Food Industry 5* (Eds. Phillips, G. O., Williams, P. A., Wedlock, D. J.), IRL Press, Oxford, **1990**, 281-285.
44. Anderson, M.E. Determination of glutathione and glutathione disulfide in biological samples. *Methods in Enzymology* **1985**, *113*, 548-53.
45. Van Handel E. Estimation of glycogen in small amounts of tissue. *Analyt. Biochem.* **1965**, *11*, 256-265.

46. Greenberg, C.G.; Gaddock, P.R. Rapid single-step membrane protein assay. *Clin. Chem.* **1982**, *28*, 1725-1726.
47. Jocelyn, P.C. Spectrophotometric assay of thiols. *Methods in Enzymology* **1989**, *143*, 44–55.
48. Rohn, T.T.; Hinds, T.R.; Vincenzi, F.F. Ion transport ATPases as targets for free radical damage. *Biochem. Pharmacol.* **1993**, *46*, 525–534.
49. Corongiu, F.P.; Banni, S. Detection of conjugated dienes by second derivative ultraviolet spectrophotometry. *Methods in Enzymology* **1994**, *233*, 303–310.
50. Yagy, K. Assay for blood plasma or serum. In: Packer L, ed. *Methods in Enzymology*. New-York: Academic Press, **1984**, *105*, 328-331.

Sample Availability: Samples are available from the authors.

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