Chapter 3

Therapeutic Potential of Seaweed Polysaccharides for Diabetes Mellitus

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Additional information is available at the end of the chapter

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Abstract

Seaweed has attracted a great deal of interest as excellent sources of nutrients. Seaweeds contain polysaccharides, proteins, amino acids, lipids, peptides, minerals, and some vitamins. Polyphenols of seaweed was used as cosmetics and pharmacological as antioxidants, protection from radiation, anti-inflammatory, hypoallergenic, antibacterial, and antidiabetic. Besides that seaweed also has a high content of antioxidant that can be used to ward off free radicals that increase due to the condition of hyperglycemia in a patient with diabetes mellitus. Hence, a great deal of attention has been directed at isolation and characterization of seaweed polysaccharides because of their numerous health benefits, especially for diabetes mellitus. This paper is expected to provide information on the effect of alginate from two seaweeds on blood glucose and lipid profiles of diabetic rats.

Keywords: *Sargassum crassifolium*, *Turbinaria ornata*, diabetes mellitus, seaweed, alginate

1. Introduction

Diabetes mellitus (DM) is a disease caused by hyperglycemia due to a relative or absolute insulin insufficiency. Chronic hyperglycemia can cause complications such as neuropathy, retinopathy, nephropathy, and cardiovascular disease [1]. Hyperglycemia can also cause impaired balance metabolism of carbohydrates, fats, and proteins [2]. International Diabetes Federation (IDF) estimates that in 2013 there were 382×10^6 people with diabetes and 316×10^6 people suffer from impaired glucose tolerance and increased risk of diabetes. These results are expected to increase to 471×10^6 in 2035 and predicted less than 25 years; there would be 592×10^6 people have diabetes without quick and precise prevention [3].

Seaweeds are the most abundant resources in the ocean. Seaweeds contain polysaccharides, proteins, amino acids, lipids, peptides, minerals, and some vitamins. Polyphenols of seaweed was used as cosmetics and pharmacological as antioxidants, protection from radiation, antibiotics, anti-inflammatory, hypoallergenic, antibacterial, and antidiabetic [4]. Polyphenol extracts from seaweed, for example, *Alaria*, *Ascophyllum*, *Padina*, and *Palmaria*, are able to inhibit the activity of α-amylase and α-glucosidase that can lower blood glucose levels [5, 6]. On the other hand, seaweed also has a high content of antioxidants that can have beneficial value for diabetes mellitus patient [7]. Research on the use of Na alginate from *Turbinaria ornata* and *Sargassum crassifolium* on in vivo studies in diabetic rats was limited. This paper is expected to provide information about the effect of Na alginate from *T. ornata* and *S. crassifolium* on the blood glucose and lipid profiles of drug-induced diabetic rats.

2. Extraction of polysaccharides from marine algae

Na alginate from *T. ornata* and *S. crassifolium* was extracted as explained by Husni et al. [8, 9]. Dried samples were weighted and were soaked in distilled water with the addition of 0.1 N HCl to pH 4 for about 24 h 1:15 (w/v). The seaweed was washed with distilled water until pH 7. The filtrate was added with 0.5 N Na_2CO_3 (pH 11) 1:10 (w/v) and then heated at 60°C for 2 h. The viscous mixture was added with distilled water 1:10 (w/v) and separated from its residue by centrifuge (3500 rpm, 5 min, 4°C) (1 rpm = 1/60 Hz). The Na alginate extract was added with 5 N H_2O_2 1:4 (v/v), stirred for 30 min before left for two h. The mixture was added with 0.5 M $CaCl_2$ and stirred for 30 min followed by adding 0.5 N HCl until pH 2. The mixture was stirred and left for 30 min at room temperature. Insoluble material (alginic acid) was separated from the supernatant by centrifuge. Alginic acid was weighed, was added with distilled water and 0.5 N Na_2CO_3 2:2:3 (w/v/v), and was stirred for one h at room temperature to obtain a solid form of Na alginate. Na alginate was precipitated with EtOH slowly 1:1 (v/v) and stirred for 30 min, after being centrifuged, followed by drying at 60°C, and the yield of alginate was determined.

3. Alginate characterizations (structural and physical properties)

FTIR spectroscopy was used to identify the polysaccharide structures. A pellet of sodium alginate was prepared with KBr. FTIR spectrum was recorded on Shimadzu-FTIR Prestige 21 with a resolution of 4 cm^{-1} in the 4000–400 cm^{-1} region, with a scan speed of 0.20 cm s^{-1}. The FTIR spectrum of sodium Na alginate of *T. ornata* showed similar bands to that Na alginate standard in 3500–1300 cm^{-1} region, while the fingerprint region has two bands at 948.98 cm^{-1} and 871.82 cm^{-1} (**Figure 1**). Sodium alginate of *T. ornata* showed eight characteristic bands which also could be found in sodium alginate standard (**Table 1**). According to literature, the band at 3400 cm^{-1} assigned to the hydrogen bonded O-H stretching vibrations and the weak signal at 2931.80 cm^{-1} due to C-H stretching vibrations [10] and the asymmetric stretching of carboxylate O-C-O vibration at 1627.92 cm^{-1} [10, 11]. The band at

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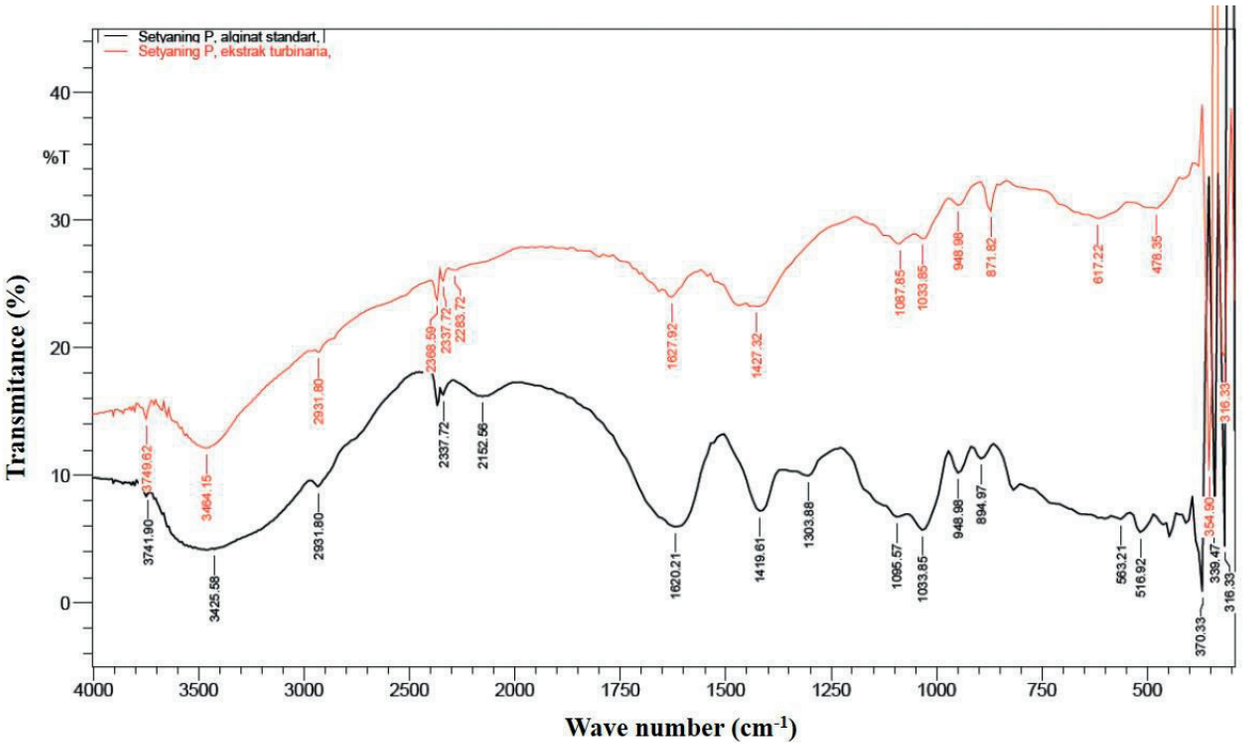

Figure 1. Infrared spectra of Na alginate standard (red) and Na alginate of *T. ornata* (black).

Wave number (cm^{-1})		Assignments
Na alginate standard [8]	**Na alginate of *T. ornata* [8]**	
3425.58	3464.15	νO–H a
2931.80	2931.80	νC–H a
1620.21	1627.92	ν asym COO- b
1419.61	1427.32	δ C–O–H, νsym COO– (carboxylate ion)[1,3]
1095.57	1087.85	ν C-O, ν C-C (pyranose ring)[1,2,3]
1033.85	1033.85	ν C-O[1]
948.98	948.98	ν C-O (uronic acid residues)[1,3]
894.97	871.82	δ C1-H (β-mannuronic residues)[1,3]

[1]Source: [10].
[2][11]
[3][12]

Table 1. FTIR spectrum of Na alginate from *T. ornata* and standard.

1427.32 cm^{-1} is assigned to C-O-H deformation vibration with the contribution of O-C-O symmetric stretching vibration of carboxylate group [10, 12]. The band 1087.85 cm^{-1} might be assigned to C-O and C-C stretching vibrations of pyranose ring [10–12], and the band at 1033.85 cm^{-1} might also be due to C-O stretching vibrations [10]. The anomeric region

of the fingerprint (950–750 cm^{-1}) showed two characteristic absorption bands. The band at 948.98 cm^{-1} was assigned to the C-O stretching vibration of uronic acid residues, and the one at 871.82 cm^{-1} was assigned to the C1-H deformation vibration of β-mannuronic acid residues [10, 12].

The peak infrared spectrum of standard alginate and *S. crassifolium* can be seen in **Table 2**. Based on the FTIR test conducted on alginate extract of *S. crassifolium* and alginate standard (**Figure 2**) on the first band of alginate extract spectra, the vibration frequency of 779.24 cm^{-1} shows the residue of guluronic acid. The second band, standard alginate spectra and alginate extracts, contained the same vibration frequency at 948.98 cm^{-1} showing the suspected vibration of C-O stretching as uronic acid. The third band detected vibrations from C-O stretching, wavelengths 1033.85 cm^{-1} at standard alginate, and 1026.13 cm^{-1} on alginate extract. The fourth band in the standard alginate detected vibrations at wavelengths of 1095.57 and 1087.85 cm^{-1} in the extra alginate indicating the presence of OCO rings. Symmetrical and asymmetrical C-O vibrations were detected in the standard alginate of the fifth and sixth bands indicating the presence of carboxylic groups at 1303.88 and 1419.61 cm^{-1} wavelengths, but this vibration was not detected in the alginate extract. The seventh band contained a vibration of 2931.8 cm^{-1} in standard alginates and alginate extracts indicating the presence of C-H stretching. O-H is stretching vibration indicating the presence of H atomic bonds detected in both alginates, 3425.58 cm^{-1} in standard alginate and 3471.87 cm^{-1} in alginate extract.

Wavelength (cm^{-1})			Type of vibration
Na alginate standard [9]	**Na alginate of *S. crassifolium* [9]**	**Reference**	
—	779.24	778.20[1]	Guluronic acid residues
948.98	948.98	950–810[2]	C-O stretching
1033.85	1026.13	1023.40[1]	C-O stretching
1095.57	1087.85	1100–1050[3]	OCO ring (shoulder)
1303.88	—	1320–1210[2]	C-O stretching
1419.61	—	1460–1400[2]	C-O asymmetric stretching
2931.80	2931.80	~2925[2]	C-H stretching
3425.58	3471.87	3600–3200[3]	O-H stretching

[1]Source: [13].
[2][14].
[3][15].

Table 2. FTIR spectrum of Na alginate from *S. crassifolium* and standard.

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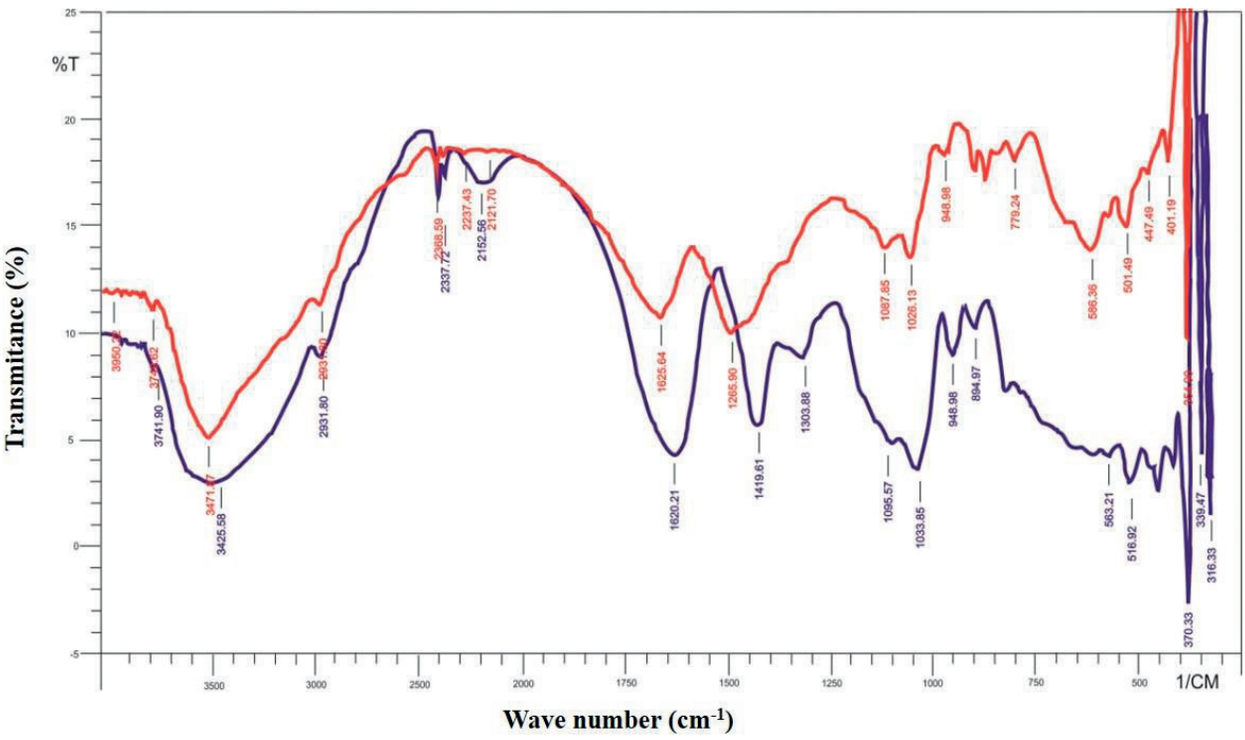

Figure 2. Infrared spectra of Na alginate standard (red) and Na alginate of *S. crassifolium* (black).

4. Biological activity of polysaccharides from marine algae

4.1. Effect of Na alginate of *T. ornata* on body weight of rats

Alloxan-induced diabetic rats did not show a significant decrease in body weight after the injection of alloxan. Five groups of diabetic rats had decreased in body weight on 15 days treatment, and there were significant differences between the groups of rats. There was no significant difference between diabetic control (negative control) compared to positive control, and the positive control was not significantly different compared to alloxan diabetic rats treated with Na alginate 200 mg/kg. Alloxan-induced diabetic rats treated with Na alginate(s) (200, 400, 600 mg/kg) did not show significant difference between each other. Administration of Na alginate(s) (400, 600 mg/kg) showed a significant difference compared to negative control. The body weight of alloxan-induced diabetic rats treated with Na alginate 600 mg/kg was not significantly different compared to normal control.

The lowering of rats' body weight treated with alginate from *T. ornata* showed lower than a study conducted by Wikanta et al. [16] using κ-carrageenan and ί-carrageenan. In those researches, κ-carrageenan increased the weight by 34.1 g, and ί-carrageenan increased the weight by 30.1 g from the body weight on alloxan-induced diabetic rats after 15 days of treatment. The significant reduction in total body weight could be attributed to the loss of fat from adipose tissue and catabolism of amino acids in the muscle tissue [17].

4.2. Effect of Na alginate of *S. crassifolium* on body weight of rats

Diabetic mice showed weight loss in all treatment groups except the normal control group. Normal control group gained weight of 24.1 g. The negative control group had a very significant weight loss of 51.6 g. The positive control group had a weight loss of 47.2 g. The treatment group of extract 200 mg/kg had a weight loss of 58.8 g. The treatment group of 400 mg/kg extract had a weight loss of 45.3 g. Meanwhile, the treatment group giving 600 mg/kg extract experienced a decrease in body weight by 43.1 g. Streptozotocin (STZ)-induced diabetic rats are one of the animal models of type 1 diabetes mellitus. It is well known for its selective pancreatic islet beta-cell cytotoxicity and has been extensively used to induce type 1 diabetes in an experimental rat model. Glibenclamide is often used as a standard antidiabetic drug in STZ-induced diabetes to compare the efficacy of a variety of hypoglycemic drugs [18].

Throughout the experiments, all the rats were monitored daily and/or weekly for the symptoms of type 1 diabetes mellitus, including polydipsia, polyuria, polyphagia, hyperglycemia, and muscle wasting leading to weight loss and insulin deficiency. **Figure 1** shows the observations of body weight of treated rats during the whole period of experiments. The body weight was continuously increased in the normal group and decreased in all diabetes groups. A severe loss of body weight characterizes STZ-induced diabetes. Due to absolute or relative deficiency of insulin and decrease of the production of ATP, protein synthesis decreases in all tissues.

5. Effect of Na alginate on blood glucose

Alloxan is a urea derivative which causes selective necrosis of the pancreatic islet β-cells [19]. Alloxan and its reduction product dialuric acid establish a redox cycle with the formation of superoxide radicals [20]. Preprandial blood glucose levels were determined as fasting blood glucose. Fasting is defined as no calorie intake for at least 8 h [1]. Diabetes is diagnosed when the fasting plasma glucose concentration is consistently ≥7 mmol/L (126 mg/dL) or when the 2 h plasma glucose concentration (after drinking a 75 g glucose load) is consistently ≥11.1 mmol/L (200 mg/dL) [21].

Administration of alloxan led to a significant increase of preprandial blood glucose levels in rats after 3 days. Administration of Na alginate(s) (200, 400, 400 mg/kg) significantly reduced the blood glucose level compared to diabetic control. The dose of 200 and 400 mg/kg of Na alginate did not show a significant difference compared to normal control and positive control (**Table 3**). The result was supported by previous studies using fiber to decrease preprandial blood glucose. Nelson et al. [22] used high indigestible fiber and low indigestible fiber diet to decrease preprandial blood glucose in diabetic dogs for 8 months which resulted in high indigestible fiber significantly that reduces preprandial blood glucose better than low indigestible fiber. Nelson et al. [23] used similar treatment in diabetic cats for 24 weeks and showed high indigestible fiber which gave a better effect on decreasing preprandial blood glucose than low indigestible fiber. Chandalia et al. [12] compared the amount of fiber that was given to diabetic patients according to the American Diet

Group	Preprandial blood glucose (mg/dL)*	
	T. ornata [8]	*S. crassifolium* [9]
Normal control	106.06 ± 11.33^{b,c}	126.30 ± 0.50^{a}
Negative control	208.57 ± 70.60^{a}	568.82 ± 46.40^{c}
Positive control	86.29 ± 13.83^{b}	316.35 ± 20.90^{b}
Alginate (200 mg/kg)	108.50 ± 11.28^{c}	279.45 ± 92.50^{b}
Alginate (400 mg/kg)	96.55 ± 15.65^{b,c}	336.63 ± 66.32^{b}
Alginate (600 mg/kg)	99.03 ± 14.26^{b,c}	257.66 ± 34.61^{b}

*Values are means ± SD. Values followed by the different superscript symbol(s) in each column were significantly different (P<0.05).

Table 3. Effect of Na alginate of *T. ornata* and *S. crassifolium* on preprandial blood glucose in alloxan-induced diabetic rats.

Association (8 g digestible fiber and 16 indigestible fiber) and fiber-rich diet (25 g digestible fiber and 25 indigestible fiber) for 6 weeks. Fiber-rich diet decreased 13% preprandial blood glucose lower than ADA diet.

Normal postprandial blood glucose level is <180 mg/dL [1]. In the normal state, the postprandial blood glucose level increases less than 50 mg/dL from the preprandial blood glucose level after carbohydrate intake [24]. Alloxan-induced diabetic rats' postprandial blood glucose level surpassed 200 mg/dL after 3 days of injection. After 15 days of treatment, the result was the administration of Na alginate(s) (200, 400, 600 mg/kg) which significantly reduces postprandial blood glucose levels on rats compared to diabetic control ($P < 0.05$). However, it failed to restore the level to that of normal control group and positive control group ($P < 0.05$). The positive control group could restore the postprandial blood glucose level at the same level as a normal control group (**Table 4**).

Wolf et al. [25] used 1.5 g sodium alginate to show its effect on postprandial glucose peak and glucose uptake reduction after 3 h which resulted in line 32.80 ± 3.40 and 1429 ± 276 mg/dL. Sodium alginate had a reduction effect better than 1.2 g gum arabic and 0.3 g gum guar with postprandial glucose peak 40.40 ± 3.30 mg/dL and glucose uptake 1717 ± 433 mg/dL. A study on the effect of a meal containing alginate compared to testing a meal without alginate by Torsdottir et al. [26] showed that postprandial blood glucose levels by meal containing alginate decrease 31% lower than a meal without alginate.

Preprandial glucose levels for all treatment groups of alginate from *S. crassifolium* were classified into normal levels ranging from 69.311 to 88.029 mg/dL and no significant difference. The streptozotocin-induced treatment group experienced very high preprandial glucose levels exceeding 200 mg/dL and can be categorized as DM. The same is also shown in Moree et al. [27]. In this study, male Wistar rats induced by streptozotocin dose 60 mg/kg increased blood glucose levels <200 mg/dL. The use of 60 mg/kg of streptozotocin in mice can trigger an autoimmune process that can produce damage to the Langerhans island beta cells [28]. Also,

Group	Postprandial blood glucose (mg/dL)*	
	T. ornata [8]	*S. crassifolium* [9]
Normal control	133.05 ± 15.81^{b}	150.416 ± 5.1^{a}
Negative control	360.48 ± 40.80^{a}	633.470 ± 27.8^{c}
Positive control	140.75 ± 9.16^{b}	333.814 ± 64.5^{ab}
Alginate (200 mg/kg)	257.08 ± 34.20^{c}	421.652 ± 21.4^{bc}
Alginate (400 mg/kg)	238.61 ± 21.48^{c}	433.333 ± 21.8^{bc}
Alginate (600 mg/kg)	196.05 ± 18.22^{d}	381.250 ± 11.4^{ab}

*Values are means ± SD. Values followed by the different superscript symbol(s) in each column were significantly different (P<0.05).

Table 4. Effect of Na alginate of *T. ornata* and *S. crassifolium* on postprandial blood glucose in alloxan-induced diabetic rats.

STZ is also capable of generating reactive oxygen that has a high role in the destruction of pancreatic β-cells and eventually occurs inhibition of insulin secretion and synthesis resulting in hyperglycemia [29].

All treatment groups of extracts of *S. crassifolium* did not differ significantly with the positive control (**Table 3**). It can be concluded that all three doses of administration of the extract of *S. crassifolium* had the same effect as the positive control group in lowering blood glucose levels in mice suffering from DM. The opposite is shown in the negative control treatment group that has a prepreg glucose level that increases from day to day due to accumulated glucose buried in the blood without treatment efforts.

In general, the viscosity of dietary fiber can reduce the rise in blood glucose levels and reduce food intake by slowing the empty stomach and slowing the absorption of nutrients in the small intestine. Based on these two mechanisms, it is still not clear what mechanisms apply to sodium alginate, perhaps one or both [30]. Different doses of alginate will affect the viscosity of the given test preparation. So, it will lead to differences in the viscosity of the fluid in the gastrointestinal tract and ultimately result in differences in the rate of glucose absorption from the gastrointestinal tract into the blood vessels [31].

6. Total cholesterol

Diabetes is associated with major abnormalities in fatty acid metabolism. The resulting disturbance results in an abnormal lipoprotein cascade from the large chylomicron through to the small HDL particle [31, 32]. Total cholesterol in the serum of negative control was not significantly different compared to positive control, Na alginate 200 and 400 mg/kg treatment, and normal control. Na alginate 600 mg/kg of *T. ornata* was a significant difference compared to negative control ($P < 0.05$). The alginate dose of 200 and 600 mg/kg of *T. ornata* did not show the difference ($P > 0.05$) (**Table 5**) significantly.

Several previous studies supported the result. Suzuki et al. [33] evaluated the effect of alginate-rich guluronic and mannuronic on cholesterol levels in rats fed with diets containing both alginates and cholesterol which resulted from reductions in liver cholesterol in rats fed with each alginate and significantly low cholesterol accumulation in mannuronic acid-rich alginate. Ren et al. [34] screened 26 species of seaweeds and six polysaccharides from algae to study their effect on lipid in rats fed with basal diet for 28 days of treatment. The six polysaccharides were sulfated glucuronoxylomannan (0.5%), fucoidin (1%), sodium alginate (1%), funorin (2.5%), porphyrin (2.5%), and agar (2.5%). Reduction effect of each polysaccharide was 64, 65, 68, 77, 88, and 95%, respectively, compared to control group. At the end of the study, the polysaccharides could restore the cholesterol level to the same level as the control group.

Total cholesterol levels of the normal control group, positive control, and alginate 600 mg/kg of *S. crassifolium* had a significant difference compared to the negative control group (**Table 5**). The three treatment groups had lower cholesterol levels than the negative control group. An extract at a dose of 600 mg/kg of *S. crassifolium* can lower total cholesterol levels as well as positive controls (glibenclamide). The opposite is shown by the treatment group giving the extract dose of 200 and 400 mg/kg of *S. crassifolium*. Both the doses are less effective in lowering total cholesterol levels in mice suffering from diabetes compared to glibenclamide and alginate 600 mg/kg of *S. crassifolium*.

Wikanta et al. [35] reported that sodium alginate could lower total cholesterol in mice with hypercholesterolemia. Administration of sodium alginate with a viscosity of 450 cps significantly reduced total cholesterol levels compared to sodium alginate with lower viscosity. Because, sodium alginate is a water-soluble fiber compound, forming a viscous solution. The stomach fluid cannot digest this compound in the gastrointestinal tract. When dissolved in water, the sodium alginate fibers form a mesh-like grid that strongly binds many water molecules in a well-defended solute. Its properties as emulgator increasingly enhance the binding ability. A similar mechanism occurs against lipid molecules in bile acids in the gastrointestinal tract. The binding or bonding of lipids by the alginate makes lipid and cholesterol unable

Group	Total cholesterol (mg/dL)*	
	***T. ornata* [8]**	***S. crassifolium* [9]**
Normal control	70.40 ± 7.12^{b}	41.55 ± 0.20^{a}
Negative control	67.75 ± 16.02^{b}	68.41 ± 12.50^{b}
Positive control	72.40 ± 15.24^{b}	45.79 ± 9.80^{a}
Alginate (200 mg/kg)	$55.80 \pm 3.42^{a,b}$	49.05 ± 20.00^{ab}
Alginate (400 mg/kg)	65.60 ± 14.47^{b}	54.46 ± 11.00^{ab}
Alginate (600 mg/kg)	47.80 ± 5.40^{a}	34.20 ± 7.50^{a}

*Values are means ± SD. Values followed by the different superscript symbol(s) in each column were significantly different ($P<0.05$).

Table 5. Effect of Na alginate of *T. ornata* and *S. crassifolium* on the total cholesterol in alloxan-induced diabetic rats.

to absorb the body through the small intestine so that it eventually comes out with the stool. Suzuki et al. [33] also reported that alginate with various mannuronic acid and guluronic acid compositions can decrease total blood cholesterol levels.

7. HDL-c

Administration of Na alginate to alloxan-induced diabetic rats for 200 mg/kg alginate of *T. ornata* did not show significant differences compared to negative control and positive control ($P > 0.05$) (**Table 6**). The alginate of *T. ornata* at a dose of 200 and 400 mg/kg was not significantly different between each other. All of the various doses of alginate were significantly different compared to normal control ($P < 0.05$). HDL-c management on type 2 diabetes is targeting for >40 mg/dL (>50 mg/dL on female) [1]. HDL particles seem to have antioxidant properties, inhibiting the oxidation of LDL cholesterol and the expression of cellular adhesion molecules and monocyte recruitment. The HDL may also reduce the risk of thrombosis by inhibiting platelet activation and aggregation [33]. Ren et al. [34] reported that three algal species showed the ability to increase HDL-c levels in blood serum of rats. Fucoidan could increase HDL-c levels up to 47% compared to the control group. Five other polysaccharides, sulfated glucuronoxylorhamman, sodium alginate, funoran, porphyran, and agar, found increased HDL-c by 31.97, 28.93, 9.14, 3.55, and 26.90%, respectively.

According to Rohman [36] HDL is a protective lipoprotein, in addition to functioning to bring fat to the liver; HDL proved to inhibit the oxidation of LDL and adhesion molecules. HDL-c levels throughout the treatment group did not have a significant difference. The same is also shown in the study of Suzuki et al. [33] that there was no statistically significant difference in HDL-c levels in mice suffering from hypercholesterolemia treated with sodium alginate in comparison with different glucuronic acid and mannuronic acids.

Group	**HDL-c (mg/dL)***	
	***T. ornata* [8]**	***S. crassifolium* [9]**
Normal control	108.00 ± 6.59^{c}	70.549 ± 1.50^{a}
Negative control	59.75 ± 9.39^{a}	75.549 ± 11.10^{a}
Positive control	58.00 ± 7.78^{a}	96.843 ± 14.10^{a}
Alginate (200 mg/kg)	61.80 ± 5.57^{a}	97.617 ± 11.50^{a}
Alginate (400 mg/kg)	74.80 ± 10.08^{b}	84.03 ± 28.20^{a}
Alginate (600 mg/kg)	78.60 ± 10.60^{b}	75.98 ± 17.70^{a}

*Values are means ± SD. Values followed by the different superscript symbol(s) in each column were significantly different ($P<0.05$).

Table 6. Effect of Na alginate of *T. ornata* and *S. crassifolium* on HDL-c in alloxan-induced diabetic rats.

8. LDL-c

LDL-c after administration of alginate(s) from *T. ornata* (200, 400, 600 mg/kg) was not significantly different between each other. Alginate of *T. ornata* 600 mg/kg showed a significant difference compared to negative control, positive control, and normal control group (**Table 7**). Ren et al. [34] studied the effect of polysaccharide extracts from algae on LDL-c in blood serums of rats given with basal diet for 28 days. The six polysaccharides used in the study decreased LDL-c levels in blood serum. Sodium alginate (1%) decreased 34.04% of LDL-c. Five other polysaccharides, sulfated glucuronoxylorhamman, sodium alginate, funoran, porphyran, and agar, decreased the LDL-c in line with 36.42, 37.66, 24.33, 36, and 14%, respectively, compared to normal control. LDL is not usually increased in diabetes. In part, this may represent a balance of factors that affect LDL production and catabolism. A necessary step in LDL production is hydrolysis of its precursor VLDL by LpL. A reduction can happen in this step because LpL deficiency or excess surface apoproteins (C1, C3, or possibly E) decreases LDL synthesis. Conversely, increases in this lipolytic step that accompany weight loss, fibric acid drug therapy, and treatment of diabetes may increase LDL levels. In diabetes, a reduction in LDL production may be counterbalanced by decreases in LDL receptors and/or the affinity of LDL for those receptors [37].

Administration of sodium alginate from *S. crassifolium* most effective in lowering LDL-c levels near the control group was 600 mg/kg followed by 200 mg/kg and 400 mg/kg. The negative control group had a very significant difference with all the other treatment groups (**Table 7**). The negative control group had higher LDL-c levels when compared to the other treatment groups. Meanwhile, the positive control group had lower LDL-c levels than the other

Group	**LDL-c (mg/dL)***	
	***T. ornata* [8]**	***S. crassifolium* [9]**
Normal control	58.80 ± 7.19^{a}	34.07 ± 0.90^{a}
Negative control	60.75 ± 16.52^{a}	55.34 ± 8.30^{b}
Positive control	65.00 ± 14.05^{a}	27.51 ± 10.00^{a}
Alginate (200 mg/kg)	$49.60 \pm 3.13^{a,b}$	31.81 ± 11.80^{a}
Alginate (400 mg/kg)	$55.60 \pm 13.13^{a,b}$	33.91 ± 5.30^{a}
Alginate (600 mg/kg)	41.00 ± 5.83^{b}	28.78 ± 5.30^{a}

*Values are means ± SD. Values followed by the different superscript symbol(s) in each column were significantly different ($P<0.05$).

Table 7. Effect of Na alginate of *T. ornata* and *S. crassifolium* on LDL-c in alloxan-induced diabetic rats.

treatment groups. Levels of LDL-c in this study are still within normal limits, i.e., <130 mg/dL, and Rachmat and Rasyid [38] reported that mice were given 50 and 250 g of alginates of *S. crassifolium* which also did not affect LDL-c levels.

9. Triglyceride

Triglyceride management on type 2 diabetes is targeting for <150 mg/dL [1]. When the glucose levels excess in the blood, glucose will be converted to triglycerides in which triacylglycerol synthesis process is known as lipogenesis. Carbohydrate-rich meal can lead to increase the process of lipogenesis in the liver and adipose tissue. However, the occurrence of insulin resistance inhibits lipogenesis process making glucose and free fatty acid levels in blood plasma increased. In the liver, triglyceride accumulation can cause malfunctioning of the liver (fatty liver) or liver cirrhosis in the long term [39]. Triglyceride of alloxan-induced diabetic rats did not show a significant difference between the groups of treatment using alginate of *T. ornata*. The triglyceride levels remained at normal levels through the given time of the study (**Table 8**).

Paxman et al. [40] reported that a drink containing alginate in the obese patient had no effect on tryglyceride level. Triglyceride levels did not show a significant difference between alginate treatment group and control group. Ren et al. [34] used six polysaccharides from algal species as a treatment for rats given with basal diet for 28 days. All of the polysaccharides used in this research could reduce triglyceride levels as good as their ability reducing LDL-c in blood serum. Funoran and sulfated glucuronoxylorhamman reduced triglyceride levels between 46 and 64% compared to the control group. Sodium alginate could decrease the

Group	**Triglyceride (mg/dL)***	
	***T. ornata* [8]**	***S. crassifolium* [9]**
Normal control	75.80 ± 10.33[a]	28.73 ± 12.20[a]
Negative control	77.75 ± 20.90[a]	77.73 ± 14.10[b]
Positive control	80.40 ± 13.14[a]	24.31 ± 9.60[a]
Alginate (200 mg/kg)	63.40 ± 25.41[a]	24.12 ± 17.70[a]
Alginate (400 mg/kg)	60.80 ± 13.80[a]	31.73 ± 2.90[a]
Alginate (600 mg/kg)	54.80 ± 10.91[a]	37.67 ± 8.50[a]

*Values are means ± SD. Values followed by the different superscript symbol(s) in each column were significantly different (P<0.05).

Table 8. Effect of Na alginate of *T. ornata* and *S. crassifolium* on the triglyceride in alloxan-induced diabetic rats.

triglyceride level to 29% compared to the control group. Fucoidan can reduce the triglyceride levels to 12–20% [34].

The levels of triglycerides during the experiment using alginate of *S. crassifolium* were decreased. The negative control treatment group had a significant difference when compared to all treatment groups (**Table 8**). This suggests that the three doses of alginate from *S. crassifolium* can lower triglyceride levels equally well with the positive control group that is close to the triglyceride levels of the normal control group.

All groups treated with DM except for the normal control group showed elevated triglyceride levels. Levels of triglycerides increased up to 574.867 mg/dL. The condition of hypertriglyceridemia can be diagnosed if the triglyceride level >150 mg/dL [41]. According to Pujar et al. [42], this can be due to direct damage from the pancreatic tissue by high free fatty acids. The concentration of high free fatty acid will decrease the pH and may activate trypsinogen. Also, high triglyceride levels can also be caused by the destruction of chylomicron which is a triglyceride carrier. This changes the acinar function and opens the pancreatic tissue to triglycerides.

10. Necrosis of pancreas

Necrosis is defined as the type of cell death caused by changing the morphology of the nucleus, including chromatin condensation and fragmentation, minor changes in cytoplasmic organelles, and overall causes of cell shrinkage (apoptosis) and autophagic accumulation of two vacuole membranes in the cytoplasm [43]. In type I diabetes mellitus, patients found changes in the pancreas in the form of the reduced size of the pancreas, atrophy in the exocrine pancreas, and atrophy of the acinar cells around the degenerated Langerhans island. On the other hand, in type II diabetes mellitus, an imbalance of exocrine secretion of the pancreas and impaired control of blood glucose occur [44].

Normal controls show normal cell conditions (**Figure 3**). Negative controls show some damage to the cell. The positive control treatment group also shows the same. The treatment group of sodium alginate extract is entirely damaged in cells (necrosis). The treatment group of *S. crassifolium* dose of alginate at 200 and 400 mg/kg had more damage than the treatment group of 600 mg/kg alginate. The results of the histological analysis showed that all treatment groups experienced cell damage (necrosis) except the normal control group. According to Holemans et al. [45], streptozotocin prevents DNA synthesis in mammals and bacterial cells. In bacterial cells, it provides a special reaction with the cytosine group that causes degeneration and destruction of DNA. This biochemical reaction in mammals causes cell death. Damage to cells in the islets of Langerhans island cells caused by streptozotocin is irreversible. Similar results were also shown in a study conducted by Elias et al. [46] and Ikebukuro et al. [47].

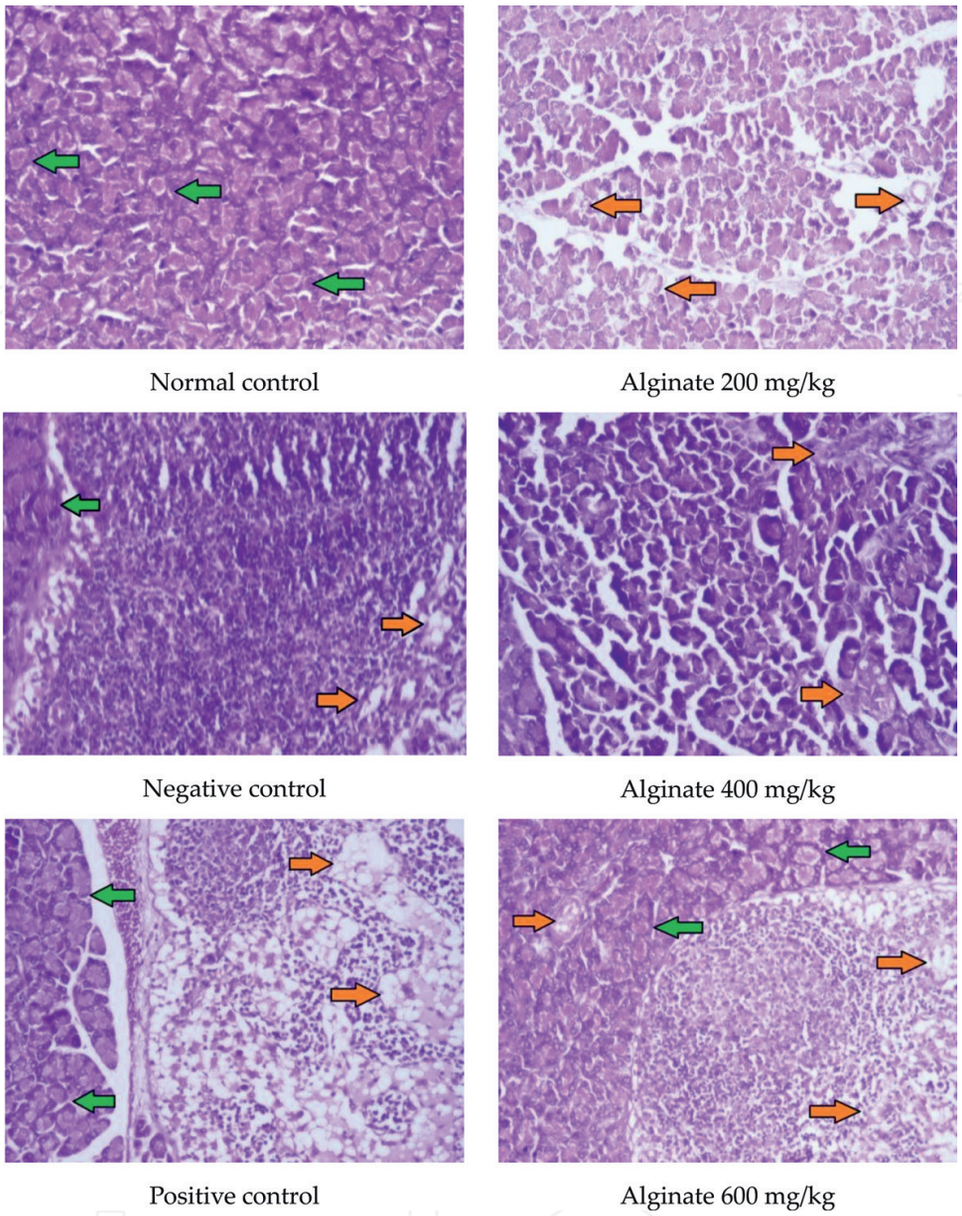

Figure 3. Histological studies of STZ diabetic rat pancreas. Normal control: pancreatic section showed the normal size of islets, and destruction was absent (Grade -). Negative control: pancreatic section showed (green arrow) occasional islets, and (orange arrow) destruction was severe (Grade ++++). Positive control (diabetic rats +5 mg glibenclamide/kg b.w.): pancreatic section showed moderate islet architecture (green arrow), and destruction (orange arrow) was moderate (Grade +++). Diabetic rats +200 mg alginate/kg b.w., and diabetic rats 400 mg alginate/kg b.w.: pancreatic section showed (green arrow) occasional islets, and (orange arrow) destruction was severe (Grade ++++). Diabetic rats +600 mg alginate/kg b.w.: pancreatic section showed (green arrow) additive improvement in the mass of islets as compared to other alginate treatments, and (orange arrow) destructions was mild (Grade ++). Grade −, normal; Grade ++++, severe destruction; Grade +++, moderate destruction; Grade ++, mild injury.

11. Conclusion

Administration of alginate from *T. ornata* in alloxan-induced diabetic rats decreased the preprandial and postprandial blood glucose, lowered total cholesterol, increased HDL-c, and lowered LDL-c in dependent dose manner. However, sodium alginate of *T. ornata* did not show any

effect on triglyceride. This result can be valuable information to discover alternative therapy to achieve and/or maintain glycemic control and lipid profile management on diabetes patient. Nevertheless, the possibility warrants further confirmation. On the other hand, the present study shows that the alginate *S. crassifolium* has potential antidiabetic action in STZ-induced diabetic rats and the effect was found to be more similar to the reference drug glibenclamide.

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Conflict of interest

The authors declare no conflict of interest.

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