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Protective effects of alginate-free residue of sea tangle against hyperlipidemic and oxidant activities in rats

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Abstract: The antihyperlipidemic and antioxidant activities of dietary supplementation of sea tangle from Goseong and the alginate-free residue of sea tangle were investigated in Sprague Dawley rats treated with a high-fat diet, streptozotocin, poloxamer 407, and bromobenzene. The alginate-free residue of Goseong sea tangle induced a significant reduction in triglycerides and total cholesterol levels, as well as a significant increase in high-density lipoprotein cholesterol levels. Alginate-free Goseong sea tangle residue reduced the activities of the phase I enzymes aminopyrine *N*-demethylase and aniline hydroxylase, which had been increased by intraperitoneal injection of bromobenzene. Pretreatment with Goseong sea tangle residue prevented a bromobenzene-induced decrease in epoxide hydrolase activity. Bromobenzene reduced hepatic glutathione content and increased hepatic lipid peroxide levels. Pretreatment with alginate-free Goseong sea tangle residue prevented lipid peroxidation induced by bromobenzene, but pretreatment with Goseong sea tangle did not. These results suggest that Goseong sea tangle residue exerted antihyperlipidemic and antioxidant activities that were higher than those induced by alginate-containing sea tangle. Therefore, the alginate-free residue may contain physiologically unknown active components, other than alginic acid, which may potentially be used to prevent hyperlipidemic atherosclerosis.

Keywords: *Saccharina japonica*, Sea tangle, Hyperlipidemia, Antioxidant activity

Background

Hyperlipidemia is considered a major risk factor for cardiovascular diseases and events such as atherosclerosis and myocardial infarction (Wald and Law 1995; Talbert 1997). Rates of hyperlipidemia-related diseases are increasing with lifestyle changes. Low-density lipoprotein cholesterol (LDL-C) is regarded as the primary risk factor for atherosclerosis and coronary heart disease (Baigent et al. 2010), and elevated circulating levels of free fatty acids and triglycerides (TG) can lead to these diseases (Pilz et al. 2006; Harchaoui et al. 2009). Therefore, modulating the dysregulation of lipid metabolism and decreasing the levels of serum total cholesterol (TC), TG, and LDL-C are considered beneficial in treating and preventing cardiovascular diseases (Derosa et al. 2006; Zhang et al. 2013). Identifying effective food sources to treat hyperlipidemia would promote this objective (Murata et al. 1999).

The brown alga sea tangle (*Saccharina japonica*) has been used in Korea to promote maternal health (Jin et al. 2004). Sea tangle is also popular in Korea and Japan as a food and has been reported to exhibit hypotensive, antioxidant, antimutagenic, and antibacterial activities (Okai et al. 1993; Han et al. 2002; Wang et al. 2006; Park et al. 2009). Moreover, aqueous extracts of sea tangle and alginate have also been shown to exhibit antioxidant activity and lower hypercholesterolemia (Torsdottir et al. 1991; Lee et al. 2004). However, the alginate-free residue of sea tangle has not been investigated for its biological activities.

In the present study, we evaluated the biological activities of sea tangle residue from which alginate had been removed. The antihyperlipidemic effects of sea tangle residue were assessed in three different experimental rat models, one in which hyperlipidemia was induced by a high-fat diet and two in which hyperlipidemia was induced by streptozotocin and poloxamer 407. In addition, the effects of sea tangle residue on lipid peroxidation and the activities of

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enzymes involved in drug metabolism were examined in the livers of bromobenzene-treated rats.

Methods

Materials

Goseong sea tangle (Goseong, Gangwon-do, South Korea; *S. japonica*) was obtained from a local supplier (Gangneung, Gangwon-do, South Korea) in March 2007. Alginate-free residue from Goseong sea tangle was also used in this study. All samples were powdered after freeze-drying.

Animals and treatments

Male Sprague Dawley rats (Daehan Biolink, Eumsung, South Korea) weighing 190–210 g were housed individually in stainless steel mesh cages in a room maintained at 22 ± 1 °C and 55 ± 3% relative humidity with a normal 12-h light/dark cycle. Rats were fed a commercial standard rat diet (AIN-76). The composition of the experimental diets is shown in Table 1. The high-fat diet-treated rats were orally administered for the last week with a high-fat diet that fed daily for 6 week. Rats were orally administered 100 or 200 mg/kg of body weight of the sea tangle powder in 5% Tween 80 daily for 1 week. During the final 2 days of the oral treatment, rats were injected intraperitoneally (i.p.) with streptozotocin (45 mg/kg in 0.1 M citrate buffer, pH 4.5), poloxamer 407 (300 mg/kg in saline), or bromobenzene (460 mg/kg in 5% Tween 80) four times at 12-h intervals.

All animal experiments procedures were approved by the Committee for Animal Experiments of Kyungsung University.

Sample preparation

At the end of the experimental period and again after 12 h of fasting, the rats were sacrificed by exsanguination under anesthesia with CO_2 and starved for 18 h before sacrifice. Blood was collected from the neck and incubated at room temperature for 30 min. The blood samples were then centrifuged at 3000×*g* at 4 °C for 10 min, after which the serum was stored at − 70 °C for further biochemical tests.

Liver tissue fat was extracted from the cystic lobe according to the method of Folch et al. (1957). The liver, which had been exhaustively perfused with ice-cold 0.9% NaCl, was homogenized with four volumes of an ice-cold 0.1 M potassium phosphate buffer, pH 7.5. An aliquot of the homogenate was used for the determination of lipid peroxide and glutathione (GSH) contents. The remaining homogenate was centrifuged at 600×*g* for 10 min, and the resulting supernatant was recentrifuged at 10,000×*g* for 20 min. The supernatant was centrifuged again at 10,000×*g* for 60 min to obtain the upper fraction as cytoplasm. The pellet was resuspended in the same volume of 0.1 M potassium phosphate buffer and centrifuged at 10,000×*g* for 60 min to obtain the microsomal fraction, which was used to measure the activities of aminopyrine *N*-demethylase (AMND), aniline hydroxylase (ANH), and epoxide hydrolase (EPH).

Table 1 Composition of the experimental diets (g/100 g)

Ingredients	Normal diet	High-fat diet
Casein	20	29
Corn starch	60	10
Sucrose	0	10
Lard	0	35
Corn oil	9	5
α-Cellulose	5.0	5.0
Mineral mixture[a]	3.5	3.5
Vitamin mixture[b]	1.0	1.0
Cholesterol	1.0	1.0
DL-Methionine	0.3	0.3
Choline bitartrate	0.2	0.2

[a]Mineral mixture derived from the rodent diet formula AIN-76
[b]Vitamin mixture derived from the rodent diet formula AIN-76

Glucose analysis

Levels of plasma glucose were determined by the glucose oxidase method using a commercially available enzymatic kit (Embiel Co., Gyeonggi-do, South Korea).

Cholesterol analysis

TG, TC, and high-density lipoprotein cholesterol (HDL-C) levels were determined by enzymatic colorimetric methods using commercial kits (Shinyang Chemical Co., Busan, South Korea).

Lipid peroxide and GSH levels

Hepatic lipid peroxidation was assessed by measuring the concentration of thiobarbituric acid-reactive substances (TBARS) in plasma using the method described by Ohkawa et al. (1979). Hepatic GSH contents were measured by a colorimetric method (Boyne and Ellma 1972).

Enzyme assays

AMND activity in liver microsomes was measured spectrophotometrically by the quantitation of formaldehyde produced from the demethylation of aminopyrine, as described by Nash (1953). ANH activity was assayed by measuring *p*-aminophenol formation from aniline (Bidlack and Lowery 1982). EPH activity was measured spectrophotometrically using the decrease in *trans*-stilbene oxide at 229 nm (Hasegawa and Hammock 1982). Protein contents of the microsome and cytoplasm were determined by the method of Lowry et al. (1951) using bovine serum albumin as a standard.

Statistical analysis

All results are presented as the mean ± SD. Data were evaluated by one-way ANOVA using SPSS (IBM SPSS, Armonk, NY, USA), after which the differences between the mean values were assessed using Duncan's multiple range test. Results were considered statistically significant at $p < 0.05$.

Results

Effects of sea tangle on serum and liver tissue lipid levels in high-fat diet-fed rats

The effects of sea tangle supplementation on serum lipid levels in rats fed a high-fat diet are shown in Table 2. Serum lipid levels were significantly reduced in rats treated with alginate-free Goseong sea tangle residue at doses of 100 and 200 mg/kg, compared with lipid levels in the hyperlipidemia control group. However, administration of Goseong sea tangle did not significantly affect serum lipid levels in rats with hyperlipidemia induced by a high-fat diet.

The effects of dietary supplementation of sea tangle on hepatic lipid levels of rats fed a high-fat diet are shown in Table 3. The rats displayed significantly higher TG and TC levels compared with rats fed a normal diet. Hepatic lipid levels in the alginate-free Goseong sea tangle residue groups were significantly lower than levels in the hyperlipidemia control group.

Effects of sea tangle on blood glucose and lipid levels following streptozotocin administration

Table 4 shows the effects of sea tangle administration on blood glucose and lipid levels in streptozotocin-induced hyperglycemic rats. The group displayed remarkably high serum levels of glucose, TG, and TC compared with normal-diet control rats. Oral administration of all sea tangle types at doses of 200 mg/kg resulted in a significant reduction in TG and TC levels, especially the administration of Goseong sea tangle, compared with the streptozotocin-induced hyperlipidemic control group.

Table 2 Effects of sea tangle on serum lipid levels in high-fat diet-induced rats

	Dose (mg/kg)	TG (mg/dL)	TC (mg/dL)	HDL-C (mg/dL)
Control		269.0 ± 68.3	93.6 ± 8.53	50.7 ± 2.16
Goseong sea tangle	100	253.8 ± 49.7	100.7 ± 9.09	49.7 ± 1.83
	200	246.0 ± 39.8	98.4 ± 7.33	47.6 ± 1.54
Alginate-free sea tangle residue	100	248.6 ± 18.9	86.3 ± 4.96	55.6 ± 3.11
	200	173.6 ± 24.5	70.8 ± 5.41	58.2 ± 1.37

Values are expressed as mean ± SD ($n = 8$) for groups of six replicates.
Different superscripts in each data column indicate significant differences ($p < 0.05$) between groups
TG triglycerides, *TC* total cholesterol, *HDL-C* high-density lipoprotein cholesterol

Table 3 Effects of sea tangle on hepatic tissue lipid levels in high-fat diet-induced rats

	Dose (mg/kg)	TG (mg/g)	TC (mg/g)
Normal diet		24.5 ± 3.38	4.17 ± 0.23
Control		50.8 ± 5.17	32.9 ± 2.48
Goseong sea tangle	100	48.7 ± 3.21	28.1 ± 2.46
	200	46.3 ± 2.55	27.9 ± 2.02
Alginate-free sea tangle residue	100	41.7 ± 3.48	25.9 ± 2.06
	200	35.2 ± 4.22	22.3 ± 1.57

Values are expressed as mean ± SD ($n = 8$) for groups of six replicates.
Different superscripts in each data column indicate significant differences ($p < 0.05$) between groups
TG triglycerides, *TC* total cholesterol

However, blood glucose levels were not elevated by sea tangle treatment in any group.

Effects of sea tangle on serum lipid levels following poloxamer 407 administration

Table 5 shows the effects of sea tangle administration on serum lipid levels in poloxamer 407-induced hyperlipidemic rats. The group displayed significantly high TG and TC serum levels compared with normal-diet control rats. Administration of the alginate-free Goseong sea tangle residue at both 100 and 200 mg/kg doses resulted in a significant, dose-dependent reduction in TG and TC levels, compared with the poloxamer 407-induced hyperlipidemic control group.

Effects of sea tangle on hepatic enzyme activity and lipid peroxidation following bromobenzene administration

Hepatic AMND and ANH activities of bromobenzene-injected rats that had been pretreated with dietary supplementation of sea tangle are shown in Table 6. In comparison with normal-diet control rats, the rats injected with bromobenzene exhibited higher AMND and ANH activities. The increase in AMND activity by bromobenzene was reduced by 8.1 and 12.9% with oral administration of the alginate-free Goseong sea tangle

Table 4 Effects of sea tangle on blood glucose and lipid levels in streptozotocin-induced hyperglycemic rats

	Dose (mg/kg)	Glucose (mg/dL)	TG (mg/dL)	TC (mg/dL)
Normal		92.6 ± 8.97	76.3 ± 7.28	65.3 ± 6.27
Streptozotocin		330.2 ± 23.8	180.7 ± 26.3	147.4 ± 5.19
Goseong sea tangle	100	339.5 ± 33.4	198.3 ± 39.3	138.6 ± 6.34
	200	330.7 ± 24.6	195.3 ± 26.2	120.5 ± 5.44
Alginate-free sea tangle residue	100	295.8 ± 29.6	170.0 ± 23.5	110.5 ± 7.69
	200	287.2 ± 36.5	153.6 ± 15.4	100.8 ± 4.24

Values are expressed as mean ± SD ($n = 8$) for groups of six replicates.
Different superscripts in each data column indicate significant differences ($p < 0.05$) between groups
TG triglycerides, *TC* total cholesterol

Table 5 Effects of sea tangle on serum lipid levels of poloxamer 407-induced hyperlipidemic rats

	Dose (mg/kg)	TG (mg/dL)	TC (mg/dL)
Normal diet		78.4 ± 7.60	69.4 ± 7.23
Poloxamer 407		1004.1 ± 58.9	780.8 ± 52.7
Goseong sea tangle	100	980.7 ± 46.5	770.3 ± 40.2
	200	916.6 ± 49.2	750.2 ± 51.3
Alginate-free sea tangle residue	100	900.3 ± 33.5	690.4 ± 49.2
	200	719.8 ± 40.0	510.3 ± 29.8

Values are expressed as mean ± SD (n = 8) for groups of six replicates.
Different superscripts in each data column indicate significant differences ($p < 0.05$) between groups
TG triglycerides, *TC* total cholesterol

residue at doses of 100 and 200 mg/kg, respectively. The increase in ANH activity by bromobenzene was reduced by 13.1% with oral administration of the alginate-free Goseong sea tangle residue at a dose of 200 mg/kg. However, no such reduction in AMND and ANH activities was observed following oral treatment with other sea tangle preparations.

Hepatic EPH activity in bromobenzene-treated rats was lower than that in normal-diet control rats (Table 7). Pretreatment with alginate-free Goseong sea tangle residue at doses of 100 and 200 mg/kg elevated enzyme activity by 31.5 and 42.6%, respectively.

Hepatic GSH and lipid peroxide contents in bromobenzene-injected rats pretreated with sea tangle are shown in Table 8. Hepatic GSH contents were significantly lower in bromobenzene-injected rats than in normal-diet control rats. No sea tangle type or dose affected GSH contents in bromobenzene-injected rats. Bromobenzene administration resulted in elevation of lipid peroxide contents to 50.0 nmol of TBARS/g from the normal value of 17.8 nmol/g. However, the increase in TBARS content by bromobenzene injection was

Table 6 Hepatic enzyme activities in bromobenzene-injected rats treated with sea tangle

	Dose (mg/kg)	Aminopyrine *N*-demethylase	Aniline hydroxylase
		Formaldehyde (nmol/mg protein/min)	*p*-Aminophenol (nmol/mg protein/min)
Normal diet		4.14 ± 0.07	0.71 ± 0.10
Bromobenzene		9.33 ± 0.52	1.34 ± 0.28
Goseong sea tangle	100	8.95 ± 0.44	1.29 ± 0.15
	200	9.28 ± 0.39	1.31 ± 0.20
Alginate-free sea tangle residue	100	8.57 ± 0.66	1.20 ± 0.14
	200	8.13 ± 0.50	1.03 ± 0.09

Values are expressed as mean ± SD (n = 8) for groups of six replicates.
Different superscripts in each data column indicate significant differences ($p < 0.05$) between groups

Table 7 Epoxide hydrolase activity of bromobenzene-injected rats pretreated with sea tangle

	Dose (mg/kg)	Epoxide hydrolase
		nmol *trans*-stilbene oxide/mg protein/min
Normal diet		13.6 ± 0.52
Bromobenzene		4.25 ± 0.17
Goseong sea tangle	100	5.10 ± 0.57
	200	4.91 ± 0.45
Alginate-free sea tangle residue	100	6.20 ± 0.30
	200	7.40 ± 0.23

Values are expressed as mean ± SD (n = 8) for groups of six replicates.
Different superscripts in each data column indicate significant differences ($p < 0.05$) between groups

inhibited in rats pretreated with alginate-free Goseong sea tangle residue, at doses of both 100 and 200 mg/kg.

Discussion

Hyperlipidemia is a major risk factor in the development of coronary artery disease and the progression of atherosclerotic lesions (McKenney 2001). Developing novel and effective antihyperlipidemia agents warrants increased attention (Sliskovic and White 1991). We investigated the effects of dietary supplementation of alginate-free extracts of sea tangle in rats with hyperlipidemia induced by streptozotocin, poloxamer 407, bromobenzene, or a high-fat diet.

Sea tangle contains alginic acid, carotenoids, xanthophylls, mannitol, and physiologically unknown active components. Aqueous extract of sea tangle has been shown to suppress hyperglycemia and oxidative stress in diabetic rats (Lee et al. 2004). However, the study suggested that dietary supplementation with sea tangle or sodium alginate did not affect plasma glucose and lipid peroxide levels.

When diabetes develops, lipid metabolism is abnormally affected and lipid peroxide and blood lipid levels increase. We found that the alginate-free residue of

Table 8 Hepatic glutathione and lipid peroxide contents of bromobenzene-injected rats treated with sea tangle

Group	Dose (mg/kg)	Glutathione	Lipid peroxide
		GSH (mg/g)	TBARS (nmol/g)
Normal		6.41 ± 0.30	17.8 ± 2.10
Bromobenzene		4.83 ± 0.39	50.0 ± 4.26
Goseong sea tangle	100	5.06 ± 0.19	47.6 ± 3.49
	200	5.18 ± 0.22	45.9 ± 4.66
Alginate-free sea tangle residue	100	5.18 ± 0.27	35.2 ± 1.69
	200	5.21 ± 0.33	31.1 ± 2.47

Values are expressed as mean ± SD (n = 8) for groups of six replicates.
Different superscripts in each data column indicate significant differences ($p < 0.05$) between groups
GSH reduced glutathione, *TBARS* thiobarbituric acid-reactive substances

Goseong sea tangle reduced serum, blood, and hepatic lipid levels in hyperlipidemic rats, although Goseong sea tangle did not. This indicates that alginate-free Goseong sea tangle residue could be used to prevent and treat complications from diabetes, in addition to its blood glucose-lowering effect. Thus, we hypothesize that the alginate-free residue of Goseong sea tangle contains components which may exert a protective effect against diabetes.

The present study also evaluated the effects of alginate-free sea tangle residue on several xenobiotic-metabolizing hepatic enzymes in rats injected with bromobenzene. Bromobenzene is a toxic industrial solvent which elicits toxicity predominantly in the liver, where it causes centrilobular necrosis (Park et al. 2005). Although formation of secondary quinone metabolites (Slaughter and Hanzlik 1991; Buben et al. 1988; Narasimhan et al. 1988) and hydrogen peroxide (Wu et al. 1994) has been proposed as a mechanism of action in the toxicity of bromobenzene, much of the chemical's toxicity is known to be associated with cytochrome P450-mediated phase I metabolism to reactive epoxide intermediates (Rogers et al. 2002).

Our results demonstrate that i.p. injection of bromobenzene modulates phase I enzyme activities in the rat liver. The activities of the cytochrome P450-dependent monooxygenases AMND and ANH increased significantly following bromobenzene injection. This increase was suppressed by treatment with Goseong sea tangle residue.

The toxic epoxide intermediate of bromobenzene, produced upon oxidation by cytochrome P450-dependent phase I enzymes, can be detoxified by several pathways, including hydration to 3,4-dihydrodiol catalyzed by EPH (Cohen et al. 1997; Pumford and Halmes 1997). Hepatic EPH activity decreased significantly following bromobenzene injection, but this decrease was inhibited by pretreatment with the Goseong sea tangle residue.

The present study shows that administration of bromobenzene induces oxidative modifications to mitochondrial proteins. Therefore, it is probable that bromobenzene-induced elevations in reactive oxygen species, lipid peroxides, and protein carbonyls may affect the integrity of the mitochondrial membrane, which would lead to mitochondrial dysfunction and, ultimately, to some of the toxic effects observed in this study. However, the alginate-free residue of Goseong sea tangle protected mitochondria against this oxidative damage.

GSH is an important cellular reductant and constitutes the first line of defense against free radicals, peroxides, toxic compounds, and chemically induced hepatotoxicity (Raja et al. 2007). A significant decrease in the level of GSH observed in the rats treated with bromobenzene may be attributable to its increased utilization, which results in increased vulnerability to free radical damage (Gopi and Setty 2010). However, administration of alginate-free Goseong sea tangle residue increased GSH levels significantly. This may be due to the increase in de novo synthesis and/or GSH regeneration. The alginate-free residue of Goseong sea tangle increased the activity of antioxidant enzymes, counteracting oxidative stress.

Lipid peroxide levels are an index of membrane damage, and increased levels can lead to alterations in membrane structure and function. In this study, elevation in lipid peroxide levels was observed following the administration of bromobenzene and is attributed to the enhanced production of reactive oxygen species (Gopi and Setty 2010). However, administration of Goseong sea tangle residue prevented these changes. The antioxidant effect of the alginate-free residue of Goseong sea tangle may not be due to the GSH-dependent removal of hydroperoxide (Park et al. 2005).

Conclusions

We showed that the alginate-free residue of Goseong sea tangle reduced the perturbation of serum and hepatic lipid levels in hyperlipidemic rats. We also showed the residue's effects on several xenobiotic-metabolizing hepatic enzymes in rats injected with bromobenzene. Altogether, our data suggest that the alginate-free residue of sea tangle contains physiologically unknown compounds which may protect against hyperlipidemic atherosclerosis.

Abbreviations

AMND: Aminopyrine *N*-demethylase; ANH: Aniline hydroxylase; EPH: Epoxide hydrolase; GSH: Glutathione; HDL-C: High-density lipoprotein cholesterol; TBARS: Thiobarbituric acid-reactive substances; TC: Total cholesterol; TG: Triglycerides; TSO: *Trans*-stilbene oxide

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Availability of data and materials

The datasets supporting the conclusions of this article are included within the article. There are no additional data and materials to disclose.

Authors' contributions

MJY carried out the hepatic enzyme analysis and drafted the manuscript. GC carried out the glucose and cholesterol analyses. JML extracted the hepatic fat and prepared the enzyme sources. SYC participated in the design of the study and helped to draft the manuscript. DSL designed the study and completed the manuscript. All authors read and approved the final manuscript.

Ethics approval

Animal experiments were performed according to the institutional guidelines for the care and the use of laboratory animals, and the protocol was approved by the ethics committee of Kyungsung University.

Consent for publication

The manuscript has been read and approved by the authors, and none of its parts have been submitted and published elsewhere. The authors also declare that no one who qualifies for authorship has been excluded from the list of authors.

Competing interests
The authors declare that they have no competing interests.

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